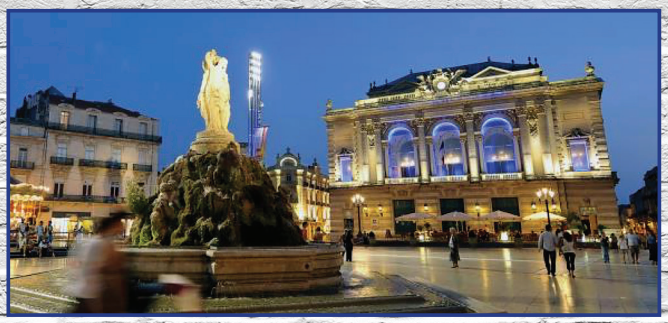
AQUA 2018

#WE R AQUACULTURE

Montpellier France
August 25-29 2018



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Welcome to AQUA18

Dear delegates and guests,

On behalf of the World Aquaculture Society WAS and the European Aquaculture Society EAS I would like to extend to you a warm welcome to AQUA 2018, our joint global aquaculture event organized every 6 years in Europe. This time the conference is taking place in the beautiful French city Montpellier thanks to the generous support of Montpellier Méditerranée Métropole, la Région Occitanie Pyrénées-Méditerranée and the University of Montpellier. A special thank you also to IFREMER in Palavas and in Sête who have been our local partners in the organization of this event and headed up the Local Organising Committee with representatives of research institutes, universities and other organisations in the region.

AQUA 2018 will celebrate the fact that aquaculture is one of the most important food production industries in the world. Aquaculture is the most efficient producer of high quality food and contributes to global health and wealth. Aquaculture respects the environment, creates employment and offers significant investment opportunities. The Steering Committee has therefore chosen the conference slogan ***#We R Aquaculture - We are the producers and investors, the scientists and technical advisors, the legislators and educators, the students, civil society organizations and consumers of farmed aquatic products*** to enshrine our activity and to show our pride in our activity and in our products.

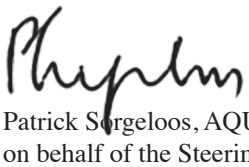
The 2 keynote speakers Robins McIntosh (Executive Vice President of Charoen Pokphand Foods PLC Thailand) and Øyvind Oaland (Global Director R&D and Technical, Marine Harvest ASA, Norway) will review their company's experience in the development of the shrimp and salmon industry respectively.

Our three Programme Co-chairs, Beatrice Chatain of IFREMER, Kjell Inge Reitan of NTNU, Norway and Michael Schwarz of Virginia Tech, USA with the help of the session chairs have organized a very attractive and diversified program covering the latest aquaculture research and technology innovation with more than 600 oral presentations and 300 poster contributions.

The tradeshow with close to 200 exhibitors from all over the world will highlight the continued growth of this exciting food production sector. This is your opportunity to inspect the latest in products and services for the aquaculture industry. I also hope that you will find the time to enjoy Montpellier, the very definition of a Mediterranean city, with its rich historical heritage, idyllic location just a few kilometers from the sea and offering an exquisite cuisine.

Last but not least our sincere thanks to the many companies and organisations that have provided support for the organisation of AQUA 2018: Gold Sponsor Biomar; Silver Sponsor AquaFish Innovation Lab; WAS Premier Sponsors Blue Aqua, Zeigler, Darling/Sonac and Tyson; EAS Premium sponsor Evonik; AQUA 2018 Granting Bodies Montpellier Métropole, La Région Occitanie and MUSE/Investissements D'Avenir; Institutional Sponsors IFREMER, INRA, IRD and CIRAD; Industry sponsors Faivre, Lallemand and Greensea; Session sponsors Phileo LeSaffre and INVE Aquaculture; WAS Gold Sponsors Aquaculture Systems Technologies LLC, Empyreal 75, Pentair Aquatic Eco-Systems and Biorgin; as well as the Media partners Fish Farming Xpert, Fish Farmer, Intrafish, International Aquafeed Magazine, FIS, Engormix, Aquafilia, Aquafeed.com, Hatchery International, Fishing News, Aquaculture Asia Pacific Magazine, MisPeces.com, Industria Acuicola, Panorama Acuicola, Aquaculture Magazine, Aquaculture Africa Magazine, Academic Journal and Efeedlink.com

A warm welcome to the 2500+ participants from all corners of the world. I hope you will enjoy AQUA 2018 and will meet with old and new friends !



Patrick Sorgeloos, AQUA 2018 Conference Chair
on behalf of the Steering and Program Committees

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ABSTRACTS

PROCESSING OF BROWN ALGAE FOR UTILISATION AS ANIMAL AND FISH FEED, AND INTEGRATION OF THE PRODUCTION IN A SEAWEED BIOREFINERY

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Cultivated macroalgae, or seaweed, is a biomass resource with potential applications in food and feed, conversion to commodity chemicals and biofuels, as well as for extraction of high value products for several markets. In Europe, cultivation of seaweed is a new industry. The production volumes are yet low and dominated by *Saccharina latissima* (sugar kelp). So far, the main market is as food. For cultivated seaweed to be significant biomass resource for non-food applications, the production costs should be reduced, in parallel with development of processing technology for production of a spectre of products, with high and low value, and from small to large volumes.

A potential application of seaweed is for fish and animal feed. In the current work, the aim has been to provide knowledge about brown algae, and brown algal protein, as a nutrient source in feed, and to develop technology for production of protein-rich products from *S. latissima*. However, since the protein content in brown algae is low, feed protein cannot be a main product. An added value is required, either as positive health effects of the feed, or by co-production of other components from the biomass in a biorefinery.

The solubility of protein from *S. latissima* has been investigated at different pH, temperature etc, and enzymes have been used to increase the extraction yields. The main conclusion is that the solubility is low (20-40 %), and the extraction yields will thus be even lower. It is therefore more feasible to enrich the protein content by removal of undesired, water-soluble components, in particular salts, than to extract the proteins. By milling and 'washing' of the biomass, the content of water-soluble components was reduced. Large scale processing of 750 kg *S. latissima* has been carried out for production of raw materials for feeding experiments. The red algae *Palmaria palmata* (dulse), with higher protein content, was used for comparison. The protein content was increased from 10 to 20 % of dry weight for *Saccharina* and from 12 to 28 % for *Palmaria*. The salt content was reduced from 44 to 26 % and from 12 to 5 % of dry weight, respectively, for the two species. The iodine content in *Saccharina* was reduced relatively more than the reduction in salt content. The protein-enriched and salt-reduced biomass have been evaluated in feeding trials with monogastric animals and fish.

The process can easily be combined with isolation of water soluble, high-value compounds, and possible flowsheets have been proposed. The acceptable biomass costs for different product combinations in a seaweed biorefinery have been calculated.

GROWTH AND SURVIVAL OF GROWTH HORMONE cDNA TRANSGENIC CHANNEL CATFISH *Ictalurus punctatus* IS INFLUENCED BY CONSTRUCT PROMOTER TYPE, FAMILY LINE AND STRAIN

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We will meet a huge challenge to feed the world in the near future so there is a great need for genetic improvement of culture fishes. Gene transfer considers a potential solution to this problem. The growth hormone (GH) gene from channel catfish, *Ictalurus punctatus*, driven by the ocean pout *Zoarces americanus* antifreeze protein promoter (opAFP), the growth hormone (GH) gene from channel catfish driven by the rainbow trout *Oncorhynchus mykiss* metallothionein promoter (rtMTP), the growth hormone (GH) gene from channel catfish driven by the Common Carp *Cyprinus carpio* □-actin promoter (cc□AP), and the growth hormone (GH) gene from channel catfish driven by the Zebrafish *Danio rerio* elongation factor promoter (ZEFP), were transferred to the channel catfish, *Ictalurus punctatus* via electroporation.

We compared growth and survival of GH transgenic channel catfish (*Ictalurus punctatus)* for three strains and family. Significant differences in body weight were found among strains. The GH transgenic channel catfish grew larger than their non-transgenic siblings. However, in some non-transgenic siblings in one family had the same size of transgenic of another family, perhaps due to the family effect ($P < 0.0001$). So, it is very important to develop the fastest growing we need to combine the GH transgenesis with family selection.

EFFECTS OF DIETARY ORANGE PEEL AS A GROWTH PROMOTER FOR NILE TILAPIA (*Oreochromis niloticus*)

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Sweet orange (*Citrus sinensis*) is a plant member of the Citrus family and principally cultivated in subtropical regions. Citrus mainly used regularly for juice and jam production which bring huge amount of by-product, such as peels. The peels contain a considerable concentration of ascorbic acid much more than the juice, and that the peel additionally contain higher concentrations of active materials. Many studies revealed the positive role of herbs that contain different bioactive components which may affect digestive processes in a positive way by enhancing enzyme activity, improving digestibility of nutrients and food absorption, consequently resulting in enhancing of fish growth.

There are no studies concerning the effects of orange peels on fish particularly as feed additives to promote the growth. Therefore, the aim of the present study was to assess the sweet Citrus peels in terms of growth induction of Nile tilapia fingerlings.

Fish were divided into 4 groups and fed for 60 days with four experimental diets (3 supplemented diets with 1 gkg^{-1}diet (OP1), 2 g kg^{-1}diet (OP2) or 4 g kg^{-1}diet (OP4) of orange peel and a control group). At the end of the experiment, the main growth, feed utilization parameters and proximate body composition were investigated.

The results showed that weight gain (WG%) of fish fed on OP1 and OP2 diets was significantly higher than those fed on other diets ($P \leq 0.05$) (table 1). Moreover, Feed conversion ratio (FCR) was also affected by the feed additive, where FCR in fish fed the orange peel supplemented diets was lower than in fish fed control diet (table 2). It could be concluded that OP can efficiently act as a growth promoter for Nile tilapia fingerlings to a certain limit.

Table (1): Effects of different levels of orange peel on growth performance of Nile tilapia

Treatment	Initial weight	Final weight	Weight Gain
OP1	4.96 ± 0.24^{a}	17.11 ± 0.16^{a}	12.15 ± 0.39^{a}
OP2	5.48 ± 0.015^{a}	17.73 ± 0.43^{a}	12.25 ± 0.43^{a}
OP4	4.99 ± 0.047^{a}	15.64 ± 0.62^{b}	10.65 ± 0.66^{b}
Control	5.21 ± 0.21^{a}	13.90 ± 0.12^{c}	8.69 ± 0.14^{c}

Table (2): Effects of different levels of orange peel on feed utilization of Nile tilapia

Treatment	Feed Intake	FCR	PER
OP1	$17.99\pm.12^{a}$	$1.48\pm.038^{b}$	$2.47\pm.06^{a}$
OP2	$18.38\pm.42^{a}$	$1.50\pm.02^{b}$	$2.34\pm.03^{a}$
Op4	$16.80\pm.608^{a}$	$1.54\pm.08^{b}$	$2.37\pm.12^{a}$
Control	$16.95\pm.85^{a}$	$1.95\pm.129^{a}$	$1.85\pm.12^{b}$

HISTOPATHOLOGICAL IMPLICATION OF ZINC OXIDE NANO-PARTICLES ON THE GILLS OF *Heterobranchus longifilis* AND AMELIORATIVE POTENTIALS OF ASCORBIC ACID

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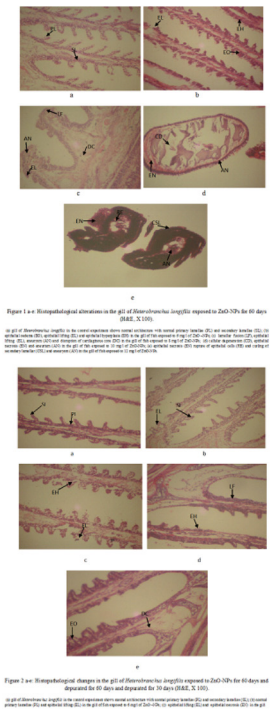
Zinc Oxide nano-particles (ZnO-NPs) are more useful in the production of commercial goods than other nano-particles because of their unique properties. The effluents of ZnO-NPs get into the aquatic ecosystems and accumulate in fish tissues causing serious health consequences. This study was therefore designed to investigate the toxicity of ZnO-NPs on histopathology of gills of *Heterobranchus longifilis*. Juveniles *H. longifilis* were exposed to chronic (6.00, 8.00, 10.00, 12.00 mg/l) concentrations of ZnO-NPs for 60 days. ZnO-NPs-exposed fish were depurated for 30 days to evaluate recovery. Fish were fed on diet supplemented with varying concentrations of ascorbic acid (AA) (50.00, 250.00, 500.00, 1000.00 mg AA/ kg diet) for 30 days to ameliorate the toxicity of ZnO-NPs. After each experiment, fish were sacrificed to remove gills for histopathological assay.

Gills of exposed fish revealed different types of histopathological alterations such as epithelial hyperplasia; lamellar fusion; lamellar disorganization; epithelial lifting; disruption of cartilaginous core; epithelial oedema; aneurysms; rupture of epithelial cell and epithelial necrosis. However, varying degrees of tissue change (DTC) were exhibited in the ZnO-NPs-exposed groups compared to control group, and severity of tissue alterations was concentration-and-time-dependent. The severity of tissue damage increased from slight (13.8) to severe (57.7) as the concentration and exposure period increased (Figure 1 & Table 2). In depurated groups, the severity of damage gradually recovered from severe (57.7) to slight (19.8) after 30 days of depuration (Figure 2 & Table 2), while in the group ameliorated with 500 mg AA/kg diet, the tissue damage were completely reversed (Figure 3 & Table 3) from severe (57.7) to normal (9.3) (Figure 3 & Table 3). The results implied that ZnO-NPs are toxic to *H. longifilis* and recovery under depuration was slow. However, 500 mg/kg of AA was able to ameliorate the pathological lesions induced by ZnO-NPs.



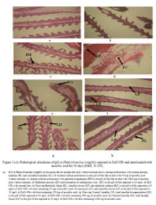


Table 1 - Stages of histopathological alterations in gills of *Heterobranchus longifilis* during exposure to ZnO-NPs, depuration and amelioration periods

Alterations mostly observed	Stages of lesions
Gill	
Epithelia hyperplasia	I
Epithelia lifting	I
Lamellar fusion	I
Lamellar disorganization	I
Curling of secondary lamellae	I
Disruption of cartilaginous core	II
Aneurysm	II
Epithelia oedema	II
Cellular degeneration	II
Rupture of epithelial cell with haemorrhage	II
Epithelia necrosis	III

Stage I- do not alter normal physiology of tissues; stage II- more severe and causing abnormal physiology of tissue; stage III- marked severe and causing irreparable damage

Table 2 - Degree of tissue change in the gills of *Heterobranchus longifilis* exposed to ZnO-NPs for the period of 60 d and depuration period of 30 d

		Concentration (mg/L)				
	Control (0.00)	6.00	8.00	10.00	12.00	
Period	0.00 ± 0.00	5.70 ± 0.66	9.60 ± 0.12	25.40 ± 0.84	31.30 ± 0.12	
	0.00 ± 0.00	9.50 ± 0.08	17.80 ± 0.40	34.80 ± 1.21	46.30 ± 0.58	
	0.00 ± 0.00	21.90 ± 0.06	36.50 ± 0.44	48.80 ± 0.84	57.20 ± 0.28	
- Period	0.00 ± 0.00	36.60 ± 0.64	50.30 ± 0.58	65.40 ± 0.66	78.50 ± 1.15	
	0.00 ± 0.00	27.40 ± 0.88	38.20 ± 0.35	44.60 ± 1.12	52.70 ± 4.88	
	0.00 ± 0.00	5.20 ± 0.01	14.30 ± 0.08	28.20 ± 0.50	31.50 ± 0.06	

Table 3 - Degree of tissue change in the gills of *H. longifilis* exposed to ZnO-NPs and ameliorated with ascorbic acid for the period of 10 days

Group						
A	B	C	D	E	F	G
0.00 ± 0.00	0.00 ± 0.00	8.2 ± 0.81	20.3 ± 1.21	25.4 ± 0.31	22.3 ± 0.39	18.6 ± 1.17
0.00 ± 0.00	0.00 ± 0.00	8.1 ± 0.82	20.2 ± 0.18	32.6 ± 1.16	28.2 ± 0.66	9.3 ± 0.12

WATER QUALITY PARAMETERS OF CATFISH (*Clarias gariepinus)* FED COMMERCIAL PELLETED FEED, SUPPLEMENTED WITH LIVE TILAPIA *(Oreochromis niloticus)* FRY AT 1% AND 2% BODY WEIGHT IN CONCRETE TANKS

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The study was conducted to assess the water quality parameters of catfish (*Clarias gariepinus*) fingerlings fed pelleted feed supplemented with live tilapia *(Oreochromis niloticus*) fry in concrete tanks. Nine hundred (900) catfish (*Clarias gariepinus*) fingerlings of mean weight 9.68+0.25g were stocked in ninety (90) units of concrete tanks at the rate of ten fingerlings/unit. The fingerlings were fed within two (2) groups of commercial pelleted feed at the rate of 0.5%, and 1%, and were replicated three times. Within each pelleted feed group, three treatment levels were established at the rate of 0.5%, 1% and 1.5% (T_1, T_2, and T_3) were fed with live tilapia (*O. niloticus)*. A control (T_0) was also setup where no tilapia fry was administered. Water exchange was continuous at a rate that replaced the entire volume within seven (7) days. The results obtained showed that dissolved oxygen (DO) ranged between 7.55±1.19mg/l to 5.77±1.25mg/l (T_0 and T_3) for catfish fed at 1% pelleted feed group and 7.13±0.17mg/l to 6.06±2.31mg/l (T_0 and T_3) for catfish fed at 2% pelleted feed group. Temperature values ranged from 29.52±2.17^{0}C to 26.99±4.64^{0}C and 29.33±4.18^{0}C to 28.42±5.64^{0}C (T_0 and T_3), for catfish fed at both 1% and 2% pelleted feed group.

Hydrogen ion concentration (pH) and Ammonia ranged between 8.13±0.44 to 7.59±1.87, 8.28±2.14 to 7.59±1.87 and between 1.011 mg/l ±0.01 to 0.13 mg/l ±0.02; 1.012 mg/l ±0.03 to 0.013±0.02 (T_0 and T_3), for pelleted feed groups of 1% and 2% respectively.

This study however reveals that the inclusion of live tilapia fry supplemented with commercial pelleted feed in the diet of *C. gariepinus* had no effect on water quality parameters during catfish culture.

PLANNING WAVE AND CURRENT MEASUREMENTS AT AQUACULTURE SITES IN EXPOSED AREAS

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The lack of sheltered aquaculture areas has motivated operators to test more exposed aquaculture areas, one of these is the bay Sandsvág in the Faroe Islands, where a test with Atlantic salmon is preformed. The bay is relatively open to incoming ocean waves; the tidal currents are limited. After a series of storms a high mortality rate occurred. It turned out, that the reason was strong currents generated by breaking waves.

The test site had two cages of the type Aqualine Midgaard System, equipped with heavy sinker rings. The measuring program included a waverider buoy near the cages, which measured waves and current, bottom mounted ADCPs, which measured current, pressure tags on the cage, which measured the lifting of the net and the sinker collar, and four pressure tags in a line on the bottom, which measured the waves, and how they changes inwards the bay.

The measurements showed that the significant waveheight was limited to around 4m, regardless of the the height of the oceanwaves. The current measurements during the planning phase was averaged over 10 minutes, and did not show dangerous high currents.

During the test, it was observed that the bottom of cage was lifted up during storms, and that this lifting occurred very quickly. The current measurements were then changed to average over only 2 minutes, and this revealed that the current in short intervals reached at least 1.1 m/s. Combined with high waves this lifted up the bottom of the cage, and damaged the skin of fish, which led to the high mortality.

The farming area is sheltered from the ocean waves by a headland and a shallow area in front of the headland. This is limiting the waves, but in the same time the breaking of the waves in in the shallow area in front of the headland transport water into the bay and generated the strong current.

In exposed areas shelter can often be found behind shallow areas. Moving the farming site inwards will reduce the wave, but care has to be taken not to place the farming site in location with strong wave generated currents. Finding the best location can be a challenge, because the phenomena only occur in severe storms, making it impossible to make on location measurements. In planning a new exposed site, measuring tidal currents or averaging the current over several minutes is not enough. A very detailed measurement of the current is necessary to detect the rip currents, which only occur in severe storms. The importance of understanding the phenomena is fast growing, as the aquaculture industry is considering more and more exposed areas.

The presentation will show the planning and results of measurements at Sandsvág, and the planning of measurements at a new test site Víkar, both in the Faroe Islands.

SPECIFIC FEATURES OF LIPID METABOLISM IN YOUNG BESTER DURING THE LPO (THE PROCESS OF LIPID PEROXIDATION) ACTIVATION

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Modern methods of artificial reproduction, with all their positive technological possibilities allowing one to control and regulate the quality of habitats and feeding of hydrobionts, as well as to carry out preventive and curative measures, do not eliminate but in some cases even exacerbate the problems associated with fish health. It is contingent on various endogenous and exogenous stress factors that might induce manifestation of some nonspecific symptoms of damage. One of these is the process of free radical oxidation in the body and associated processes of lipid peroxidation (LPO), uncontrolled amplification of which is the cause of many diseases. At the same time, against the background of the hydroperoxides' increase in the body, the antioxidant protection of an organism decreases, however, energy expenditures become greater.

We have considered changes in the ratio of phospholipids to triacylglycerols (P/TAG), cholesterol to phospholipids (C/P ratio), phosphotidylcholines to phosphotidylethanolcholines (PC/PEC) in young bester cultivated in flowing-water tanks.

The increased LPO activity in fish is caused by disorder in symbiotic nutrition and by ammonia toxicity of exogenous and endogenous origin due to the disruption of normal nitrification processes under high temperature conditions. Despite the different nature of stresses, the reaction of the organism was identical pertaining to the character of lipid metabolism (Fig. 1).

Thus, in both cases, the value of P/TAG decreased in comparison with physiologically healthy fish by 43.2% and 56.8%, PC/PEC lowered by 36.0% and 42.2%, and C/P increased by 42.4%-70.0%.

The decrease in P/TAG is probably due to the higher synthesis of TAG requiring less energy, which compensates for the lack of energy reserves necessary for the phospholipid synthesis. An increase in C/P may indicate reduced strength of the cell membrane and the possible destruction of body tissues. A decrease in the value of PC/PEC gives evidence of an increased utilization of PC and a lower rate of PEC oxidation, which inevitably entails the LPO activation.

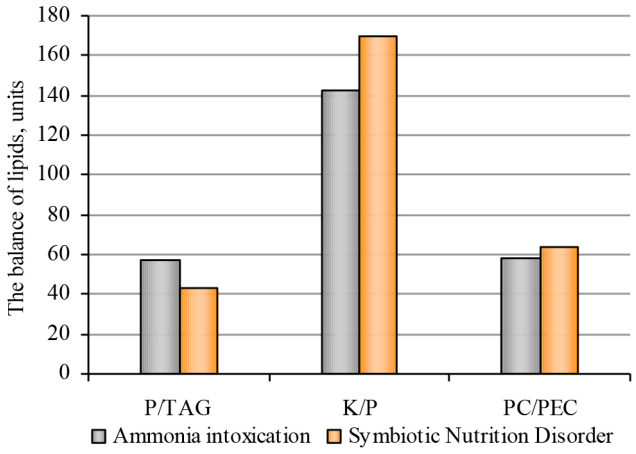

Fig.1. Lipid metabolism under stress

EFFICACY OF NANO-ADJUVANTED IMMERSION STREPTOCOCCUS VACCINE IN NILE TILAPIA (*Oreochromis niloticus*) FINGERLINGS

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The objective of this study was to evaluate the efficacy and potency of (ME-VAC Aqua Strept II) ®; Inactivated polyvalent vaccine against *Streptococcus agalactiae, Lactococcus gravieae and Enterococcus faecalis*. The vaccine was formulated with nano-adjuvant designed precisely to be used for immersion vaccination of fingerlings.

A total of 1000 apparently healthy Nile tilapia (*O. niloticus*) with an average body weight of 20 ± 2gm were randomly distributed in 10 tanks, 100L capacity. To formulate the vaccine, an equal volume (50/50) of antigenic aqueous medium was mixed with adjuvant at room temperature under moderate agitation, as recommended by adjuvant manufactures.

In a separate tank, one liter of the vaccine was mixed with 9L water to vaccinate Five hundred fish under aeration. Fish were immersed in the vaccine bath using a knot-less hand net for 5 min then returned to the rearing tanks. 500 fish were used as control none vaccinated fish. Antibody titration was determined every two weeks post vaccination for two successive months. Relative percent of survivability (RPS) was determined every month by challenging fish groups against *S. agalactiae, L. gravieae and E. faecalis*. SPSS version 21 was used to analyze the data. Results were expressed as mean ± SE. Un-paired one-way ANOVA was used to test for significant differences among groups at $p \leq 0.05$. Post hoc test (Tukey's) was used for pairwise comparisons. All non-vaccinated fish challenged with *Streptococcus* species showed more than 60% mortality. Protection against Streptococcus species was associated with antibody titers (> 4 log2) that lasted for 8 weeks post vaccination and mortality % not exceed 35%

Figure 1: *O. niloticus* showing unilateral exophthalmia and cataract after infection with *S. agalactiae*

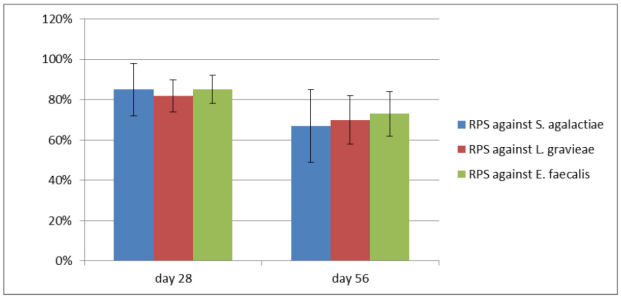

Figure 2: RPS of vaccinated *O. niloticus* after challeng with *S. agalactiae, L. garvieae* and *E. faecalis*

Table 1: Agglutination titers ($\log_2$ (x+1) of Nile tilapia vaccinated with inactivated *Streptococcus* immersion vaccine 8 weeks post vaccination.

Time	Vaccinated group		
	S. agalactiae	*L. gravieae*	*E. faecalis*
Log2 antibody titer 2w	7.4 ± 0.3	7.3± 0.29	7.9± 0.26
Log2 antibody titer 4w	7.3 ± 0.29	7.00 ± 0.22	7.8 ± 0.29
Log2 antibody titer 6w	6.00 ± 0.3	6.3 ± 0.26	6.4 ± 0.2
Log2 antibody titer 8w	5.3± 0.3	5.6 ± 0.3	6.0± 0.3

GREENSHELL™ MUSSEL *Perna canaliculus* SPERM CRYOPRESERVATION: APPLICATION IN SELECTIVE BREEDING

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With over 100,000 t of harvested product (representing a total export value of NZ$ 260 million in exports), the Greenshell™ mussel (*Perna canaliculus*) is the main shellfish species farmed in New Zealand (Aquaculture New Zealand, 2011). Until recently, the mussel industry relied upon wild-caught spat but research and commercialization of hatchery technologies is enabling reliable production of selectively-bred hatchery-produced spat.

Cryopreservation of bivalve gametes can be a powerful tool in selective breeding programmes as it gives breeders more flexibility (i.e., make parental crosses on demand or gene banking) allowing faster genetic gains. It also manages the biological and commercial risks associated with selective breeding such as broodstock losses through disease or human error and changes in breeding programme direction.

Here, we present the recent results obtained from a series of experiments conducted as part of our cryopreservation method optimisation for Greenshell™ mussel (*Perna canaliculus*) sperm. Using fertilisation and early D-larvae assays, different cryoprotectant (CPA) combinations and the use of antioxidants (e.g. EDTA) were investigated. In addition, post-thaw procedures (e.g. sperm:egg ratio, egg density, contact time) were refined, and post-thaw fertility associated with inter-individual variability was further studied. Optimal CPA combinations and fertilization conditions were determined. Overall, these refinements yielded high post-thawing fertility. In general, between 100x to 300x more sperm were required to achieve an equivalent level of fertilization to that of fresh sperm (Fig. 1). D-larval yields resulting from post-thawed sperm varied considerably (0.5 to 97% of controls; Fig. 2). Nonetheless, given that tanks are generally stocked with 500 000 to 1 M D-larvae per cross, sufficient numbers were obtained for selective breeding purposes from most of the males evaluated and the proposed cryopreservation method can therefore be implemented.

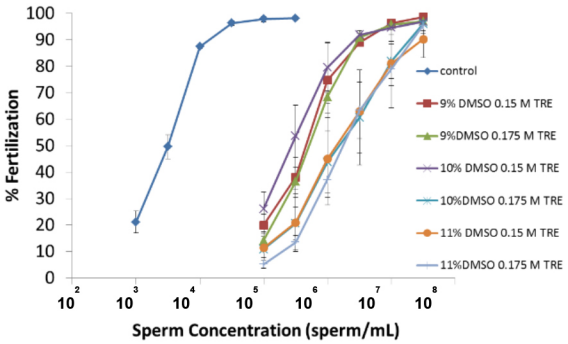

Figure 1. Effect of different cryoprotectant solutions on post-thaw fertility of Greenshell™ mussel sperm (n=3 separate pools of sperm from several males). Legend shows final concentrations of dimethyl sulphoxide (DMSO) and trehalose (TRE) used during freezing.

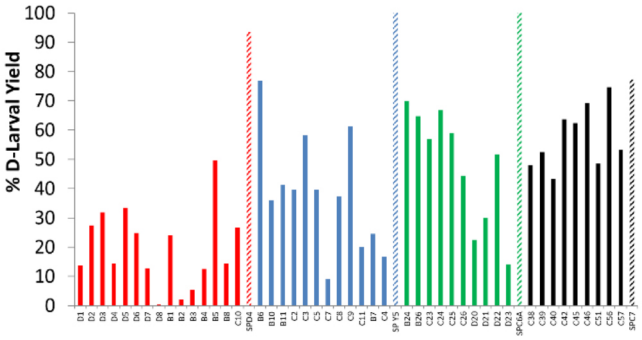

Figure 2. D-larval yields produced with cryopreserved sperm from individual males (solid bars). Sperm were thawed on 4 separate occasions. Fresh controls for each occasion are shown as stripped bars of same colour.

EFFICIENCY AND SELECTIVITY OF GILLNET MESH SIZES FOR ASSESSING FISH COMPOSITION OF EGBE WATER RESERVOIR, EKITI STATE, NIGERIA

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Comparism of five (5) different gillnet mesh sizes ranging from 50.8mm, 63.5mm, 78.2mm, 88.9mm, and 101.6mm was done at four (4) different sampling stations along the banks of the reservoir to evaluate the efficiency and selectivity of gillnet mesh sizes for assessing fish composition in Egbe water reservoir, between April, 2014 and January, 2016, they were designated as A (Odode), B (Church), C (Ayepe) and D (Dawodu). The nets were set at 6.00pm and hauling done in the morning at 8.00am, fishermen were well monitored to avoid sampling bias and ensure a constant fishing effort. The fishes were sorted into separate container based on gillnet mesh sizes and also species. The total length and weight of individual were taken, Simpson's index, Simpson reciprocal index and Evenness were determined. The percentage composition by biomass fish landing was recorded. 31,905 fishes belonging to 7 species and 5 families were recorded. The most abundant family was the Cichlids represented by 3 major species, *Oreochromis niloticus*, *Coptodon zillii*, and *Tilapia guineensis*. All the net types captured all the fish species but the smallest gill net mesh size (50.8mm) captured a greater number than others, this shows that the smaller bar mesh nets were more efficient for capturing more fishes than other graded mesh nets, but should be discouraged for artisanal fishing activities in the study area, other gears like hook and line, cast nets and traps should be encouraged.

INSECT PROTEIN MEAL AS ALTERNATE PROTEIN SOURCE IN THE DIETS OF AFRICAN CATFISH *Clarias gariepinus*

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A study was carried out to evaluate the effects of black soldier fly larval protein meal (BSF) in the diets of African catfish (*Clarias gariepinus*). The catfish (2.67±0.0 g fish^{-1}, 30 34.8L tank^{-1}) were fed to apparent satiation with 0% IM diet (containing 0% BSF), 25% IM diet (containing 25% BSF), 50% IM diet (containing 50% BSF) or 100% IM diet (containing 100% BSF). After 42 days, there were no significant differences ($P > 0.05$) in the growth performance of catfish fed 0% IM, 25%IM and 50%IM diets. However, the catfish fed 100% IM diet displayed significantly ($P < 0.05$) lower performance than others. The somatic indices and survival of the fish showed no significant difference ($P > 0.05$). This study indicates that BSF (up to 50% inclusion level) can be included in the diets of African catfish without any adverse effect on the fish growth performance, survival and health.

UTILIZATION OF DIFFERENT PORTIONS OF CASSAVA ROOT AS ENERGY SOURCE IN THE DIET OF THE NILE TILAPIA, *Oreochromis niloticus*

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This investigation was done to determine the effects of substituting maize with bfc sweet cassava (*Manihot palmata*) root meal portions, as energy sources, on the growth, nutrient utilization and haematological parameters of *Oreochromis niloticus* fingerlings. Five iso-caloric and iso-nitrogenous diets were formulated containing the maize only as energy source, and cassava peel, tuber, starch, and chaff meals as replacements for maize meal respectively. The dietary treatments were tagged, control, peel, tuber, starch, and chaff respectively. The experimental fish were distributed randomly in a completely randomized design into 15 rectangular glass tanks (60 x 35 x 30 cm^3) at a density of 20 fish per tank representing five dietary treatments. Fish were fed at 3% of their body weight per day in two equal meals at 0800-1000 and1700-1800hr. The meals and fish carcass biochemistry, fish growth performance and haematological parameters were determined accordingly.

Results showed that the crude protein (0.42±0.07) of the chaff meal was significantly lower ($p<0.05$) than the other cassava based meals, while the peel meal had the highest crude protein (6.84±0.01) and energy (383.64 ±0.04) content. The highest specific growth rate (0.39 ±0.01%/d) and weight gain (8.98 ±0.20), comparable to the control diet, were recorded in *O. niloticus* fed peel diet while the lowest (0.30 ±0.01; 1.61 ±0.15) was observed in fish fed tuber diet. The least FCR, which favourably compares with the control, was recorded for the peel diet. However, these growth indices were not significantly different ($p>0.05$) from one another. Haematological parameters indicated that the PCV, RBC and haemoglobin of the fish fed starch diet were significantly higher ($P<0.05$) than the control and the other diets. The WBC and neutrophils of the fish fed the peel diet were significantly higher ($P<0.05$) than the control and the other diets.

The positive growth response of tilapia to cassava peel diet could be attributed to its high gross energy, crude protein and lipid as well as ready acceptability and utilization by the fish. Further processing of the cassava peel may reduce or completely remove all inherent anti-nutritional factors to improve the health of the fish.

Table 1: The biochemical composition of the carcass of the fish fed the different diets

Parameters		Experimental Diets			
	Control	Peel	Tuber	Starch	Chaff
Ash	18.36^{a}	19.63^{a}	17.71^{a}	21.78^{a}	17.85^{a}
Moisture	2.80^{a}	2.63^{a}	2.14^{a}	2.09^{a}	4.97^{b}
Crude Lipid	13.73^{a}	14.16^{a}	13.40^{a}	15.91^{a}	14.67^{a}
Crude Protein	46.16^{c}	40.18^{a}	43.21^{b}	47.79^{c}	45.03^{ab}
NFE	18.95bc	23.41c	23.54^{c}	12.42^{a}	17.48^{ab}

Mean ± S.E with different superscript are significantly different from each other ($p<0.05$)

NFE = Nitrogen Free Extract

Table 2: Haematological parameters of the fish fed the various diets

Parameters		Experimental Diets			
	Control	Peel	Tuber	Starch	Chaff
PCV (%)	26.33^{ab}	22.33^{a}	24.00^{ab}	31.00^{b}	19.33^{a}
Hb (g/100ml)	8.71^{c}	7.37^{a}	8.01^{b}	10.31^{e}	9.31^{d}
WBC ($x10^6/\mu L$)	7.8^{a}	9.6^{e}	5.4^{e}	6.7^{d}	8.8^{b}
RBC ($x10^6/\mu L$)	2.85^{c}	2.41^{a}	2.61^{b}	3.63^{e}	0.07^{d}
Neutrophil ($x10^6/\mu$L	62.00^{c}	70.33^{e}	68.33^{d}	60.33^{b}	57.00^{a}

Means with different superscript are significantly different from each other ($p<0.05$). PCV = Pack Cell Volume; WBC = White Blood Cell; RBC = Red Blood Cell, Hb=haemoglobin

REDUCING FLOOD- WASHED DEBRIS INFLOW INTO INLAND AND MARINE ECOSYSTEM FOR IMPROVED AQUATIC ENVIRONMENT

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The presence of plastics and other organic wastes from municipal wastes carried in storm flow and flood water is a growing global concern and major threat to fish and other aquatic lives. The proliferation of *Eichornia crassipes* (Water hyacinth, WH) a prominent waterway weed in aquatic bodies not only reduce available nutrients to aquatic lives but economically reduce navigation and fishing activities. The need for engineering and scientific control of such water-borne pollutants is the focus of this paper.

Production of particle boards and organic fibres from harvested WH was modeled and tested using engineering principles in a recent study. The WH collected were washed, weighed and shredded and air dried. Varying concentrations (10%, 20%, 30%) of organic starch from cassava and maize were prepared and used as organic binding material for the fibres derived from the WH and pressed in a mould. The extracted board was then properly dried and tested for its tensile strength using tria-axial machine, and water absorption and swelling for exposure periods of one day and three days to simulate flood situation when used internally as partition boards or ceiling materials. For the harvesting of WH and other floating materials such as plastic wastes, hydraulic flow analysis on selected materials were modeled for the purpose of designing appropriate process for removal of such debris from water bodies for recycling or disposal.

The results showed good quality board was made from the 20% maize starch and the board samples had negligible swell that returned to initial shape even in 3-day soaking. This recommends it to internal use only where water exposure is limited. The von Karman constants for both plastic wastes and WH are within the 50% and 100% suspended flow range. These results recommend the design of a combination of *groynes* and sluice gates as hydraulic structures for diversion and deposit of the debris to the river bank from where the materials could be mechanically or manually harvested for final processing. While the harvested plastic wastes could be taken to a recycling plant, the WH would be taken for valorization into fibres or used as substrate feed material in a biogas digester plant for producing cooking gas and organic fertilizer. The laboratory scale results favor development of a full scale prototype for protecting the aquatic environment and improving the sanitation levels and socio-economic lives of riverine communities.

BURBOT LARVICULTURE: TO ENRICH OR NOT?

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Introduction

The enrichment of *Artemia* has a lot of benefits but is also more laborious and costly, especially in larviculture where there is a long period of *Artemia* feeding. This is the case for burbot, *Lota lota*, where almost 60 days of *Artemia* feeding is performed. In a previous trial (Adriaen et al. 2013) we observed that larvae receiving only non-enriched *Artemia* Instar I nauplii, but at a higher number per larvae, were larger and heavier than larvae receiving enriched *Artemia* instar II. However, larvae of latter treatment showed higher survival after weaning. The hypotesis for these results was the possible influence of the number of preys that were available for the larvae rather than the type of prey. A more elaborated trial, including a salinity stresstest, was set up to investigates these differences.

Materials and Methods

Burbot larvae of 45 days after hatching (DAH) (0.0025 mg; 11,7 mm) were stocked at random at a density of 27.5 larvae per liter over twelve experimental eight liter zugler-bottles connected to a recirculating aquaculture system. Four feeding protocols (E1; E1.5; N1.5; N2.5;) were applied in triple. At the start of the trial all larvae were fed 50% *Artemia* Instar I nauplii and 50% *Artemia* instar II enriched with Easy Dry Selco (Inve Technolgies). Three days after the start, larvae of treatments N1.5 and N2.5 where only given *Artemia* Instar I nauplii and larvae of treatments E1 and E1.5 were given enriched *Artemia* instar II. Treatment E1 is considered the control and the other treatments received 1.5 or 2.5 times the number of *Artemia* gift of the control (E1). Larvae were fed manually three times a day. Before feeding, dead larvae were removed and the amount of feed given adjusted to the number of larvae. All larvae were weaned after 28 days of experimental rearing (76 DAH), without a co-feeding phase; *Artemia* supplementation was stopped on the same day artificial feed was introduced. Larvae received 200-300μm dry feed (Aglonorse) during two weeks after which the trial was ended. Water temperature was maintained around 16°C during experimental rearing and this under constant light conditions. Mortality was recorded every day. During the trial larvae samples were collected on four specific dates for biometric analyses: before stocking, after 10, 20, 27 days and at the end of the trial (41 days). For each sample, fifteen larvae per tank were used to measure length, wet and dry weight. At the end of the trial all larvae were graded per replica. One day before weaning of the larvae a salinity stress test based on Wolnicky et al. (2011) was performed with 12 larvae/replica.

	A	A1,5	N1,5	N2,5
DW (mg)	6,38+1,02 (a)	8,42+1,61 (a,b)	9,22+0,83 (b,c)	11,56+2,20 (c)
WW (mg)	44,4+1,5 (a)	59,1+1,6 (a,b)	64,5+0,8 (b,c)	83,4+2,2 (c)
L(mm)	17,14+1,97 (a)	19,35+2,21 (a)	20,69+1,91 (b)	23,52+2,18 (c)
survival (%)	44,1+2,7 (a,b)	63,7+8,7 (a)	29,7+2,0 (b)	41,0+4,8 (a,b)

Results

Table I shows the results obtained for biometric parameters measured at the end of the trial. The average calculated percentage of survival per treatment is also presented. Different superscript are statistically different.

Discussion and Conclusion

At the end of the trial larvae fed with only *Artemia* Instar I nauplii where larger and heavier than those fed with enriched *Artemia*. However survival was significantly higher for those fed with enriched *Artemia*. Grading results showed a higher percentage of big larvae in the N-treatments indicating a size shift in the larval population that could be related to prey size. There was no significant differences to be found in the salinity stress test. These results will probably persuade farmers to use enriched *Artemia* as the gain in survival will compensate for the extra costs of enrichment.

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TWO-GENERATION SELECTIVE BREEDING PROGRAM FOR PRAWN GENDER SPECIFIC GROWTH

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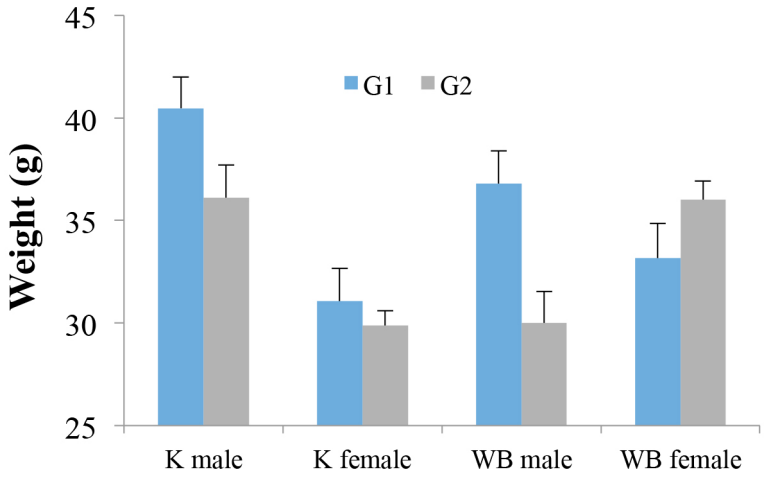
Culture of monosex giant freshwater prawn (*Macrobrachium rosenbergii*) presents one of the most promising avenues for increasing yield and income. Recently, sex reversal technologies, realized through androgenic gland (AG) manipulation, were introduced as a source of all-male and all-female producing broodstocks. For both, genetic improvement via a breeding program and prevention of inbreeding are needed to ensure the sustainability of such technologies. The current study is set for two consecutive generations (G1 and G2) to test the potential and as a preparatory step towards a long-term breeding program. The study is based on promising results obtained in a diallel experiment held in India showing highest growth performance of the West Bengal and Kerala strains and pointing on their genetic potential for a selective breeding program.

The above two wild strains of prawns originating from geographically isolated locations in India Kerala (K) and West Bengal (WB) were assessed through a comparative evaluation of growth performance and population structure. The prawns were stocked in earthen ponds with offspring representing different families. Prawns were stocked in 4 replicates for 200 days of culture at a density of 4 prawn/m^2. At the end of growout period, biometric data were recorded from each of the harvested prawn and included: sex, weight, body length, male morphotypes and female reproductive stages. 20% of each pond, representing the best performing males and females were selected (~185 ind.) as potential brooders for the second generation.

In G1, significant higher weights were observed in males of both strains (K and WB) compared to females of the same strain while in G2 WB strain showed opposite growth pattern in which females showed higher weight compared to the males of the same strain. These preliminary results pave the way toward a tailored selective breeding program favoring improved performances of an all-female strain suitable for intensified aquaculture practices.



STUDIES ON THE BEHAVIOUR OF *Neocaridina davidi* VAR RED WHEN EXPOSED TO DIFFERENT COLORED LIGHT SPECTRUMS

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Freshwater shrimp farming has become a highly valuable business, with Asian countries being the biggest producers. Almost all ornamental shrimp are produced from aquaculture. Guaranteeing the well-being of these animals has the direct consequence of increasing production.

Two experiments were elaborated with the objective of studying the behaviour of Neocaridina davidi var. red when exposed to different coloured light spectrums.

In the first experiment, adult shrimp Neocaridina davidi var. red (n=120) were distributed in four tanks. Changes in behaviour when exposed to different colored light spectrums were studied during four weeks. Each of these tanks contained a group of fifteen shrimp and a different coloured LED light spectrum, white, blue, red and green. Every week different groups of fifteen shrimp were placed in each tank.

In the second experiment, adult shrimp Neocaridina davidi var. red (n=6) were distributed in four tanks. Changes in behavior when exposed to different colored light spectrums were studied during twenty days. Each of these tanks contained a group of fifteen shrimp and a different coloured LED light spectrum, white, blue, red and green. Every five days the LED lights were changed in each tank and the group of shrimp remained the same.

Significant differences ($p<0,05$) were found between the mean number of shrimp observed in each light spectrum used in both experiments. These results indicate that the light spectrum used influences Neocaridina davidi var. red's behavior, having preference for a light in the visible spectrum corresponding to the colour red.

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PREVALENCE OF *PERKINSIOSIS* IN THE CLAMS *Ruditapes decussatus* AND *Ruditapes philippinarum* IN PORTUGAL

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The culture of bivalve molluscs is an activity with high expression in Portuguese aquaculture. It represents 55% of whole production, being the main species clams, oysters and mussels. Of the total produced, about 24% corresponds to the production of clams.

The European clam, *Ruditapes decussatus*, is the species with the major economic importance in Portuguese aquaculture. However in recent years the production has been decreasing associated with several factors, namely overfishing and diseases like the Perkinsiosis which has been associated with high mortality rates.

To face the decrease of *R. decussatus*, producers started to substitute this clam for another one from the same genus, the Japanese clam, *Ruditapes phillippinarum*, which appears to have more resistance to diseases and greater growing rates. Nevertheless the fast and overwhelming expansion of the Japanese clam in Portuguese waters, the native clam stills being commercially more important and appreciated than the Japanese clam, mainly because its quality and market prices.

In order to study the impact of the *Perkinsiosis* in clams production we collected samples of the two species from different estuaries and lagoons of Portuguese coast. To survey the presence of the pathogen, *Perkinsus olseni,* we use as main diagnostic methods, histopathology and RFTM test (Ray's fluid thioglycolate médium). Samples were collected in October 2017 and April 2018 from 12 different sites with an n=25 for each sampling corresponding to a total of 300 specimens examined.

Preliminary results showed that the major prevalence of *Perkinsiosis* correspond to the European clam produced in Formosa lagoon (Olhão). The Japanese clam is also affected by this parasite, although with a lighter level of infection than the other species. Our results, also suggest that to diagnose *Perkinsiosis*, the RFTM test should always be complemented by the analyses of histological slides or molecular biology tools, especially in animals with lighter or in early stages of the infection.

Taking in account the environmental conditions in the different sites it is clear that environmental stress caused by high salinities and water temperatures, low oxygen concentration in sediments, high densities and physiological stress due to spawning season, contributing to increase the prevalence and the spread of the disease.

This work was supported by SNMB-MONIT," Sistema Nacional de Monitorização de Moluscos Bivalves", 16.02.01-FMP-0043

PRESENCE OF THE OSTREID HERPSVIRUS IN PORTUGUESE OYSTER, *Crassostrea angulata,* IN SADO ESTUARY AND IN MIRA RIVER, PORTUGAL

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The first mortality outbreak observed in Portugal, occurred in populations of Pacific oyster, *Crassostrea gigas*, related with the presence of a herpes-like virus, Ostreid Herpsvirus (OsHV-1), reported during the summer of 2012 in an off shore long line culture systems, settled near S. Vicente Cape, in south coast of Portugal. Associated with high mortality rates, was detected for the first time in 2011, the presence of the µvar genotype of OsHV-1 in *C. angulata* produced from a broodstock collected in Sado River and then transferred to the Formosa Lagoon (Batista et al., 2015).

The presence of OsHV-1 in wild and farmed populations of the Portuguese oyster in Sado estuary (n=131) and Mira River (n=150) were surveyed from June 2017 until May2018. Following the protocol set out in Regulation (EC) No 175/20 of 10 March 2, Part B for the DNA extraction method and PCR analysis, we detected the presence of that pathogen in Portuguese waters, since 2010. The tissue samples for histopatholgy were fixed in Davidson's fixative for 48h, dehydration, embedding in paraffin and cutting with a microtome in sections less than 5 μm thick, than stained with Hematoxylin and Eosin (H&E).

In wild and farmed populations of the Portuguese oyster, *C. angulata,* in Sado estuary the presence of the virus was present in low prevalence (10%) and without any mortality episode associated with. However, at Mira River in wild beds of Portuguese oyster and farmed populations the virus was detected with a high positive prevalence (60%), associated with mortalities reported by farmers. The overall appearance of the soft tissues in most of the illness specimens shows a poor flesh condition, a light cream and watery color and a discoloration of the digestive gland. The pathological picture, in terms of histopathological observations, can be described highlighting the following lesions: large areas of the digestive gland diverticula epithelia are heavily affected showing important cellular alterations; hypertrophic cells with intense vacuolisation and, membrane disruptions are present in a large number of ducts and tubules; the nuclear alterations in shape and size are also dramatic. All this lesions suggests an acute necrotic process, affecting also, but no so dramatically, the gills epithelium and connective tissue. The hemocitosis has however a reduced expression. In the most affected tissues, both in digestive gland and gills, the presence of intracytoplasmic inclusion bodies, densely coloured, is also frequent.

The identification, the characterization and the registration of pathologic processes in oysters constitutes important measures for sanitary control. Furthermore, the high prevalence of Ostreid Herpsvirus (OsHV -1 μvar) in *Crassostrea angulata* from Mira River and its low presence in Sado estuary, suggests specificity in relation to environmental factors that plays an important role in the spread of the virus.

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

HOW DIETARY PHOSPHOLIPIDS EFFECT THE GROWTH PERFORMANCE, BLOOD CHEMISTRY AND IMMUNITY IN JUVENILE STELLATE STURGEON (*Acipenser stellatus*)

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Lipids play an important role in the immune system. Among lipid components, phospholipids (PL) are important components for maintaining the structure and function of cellular membranes, emulsifying lipids in the gut and improving intestinal absorption of long chain fatty acids. Phospholipids are a source of fatty acids for the synthesis of eicosanoids, a wide range of bioactive compounds with multiple functions.

During this research an eleven weeks feeding trial was conducted to determine the effects of different levels of dietary soybean lecithin, as an important sources of phospholipids, on growth performance, blood chemistry and immunity in juvenile stellate sturgeon (*Acipenser stellatus*). Fish were fed seven isoproteic (44% crude protein) and isolipidic (17% crude fat) diets containing graded levels of soybean lecithin (SBL) levels: 0 (control), 1, 2, 4, 6, 8 and 10%.

Results showed that dietary SBL supplementation significantly improved the final body weight (BW) and weight gain (WG). Fish fed 6% SBL showed the highest BW and WG values in comparison to fish fed the control diet ($P < 0.05$). Significantly improved humoral immune system; alternative complement, serum lysozyme activity and serum total antibody were observed in fish fed 8, 7 and 6.5% SBL compared to control, whereas highest bactericidal activity and phagocytosis rate were detected in fish fed 8% SBL ($P < 0.05$). Red blood cells, hemoglobin and hematocrit levels increased with increasing dietary SBL levels, especially in those sturgeons fed the diet with 6% SBL ($P < 0.05$). In addition, white blood cell counts significantly increased as dietary SBL levels increased from 4 to 8% in comparison to the control group. Blood biochemistry was also affected by different dietary SBL levels. In particular, significantly higher levels of glucose, cholesterol, HDL and triglycerides were detected in fish fed >6%, >4%, >2%, and 2% SBL respectively ($P < 0.05$). Based on somatic growth parameters, blood chemistry and systemic immunity parameters, diets containing 4 to 6% SBL are recommended for juvenile stellate sturgeon.

AMENDING REDUCED FISH OIL WITH LYSOLECITHIN TO IMPROVE PERFORMANCE OF STELLATE STURGEON (*Acipenser stellatus*)

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The present study was designed to test the effects of dietary lysolecithin (sources of phospholipids) on the growth performance of stellate fed reduced fish oil. We evaluated the growth performance of *Acipenser stellatus* fed diets containing 0.0, 0.5, 1 and 2% lysolecithin for 8 weeks. Juvenile fish (45 ± 0.1 g, mean ± SED) were stocked in 12 polyethylene tanks (300 L) supplied with freshwater at a flow rate of 1 L min^{-1}.

Growth performance of stellate fed test diets for 56 days is shown in Table 1. According to the results fish fed diet supplemented with 1% lysolecithin showed significantly greater final weight, weight gain rate, and specific growth rate compared to other treatments ($p<0.05$). Our study showed that lysolecithin could enhance growth performance and needs further research.

Table 1: Growth performance of stellate fed test diets for 56 days.

	Lysolecithin (%)			
	Control	0.5	1	2
Initial weight(g)	45.27±.1	45.27±.1	45.27±.1	45.27±.1
Final weight(g)	73.32±1.8^{a}	74.54 ±.2.7^{a}	84.23 ± 1.2^{b}	71.22 ± 1.4^{a}
WGR (%)	28.05±2.01^{a}	31.26±1.4^{a}	38.95±1.08^{b}	25.95±1.3^{a}
SGR(%/day)	0.85 ± .05^{ab}	0.97± 0.01^{bc}	1.1 ± 0.02^{c}	0.80 ± 0.03^{a}
K factor (%)	69.02 ± 5.8	59.87 ± 8.2	75.29 ± 4.33	71.87± 5.5
FCR	0.97 ± 0.07	1.03 ± 0.07	0.90± 0.02	1.08± 0.006
VSI	7.7 ± 1.03	7.05± 0.17	7.11± 0.03	6.40 ± 0.65
HSI	2.62± 0.47	2.27± 0.24	2.05± 0.27	2.06± 0.08

Values are means ± S.E.M. from triplicate groups. Means in each row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant difference between treatments.

GUIDANCE ON SPATIAL TECHNOLOGY FOR EMERGENCY PREPAREDNESS AND RESPONSE FOR AQUACULTURE

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This new Guide describes the application of spatial technology to improve disaster risk management (DRM) within the aquaculture sector. DRM requires interrelated actions and activities to ensure preparedness, response, and recovery for a wide range of natural (weather or geological related), biological, technological, or complex (e.g. military conflict or civil strife) disasters that can impact aquaculture operations and livelihoods. Timely disaster response activities are required when an emergency or disaster event commences and continue throughout the event. The response phase could last from days to months depending on the magnitude of the disaster, after which the focus is on recovery and building resilience to future events.

Spatial technology refers to systems and tools that acquire, manage and analyse data that have geographic context. Some of the technologies include satellite remote-sensing, aerial surveys, global positioning systems, geographic information systems, information and communication technology, and other data gathering sensors used in meteorology. Spatial technology, like many other information technologies, is developing rapidly. The revolution in computing and communications technology has changed the risk paradigm through widespread usage of location-enabled and Internet-connected devices establishing a global network of interconnectivity.

Spatial technology supports activities across all phases of the DRM cycle within the aquaculture sector. These activities include: disaster risk reduction through preparedness, prevention, mitigation and early warning; emergency response to assess immediate damage and needs; and recovery for rehabilitation, building back better, and monitoring and assessment of the recovery progress and impact. Spatial technology can make a powerful contribution by communicating the outcomes and lessons from rehabilitation and recovery efforts, and by developing and implementing a programme to build back better, strengthen resilience, improve adaptive capacity, and further enhance preparedness, so that the socio-economic and ecological systems can better deal with risks from future disaster events.

This Guide identifies suitable spatial technology within DRM for aquaculture, which considers factors such as accessibility, limitations, complementary data and tools, human resources, and financial resource requirements. Selected country case studies in Bangladesh, Gulf of Mexico and the Caribbean, Indonesia, and the Philippines illustrate the application of spatial technology in DRM for aquaculture at the national level within local contexts. Take-home messages from each case study include recommended steps for using spatial technology to support the DRM process, or parts of the process.

Best practices at the farm and area management levels supported by spatial technology will reduce volatility and risks to facilitate investment. Countries that want aquaculture to grow sustainably and reliably should consider using this Guide in order to support spatial planning approaches and protect responsible investors.

IMPLEMENTED ECOLOGICAL FOOTPRINT ANALYSIS FOR AQUACULTURE: CONCEPTUAL MODEL DEVELOPMENT AND CASE STUDY APPLICATION

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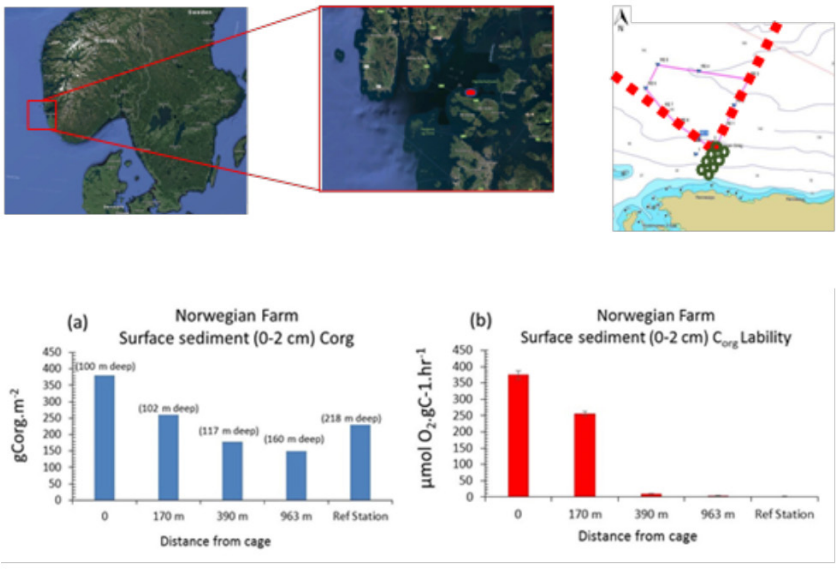
The main goal of the co-funded COFASP / ERA-net ECOAST project is to identify, develop and test new methodologies for spatial and temporal management of aquaculture and other activities in coastal areas. The project aims to implement methodological criteria to assess the ecological footprint of the aquaculture industry, using a holistic approach which combines ecological, ecotoxicological and chemical analyses to evaluate the real carrying capacity of aquaculture activities in an increasing competition for space in marine and coastal areas.

To quantify the fluxes nutrients and chemicals as well as their impact on the ecosystem, it is necessary to integrate several parameters such as the mass balance between supplied and uneaten food in a cage, the biotic analysis the organic and inorganic sediments chemistry, the estimation of parasite treatments and the quantification of organic compounds and nutrients computed from supplemented food and excretion products. In our analysis, a special focus is dedicated towards the nutrients' fluxes from medicated and/or supplemented feed used in the marine aquatic production, affecting the seabed and the water column. The project will initially implement an integrated ecological-chemical and ecotoxicological biomonitoring to assess both the short and long term environmental impact of aquaculture farm site within different stages of the production. Obtained results will be then parametrized in the EFA framework. The implemented EFA will be applied to a case study located in Boknafjord (South-Western Norway see figure below). The spatial distribution of chemicals has been used to track the cumulative-long term (trace elements; fig. 3A) versus short term (polyaromatic molecules; fig. 3B) impact of the aquaculture in the surrounding environment. Results have been integrated with the biological responses obtained by exposing collected sediments with representative organisms of the microbenthic community (fig. 3C). The flux of energy is being monitored, parametrized in terms of characterization and quantification and linked with the recorded mass production. Early results show that a direct measure of sediment organic matter reactivity represents a superior discriminator of fish-farm derived organic matter. To verify these results, the sediment organic matter reactivity and the analysis of surface sediment molecular based biodiversity, will be conducted at selected sites of the project's partners, and applied to sites of contrasting trophic status.



EFFECTS OF LOCAL AND IMPORTED FEED ON GONADAL DEVELOPMENT OF AFRICAN CATFISH *Clarias gariepinus*

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The study was conducted to determine the effects of local and imported feeds on gonadal development of *Clarias gariepinus* in plastic tanks for 80 days. CHI, imported feed; Treatment 1 (T_1), Afe Babalola University, Ado Ekiti (ABUAD) local feed; Treatment 2 (T_2), Durante, imported feed; Treatment 3 (T_3), and formulated feed; Treatment 4 (T_4) were fed to *C gariepinus* in (3) three replicates using plastic tanks (55 x 35 x 40 cm) stocked with 10 (ten) fish. Each *C. gariepinus* juvenile average weight was 14.20±0.02g. Fish were fed twice daily at 4% body weight. There were significant differences (P>0.05) in female fish weight gain when T_2 (44.87g) was compared to T_1 (19.7g) and T_4 (11.5g). ABUAD feed had the best Feed conversion ratio, protein efficiency ratio, ovary weight, egg size, fecundity, milt count and milt volume. Histological analysis in male fish fed ABUAD feed when compared with other feeds, revealed advanced adult stage with many cysts containing spermatocytes undergoing cell division while the female fish showed late peri-nucleolar stage. It could be concluded that local feed (ABUAD) compete favorably with imported feed.

Table 1: Growth and reproduction analysis of *C. gariepinus*

PARAMETERS	T_1	T_2	T_3	T_4
Weight gain (male)	22.33 ± 0.5^a	47.59 ± 3.5^b	41.60 ± 7.8^b	11.30 ± 3.1^a
Weight gain (female)	19.7 ± 2.7^a	44.87 ± 4.7^b	37.16 ± 5.9^b	11.5 ± 1.43^a
FCR (male)	2.03 ± 0.3^a	1.11 ± 0.1^a	1.27 ± 0.2^a	3.78 ± 0.8^b
FCR (female)	2.04 ± 0.3^a	1.14 ± 0.1^a	1.27 ± 0.2^a	3.32 ± 0.5^b
Milt volume	0.20 ± 0.0^b	0.43 ± 0.3^d	0.29 ± 0.0^c	0.10 ± 0.0^a
Milt count	3.05 ± 0.3^b	3.96 ± 0.3^c	3.17 ± 0.1^b	2.55 ± 0.4^a
Egg size	1.64 ± 0.3^b	1.84 ± 0.2^c	1.74 ± 0.6^{bc}	1.40 ± 0.6^a
Fecundity	153 ± 3.3^a	207 ± 3.7^d	186 ± 3.3^c	125 ± 5.0^a

EFFECTS OF STARVATION ON THE HUMORAL IMMUNE ACTIVITIES OF GILTHEAD SEABREAM

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Although different studies have been done on the immune system of teleosts and on the metabolic effect of starvation, very little information is available on how starvation affects the fish immune system. The aim of the present work was to verify how different periods of starvation affect several humoral immune activities of gilthead seabream (*S. aurata* L.). To do this, juvenile specimens of gilthead seabream were subjected to 0 (control), 1, 2, 4 and 20 days of starvation. After the respective periods of starvation, samples of mucus and serum were taken from each specimen. Immunoglobulin M, protease, antiprotease, peroxidase activities and bactericidal activity against *Vibrio anguillarum* were determined. In addition, the following blood biochemical parameters were determined: albumin (ALB), aspartato-aminotransferase (AST), bile acids (BA), calcium (CA), creatin kinase (CK), globulin (GLOB), glucose (GLU), potassium (K+), sodium (NA+), phosphate (PHOS), total protein (TP) and uric acid (UA).

With respect to the humoral defense parameters, statistically significant differences were observed between mucus and serum, being the seric levels of IgM, protease, antiprotease and peroxidase higher than those obtained in skin mucus. On the contrary, the bactericidal activity was always higher in mucus than in serum. In addition, for all the humoral defense parameters studied we found statistically significant differences for each period of starvation, finding a general pattern by which the activity found in control samples was always statistically similar to that found in samples from specimens starved during 20 days. Furthermore, serum and mucus IgM and mucus protease decreased in starved fish finding their minimum in samples from fish starved for two days. In contrast, serum peroxidase and antiprotease and bactericidal and peroxidase activity in mucus increased, peaking at 2 days of starvation. On the other hand, a great increase was observed in the protease activity from specimens subjected to one day of starvation. Regarding bactericidal activity in serum, it reached its minimum and maximum levels in fish starved for one and two days, respectively. With respect to the measured blood biochemical parameters, there were only significant differences between the different periods of starvation for CK and TP, so that CK decreases significantly and reaches its minimum in the 2 days of starvation, as does TP.

In conclusion, we may affirm that there are important differences in the immune parameters of starved fish determined in serum and skin mucus, being the values higher in serum than in skin mucus, except in the bactericidal activity. The most notable influence of starvation on the analyzed parameters was detected in fish starved for two days and all the parameters tend to recover their normal values after 20 days of starvation.

Acknowledgements: This work was supported by the MINEICO (grant no. AGL2017-83370-C3-1-R) co-funded by the European Regional Development Funds (ERDF/FEDER) and *Fundación Seneca de la Región de Murcia* (Grupo de Excelencia grant no. 19883/GERM/15).

FATTY ACID PROFILE OF MARINE AMPHIPODS FED TERRESTRIAL FEEDSTUFFS AND ITS IMPLICATIONS FOR AQUACULTURE

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In aquaculture, marine specimens from lower trophic levels have the potential to serve as alternative food source for farmed fish and invertebrates, replacing traditional meal and fish oils. Marine amphipods are a natural food source for finfish and are rich in essential fatty acids hence strong candidates as live feeds for culture.

Diets can heavily impact fatty acid profiles of both fish and invertebrates and thus marine amphipods. To estimate the potential of marine amphipods as alternative feed source for aquaculture species, a better knowledge on the manipulation of their fatty acid profile is needed. In this study two species of Gammaridea (Amphipoda, Crustacea) and their nutritional value in response to three different feedstuffs were investigated.

A three-month feeding trial was conducted with the species *Gammarus locusta* and *Echinogammarus marinus*. Newly hatched juveniles of were randomly divided into three experimental groups by species, each group feeding on different plant diets, one marine algae (*Ulva lactuca*) as natural diet and two terrestrial feed sources: carrot (*Daucus carota*) leaves, and lupine (*Lupinus* spp.) meal.

The amphipods were reared in 750 ml Kautex-containers filled with artificial seawater with a salinity of 30 ppt and water temperature of 15°C.

Growth and mortality was recorded weekly and fatty acids analyzed at the end of the three-month trial using gas chromatography.

We expect a nutritional profile that can be used for rearing fish in aquaculture. We expect a fatty acid content of 10 – 20 % mg/g DW, high in polyunsaturated fatty acids (PUFAs). Former studies (Moe, unpublished) indicate a strong impact of diet on the fatty acid profile of amphipods. Also species selection should result in differences in PUFA composition and content.

Our results (pending!) can help to adjust species and feedstuff in regards of the fatty acid demands of the target species in aquaculture, both as life feed in rearing juvenile fish and as dry feeds for fish after weaning.

Table 1. Fatty acid composition of selected species used as feedstuff in aquaculture. PUFAs of gammarid species initially manipulated by different diets (soybean (SB) and coconut (CN)), soldier fly manipulated by feeding macroalgae (MA).

Fatty acids	*Sardina pilchardus*[1]	*Gammarus oceanicus*[2]	*Gammarus locusta*[2]		*Hermetia illucens*[3]
		SB	SB	CN	MA
total lipids (% DW)	18,4	8,7	13,6	12,5	25,4
ARA (20:4n-6)	0,56	2,55	0,74	0,93	0,7
DHA (22:6n-3)	16,16	9,09	8,44	15,21	0,0
EPA (20:5n-3)	4,87	6,22	3,3	9,52	0,4
Sum PUFA	26,60	28,61	26,84	35,85	22,2

([1]Saglik&Imre 2001; [2]Moe, unpublished 2010;[3]Liland et al., 2017)

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ASSESSMENT OF THE REPRODUCTIVE CYCLE AND PRESENCE OF PARASITES IN MOLLUSCS CULTURED IN OFFSHORE WATERS OF THE SOUTHEASTERN BAY OF BISCAY

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Efforts to develop offshore oyster and mussel aquaculture have been made in the Basque region since 2007. It is well known that differences in the temporal pattern of gonad condition is found in molluscs from different geographical origins. Thus, in new shellfish farming initiatives it is crucial to characterize the reproductive cycle to ascertain the optimal season for marketability of the best meat quality as well as to predict the timing for harvesting seeds. On the other hand, another relevant parameter that can also significantly affect bivalve production is the presence of parasites; therefore, the evaluation of the incidence of parasites in potential shellfish farming areas is essential to take adequate management decisions.

In the present work, Pacific oysters (*Crassostrea gigas*), flat oysters (*Ostrea edulis*) and mussels (*Mytilus galloprovincialis*) were collected monthly during 18 months from an experimental offshore farm of the Basque coast (SE Bay of Biscay). Tissue was routinely processed for histological examination: cross-sections were dissected, fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin. Histological sections were examined under light microscopy for the evaluation of the gamete developmental stage and the presence of parasites.

Results indicated that spawning started in June for Pacific oyster and continued until August while flat oyster exhibited a shorter spawning period in early summer. Spawning occurred from spring to summer in mussels. On the other hand, this study did not detect any pathogen of concern. Rickettsia-like prokaryotes, *Ancistrocoma*-like ciliates and copepods of the genus *Mytilicola* were detected in oysters, but no associated tissue level damage was observed. *Bonamia sp*. was found in the flat oyster, but at very low prevalence (0,6%). Overall, results indicated a possible difference in the optimal marketability and seed harvesting in the studied species. On the other hand, pathogen prevalence was considered low, but as higher mollusc densities are foreseen, further studies should be performed.

This work was partially supported by the Department of Agriculture, Fisheries and Aquaculture from the Basque Government through the projects “IM16SIMMA and IM17MUSSELS”.

U. Aldalur was benefited from a PhD Scholarship granted by the Iñaki Goenaga Technology Centres Foundation.

MODELLING SEAWEED BIOMASS YIELDS AND COMPETITION FOR NUTRIENTS AND LIGHT BETWEEN FARMED SEAWEED AND PHYTOPLANKTON: IMPLICATIONS FOR MUSSEL PRODUCTION

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Coupled macroalgal-phytoplankton and shellfish models were used to investigate the effect of macroalgal cultivation on phytoplankton concentrations and shellfish growth. Results were compared with available nutrient and chlorophyll observations and measurements of frond area growth for the kelp species *Saccharina latissima* from rope deployments at an experimental seaweed farm in in Strangford Lough. When calibrated, the model was able to produce good agreement with observed nutrient and chlorophyll concentrations. The model compared well with measurements of specific growth rate, and frond area increase relative to the first observation, for the highest yielding kelp deployments. However, even small underpredictions in growth rates can lead to marked underprediction (~50%) in predicted frond area (see figure).

Nutrient competition between phytoplankton and a hypothetical large-scale kelp farm was investigated and the effect on phytoplankton found to be relatively modest, with < %15 decrease on phytoplankton chlorophyll concentrations. Based on the shellfish growth model, the consequent decrease in food availability for mussel growth was predicted to lead to only a small decrease (< 7%) in the harvested dry weight of mussels. Results will also be shown for the effect of shading at high kelp densities on phytoplankton growth and consequent effects on the food available for shellfish.

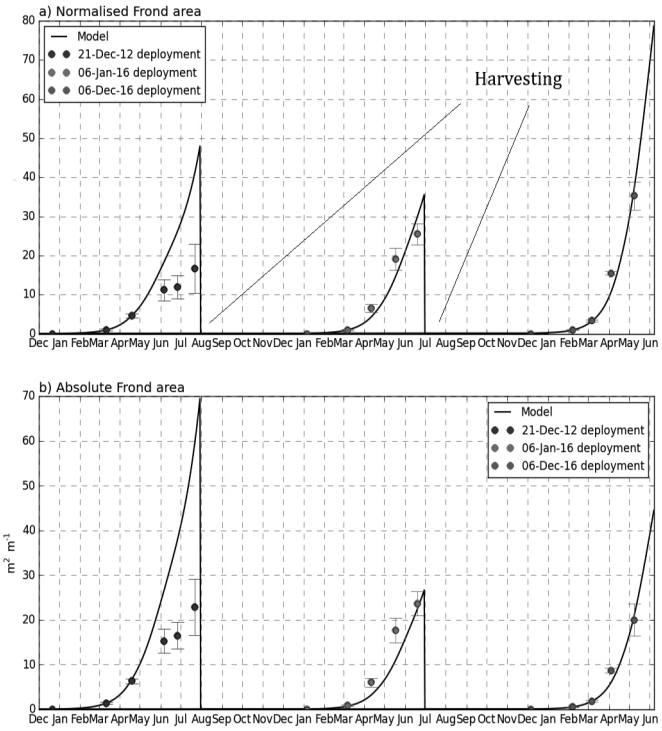

Figure: a) Normalised frond area, error bars are standard errors; b) absolute value of Frond area.

THE DEVELOPMENT OF THE REPRODUCTIVE SYSTEM OF AFRICAN CATFISH *Clarias gariepinus* IN RAS

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Due to the current economic situation in the country, there is a growing demand for domestic aquaculture products under the import substitution program. One of the prospective objects of cultivation can be considered African catfish (*Clarias gariepinus* Burchell, 1822). This is heat-loving fish, which in Russia is bred in recirculation aquaculture systems (RAS).

In the aquacomplex of the Southern scientific center of RAS researches were conducted to study the development of the reproductive system of African catfish in artificial conditions. Fish cultivated from eggs to eggs. The basic parameters of the environment in RAS were maintained at a constant level. During the growing period, the water temperature was in the range of 21-28,5^{0}C, and its average value was 24.4^{0}C. The oxygen concentration was within the normal range of 7.31-11.4 mg/l, the active reaction of the medium 6.5-8.2 units. The study of hydrochemical samples from the basins revealed that they conform to the norms and slight deviations do not have a significant impact on the growing facilities.

At the first stage, we carried out the diagnosis of the reproductive system of catfish using ultrasound (ultrasound scanning). Then caviar samples were taken for analysis. Size and weight of eggs at 2, 3, 4 stages of maturity were determined.

At the age of 3-4 months gonads of African catfish were at stage 2 of maturity. In this case, the diameter of the eggs was 0.36±0.02 mm, and the weight of 0.86±0.02 mg. At the onset of the 3rd stage of maturity gonad diameter changed to 0.68±0.06 and weight of 1.19±0.04 respectively. The age of the specimen, which reached the 3rd stage of maturity, was set at 4-5 months. At the age of 5-6 months reproductive system is the most formed, females at this age are ready to spawn. The size of the caviar was 0.94±0.04 mm and weight - 1.36±0.05 mg (table. 1).

In assessing the quality of oocytes of the catfish any deviations were not revealed. Oocytes' membrane formed without any violations at the stage of trophoblastic growth, the size of the cells corresponds to biological characteristics for this species in the cytoplasm is not marked abnormal inclusions.

From the research findings it follows that sexual maturity of African catfish comes rather quickly. After 5 months of cultivation it is possible to obtain the first offspring. Accelerated maturation of the reproductive system of producers can be achieved by optimizing feeding, stabilization of temperature and oxygen regimes.

The publication was prepared with the use of the UNA MUK SSC RAS and Bioresource collections of rare and endangered species, SSC RAS №73602.

Table 1 - Size indices of African catfish eggs

Age, month	Stage of maturity	Diameter of eggs, mm	Weight of eggs, mg
3-4	2	0,36±0,02	0,86±0,02
4-5	3	0,68±0,06	1,19±0,04
5-6	4	0,94±0,04	1,36±0,05

BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO HYPOXIA, HIGH TOTAL AMMONIA NITROGEN (TAN) CONCENTRATION IN EUROPEAN SEA BASS, *Dicentrarchus labrax*

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In modern aquaculture, production costs are the major driver. This has resulted in culture practices and rearing environments aimed at maximizing production capacity. Consequently, fish are exposed to unavoidable stressors, which can be detrimental to animal welfare. This work aims at bringing a better understanding of behavioural and physiological fish responses to stressors such as oxygen and TAN variations. Groups of ten fish (n=3) were exposed either to hypoxia (20 % O_2), hyperoxia (200 % O_2) or high TAN (0.9 mg.L^{-1}) during 2 hours.

Videos were recorded to assess group (social cohesion) and individual behaviour (distance moved, swimming speed) in comparison to control condition. After this challenge, blood samples were taken in a subsample (n=10 fish/condition) to analyse plasma cortisol concentration as physiological stress indicator. Other fish exposed to the same three conditions (n=20) were analysed individually using a novel tank challenge during 25 minutes to evaluate anxiety state *post*-stress situation. No behavioural differences were observed in hyperoxia exposed fish while hypoxia fish showed lower group cohesion and TAN fish higher group cohesion compared to control condition. Both in hypoxia and TAN condition, fish travelled less distance and showed higher plasma cortisol concentration than control condition (**Fig. 1A,B**). During novel tank challenge, a lower activity was observed in all conditions compared to control (**Fig. 1C**). and only fish exposed to TAN seem less anxious than control fish. This study indicates that stressors found in aquaculture condition may quantitatively impact behavioural and physiological stress responses of farmed fish. ERANET ANIHWA and French National Research Agency funded the project WIN-FISH, ANR-14-ANWA-0008.

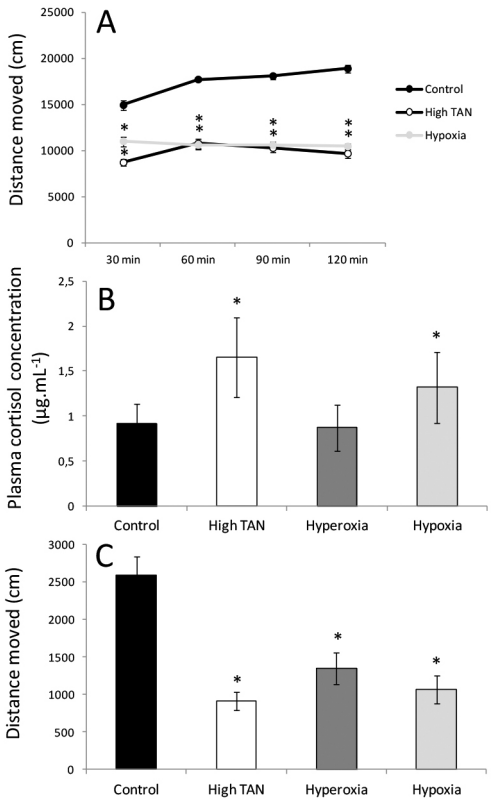

Fig 1. (A) Distance moved by fish exposed to stressors in group; **(B)** Plasma cortisol concentration in fish *post*-stress; **(C)** Distance moved in novel tank by fish *post*-stress. * indicates statistical difference between group and respective control (mean+SEM).

STRESS COPING-STYLE IN EUROPEAN SEA BASS, *Dicentrarchus labrax*: FROM GENES TO PHYSIOLOGY AND BEHAVIOUR

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Stress coping styles (SCS) are defined as a coherent set of individual physiological and behavioural differences in stress responses consistent across time and context. This work aims at understanding the mechanisms underpinning SCS in European sea bass, *Dicentrarchus labrax*, through the combined measures of physiological and behavioural responses.

Individually PIT tagged fish were challenged twice (four months apart) in a group risk taking test to assign an individual boldness score (n=1000). The risk taking test consists in grouping the fish into a sheltered area and measuring the latency to leave it towards an open area. Fish leaving the shelter during the two tests were classified as proactive, whereas fish staying were described as reactive. One year later, 30 proactive and 30 reactive fish were challenged using an Open Field Test (OFT). The OFT consists in placing a single fish in an observation arena (75x75 cm) with a shelter (**Figure 1**). After 5 min of habituation, fish are free to exit the shelter and explore the arena during 20 minutes. Behavioural variables (latency to exit shelter, time spent in shelter or distance travelled) were recorded. Directly after the OFT, blood and brain samples were taken to measure blood plasma cortisol concentration, brain neurotransmitter levels (serotonin, dopamine), expression of genes involved in stress regulation (*gr1, gr2, mr, crf*) and neurogenesis (*egr1, neurod1, pcna*).

Correlations between behavioural responses, stress regulation processes, neurotransmitters and neurogenesis were evaluated to bring a better understanding of SCS in European sea bass with the goal of contributing to fish welfare improvement in aquaculture. ERANET ANIHWA and French National Research Agency funded the project WIN-FISH, ANR-14-ANWA-0008.

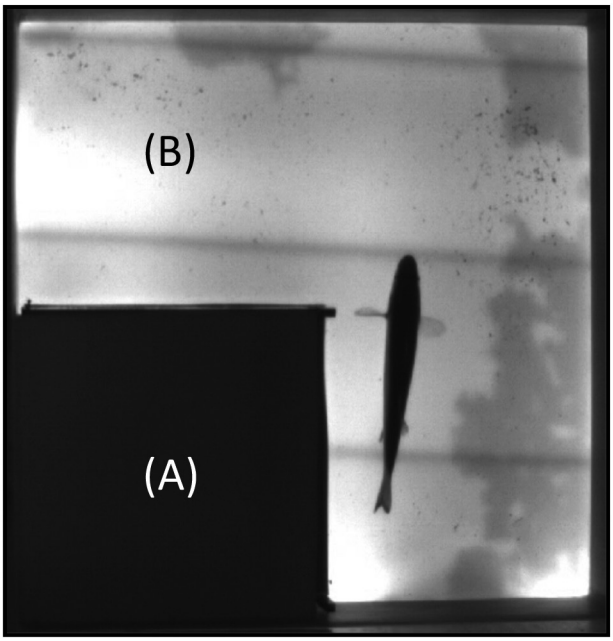

Fig 1. Open Field Test (OFT) experimental set-up. **(A)** Sheltered area; **(B)** Exploration area

IMPACT OF THE METAMORPHOSIS ON ONTOGENESIS OF THE DIGESTIVE TRACT IN A DISCOID FISH SPECIES *Platax orbicularis* A MORPHO-ANATOMICAL CHARACTERIZATION

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In teleosts, metamorphosis is one of a key developmental event. It represents a crucial transition between the larval and juvenile periods but it is still difficult to precisely determine some of the processes occurring. This phenomenon can induce strong morpho-anatomical, physiological, behavioral modifications depending on the species and, sometimes, ecological challenges. Several organs can be impacted, particularly the digestive tract which is an essential crossroad between several functions. It is a highly plastic and multifunctional organ throughout development, at the interface between internal and external medium. It is involved in metabolism and various physiological functions such as immunity, absorption, osmoregulation.

Several studies already described digestive tract development during larval growth in various fish species. However, until now, no work has focused on the digestive system and its ontogenesis in a discoid fish, especially through metamorphosis. Therefore, the main goals of this study are to understand the organization of the digestive system in this species (i) at the cellular and organ levels and (ii) through the identification of different anatomical and functional sections, during larval and juvenile periods.

The ontogenesis of *P. orbicularis* digestive tract was characterized using classic histological and immunofluorescence techniques at 3, 9, 15, 20 and 56 days post-hatching (dph). We revealed that the digestive system is located all along the body in the early larval fish (3 dph) but this location changes during metamorphosis to finally be in the anterior part of the juvenile fish (56 dph). Moreover, the organization of the digestive tract in the early larvae is already complex with different intestinal loops and becomes even more intertwined in adults with typically an elongated stomach, a pyloric zone composed of 4 pyloric caeca, a proximal, medium and distal intestine and a rectum. We identified variations in mucus production and mucin types related to the different developmental stages and according to the digestive areas previously described. Before metamorphosis, mucins are mainly acidic but are mostly neutral in later stages. Finally, the analysis of Na-K-ATPase immunostaining a key marker of osmoregulatory processes will be discussed.

Therefore, the organization of the digestive system of *P. orbicularis* is similar to other teleosts. Nevertheless, the morphological development of this discoid fish leads to an anterior localization of a complex digestive system made of numerous folds and loops that can bring some uncertainty regarding the different functional sections. The acidic mucin composition during the early larval development could allow the fish to optimize nutrient assimilation in order to have enough energy for metamorphosis. In conclusion, by characterizing the morpho-anatomy of *P. orbicularis* digestive tract, this preliminary work allows the exploration of the different roles of this organ such as absorption, osmoregulation or immunity. Since metamorphosis represents a drastic change in this discoid species, additional molecular analyses at this transition time could help describing the successive steps involved during the indirect development of the digestive tract in other fish species.

THE TENACIBACULOSIS IN THE TROPICAL ORBICULAR BATFISH *Platax orbicularis* HIGHLIGHTS ON THE TRADE-OFF IN THE GUT BETWEEN IMMUNITY, OSMOREGULATION AND DIGESTION

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The marine tenacibaculosis is an important disease caused by a widespread filamentous bacterium *Tenacibaculosis maritimum*, inducing considerable economic losses in a large number of aquaculture species (i.e. turbot, Senegalese sole, sea bass, Atlantic salmon). Even if this disease has a great impact in fish farms, little is known about immune responses of fish to this pathology. One of the key organ involved in this response and in protection against pathogens is the gut. It regulates nutrient absorption, immunity and hydromineral balance mainly through mucus secretion. The mucus quality and quantity modulate this trade-off between assimilation and protection. The orbicular batfish (*Platax orbicularis*) is an emerging and valuable species for French Polynesian aquaculture. However, its rearing is threatened because of *T. maritimum*. Indeed, high mortality occurs when 2-months old juvenile batfish reared in bio-secured hatchery tanks (*T. maritimum* free) are transferred to floating net-cages in the lagoon.

Therefore, the present study aims at identifying the trade-off between the different functions of *P. orbicularis* gut in healthy fish and at different time after an experimental *T. maritimum* infection (T1 to T6, from beginning of experiment to 112 hours post-infection). To do so, mortality rates and morpho-anatomy of the gut in naïve and contaminated 56-dph juveniles were characterized. Moreover, mucus production, mucin types, marker of immune cells (B-lymphocyte differentiation, dendritic cells) and osmoregulation were determined using various histological techniques.

We show that mortality rates increase from 0 to 55% at 40 hours post-infection. Globally, in infected fish, gut appears more transparent and thinner than in non-infected fish. Moreover, mucus production and mucin types are significantly modified after infection. Indeed, mucus production increases whereas mucins, which are mainly neutral in non-infected juvenile fish, will become acidic 40 hours post-infection. Moreover, we identified a significant difference of immune cells expression between the two conditions. In infected fish, immune cells are localized in the enterocytes whereas in non-infected fish, no staining has been detected. Finally, the analysis of Na-K-ATPase immunostaining a key marker of osmoregulatory processes are still in progress.

T. maritimum infection induces various changes at different levels. Firstly, digestive wall appears thinner in infected fish which could be a direct impact of infection (such as lesions on skin) or indirect impact due to increasing mucus production. This production could lead to reduction in nutrients assimilation and consequently, to a decrease in digestive wall thickness. Secondly, the variations in mucin composition following primo-infection, from neutral to acidic mucin, which may indicate (i) either an effective immune defense towards the attack or (ii) that orbicular batfish immunity is compromised. The presence of immune markers in enterocytes support the first hypothesis. Nevertheless, further molecular studies are needed targeting known innate and adaptive immune factors.

EFFECTS OF FASTING AND REFEEDING ON THE DIGESTIVE PHYSIOLOGY OF SELECTED SEA BASS POPULATIONS

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For teleost, the energetic demands for maintenance of the gut is at least five- to eightfold higher than corresponding mass-specific whole animal consumption rates. This is due to the multifunctionality of the gut involved in energy consuming roles such as digestive, immune and osmoregulatory processes leading to energetic trade-offs. The nutritional status of fish can directly impact this trade-off and metabolic response to an osmotic stress, for example. However, whilst some individuals cope better with food deprivation, others recover more efficiently from prolonged fasting.

Therefore, the aim of this study was to analyse, using the European sea bass *Dicentrarchus labrax* L., a strictly carnivorous teleost, the effects of fasting and re-alimentation on the postprandial osmoregulatory capacities and intestinal morphological changes. To do so, two genetically selected populations (F2 generation) of sea bass ("West Mediterranean wild type") representing phenotypes from the opposite ends of the trade-off between tolerance to food deprivation and compensatory growth rate were used. These two selected phenotypes show different degrees of body mass loss during food deprivation: one group losing body mass rapidly during fasting (F+) and the other one limiting body mass loss during the same period (F-). Blood osmolarity, gill and intestinal morphology and expression of the sodium pump (Na^+, K^+-ATPase, NKA subunit $\alpha 1$) were studied from these populations during fasting condition (3-week feed deprivation) and throughout the postprandial period (at 4, 24, 48 and 72 hours).

We show that blood osmotic pressure significantly decreases due to re-alimentation in both groups, but this is compensated in the F+ group after 72 h after re-alimentation. In this group, gill ionocytes are smaller and less numerous, but a significantly higher NKA gene expression is observed in the gills in comparison to fasting individuals, to the F- individuals 48 and 72h after re-alimentation, and also in the posterior intestine 72h after re-alimentation. Globally, proximal anterior intestine morphology changes between fasting and re-alimented animals with an elongation of primary and secondary folds. Moreover, for both populations, we noted a significant elongation of enterocytes in the same part of intestine. Nevertheless, at 48h post-re-alimentation, the F- group has significant longer cells. Furthermore, refed F- fish absorb more lipids along the proximal anterior intestine and take longer to digest than the F+ group and show enterocyte vacuolization in the posterior intestine.

Significant higher NKA gene expression observed in F+ group may compensate for a higher salt intake during nutrient absorption in comparison to the F- group. Therefore, the two selected populations have different postprandial digestive strategies: the F- group optimize feed efficiency first at the cost of optimal hydromineral adjustment, while the F+ group invests in osmoregulatory performance at the expense of digestive physiology. These preliminary physiological data could help in management practices of farmed fish in the future. Nevertheless, further studies such as NKA activity measurements or body composition are necessary to fully characterize the phenotypic differences.

DEVELOPMENT AND CHARACTERIZATION OF A 57K SINGLE NUCLEOTIDE POLYMORPHISM ARRAY FOR EUROPEAN SEA BASS

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In this study, we describe the development and characterization of the first high-density single nucleotide polymorphism (SNP) genotyping array for the European sea bass (*Dicentrarchus labrax*). This Axiom® sea bass Genotyping Array will become publically available in 2018 (Affymetrix - ThermoFisher), includes 56730 markers and is available in 384 format.

To design this array, SNPs were selected from ~2.6 million SNPs identified by haplotype-resolved whole-genome resequencing of 24 fish of wild sea bass from Atlantic (N=6), western Mediterranean (N=12) and eastern Mediterranean (N=6). After alignment to the sea bass reference genome (Tine et al. 2014), duplicate reads filtering, base quality and variant quality score recalibration, variant calling and filtering, a total of 2,628,725 phased SNPs without missing data were generated. From this database, only SNPs located more than 30bp away from another known variant were selected to ensure a high probe specificity. The final set of variants were chosen to cover the whole genome (including ungrouped scaffolds), but with a variable density of SNPs depending on the estimated local nucleotide diversity (π) reported by Tine et al. (2014). This strategy aimed at increasing the density of SNPs within chromosome regions displaying a higher recombination rate.

To evaluate the SNP array, 2104 fish from two selected lines of FMD (Ferme Marine du Douhet, France) and EMG (Ecloserie Marine de Graveline, France) were genotyped at the Gentyane genotyping platform (INRA, France). Results reported a large number of polymorphic SNPs with high clustering resolution (PolyHighResolution SNPs) respectively 51,195 (90.25%) and 51,710 (91.15%) and for passing samples, an average genotype call rate >99.7% for FMD and >99.8% for EMG. The minor allele frequency (MAF) distribution for PolyHighResolution SNPs was similar in both populations (figure 1) with an average at 0.30 for FMD and 0.29 for EMG. These results highlight SNP array design quality and demonstrate the high level of genetic variability in the selected broodstock of these two French hatcheries.

Acknowledgements
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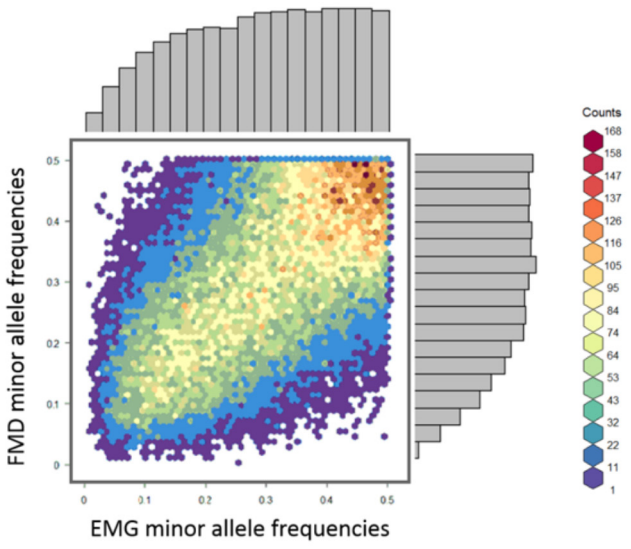

Figure 1. Bar plots of minor allele frequencies (MAF) in the two commercial populations of FMD and EMG, and comparative scatter plot of MAF coloured by counts of SNP markers.

GENOME-WIDE ASSOCIATION STUDY AND GENOMIC EVALUATIONS FOR RESISTANCE TO VIRAL NERVOUS NECROSIS IN WILD POPULATIONS OF EUROPEAN SEABASS *Dicentrarchus labrax*

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Viral Nervous Necrosis (VNN) is one of the most serious threats for European sea bass culture in the Mediterranean Sea (Doan et al. 2017a). One possible route to obtain more resistant populations is to use marker-assisted or genomic selection. These breeding strategies are gaining importance in Salmonid aquaculture, but are not yet practical in sea bass.

After an experimental infection of 1472 offspring derived from a full factorial mating of nine dams with 60 sires from four different wild populations of European sea bass, we demonstrated large differences in the survival of sea bass exposed to VNN, from 44% to 99% (Doan et al 2017b). Parents and offspring were genotyped on a custom Illumina Iselect® 3K SNP array. 1274 informative SNPs were retained to detect QTLs of NNV resistance by a weighted (wGWAS) with BLUPF90, based on a single trait linear mixed model. The wGWAS revealed 3 potential QTL located in LG9, LG12 and LGx, explaining respectively 3.7%, 6.2% and 3.4% of the resistance (Fig 1).

Genomic evaluation was done with GBLUP and SNP-BLUP models fitted in BLUPF90 and GS3, respectively, in order to assess the accuracy of genomic prediction, compared to pedigree-based (PBLUP) prediction. The accuracy of breeding values predicted with genomic models (R_{GBLUP} = 0.76 for GBLUP and $R_{SNP\text{-}BLUP}$ = 0.74 for SNP-BLUP for survival) were similar to those of PBLUP (R_{BLUP} = 0.78 for survival) even though the genomic heritability (0.14±0.05 for binary survival and 0.08±0.03 for time to death) we estimated only equals approximately half of the polygenic heritability (0.23±0.08 and 0.14±0.05, respectively). This may suggest that our experimental setup was not optimal to account for the variation in VNN resistance using genomic information, and that VNN resistance may be more efficiently improved with genetic evaluations incorporating dense SNP genotype information in appropriate designs using larger families within one population.

Acknowledgements
This work is part of the RE-SIST project selected at the 15th "Fonds Unique Interministériel", project labelled by the competitiveness clusters AQUIMER, Agrimip Innovation, Mer Méditerranée and Mer Bretagne, and funded by the French Government, BPI France; Bretagne, Languedoc-Roussillon and Aquitaine regional authorities; Landes and Pyrénées Atlantiques regional councils.

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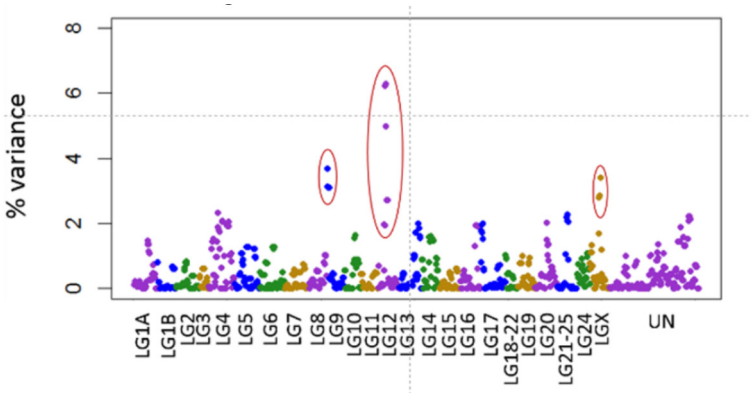

Figure 1. Percentage of variance explained by 5 adjacent for NNV resistance in a weighted Genome-Wide Association Study (wGWAS)

ANALYSIS OF THE LONG-LIVED RESPONSE INDUCED BY IMMUNOSTIMULANTS AND EFFECT ON A VIRAL INFECTION IN ZEBRAFISH *Danio rerio*

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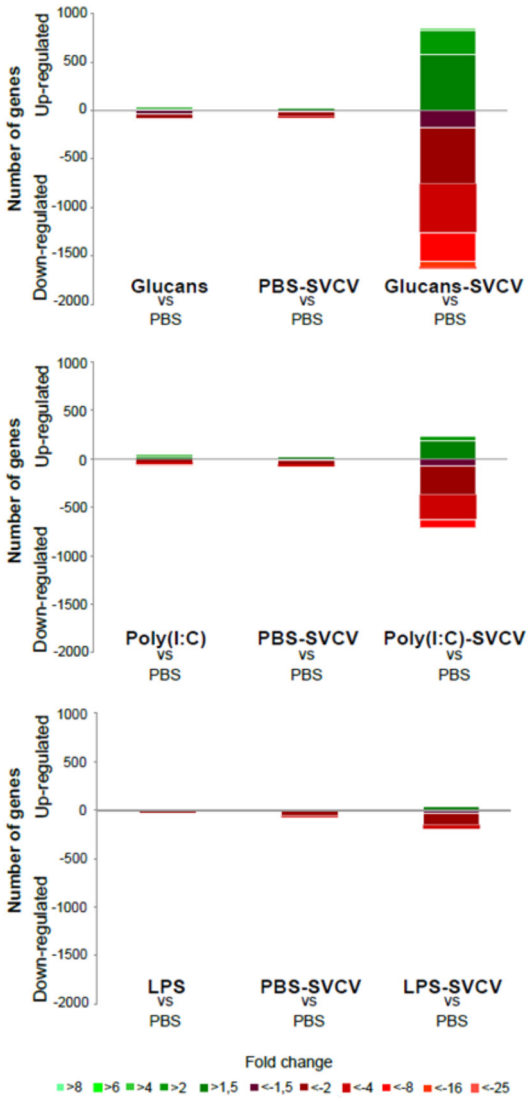
Recently, the innate immune response has gained importance since evidences indicate that, after an adequate priming protocol, it is possible to obtain some prolonged and enhanced immune response. An inappropriate protocol can transform the treatments into a double-edged sword for the teleost immune system, resulting in a stressful and immunosuppressive status.

In this work, we analyzed the long-term effect of different stimuli (β-glucans, lipopolysaccharide and Polyinosinic:polycytidylic acid) in the transcriptome modulation induced by with Spring Viraemia Carp Virus (SVCV) in adult zebrafish (*Danio rerio*) (Fig.1) and in the mortalities caused by this infection. At 35 days post-immunostimulation the transcriptome was found to be highly altered compared to the control fish, and these stimuli also conditioned the response to SVCV challenge, especially in the case of β-glucans. No protection against SVCV was found with any of the stimuli and even non-significant higher mortalities were observed, especially with β-glucans. However, at short-term (a pre-stimulation with β-glucan and infection after 7 days) a slight protection was observed after infection. The transcriptome response in zebrafish kidney at 35 days post-treatment with β-glucans revealed interesting aspects that could be related with stress and immunosuppression. The identification of genes differentially expressed before and after the infection seem to indicate a high energy cost of the immunostimulation prolonged in time that could explain the lack of protection against the SVCV. The differential response to stress, alterations in the lipid metabolism, the tryptophan-kynurenine pathway and the interferon-gamma signaling seem to be some of the mechanisms involved in this particular response, which means the end of the trained immunity and the beginning of a stressful status characterized by immunosuppression.

Figure 1. Analysis of the gene modulation at 35 days post-stimulation with the three PAMPs.



COMPARATIVE PATHOGENICITY AND VIRULENCE OF *Vibrio parahaemolyticus* ISOLATES DERIVED FROM AN AHPND OUTBREAK IN THE PHILIPPINES

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The shrimp farming industry in Southeast Asia has been heavily impacted by acute hepatopancreatic necrosis disease (AHPND) caused by a unique strain of *Vibrio parahaemolyticus* that produces toxins responsible for the primary pathology of the infected shrimp. In the Philippines, the status of AHPND was uncertain until its detection in two separate occasions in 2015.

In this paper, we describe the isolation, biochemical characterization, PCR testing, and whole genome sequencing (WGS) of bacteria isolated from the hepatopancreas of shrimp collected from an outbreak in the Philippines. Eleven isolates (1212, 1213, 1214, 1216, 1217, 1218, 1220, 1222, 1329, 1337, and 1339) were found to contain the PirAB^{vp} toxin gene by PCR. Also, the isolates were identified by WGS to be mainly two strains of *Vibrio parahaemolyticus* (*V. parahaemolyticus* S167 and *V. parahaemolyticus* S171). Moreover, pathogenicity tests using a previously published laboratory infection model, caused 100% mortality after 24 hours, accompanied by characteristic AHPND histopathology and presence of the bacteria in the hepatopancreas which was confirmed by PCR analysis. Tests with three of these isolates (1213, 1214, and 1216) revealed that mortality rate was dose-dependent and varied per isolate (Fig 1). Using isolate 1213 to infect shrimp of different weights, we found that the susceptibility of shrimp to AHPND differed accordingly (Fig 2).

These results revealed that the *Vibrio parahaemolyticus* isolates from a shrimp farm outbreak in the Philippines are pathogenic and could induce mortalities in healthy shrimp by the secretion of PirAB^{vp} toxins. Further studies to elucidate the regulation of virulence in AHPND-causing *V. parahaemolyticus* are needed to develop practical disease prevention and control strategies.

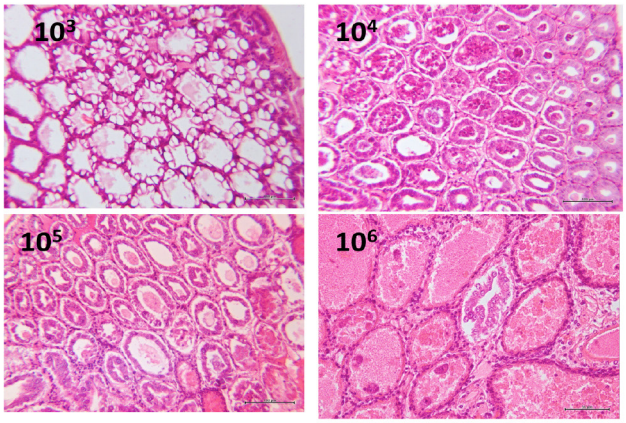

Fig 1. Histopathological lesions in the shrimp hepatopancreas induced by different doses (cfu/ml) of *V. parahaemolyticus.*

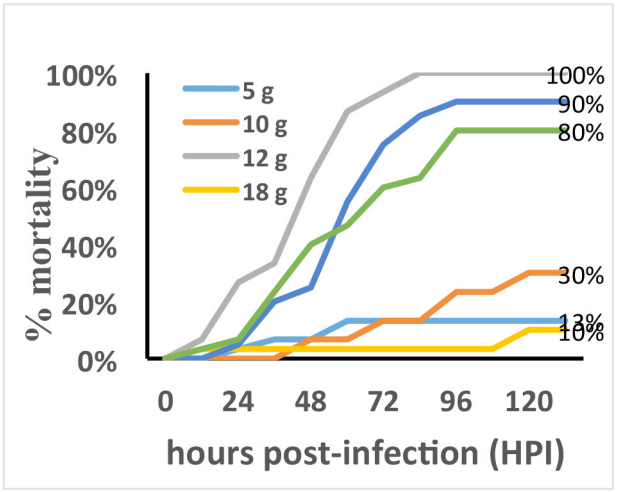

Fig 2. Ssusceptibility of different sizes of shrimp to *V.parahaemolyticus* at 5x10^4 cfu/ml.

VIRTUALLY THE REALITY: NEGOTIATING THE DISTANCE BETWEEN STANDARDS AND LOCAL REALITIES WHEN CERTIFYING SALMON AQUACULTURE

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As has been the general trend for many larger industries, salmon aquaculture has seen an increased demand for standardization to ensure a degree of global transparency and accountability. This entails both practices and protocols that are quantitative, transferable and comparable. A proliferating approach to achieving this is the implementation of sustainability standards through certification schemes. These standards comprise a list of requirements to which the aquaculture companies must demonstrate compliance. Though often projected and treated as such, these standards are by no means precise representations of reality, as they are a simplified generalization meant to cover a manifold of local realities; realities which will vary across nations, fjords, and even neighboring sites. Consequentially, standards will necessarily provoke different local responses and adaptations in how they are implemented.

The distance between the standard and any given local reality is often negotiated through a third-party auditor, who periodically evaluates whether the aquaculture company is in compliance with the list of requirements, through inspections and thorough review of documentation. Based on fieldwork conducted at audits for three different certification schemes and numerous interviews in some of the major aquaculture companies in Norway and Chile, this paper explores the role and capabilities of the auditor in negotiating compliance between local realities and standards.

While standards emanate from the idea of objectivity, it is important to keep in mind that they are both made and managed by people. The auditor plays an important role in bridging the gap between global ideals and local realities, as the possibility for negotiation occurs at their discretion, granted within a set framework. While the aquaculture companies seek to influence the standard to better fit their reality, the auditor is managing the difficult balance between considering the local context and upholding the standard. This reflects the embedded dilemma of standards and certifications: being sufficiently stringent to be considered credible, while also not being so stringent as to discourage companies from attempting to become certified.

Our findings show that while auditors are obligated to operate within a set framework defined by the standard owners, there is some flexibility in how criteria compliance is evaluated. This illustrates the significance of the particular appointed auditor, demonstrating the subjectivity in what is intended (and viewed) as objective. We find that the auditors' capability to negotiate between the reality and the standard is shaped by their familiarity with the industry and company in question. More specifically, through such aspects as experience, expertise, knowledge of the specific industry, relational trust, and language proficiency. While the assumed intrinsic condition of a 'neutral' third-party proves somewhat absent in our material, this is not necessarily unfavorable, as these standards cannot capture the many idiosyncrasies of different sites. It is, however, important to acknowledge that this perceived objectivity is made and managed by man, and this has implications for how we deal with standards as a route towards sustainability.

MONITORING THE EFFECT OF SEA PLASTICS IN MADEIRA ISLAND OFFSHORE FISH FARMS: IS THE THREAT REAL?

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Offshore fish farms from the North Eastern Atlantic Islands such as the Archipelagos of Madeira and Canaries are situated near the coast lines. Steep seafloors is the major reason for it. Regarding the location there are considerable advantages for the fish farms, including the easier logistics and operations due to the proximity to major ports.

However, the proximity to land may be detrimental to water quality and consequently, to the rearing of fish. Despite the small size of rivers in the islands, they are mostly seasonal and may carry considerable amounts of debris and suspended solids to the coast. Therefore a considerable distance to river mouths is taken into consideration for the site selection of the farms and for aquaculture development plans. This might be effective to avoid sediments where historic data is available, but little is known about plastics. This is the reason for a monitoring programme of sea plastics that was initiated in a fish farm on the South coast Madeira Island.

A convergence of patterns are shown from the preliminary evidence of plastics in the farm:

- Plastics are more common during the Winter/rain season;
- Ocean currents from South West play a major role in the transport of plastics to the farm, evidenced by the more exposed fish cages;
- Plastics from agriculture activities, particularly from banana plantations are among the more common found in both the water column and fish tract;
- Ingested plastics are occasionally the cause of highest mortality of farmed fish (up to 63.3% of total).

CHARACTERIZATION OF CONTAMINATION LEVELS AND SOURCES IN FRESHWATER FISH FROM ANTANANARIVO, MADAGASCAR

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Freshwater fish is a major source of animal protein and essential nutrients for human diet in developing countries such as Madagascar. Among animal proteins usually consumed by the population, freshwater fish is the only one available in both natural environment and aquaculture farms. However, the potential health benefit of dietary freshwater fish intake is in close relationship with water quality of aquatic environments from which fish belongs. In large cities such as Antananarivo, capital of Madagascar, anthropogenic pollution affects aquatic ecosystems. Therefore, freshwater fish caught in these waters may display a poor quality and a potential source of environmental contaminants including trace metals.

The aim of this study was therefore to characterize levels of freshwater fish contamination from three areas in Antananarivo. This study will also access the potential sources of these contaminations in the environment. Fish were sampled in the three areas, which display part of the diversity of freshwater areas in Antananarivo, including a floodplain, a swamp and a lake. Concentration of trace metals and stables isotopes ($\delta^{13}C$ & $\delta^{15}N$) was measured in white muscle of tilapia to characterize fish quality. Also, particulate organic matter and sediments stables isotopes ($\delta^{13}C$ & $\delta^{15}N$) were analysed to trace anthropogenic contamination from wastewaters. Afterward, a survey was carried out to identify sources of contamination according to identified trace metals.

Trace metals in tilapia muscles from this study displayed a trend in concentration (Fe>Zn>Hg>Cu>Pb>Cr>Mn>Ni>As). However, in the three selected areas, concentrations of trace metals were beyond allowable limits for human consumption. For instance, the Masay Swamp was the most polluted area and stable isotopes analyses permitted to evidence the occurrence of anthropogenic wastewater being usually enriched in $\delta^{15}N$ (Fig. 1). However, water contamination in Tanjona Floodplains is rather belonging to agricultural weed and pests chemicals.

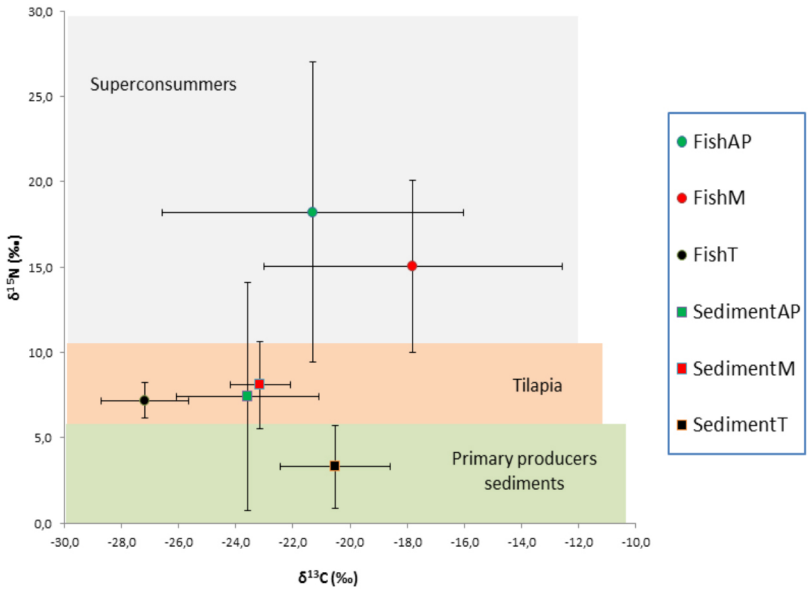

Fig 1: Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope biplot of fish and sediment from the three study areas. Coloured box corresponds to theoretical nitrogen signatures

IMPACT OF ANIMAL WELFARE AND ENVIRONMENTAL INFORMATION ON THE CHOICE OF ORGANIC FISH: EVIDENCE FROM GERMAN CONSUMERS

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Unlike organic labeling of plants, organic labeling of animal based products claims both environmental protection and animal welfare issues. This article examines the effect of information on these to or their combination thereof on consumer preferences in the setting of the EU organic aquaculture production principles. A choice experiment was used to examine German consumers' preferences for farmed rainbow trout, where respondents were split into groups receiving different levels of information. The results show significant preference for organic labeled fish products among majority of consumers. Informing consumers about animal welfare consequences associated with the label significantly increases the likelihood of choosing a labelled product. A similar effect was not found when informed about environmental consequences. When being informed of both environmental and animal welfare consequences, the priority for choosing organic was a little lower than when being informed of health alone. Thus, combining both sets of information may overwhelm consumers and leads to a lower marginal utility of organic products compared with the animal welfare effect.

MARKET INTEGRATION BETWEEN FARMED AND WILD SPECIES IN BANGLADESH

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The study examines market integration between farmed and wild species in Bangladesh. Farmed species including Tilapia and Pangas are tested against the wild-caught species Ilish, Puti, Chotochingri, Boal and Ahire in the Bangladesh terminal market. A time series data on wholesale prices during January 2010 to May 2017 are considered for the study. It was found that there is market integration between farmed and wild species based on the Johansen cointegration framework. Market leadership and follower roles are however mixed and the law of one price is rejected in all market pairs but for Pangas and Ahire. Interaction between the wild and farmed species implies that fisheries management has implications for aquaculture production and vice versa.

EFFECT OF EUTHANASIA WITH ESSENTIAL OILS *Eugenia aromatica* AND *Cinnamomum zeylanicum* ON STRESS RESPONSE OF GILTHEAD SEABREAM (*Sparus aurata* L.)

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The basic principle of bioethics demands the instant death of animals in order to minimize the stress response. Studies addressing fish welfare at slaughter have started much later compared to land animals. In case of Mediterranean farmed fish, asphyxia in ice water is the preferred method. The cold environment leads eventually to the loss of brain function, however that rapid temperature changes are stressful for fish. Overdose of anaesthetic used for euthanasia is the proposed slaughtering technique for fish used for scientific purposes, according to directive 2010/63/EU. Use of essential oils is an alternative option for fish anesthesia, which is necessary for fish welfare. Essential oils may be safe, but display toxicity at specific concentrations.

Essential oils *Eugenia aromatica* and *Cinnamomum zeylanicum* known for their anaesthetic properties were presently applied for euthanasia in comparison to slaughtering by icewater. Stress response was determined by measuring fragmented DNA in hepatocytes using the molecular technique, comet assay (parameter: Tail Moment). Seabreams (mean weight: 14.97±5 g) were acclimated for a month in 150L tanks. Throughout the experiment, the water temperature was stable at 21±1 °C. Slaughtering methods of icewater and over-dose (0.4ml, 0.5ml and 1 ml per 2L of water) of essential oils *Eugenia aromatica* and *Cinnamomum zeylanicum*, were tested.

Results revealed a significant reduction ($p<0.05$) in DNA damage in case of essential oils (Fig.1). However, DNA damage in hepatocytes after application of 0.4 ml was higher compared to doses of 0,5ml and 1ml. This is probably due to the fact that this dose was too low for fish to lose consciousness in a short time. Fish stunning and slaughter techniques urgently need further studies, for existing as well as for newly farmed or consumed fish species or sizes. Various stunning and slaughter methods may be chosen, depending on context and species, for practical and physiological reasons and consumer requirements.

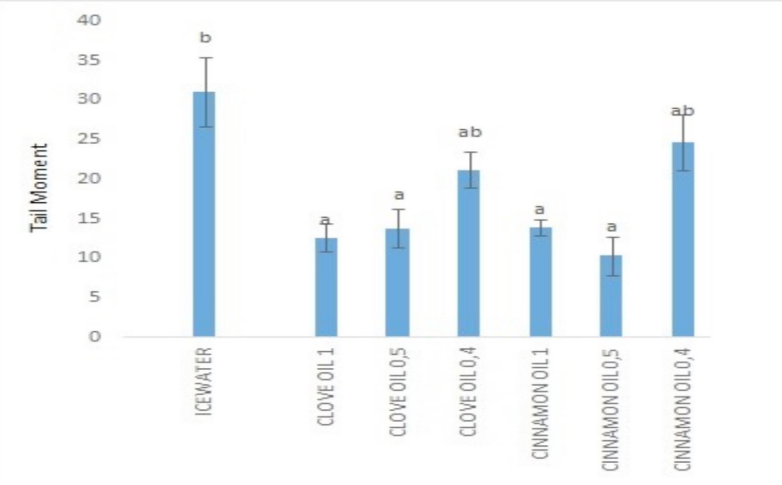

Figure 1. Tail Moment values in hepatocytes

ENDOGENOUS PROBIONTS IMPROVE SURVIVAL AND IMMUNITY IN BLACK TIGER SHRIMP *Penaeus monodon*

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The use of probiotics is employed as a disease management strategy in shrimp farming worlwide, including the Philippines. In the early years of probiotics research, the application of bacteria already available in the human health, bioremedation, and veterinary fields is a common theme of many researches on shrimp farming. However in recent years, use of bacteria isolated from the host itself or from its rearing environment has become essential in efforts to increase efficacy. For bacterial disease control strategies, studies indicate that green-water from saline-tolerant tilapias and their skin extracts are capable of inhibiting pathogenic *Vibrio* spp. in shrimp.

In this study, probionts 1.1 and 2.1 isolated from Tilapia mucus were biochemically identified as *Bacillus* sp. and exhibited inhibitory activity against *Vibrio harveyi* and *V. parahaemolyticus in vitro*. A pathogenicity test was conducted to check if the isolates were pathogenic to the shrimp. Subequently, an 8-week feeding trial was conducted to evaluate the effects of the probionts on growth, survival and immunity of *P. monodon*. A formulated shrimp feed was supplemented with either of the probionts and fed to the shrimp. Thereafter, the shrimp were experimentally infected with *Vibrio harveyi* and mortality was observed for 2 weeks.

The pathogenicity test demonstrated that both isolates were not infective to the shrimp. Although growth was not significantly affected, survival was improved with the probiont supplementation (Figure 1). Phenoloxidase (Figure 2) and bactericidal activity were enhanced with the dietary application of the probionts. Moreover, both isolates showed protection to the shrimp against *V. harveyi* infection.

The results indicated that isolates 1.1 and 2.1 are potential probionts for *P. monodon*.

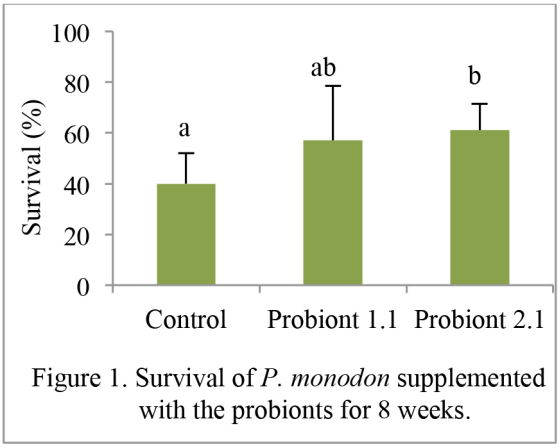

Figure 1. Survival of *P. monodon* supplemented with the probionts for 8 weeks.

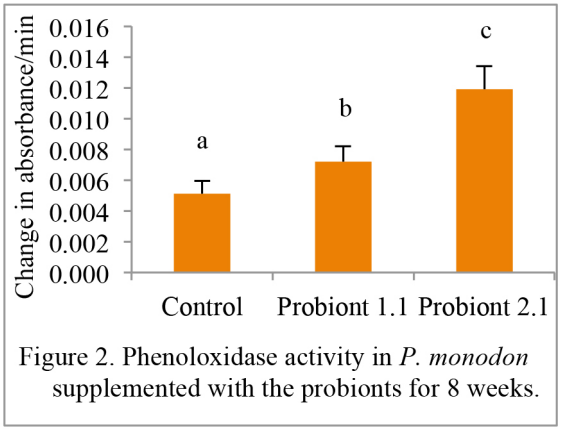

Figure 2. Phenoloxidase activity in *P. monodon* supplemented with the probionts for 8 weeks.

EFFECT OF DENSITY ON THE GROWTH AND SURVIVAL OF PACIFIC OYSTER *Crassostrea gigas* REARED IN WATERS OF THE BASQUE COAST (SE BAY OF BISCAY)

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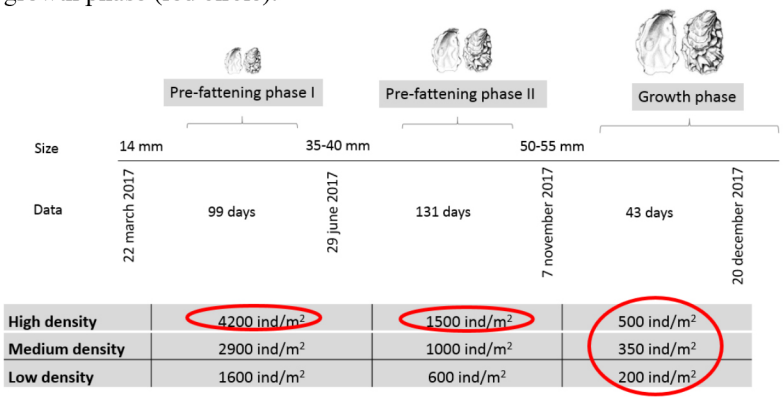
An important question in bivalve aquaculture is how many individuals of a certain species can be grown on a lease before growth is limited or before mortality becomes unacceptable. Thus, in the present study effects of stocking density on changes of total weight, length and survival were evaluated monthly along the production cycle of the Pacific oyster (*Crassostrea gigas*) deployed in Mutriku, a small harbor located in the Basque coast (SE Bay of Biscay). The experiment ran for 10 months, from March to December 2017. In each cultivation phase oysters were divided into three density groups referred as low, medium and high densities. The oceanographic variables monitored monthly were those routinely registered in the study area.

During the first pre-fattening phase (99 days duration) oysters cultivated at low densities presented significantly higher growth in length and weight (36.6 mm and 3.6 g) than oysters cultivated at medium (34.1 mm and 2.7 g) and high (32.6 mm and 2.6 g) densities. However, no significant differences were detected in survival values among the oysters of different density groups. Survival was 99% in all density groups. In the second pre-fattening phase (131 days duration), the same trend was observed indicating that oysters cultivated at low densities showed higher size and weight (56.2 mm and 16 g) than oysters cultivated at medium (48.4 mm and 13.1 g) and high (45.2 mm and 10.5 g) densities. Accordingly, the mortality was low since survival values were higher than 97% in oysters from the three density groups. In the growing phase (43 days duration), no significant differences were observed in the size of oysters cultivated at different densities (47.9-50.4 mm) but oysters cultivated al low density presented higher weight (14.2 g) than oysters cultivated at medium (10.6 g) and high (11.1 g) densities. Survival values were higher than 95% in all density groups, but oysters cultivated at low (98.1%) and high (97.8%) densities presented higher survival values than oysters cultivated at medium density (95.3%).

The best oyster culture densities selected for each growth phase are shown in Figure 1.

Figure 1: The best densities selected to obtain the best growth in each growth phase (red circle).



Overall the effect of density on the growth was critic, but especially in the pre-fattening phase, when the best growth rates were obtained at low densities. On the other hand, the density had no effect on survival values possibly because mortality is associated with other variables (i.e. pathogens) not measured herein.

Acknowledgements: This work was partially supported by the Department of Agriculture, Fisheries and Aquaculture from the Basque Government through the projects “SIMMA and MUSSELS”.

DEVELOPMENT OF A METHOD FOR LIVE OCTOPUS TRANSPORTATION FOR LONG DISTANCE AT HIGH DENSITIES

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Fishing is one of the most important economic sectors in Portuguese social economic history, being particularly relevant in the Algarve region. The development of new octopus based products with a growing economic value ensures the commercial interest of this species to a national and international level. In countries like Japan and Korea the consumption of live octopus is very traditional. These countries have shown interest on live octopus importation and the possibility of integration of the Portuguese octopus (*Octopus vulgaris*) in this market has generated great enthusiasm among ship owners and octopus fishermen.

Methods for long distances transportation of live octopus at high densities have been tested. The system was composed of 220 l tanks equipped with a cooling and aeration system, where the animals were kept isolated in pvc pipes of 14 and 16 cm in diameter covered with 20 mm mesh network. The water temperature was maintained at 10° C, after a controlled decreasing rate of 1° C/hour. Before the trial, animals were fasted for two days. Live Octopus transportation was tested for a 48 hours period at two densities: 50 kg/m^3 and 100 kg/m^3. During this period water temperature, dissolved oxygen, pH and nitrogen compounds were monitored. Animals behavior and mortality were also recorded. Octopus stress response was evaluated through the analysis of hemolymph samples, muscle and brain tissues. For both densities (50 and 100 kg/m^3) a 100% survival was registered after 48 hours. In all trials, water quality remained within the normal limits considering the dissolved oxygen and pH. In both densities, there was however an increase in the levels of ammonia, far above the recommended by literature. Ammonia, dopamine and Hsp70 levels were analyzed in the beginning and at end of the experiment for both densities, however no significant differences were found among them. In general, this study indicates that this system is a viable solution for live Octopus transportation during 48 h trip at a density of 100 kg/m^3.

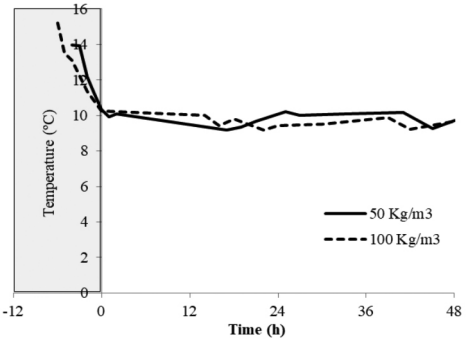

Fig. 1 Experimental temperature variation for both densities.

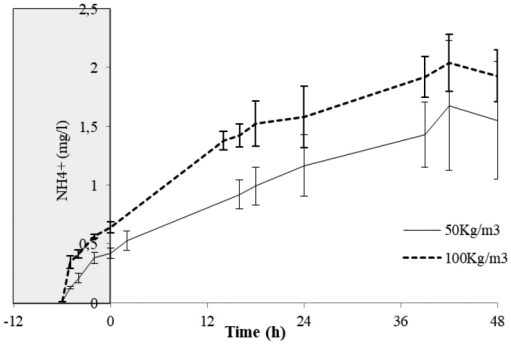

Fig. 2 Water ammonia levels variation for both densities.

Table 1. Mean values±SEM of Dopamine (ng/ml hemolymph), Ammonia (g/100g brain) and relative Hsp70 protein expression in octopus muscle.

	Dopamine (ng/ml)	Ammonia (g/100g)	Relative Hsp70 expression
Initial	1,35±0,6	0,082±0,003	0,17±0,03
50 Kg/m3	1,3±0,9	0,085±0,005	0,27±0,06
100 Kg/m3	1,54±0,7	0,082±0,011	0,28±0,09

IMPACT OF PROBIOTICS ON GROWTH PERFORMANCE AND SHRIMP SURVIVAL: A META-ANALYSIS

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Probiotics have emerged as promising alternatives for improving resistance to diseases and stimulating growth of farmed shrimp. The aim of the present work was to assess the effect of probiotic addition on shrimp survival and growth performance through meta-analysis. PubMed, SciELO and Google Scholar databases were searched in all languages from 1980 to 2017. We only selected the experiments using penaeid shrimps without apparent disease, with the results published in peer-reviewed journals with any dispersion measure. Survival, specific growth rate (SGR) and feed conversion ratio (FCR) were selected as outcomes. We evaluated experimental design, shrimp species, developmental stage, probiotic strain, modes of administration among others. A total of 100, 60 and 49 studies were included to assess probiotic effects on survival, SGR and FCR, respectively. Probiotics increased survival compared to controls (SMD = 3.068, 95% CI 2.594 to 3.543) and improved SGR (SMD = 3.811, 95% CI 3.269 to 4.354) and FCR (SMD = -3.194, 95% CI -3.469 to -2.920) in the pooled standardized mean difference random effect model, considering the source of heterogeneity and publication biases (i.e. where the decision to publish or not depends on the outcome of the experiment, e.g. withhold negative outcomes). Survival, SGR and FCR were improved in shrimps treated with probiotic. To our knowledge, this is the first report of a meta-analysis designed to investigate the effects of probiotics on shrimp survival and growth performance.

The wide variety of experimental designs detected in this meta-analysis, is a source of heterogeneity that affects the results and reduces the consistency of the findings. However, this meta-analysis allowed us to identify certain components of the experimental designs that could affect the probiotic effect on shrimp farming (Figure 1). This, in turn allowed us to define guidelines to standardize the experimental designs of future trials. Some of these guidelines are that: (1) there are more chances of finding beneficial effects on shrimp farm indicators by including probiotics in the feed; (2) probiotic viability should be maintained during all the trial; (3) trials should focus on the use probiotics with more than one strain (4) increasing the numbers of animals can improve the success of probiotics use and (5) trials designed as experimental growth models can be useful to assess the effectiveness of the probiotic to induce enzyme such as amylase, lipase, protease and gut probiotic colonization.

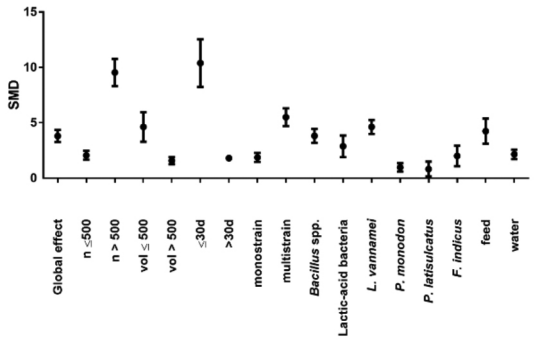

Figure 1.Subgroup analysis comparing the effect of probiotic supplementation on specific growth rate (SGR) of penaeid shrimps.

STOCKING ACCENTUATES GENETIC INTROGRESSION OF FARMED ESCAPED SALMON IN A WILD SALMON POPULATION

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Supplementary stocking is an important conservation strategy in many species, one example being the Atlantic salmon (*Salmo salar*). However, stocking can have unwanted population genetic effects on the recipient population, particularly in systems where admixture between domesticated and wild conspecifics occurs and where broodstock may be introgressed with farmed escapees.

To investigate how stocking affects farmed genetic introgression in a wild recipient population, we looked at two independent datasets from the same stocked river. These datasets comprise 1) individual estimates of farmed genetic introgression in the broodstock from seven brood years and their returning offspring, and 2) individual estimates of farmed genetic introgression in returning spawners of wild and hatchery origin from 20 run years over a 30-year period.

Our results show that severely introgressed broodstock produce more than twice the number of adult offspring compared to wild broodstock (Figure 1) and that spawners of hatchery origin are more introgressed than wild individuals (Figure 2). Stocking may therefore accentuate genetic introgression of escaped farmed salmon in wild salmon populations.

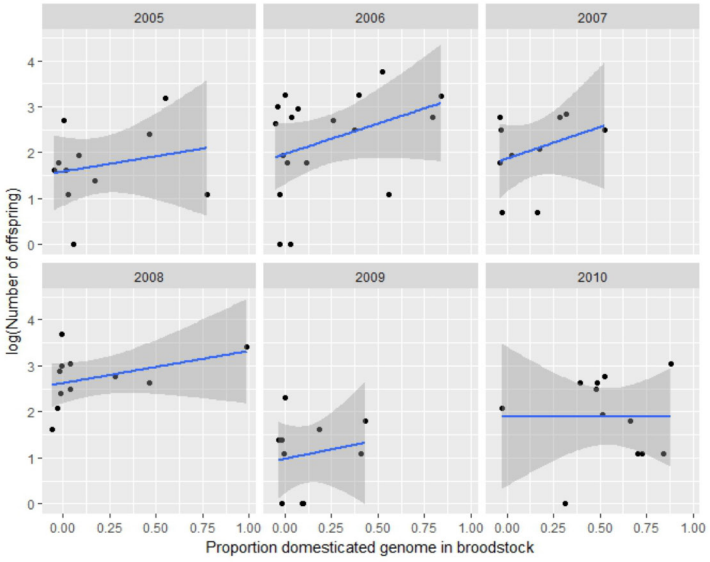

Figure 1: Proportion domesticated genome averaged across the broodstock pair for six of seven analysed brood years. More introgressed broodstock pairs produce a larger number of recaptured adult spawners (p = 0.03).

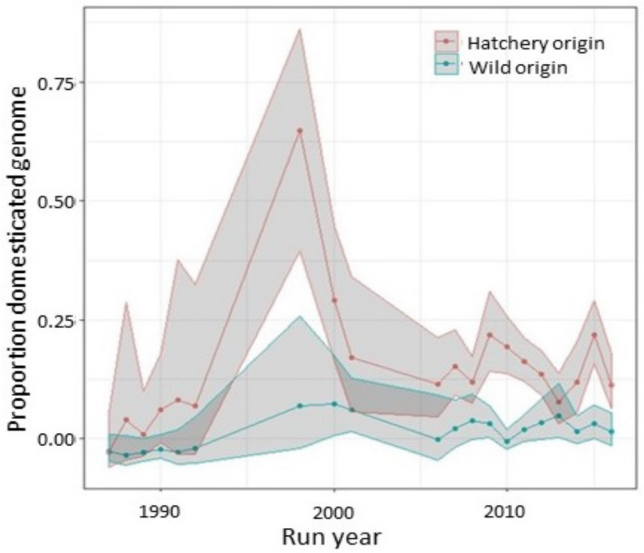

Figure 2: Introgression in returning adult spawners of wild and hatchery origin. Individuals of hatchery origin (n = 1567) were more introgressed than wild conspecifics (n = 1347) across all years compared.

MUSCLE EPA AND DHA LEVELS CORRELATE WITH DIETARY LINOLENIC ACID IN VEGETABLE OIL FED RAINBOW TROUT

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Having long chain (20-22 carbon) omega-3 fatty acids, which are very useful for human health, fish is very important food source. As the global fish catches reached plateau, aquaculture activities accelerated. Growing aquaculture resulted in a remarkable need for aquafeeds globally and locally. In the current situation, finding alternative to fish meal and fish oil, which are the base for aquafeeds, is the major issue that aquaculture sector is facing. As the natural resources for fish meal and oil are limited, the requirement for the research on the suitable vegetable sources was urged. In general, fish producers focus on using the cheaper aquafeeds with no detrimental effect on fish growth while consumers are concern about product quality in the sense of long chain omega-3 fatty acids and other nutrients.

In the present work, alternative dietary lipid sources for the rainbow trout (*Oncorhynchus mykiss*) were investigated. Defatted fish meal based 5 iso-nitrogenous (~%45) and iso-lipidic (~%20) experimental diets were supplemented with fish oil (control; FO), hazelnut oil (HO), linseed oil (LO), soybean oil (SO) and a blend of linseed oil and soybean oil (1:1) (LO+SO). Fish with 5g initial weight were fed experimental diets for twenty-four weeks to the market size (~250g). At the end of the feeding trial, significant increase was observed in Δ6 desaturase and elongase gene expressions in fish fed diets with vegetable oils. Accordingly, EPA (20:5n-3) and DHA (22:6n-3) in fish muscle increased with the increasing amount of dietary LNA (18:3n-3) (Fig. 1). Our results suggested that rainbow trout has the ability to convert 18C fatty acids to 20-22C fatty acids depending on dietary lipid resources.

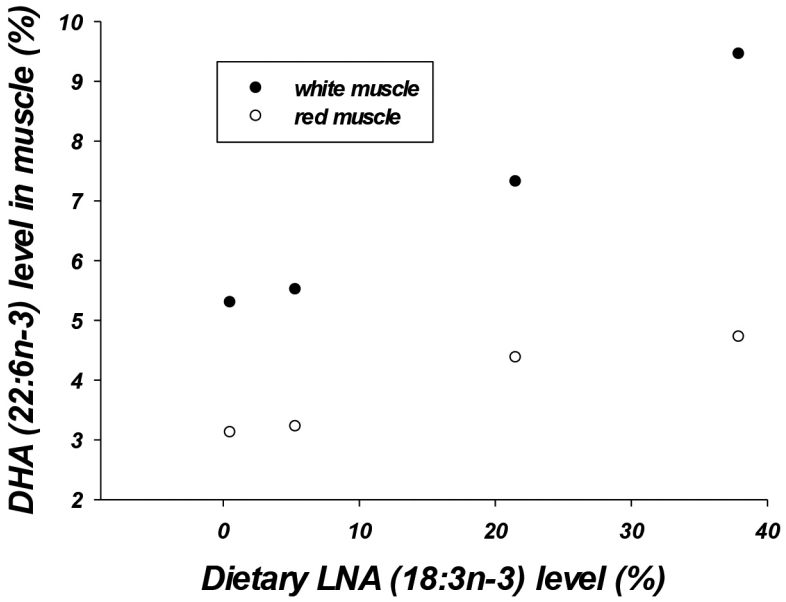

Fig. 1. Relationship between dietary LNA and muscular DHA in rainbow trout

BLUE MINER CLOUD BASED PLATFORM FOR AQUACULTURE ANALYTICS

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Introduction

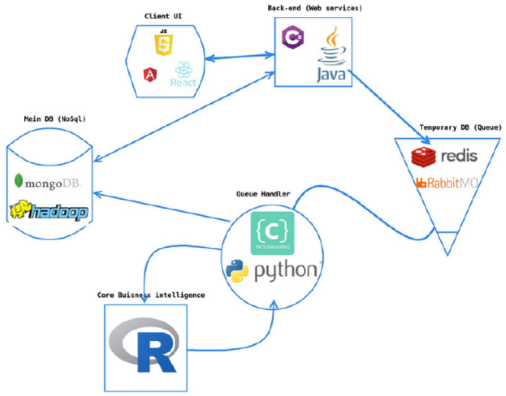
Aquaculture is probably the fastest growing food-producing sector and now accounts for more than 50 percent of the world's fish that is used for food. With the world population expected to reach nine billion by 2050, the aquaculture sector will play a key role in ensuring food and nutrition security. However, this growth is not without challenges; in order to satisfy the demand and minimize the impact on the environment, the sector has to use new technologies to intensify, diversify and produce in a more efficient, sustainable and environmental friendly way.

The work to be presented is a cloud-based platform that provides state of the art technologies to transform aquaculture production data into knowledge. Companies can then use this knowledge to boost performance, reduce costs and gain a competitive advantage. The platform combines descriptive statistics and machine learning technologies that companies can use to

- Explore your data
- Create independent models and use these models as a recommendation engine
- Benchmark fish performance
- Spot outliers
- Predict performance (FCR, growth, harvest result, etc.)
- Optimize harvest outcome to match customer's needs
- Improve monitoring of new species performance and brand planning.
- Reveal how the environment, fish feeding, production management and practices affects the production
- Optimize feeding strategies

It is based on cutting edge technologies to support performance and scalability. It supports data preparation, cleaning, advanced descriptive statistics and machine learning. It speaks the language of the aquaculture industry and it is the first time such technologies become available to the sector.

In addition to the platform features, real-life business cases and practical benefits of the application of such technologies in European aquaculture companies will be presented.



DIVERSITY OF THE PROTOZOAN PARASITE *Marteilia refringens* IN MARINE BIVALVES IN EUROPE

Isabelle Arzul*, Delphine Serpin, Bruno Chollet, Juliette Gaillard, Christine Dubreuil, Mathilde Noyer, Lydie Canier, Céline Garcia

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Marteilia refringens is a protozoan parasite belonging to the Paramyxida order (Ward et al. 2016). Its impact on flat oyster *Ostrea edulis* populations in Europe has contributed to include the related disease in the list of diseases notifiable to the EU and to the Word Organisation for Animal Health (OIE). The parasite host range includes other bivalve species among which mussels *Mytilus edulis* and *M. galloprovincialis*. *M. refringens* types O and M, preferentially detected in *O. edulis* and mussels respectively, were identified based on a polymorphism in the Internal Transcribed Spacer-1 (ITS-1). Recent phylogenetic analyses suggest that both types should be considered as two distinct species: *M. refringens* and *M. pararefringens* (Kerr et al. 2018). The parasite genome is still poorly known and only sequences belonging to the ribosomal gene cluster are currently available including 18S rRNA gene, ITS-1 and Intergenic Spacer (IGS).

Flat oysters and mussel samples collected in different European countries and confirmed to be infected with *M. refringens* were selected for further characterization work in PCR and sequencing on the three previously mentioned regions of the parasite genome. Obtained ITS-1 sequences reveal a relative but not strict host specificity of types O and M: type O was indeed mostly detected in flat oysters and type M in mussels, however rare co detection cases could be observed. IGS sequence analysis also supports this host type association.

The respective geographic distribution of *Marteilia refringens* types in Europe (Figure 1) questions the factors driving the development and transmission of the disease.

Ward, G. M., Bennett, M., Bateman, K., Stentiford, G. D., Kerr, R., Feist, S. W., Williams, S. T., Berney, C., & Bass, D. (2016). A new phylogeny and environmental DNA insight into paramyxids: an increasingly important but enigmatic clade of protistan parasites of marine invertebrates. *International Journal of Parasitology, 46*(10), 605–619

Kerr, R., Ward, G.M. , Stentiford, G.D., Alfjorden, A., Mortensen, S., Bignell, J.P., Feist, S.W., Villalba, A., Carballal, M.J., Cao, A., Arzul, I., Ryder, D., Bass. 2018. Marteilia refringens and M. pararefringens sp. nov. are distinct parasites of bivalves and have different European distributions. Parasitology In Press

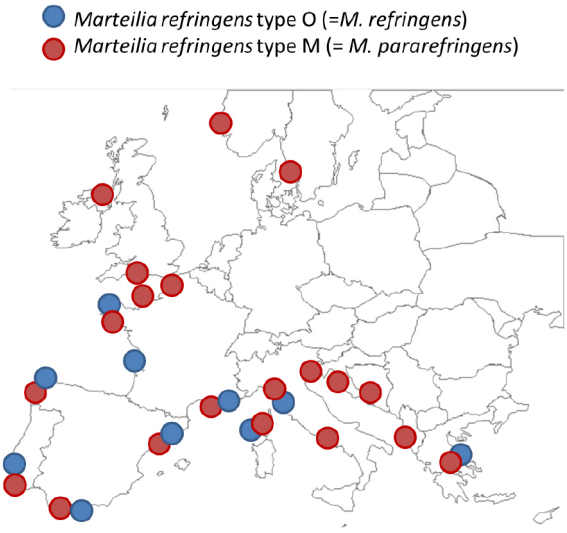

Figure 1. Distribution of *Marteilia refringens* types O and M in Europe (according to our current knowledge)

UV-INACTIVATION OF THE PROTOZOAN PARASITE *Perkinsus olseni*

Isabelle Arzul*, Clément Provot, Cyrielle Lecadet, Sergio Fernandez-Boo, Christophe Stavrakakis, Mathias Papin, Bruno Chollet, Jean-François Auvray

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Although the impact of the protozoan parasite *Perkinsus olseni* on clams *Ruditapes philippinarum* and *R. decussatus* is under debate, infection with this parasite is a notifiable disease to the Word Organization for animal health. Additionally, clams are listed as vector species for two endemic EU diseases, infections with *Bonamia ostreae* and *Marteilia refringens*. In that context, efficient water treatment could be useful to ensure the absence of these protozoan parasites within clam producing hatcheries/nurseries. No data is currently available regarding the efficiency of classical marine water treatment against known marine mollusc parasites. Considering that *P. olseni* is cultivable and can multiply in artificial media we have used this model to test the efficiency of UV to inactivate mollusc protozoan parasites in sea water.

For that purpose bench and hatchery scale approaches have been followed to test different doses of UV on suspensions of *P. olseni*. The survival of exposed parasites was estimated and compared with non-exposed parasites using neutral red in *P. olseni* suspension maintained in culture conditions (DMEM:HAMS' at 20°C) up to 21 days.

Bench scale experiments demonstrated the effect of UV on *P. olseni* survival (Figure 1). Three weeks after a 10 minutes UV exposure (188 mJ/cm2) less than 5% of parasite cells could be detected alive. Hatchery scale experiments confirmed the effect of UV and showed that 4 passages (corresponding to an UV dose of 168 mJ/cm2) were necessary and efficient enough to inactivate 100% of parasites.

The cultivable parasite *P. olseni* appears as an interesting model to test the efficiency of water treatment such as UV inactivation in conditions mimicking conditions used by producers.

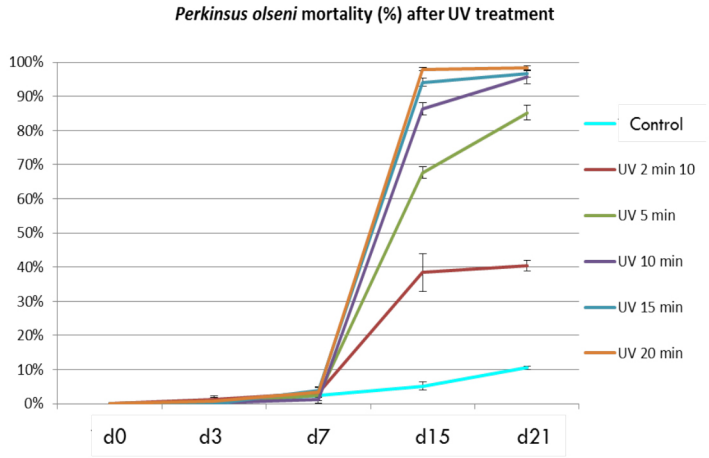

Figure 1. Bench scale experiment: P. *olseni* mortality at different times post UV exposure depending on UV doses.

ECOLABELS AND PRODUCT LONGEVITY: THE CASE OF WHITEFISH IN UK GROCERY RETAILING

Geir Sogn-Grundvåg, Frank Asche, Jimmy Young and Dengjun Zhang

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For the past twenty years the role of eco-labels and certification schemes has played an increasingly import role in food markets (Blend & van Ravenswaay, 1999; Onozaka & McFadden, 2011; Roheim, Asche, & Insignares, 2011). For example, the ASC label of the Aquaculture Stewardship Council provides assurance to the consumer regarding the environmental sustainability of the fishery from which the products originate. Similarly, in some markets both retailers and brand manufacturers emphasize the environmental credentials of the production method applied.

Recent studies based on hedonic price modeling and revealed preference data conducted in various seafood markets such as the UK, Germany, and Sweden indicate that some eco-labels such as that of the MSC and ASC labels obtain price premiums in most cases, but with substantial variation (Bronnmann & Asche 2017). The literature has largely focused on increased revenues to cover the cost of the ecolabel and possibly provide additional incentives for sustainable production processes. An unexplored question is whether ecolabels also might influence profitability in a supply chain by reducing cost. One example of this is product longevity. Intense competition for shelf space leads to relatively short product life cycles for many products (Asplund & Sandin, 1999). This seemingly never-ending spiral of creative destruction implies that at least some of the substantial resources invested in product development and marketing may be expendable. An extended product life implies lower cost and more efficient use of resources (Cooper, 2012). Eco-labels may play a part in the marketing strategies of retailers and national brand manufacturers in reducing cost by prolonging product life cycles. This is important because it sheds additional light on the largely unanswered question of whether price premiums for eco-labels are sufficiently large to cover the costs of a sustainable fishery and its certification. Due to lack of cost information, this is an important omission in the hedonic price function literature investigating ecolabel premiums (Roheim, Asche, & Insignares, 2011).

In this paper the effect of eco-labels on product longevity for seafood products is tested using duration analysis on a data set covering retailers in Glasgow, Scotland. Preliminary results indicate that ecolabeled seafood product have a longer product cycle, but that it varies by retailer.

COMBINATION OF ANTIMICROBIALS AS AN APPROACH TO REDUCE THEIR USE IN AQUACULTURE: EXAMPLE OF USING THIAMPHENICOL WITH FLORFENICOL AGAINST *Aeromonas hydrophila*

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

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

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

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

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

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

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

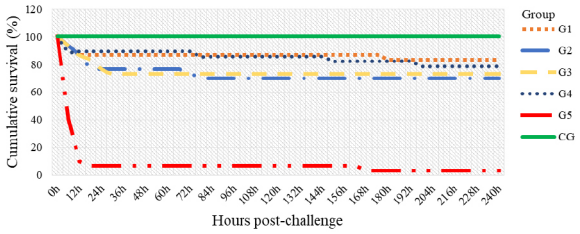

Figure 1. Cumulative survival (%) of Nile tilapia challenged with *A. hydrophila* and medicated with thiamphenicol (TAP) or florfenicol (FFC) alone and combined. G1: TAP (10 mg kg^{-1}); G2: FFC (10 mg kg^{-1}); G3: [TAP + FFC] (5 + 2.5 mg kg^{-1}); G4: [TAP + FFC] (2.5 + 1.25 mg kg^{-1}); G5: unmedicated;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

INFLUENCE OF AERIAL EXPOSURE AT LOW TIDE ON CONCENTRATIONS OF HUMAN-PATHOGENIC VIBRIOS IN OYSTERS CULTURED IN THE INTERTIDAL ZONE

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Aerial exposure of oysters cultured within the intertidal zone can lead to increased internal oyster temperature, with potential for increased vibrio levels and an associated human health risk. To investigate this potential, eastern oysters, *Crassostrea virginica*, were grown under intertidal or subtidal conditions at low (12-15 psu), moderate (18-25 psu), and high (27-35 psu) salinity sites within the mid-Atlantic region of the US, then examined monthly to assess concentrations of total *Vibrio parahaemolyticus* (*tlh+ Vp*), pathogenic *V. parahaemolyticus* (*trh+ Vp* and *tdh+ Vp*) and total *V. vulnificus* (*Vv*). Relative effects of salinity, tidal position, and duration of aerial exposure are shown in Figure 1. Vibrio concentrations were strongly associated with salinity. Total and pathogenic *Vp* demonstrated unimodal responses with the highest concentrations found at moderate salinity. In contrast, highest concentrations of *Vv* were at low salinity sites, and declined from this baseline across the moderate and high salinity sites. All sampled markers increased during exposure to aerial conditions, but the magnitude was dependent on vibrio species, strain and duration of exposure. *Vp* concentrations increased from two to four hours of aerial exposure, the maximum exposure time in the region, but *Vv* concentrations did not. We did not see a strong effect of gear type (subtidal vs. intertidal) at the initial emergence of the outgoing tide, suggesting that the elevated vibrio concentrations in intertidal oysters we observed when exposed returned to levels found in subtidal oysters by the following tidal exposure, if not sooner. These data reinforce regulations using the time of initial aerial exposure as the time-to-temperature starting point for oysters grown in the intertidal. Best management advice should recommend that growers harvest intertidal oysters as close to initial aerial exposure as possible.

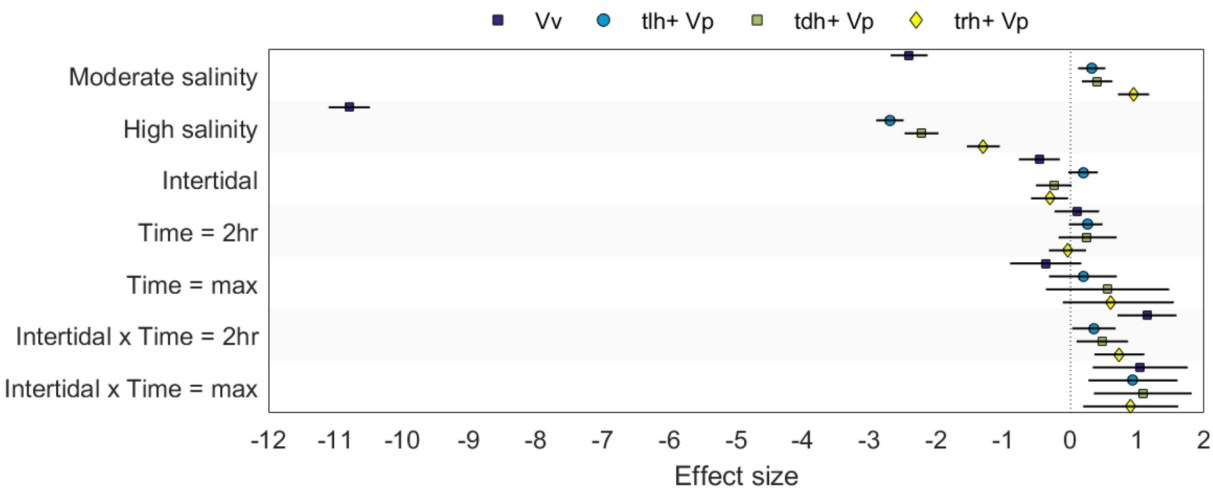

Fig. 1: 95% highest posterior density intervals of modeled effects describing the relative change in vibrio concentrations given the measured covariates (y-axis). Moderate and High salinity effects relative to low salinity levels. Intertidal effect relative to subtidally cultivated oysters. Time effects are relative to levels at initial emergence.

AQUACULTURE LIVESTOCK INSURANCE

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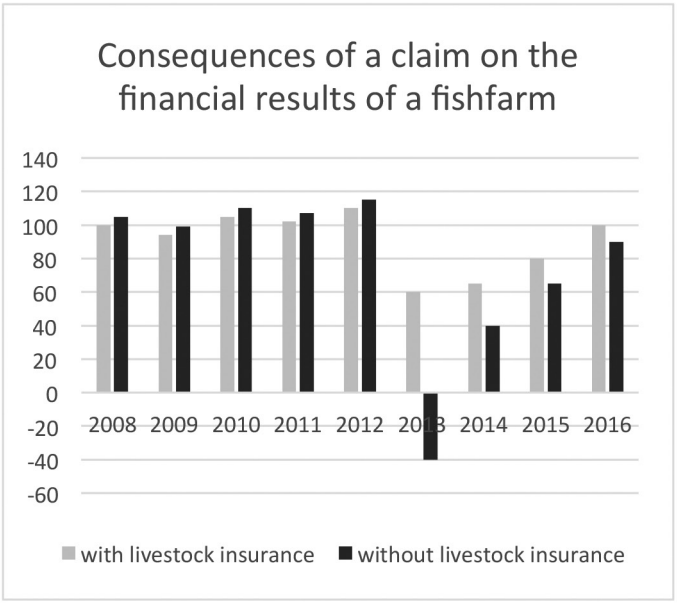
With aquaculture, the fish, the crustaceans or even the molluscs you are farming represent the future income of your business. Losing this source of income as a result of a mechanical fault, bad weather, disease or whatever will deprive your business of its livelihood. Given these risks, aquaculture insurance is not something you can afford to ignore. It will guarantee your future income and ensure the long-term survival of your business. The risk calculated by the insurer will therefore be specific to each business and will naturally depend on many factors such as the type of farming, the species, the experience of the staff and the location amongst other things.

A real case study with loss on offshore Seabream farm will show the importance of being covered for the farmers to guarantee the future of their business. Furthermore, what is the evolution of the aquaculture insurance trade after the huge losses in Chile in 2016 or in front of all the new technologies today developed?

Using a specialist broker and a specific policy is essential as choosing a policy working strictly on agreed value. We will use examples on all these points based on real cases (non-specific policy proposed in some geographic area & non-agreed value policy) will demonstrate the consequences of those two last points on the quality of the coverage for the farmer.



INPUT OF NEXT GENERATION SEQUENCING INTO THE ANALYSIS OF POORLY STUDIED FISH VIRUSES: EXAMPLE OF CYPRINID HERPESVIRUS-3

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Koi herpesvirus disease (KHVD) is an emerging disease that causes mass mortality in koi and common carp, *Cyprinus carpio* L. Its causative agent is the Cyprinid herpesvirus 3 (CyHV-3), also known as koi herpesvirus (KHV). If data on the pathogenesis of this deadly virus is relatively abundant in the literature, still little is known about its genomic diversity and about the molecular mechanisms that lead to such a high virulence. In this context, we developed a new strategy for sequencing full-length CyHV-3 genomes directly from tissues of infected fish. Total genomic DNA extracted from carp gill tissue was specifically enriched with CyHV-3 sequences through hybridization to a set of nearly 2 million overlapping probes designed to cover the entire length of the genome. Even though the rate of enrichment was directly correlated to the initial viral load, results revealed that full genomes could be recovered from gill samples containing as little as 5,000 CyHV-3 copies, with a high depth (>100x) almost all along the genome. Full genome sequences of 18 CyHV-3 specimens or isolates from all over the world as well as 9 nearly-full or partial genomes of atypical specimens were further obtained. These latter included specimens that could not be detected by the OIE-recommended primers and/or that did not elicit the clinical signs classically associated with KHVD. First analyses highlighted a high proportion of intra-specimen sequence heterogeneity, suggesting the presence of mixed infections with different levels of virus variations in most specimens. Comparison of typical specimens showed that genetic diversity at the genome scale was very low (Fig. 1) and confirmed the existence of only two lineages, *i.e.* one Asian and one European. However, some atypical samples exhibited a marked divergence (~3%), which translated into significant alterations of many predicted open reading frames. Analyses are still ongoing, and results will undoubtedly i) help shed new light on the evolution patterns and replication strategies of this deadly virus, and ii) contribute to the establishment of suitable measures to reduce its incidence (*e.g.* vaccination).

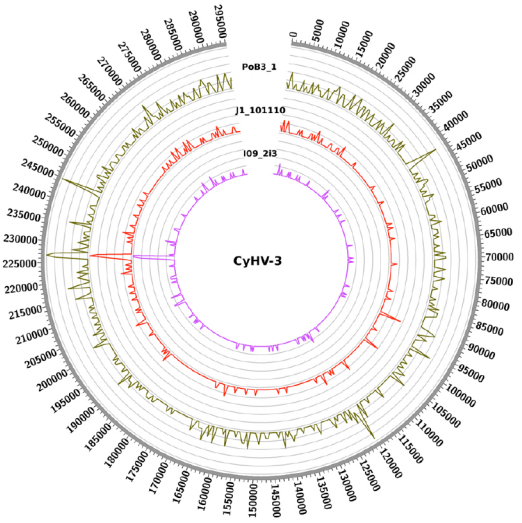

Figure 1. Distribution of variations along CyHV-3 genomes. The curves depict the variations (SNPs, Indels) of 3 genomes compared to KHV-J. This circular visualization was created with CIRCOS software.

SUSPENDED OR LOCALIZED BIOFLOC SYSTEMS: DEFINITION, DATA AND NEED FOR FURTHER RESEARCH

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Biofloc technology systems are based upon the minimal exchange of water, and a subsequent development of high microbial biomass that can support production in those systems. The microbial biota degrade organic residues in the water, encourage spontaneous TAN removal and control TAN concentration by the control of C/N ratio in the feed and a subsequent control of TAN in the water. In addition, the presence of the rich biota raise feed value and utilization, increase immunity against diseases and improve production and profit. Most existing BFT ponds are highly mixed and aerated, leading to suspension of the bioflocs in the water. Such systems can be effective when fish (or shrimp) biomass is high (shrimp stocking rate >> 100/m^2) and feeding rate above 20 g/m^2, supplying ample organic substrates needed to the development of the bioflocs. Thus, only intensive and properly equipped ponds can be properly effective as biofloc systems.

A different approach was demonstrated recently by Suryakumar and Avnimelech (2017). Vertical substrates (plastic nets) were fixed in the pond enabling attachment of organic residues and the resultant microbiota on these nets and by this, lowering sedimentation of organic particles on the pond bottom. The attached microbial biota was managed, similar to management of suspended bioflocs, by locally directed water movement and aeration of the vertical substrates and through the adjusted supply of carbohydrates to induce TAN assimilation by the attached heterotrophic biota. Such ponds are operating as efficient biofloc system, even when stocking rates are lower than those needed for suspended BFT (e.g. stocking with 25 PL/m^2).

It is possible to properly manage fixed biofloc systems using limited aeration and mixing devices, probably without lining all pond area, in a way enabling traditional farmers to use biofloc approach and advantages. Different formulations of fixed supports for microbiota development are possible.
More R&D of different biofloc systems is needed.

IDENTIFICATION OF BACTERIA ISOLATED FROM CATFISH (*Clarias gariepinus*) FROM SELECTED FISH FARMS IN ODOGBOLU LOCAL GOVERNMENT, OGUN STATE, NIGERIA

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The need for proper identification of bacterial organisms from catfish cannot be overemphasized as it provides update information on emerging and existing organisms thereby enriching the gene bank for our catfish.

Samples of *C. gariepinus* were collected from Elegbeji, Sanni, Kunle, Johnson, Adewale and Awosanya fish Farms for analysis at the Microbiology laboratory, Federal University of Agriculture, Abeokuta, Ogun State. Specimens collected from flesh, gills and intestine were subjected to microbiological examination for colonial, morphological characteristic, biochemical tests and molecular tests. Bio Edit was used for importing and mining nucleotides sequences into Gene Bank. The results revealed the following bacterial organisms: Elegbeji and Sanni fish farms (1 and 2) *Staphylococcus aureus, Pseudomonas aeruginosa, Aeromonas veronii, Bacillus subtilis* were identified from the skin and Gills while *Enterococcus feacium* was also identified from the intestines. Kunle and Johnson Farms (3 and 4) , the Major bacterial organisms identified from skin, gills and Intestines of the fish were *Staphylococcus aureus, Pseudomonas aeruginosa, Aeromonas veronii, Bacillus subtilis,* while Adewale and Awosanya Farms (5 and 6) had *Enterococcus feacium* and *Streptococcus agalactiae*. The BLAST result is a confirmation of the bio-chemical test earlier carried out with percentage similarity ranging from 78-98% and their accession number, the bacterial organisms identified were *Aeromonas veronii, Enterococcus feacium, Pseudomonas aeruginosa, Bacteria subtilis, Staphylococcus aureus, Streptococcus agalactiae*. The bacterial organisms are present on the fish, but not invasive and only become dangerous if conditions necessary for disease initiation are present such as susceptible host, virulent pathogen and favourable environment are present at the same time. Therefore, preventive medicine is the solution for fish farm management, but the blast result can be build into Nigeria Gene bank data for catfish.

Table 1: **BLAST Results of bacteria isolated from catfish *Clarias gariepinus* from selected farms**

Site of Infection	Name of Bacteria	Description	Accession no.	% similarity
Skin S9	*Aeromonas veronii*	G18	KF853564.1	81%
Gills 3G5	*Bacillus subtilis*	MJ4	KF933349.1	93%
Intestine I10	*Pseudomonas aeruginosa*	strain MBL	KF811604.1	80%
Skin 3S2	*Staphylococcus* sp.	38MP	FR719724.1	89%
Intestine 2I5	*Pseudomonas aeruginosa*	MBL	KF811604.1	83%
Skin S2	*Staphylococcus* sp.	38MP	FR719724.1	90%
Skin S7	*Bacillus subtilis*	strain TO-AJPC	CP011882.1	87%
Gills G2	*Staphylococcus aureus*			
Intestine 2I1	*Streptococcus agalactiae*	15-92MP	EU075070.1	98%
Intestine I6	*Enterococcus faecium*	strain E1	HG798651.1	90%
Gills 3G5	*Bacillus subtilis*	isolate B-20091009	AM110937.1	85%
Intestine I10	*Pseudomonas aeruginosa*	strain 3.5.2	HM192785.1	93%
Intestine I7	*Bacillus subtilis*	gene 1631	HE 612877.1	95%
Intestine I9	*Pseudomonas aeruginosa*	strain MBL	KF811604.1	80%
Intestine I14	*Aeromonas veronii*	strain R9	KF853563.1	79%
Intesine I13	*Enterococcus faecium*			
Intestine I8	*Pseudomonas aeruginosa*	strain MBL	KF811604.1	81%
Intestine 2I3	*Pseudomonas* sp	CfO-4	JN836274.1	97%
Gills 2G1	*Aeromonas veronii*	strain G8	KF853564.1	81%
Skin S4	*Pseudomonas aeruginosa*	NCTC	LN831024.1	86%
Skin 2S1	*Aeromonas veronii*	strain G18	KF853564.1	79%
Skin 2S2	*Aeromonas veronii*	strain G18	KF853564.1	78%
Skin 2S4	*Aeromonas veronii*	strain R9	KF853563.1	80%
Intestine 2I2	*Staphylococcus aureus*			
Intestine 2I4	*Pseudomonas aeruginosa*	NCTC 10332	LN831024.1	96%
Gills 3G2	*Pseudomonas aeruginosa*	NCTC 10332	LN831024.1	97%
Gills 3G6	*Pseudomonas aeruginosa*	NCTC 10332	LN831024.1	94%
Gills 3G8	*Pseudomonas aeruginosa*	NCTC 10332	LN831024.1	95%
Gills 3G10	*Pseudomonas aeruginosa*	NCTC 10332	LN831024.1	93%
Gills 3G15	*Pseudomonas aeruginosa*	NCTC 10332	LN831024.1	97%
Intestine 3I10	*Pseudomonas aeruginosa*	NCTC 10332	LN831024.1	94%
Skin 3S1	*Pseudomonas aeruginosa*	NCTC 10332	LN831024.1	96%

ANTIMICROBIAL RESISTANCE IN BACTERIA ISOLATED FROM MEDITERRANEAN FISH FARMING

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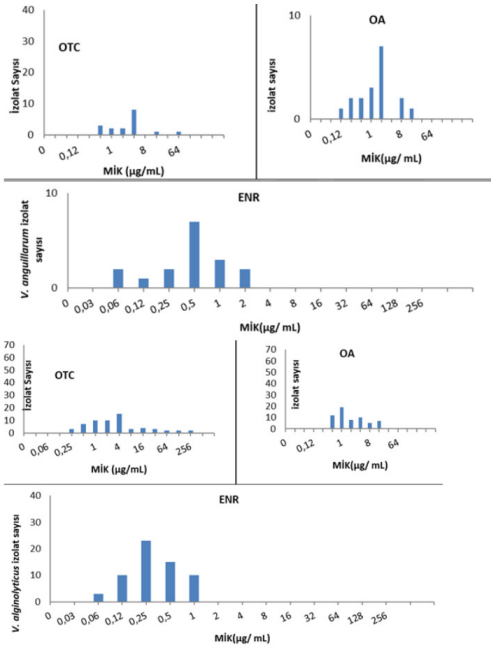
Fish is an important source of food in human health, due to its rich content in polyunsaturated fatty acids. According to data from Turkish Statistics Institute in 2016, the production rate of aquaculture products is 558.715 tonnes (TUIK, 2018), according to Federation of European Aquaculture Producers (FEAP), Turkey stands as the main producer among Mediterranean region in the production of seabass (Dicentrarchus labrax) and gilt-head seabream (Sparus aurata); also first in the production of rainbow trout (Oncorhynchus mykiss).

The most important loss in aquacultural production is related to the economic loss due to diseases. For the treatment of fish diseases, 7 active compounds and several antibacterial preparations are licensed in our country and European Union. Among these compounds, quinolone and tetracycline are the most well-known and important ones. Meanwhile, due to the imprudent and widespread use of antibiotics, lead accumulation of antibiotics in water environment and sediment for a long period of time leading to resistance in the target and non-target microorganisms. Resistant microorganisms are transferred to other fish species and aquatic organisms leading to alteration of the ecological balance, causing health problems among the consumers

In this research, samples of sea bass and gilt-head seabream as the most common cultured fishes in Aegean region and wild fishes are collected for the isolation of *Vibrio (Listonella) anguillarum* and *Vibrio alginoliticus* to detect the oxytetracycline, enrofloxacin and oxolonic acid resistance using conventional and moleculer methods. In this study, 240 samples (120 seabass and 120 gilt-head seabream) were collected from cage breeders and 82 samples (40 seabass and 42 gilt-head seabream) of wild fishes were caught between 30.10.2014 to 07.09.2015.

Drug resistance were tested with disc diffusion, minimum inhibitory concentration method(MIC-microplate dilution) according to CLSI and also molecular methods were used to determine resistance gene.

Results of this study and identification of the resistance gene in natural environment fish and cage fish in a lesser extent confirms the research hypothesis. This situation shows the importance of the matter and makes it important to take necessary precautions. Detection and implementation of measures should be carried out not only at the national level but also at the international level, considering the international status of the seas.



ASSESSMENT OF METHIONINE AND THREONINE DIETARY SUPPLEMENTATION IN RAINBOW TROUT (*Oncorhynchus mykiss*) EXPOSED TO ACUTE THERMIC STRESS

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Similar to other stress factors, seasonal temperature patterns might lead to severe biological consequences such as growth impairment, delayed reproductive development and compromised immune performance. Nutritional strategies to enhance health and stimulate disease resistance are currently being adopted and amino acids have been shown to have immune-stimulating properties. Methionine (MET) metabolic pathways are also associated to key immune mechanisms such as cell proliferation and antioxidant activity. Differently, threonine (THR) is a major component of intestinal mucin and promotes antibody production in lymphocytes. Farmed rainbow trout (*Oncorhynchus mykiss*) is particularly exposed to thermic stress due to the wide range of temperature in inland water reservoirs throughout the year. The present study aimed at evaluating individual and synergistic effects of MET and THR dietary supplementation in rainbow trout exposed to increasing water temperature. Four diets were formulated based on a control diet (CTRL) which formulation met the amino acid requirement levels established for rainbow trout. To do so, MET and THR were added individually or in combination to final concentrations of 2 × the respective requirement level and were named MET2, THR2 and MT2. Dietary treatments were randomly assigned to triplicate tanks and fish were fed for 15 days. Water temperature was maintained at 15 °C for 6 days from which point it gradually increased to 24 over the next 9 days (1 °C per day). Fish were sampled at day 1 (T0–15 °C), day 7 (T1-16 °C), day 11 (T2-20 °C), day 13 (T3-22 °C) and day 15 (T4-24 °C). A control group fed CTRL was held at a stable temperature of 15 °C (CTRL15) throughout the feeding trial. Blood and head-kidney samples were collected at every time-point for cellular and humoral parameters and gene expression analysis, respectively. Preliminary results showed that lysozyme along with neutrophils, monocytes and thrombocytes increased with temperature, regardless of dietary treatment. When no signs of immune activation are observed, increased innate immune defenses such as lysozyme and phagocytes are indicators of a primed immune system. Since peroxidase plasma content did not increase above levels measured at the beginning of the feeding trial, the observed enhanced immune condition might have been the result of thermic stress-mediated priming effect. Despite the temperature-induced increase in cell numbers, neutrophil and lymphocyte percentages were lower in MET2-fed fish than in CTRL, which seems to suggest that MET might be involved in negative feedback mechanisms to counteract the increasing leucocyte numbers. Despite no apparent effects of synergism or individual THR were observed, molecular analysis is currently being carried out to evaluate possible genomic modulatory effects of these diets not detected at the metabolic level. Nonetheless, present results expose important aspects of MET role in regulating stress and immune responses, highlighting its potential as a functional ingredient to farmed fish.

EVALUATION OF *Moringa oleifera* SEED MEAL AS REPLACEMENT FOR FISHMEAL IN *Clarias gariepinus* FINGERLING DIETS

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Adequate supply of food that is balance in energy, protein, minerals and vitamins is one of the limiting factors to fish production. Availability of cheap and readily available ingredients for fish feed formulation is essential in any intensive fish culture system. Scarcity and high cost of fishmeal has necessitated the research into unconventional feed resources for aquafeed. *Moringa oleifera* seed meal is a potential unconventional feed resources in aquaculture. It contains high protein (68%), high levels of essential amino acids, minerals and vitamins. The potential of this feed resources is not yet fully harnessed in aquaculture. This study was conducted for six weeks to determine the optimum levels of *Moringa oleifera* seed meal (MSM) inclusion in the diet for *Clarias gariepinus* fingerlings based on growth performance. Five treatments each with three replications were used, Treatment 1 (T_1) contains 25% fishmeal, Treatment 2 (T_2) contains 18.75% fishmeal and 6.00% MSM, Treatment 3 (T_3) contains 12.50% fishmeal and 12.00% MSM, Treatment 4 (T_4) contains 6.25% fishmeal and 18.00% MSM, Treatment 5 (T_5) contains 24.00% MSM. Fishmeal was replaced by MSM for an equal amount of crude protein in the diets. Feed was supplied at a rate of 5% of the fish body weight. The SGR, FCR and PER in T_2 was significantly ($P<0.05$) higher than that of other treatments. However, the SGR, final weight and weight gain of T_1 and T_2 were not significantly ($P>0.05$) different, but were higher than that of the other treatment groups. The results of this trial suggest that MSM has the potential to replace fishmeal and could be used up to 6% (25% fishmeal protein replacement) without any negative effects on growth and feed utilization of *C. gariepinus* fingerlings.

Table 1. The effects of *Moringa oleifera* seed meal levels on growth parameters of *C. gariepinus* fingerlings (mean ± standard error of triplicate values)

Growth parameters	Treatments				
	T_1	T_2	T_3	T_4	T_5
Initial weight (g)	8.40 ± 0.05	8.35 ± 0.03	8.39 ± 0.01	8.40 ± 0.02	8.40 ± 0.01
Final weight (g)	19.03 ± 0.11^{d}	18.92 ± 0.13^{d}	12.47 ± 0.04^{c}	11.50 ± 0.02^{b}	11.33 ± 0.02^{a}
Weight gain (g)	10.62 ± 0.07^{d}	10.57 ± 0.16^{d}	4.08 ± 0.04^{c}	3.10 ± 0.01^{b}	2.93 ± 0.03^{a}
Feed intake (g)	18.78 ± 0.16^{e}	16.30 ± 0.23^{d}	8.02 ± 0.33^{c}	6.02 ± 0.09^{b}	4.91 ± 0.06^{a}
SGR (%)	1.94 ± 0.02^{d}	1.95 ± 0.03^{d}	0.94 ± 0.01^{c}	0.75 ± 0.00^{b}	0.71 ± 0.01^{a}
FCR	1.77 ± 0.01^{b}	1.54 ± 0.04^{a}	1.97 ± 0.10^{c}	1.94 ± 0.02^{c}	1.68 ± 0.04^{b}
PER	1.42 ± 0.01^{b}	1.62 ± 0.04^{d}	1.27 ± 0.06^{a}	1.29 ± 0.02^{a}	1.49 ± 0.04^{c}

MACROBENTHIC ASSEMBLAGE STRUCTURE AND DISTRIBUTION AT THE BOOJAGH MARINE NATIONAL PARK, SOUTHERN CASPIAN SEA, IRAN

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Although macrobenthic assemblages are considered as major players in many ecosystems around the world, the ecology of Caspian Sea macrobenthos is currently understudied. This study describes the species composition and quantitative distribution of macrobenthos in the southern Caspian Sea and relates the distribution to seasonal changes at three depths (1, 5 and 10 meter) on the Boojagh Marine National Park (BMNP) coast in southern Caspian Sea between summers 2015 and 2016. To investigate the distribution of macrobenthos in BMNP, the data of the 450 samples were analyzed. In this study sixteen species were identified: *Cerastoderma glaucum*, *Mytilaster lineatus*, *Pyrgula grimmi, Anisus kolesnikovi, Stenogammarus carausui, Paraniphargoides motasi, Onisimus caspius, Pterocuma pectinatum, Pterocuma sowinskyi, Pseudocuma (Stenocuma) gracile, Nais sp., Hypania invalida, Manayunkia caspica, Streblospio gynobranchiata, Hediste diversicolor, Amphibalanus improvisus*.

Among them, the non-indigenous *C. glaucum* was the dominant species, accounting for 27% of the total abundance and in order *P. grimmi* with 14.4%, *A. improvisus* with 8.7%, *M. lineatus* with 7.9%, *Nais sp*. with 7.5%, *N. carausui* with 5.2%, *P. motasi* with 5%, *S. gynobranchiata* with 4.5%, *H. invalida* with 5%, *M. Caspica* with 3.1%, *P. sowinskyi* with 2.5%, *O. caspius* with 2.4%, *A. kolesnikovi* and *H. diversicolor* with 1.8%, *S. gracilis* with 1.6% and *P. pectinatum* with 1.5% were in the next rank. Significant differences in abundance across the sixteen species were observed among depths and seasons. This study highlights the potential consequences of established non-indigenous species in the southern Caspian Sea.

LONG-RANGE PCR AND HIGH-THROUGHPUT SEQUENCING OF *Ostreid herpesvirus 1*

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Ostreid herpesvirus 1 (OsHV-1) is an important pathogen associated with mass mortalities of cultivated mollusks worldwide. Since no cell line allows OsHV-1 replication *in vitro*, it is difficult to isolate enough high-purity viral DNA for High-Throughput Sequencing (HTS). We developed an efficient approach for the enrichment of OsHV-1 DNA for HTS with long-range PCR. Twenty-three primer pairs were designed and examined on ten OsHV-infected samples. Amplicon mixtures from six successfully amplified samples were sequenced with Illumina platform, and one of them (ZK0118) was also sequenced with PacBio platform. PacBio reads were assembled into 2 scaffolds compared to 9 - 68 scaffolds recovered from the Illumina reads. Genomic comparison confirmed high genetic diversity among OsHV-1 variants. The present study provided us a rapid method for determination of OsHV-1 genomes, which enables the study of viral population structures and their diversity.

Ten OsHV-1 positive samples determined by qPCR and collected during mortality events in Shandong Province, China were employed in the present study (Table 1). Primer pairs of long-range PCR (LR-PCR) were designed with the online version of GenoFrag using the genome sequence of OsHV-1 reference. Each amplicon was set 9-11 kb in length, and overlapped the adjacent amplicons by 500-1500 bp. All LR-PCR reactions were performed using the TaKaRa PrimeSTAR GXL DNA polymerase, which showed high fidelity and stability in amplifying long PCR products as reported previously.

All 23 amplicons were amplified with long-range PCR for supernatants with viral DNA loads ≥ 8.8×10^3 copies per µL . While for specimens with viral DNA loads ≤ 4.8×10^3 per µL, less than 13 amplicons were amplified despite optimization.

TABLE 1. *Ostreid herpesvirus 1* (OsHV-1) infected samples used for long-range PCR

Sample ID	Date of sampling	Age*	Viral loads
ZK0118	**August 2001**	**Adult**	**3.4×10^4**
ZK2002	**August 2002**	**Adult**	**2.1×10^4**
ZK2003	**July 2003**	**Adult**	**1.6×10^4**
ZK2004	**August 2004**	**Adult**	**3.2×10^4**
ZK2006	July 2006	Adult	8.5×10^2
ZK2007	September 2007	Adult	4.8×10^3
ZK2008	**August 2008**	**Juvenile**	**8.8×10^3**
ZK2011	August 2011	Adult	7.3×10^2
KH2015	**June 2015**	**Adult**	**4.6×10^4**
ML2015	May 2015	larva	2.3×10^3

TABLE 2. Summary of sequencing results.

Sample ID	No. of Scaffolds	Mean length of Scaffolds	Coverage on OsHV-1 (%)
ZK0118.3	2	984,189	95.50
ZK0118.2	9	21,046	93.64
ZK2002	36	5,019	92.71
ZK2003	42	4,353	91.46
ZK2004	17	1,1200	95.23
ZK2008	68	2,547	88.09
KH2015	16	1,2042	91.35

THE OPTIMUM LEVELS FOR THE MIXTURE OF ANIMAL AND PLANT PROTEIN AS THE FISH MEAL REPLACERS IN OLIVE FLOUNDER *Paralichthys olivaceus*

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A farm scale feeding trial was conducted to investigate the mixture of plant and animal protein, as the dietary fish meal replacer in sub-adult olive flounder, *parlichthys olivaceus*. Four experimental diets with a mixture containing equal amounts of animal and plant proteins were used to replace fish meal at 0% (control), 20 (APP20), 30 (APP30), and 40% (APP40). Fish averaging 478±4.7g (mean±SD) were randomly distributed into 12 tanks in the farm, and fed one of the four experiment diets in triplicate groups of 90 fish for 16 week. After the feeding trial, weight gain, specific growth rate, protein efficiency ratio and feed efficiency were not significantly different among fish fed the experimental diets ($P > 0.05$). However, feed efficiency of fish fed APP40 diet were significantly lower than those of fish fed the other diets ($P < 0.05$). Survival rate, whole-body proximate composition, hematological parameters, and non-specific immune responses were not significantly affected by the experimental diets. But intestinal villi length of fish fed APP20, APP30, and APP40 diets were significantly lower than those of fish fed control diet ($P < 0.05$). Results indicated that, based on growth performance and non-specific immune responses, the dietary animal and plant protein mixture could replace up to 30% of fish meal in sub-adult olive flounder.

POTENTIAL USE OF THE SYNBIOTICS AS THE ANTIBIOTIC REPLACERS FOR JAPANESE EEL *Anguilla japonica* AQUACULTURE IN KOREA

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Three experiments were conducted to investigate the potential use of synbiotics as the antibiotic replacers for Japanese eel (*Anguilla japonica*) aquaculture in Korea. For the first feeding trial, three different concentrations of *Bacillus subtilis* and Lactobacillus *plantarum* (10^6,10^7 and 10^8 CFU/g diet) were tested. Results indicated that based on growth performance, immunological parameters, gut morphology and disease resistance, *B. subtilis* at 10^8 and 10^7 CFU/g diets could be better effective probiotic than *L. plantarum* in Japanese eel. In the second feeding trial, three different concentrations of *B. subtilis* (0, 0.5×10^7 and 1×10^7 CFU/g diet) with two different concentrations of MOS or β-glucan (0 and 5 g/kg diet) were tested. Results indicated that combination of *B. subtilis* + MOS (0.5×10^7 CFU/g + 5 g/kg diet) group had better growth performance, immunological status, gut morphology and disease resistance than did the other combinations including control and antibiotics groups. In the third feeding trial, two probiotics, *Bacillus subtilis* and *Bacillus licheniformis*, were tested with MOS or FOS as the prebiotics. Results showed that *B. subtilis* (10^8 CFU/g diet) with either MOS or FOS (5 g/kg diet) could have the beneficial effects on growth performance, immunological parameters and disease resistance of Japanese eel. Therefore, the synbiotics *B. subtilis* (10^8 CFU/g diet) with either MOS or FOS (5 g/kg diet) could have the potential as the antibiotic replacers for enhancing growth performance, immunity and disease resistance.

IMTA ECOPATH MODEL METHODOLOGY: FOCUS ON A POND POLYCULTURE SYSTEM

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Fish farming is increasing worldwide and faces number of different social and environmental issues. Integrated Multitrophic Aquaculture (IMTA) is a production system using trophic interactions in order to limit input uses and environmental impacts. Using modelling might be an interesting way to support management and optimization of complex multitrophic systems. The aim of this study is to provide a methodology for model building, using Ecopath software, applied on a polyculture system based on common carp (*Cyprinus carpio*) culture.

In our study, Ecopath modelling methodology was supported by experimental data gathered from small pond (500m^2) polyculture system. Dataset concerned a polyculture of different species: common carp, roach (*Rutilus rutilus*), and Perch (*Perca fluvatilis*) (140 kg/ha of introduced fish) raised for 280 days, fed with 108.5 kg of commercial pellets. Information on chlorophyll, zooplankton and macroinvertebrates biomasses was also recorded.

A mass balance snapshot model of the system was built in Ecopath following six steps: (1) Defining trophic groups allocated as consumers, producers or detritus; (2) Determination of biomass and production using *in situ* data for fish and detritus or estimation methods for macroinvertebrates, zooplankton and phytoplankton; (3) Estimation of consumption over biomass ratio for all consumers groups; (4) Description of the diet of each consumer group based on published data (Fishbase mainly) and available food sources in the system; (5) the results were refined by stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of the different species and sources; (6) Interpretation of key outputs of Ecopath model.

IMTA are complex production systems, they require precise knowledge of trophic interactions. Ecopath software provides an interesting tool to represent what occurs in the rearing environment. This method enlightens the use efficiency of each trophic compartment and possible improvement solutions in terms of species stocking. Modelling can help maximising the contribution of each trophic groups and thus fish productivity. However, running Ecopath software requires a large variety of data, missing information can be estimated but degrade the reliability of the outputs of the model.

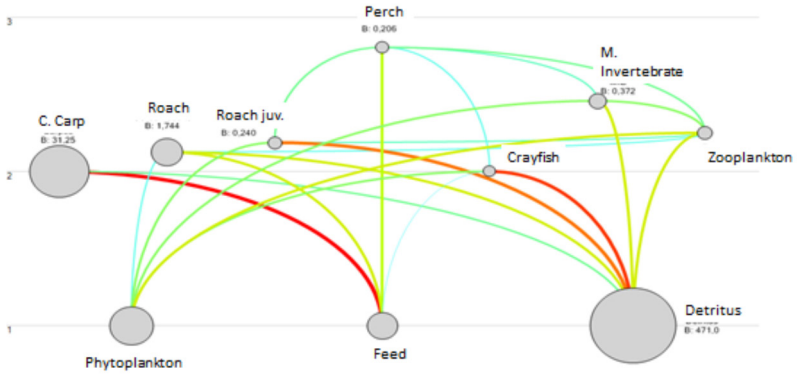

Figure 1: example of trophic interactions in a polyculture pond system under Ecopath software

PHYLOGENETICS AND EXPRESSION ANALYSIS OF TRANSCRIPTIONAL REGULATION FACTORS SOHLH IN *Crassostrea gigas*

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Self-renewal and differentiation of germ cells are essential for gametogenesis and reproduction. In mammals, transcriptions factors Sohlh1 and Sohlh2, two members of bHLH family, are specifically expressed in gonads, and play an important role in spermatogonia differentiation, oocyte differentiation and primordial follicle development. Here we analyzed the phylogenetics of Sohlh genes based on species that have completed genome sequencing. Sohlh were ancient ancestral genes which exist in protozoan, but they had lost in many species including most of lower invertebrates, suggesting multiple losses of SOHLH genes in species evolution. Sequence multiple alignment results also indicated that SOHLH1, invertebrate Sohlh2 and vertebrate Sohlh2 showing very low (12-15%) similarity. These data suggest that SOHLH1 and SOHLH2 originated from different ancestral genes or they separated early in evolution.

Using RNA-seq, immunohistochemical and RT-PCR, we found that Cg-Sohlh1 gene specifically expressed in male gonad. In five development stages, Cg-Sohlh1 expression increased from proliferative stage to growth stage and reached highest in growth stage ($P<0.05$). The data indicated that Cg-Sohlh mainly distributes in gonad, and suggest that Cg-Sohlh1 might be involved in the regulation of spermatogonial differentiation in spermatogenesis. Further study of Sohlh function would be explored by RNAi and ChIP approaches.

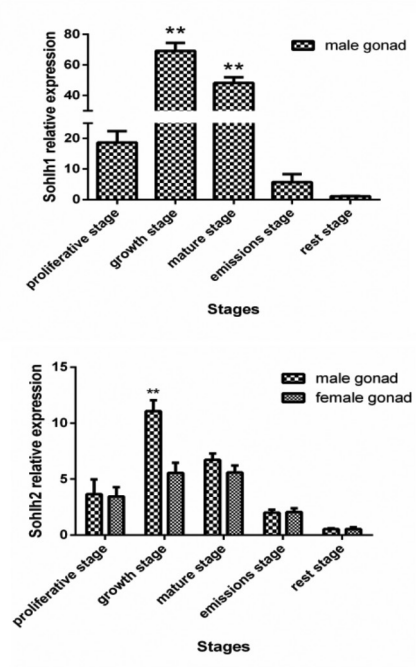

Figure qRT-PCR analysis of Sohlh expression in the gonad development stages.

STIMULATION OF SUPEROXIDE DISMUTASE EXPRESSION IN THE SKIN OF ATLANTIC SALMON Salmo salar RECEIVING A SOD-RICH MELON PULP CONCENTRATE DIETARY SUPPLEMENT AND UPON H_2O_2 EXPOSURE

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The Atlantic salmon (*Salmo salar*) farming industry is facing significant losses from sea lice and amoebic gill disease (AGD), against which hydrogen peroxide (H_2O_2) baths are frequently applied. H_2O_2 is a powerful oxidizer documented to induce oxidative stress and mucosal damages. The present study investigated the effect of a melon pulp concentrate (MPC), naturally rich in antioxidant enzyme superoxide dismutase (SOD), on skin SOD levels in Atlantic salmon exposed to a standard H_2O_2 challenge.

Three experimental diets were prepared: a basal diet (control, C) and two MPC-containing diets, in which the MPC was top coated at two levels (low dose: 50 mg/kg diet and high dose: 100 mg/kg diet). Atlantic salmon (~ 1.7 kg) were allocated to these 3 dietary groups (triplicate tanks/group, 12 fish/tank) and maintained on the diets during 7 weeks prior to application of a standard H_2O_2 bath challenge (1500 ppm, 30 min) mimicking commercial protocols. Following the challenge, the fish were anaesthetized and netted out for collecting the skin samples (2 cm^2 portion just below the dorsal fin). Endogenous SOD (cytosolic Cu/Zn-SOD and mitochondrial Mn-SOD) levels were assessed in the skin by Western Blot immediately before (0h), 24h and 72h after the H_2O_2 challenge.

The H_2O_2 challenge induced a significant increase in skin SOD levels (Fig 1: Mn-SOD: +94% from 24h; Cu/Zn SOD: +86% at 72h post-challenge) compared to pre-challenge levels. Supplementation with a high MPC dose significantly increased skin SOD levels prior to and after the H_2O_2 challenge compared to the control diet (+ 26 % to + 44 %). In comparison, the low MPC dose induced an intermediary increase in Cu/Zn SOD and Mn-SOD at 72h only. Statistical analysis was performed with non-parametric Mann-Whitney tests and error bars are SEM.

Exposing Atlantic salmon to H_2O_2 increased skin SOD levels, indicating the recruitment of endogenous antioxidant defence in response to the pro-oxidative effect of H_2O_2. Following 7 weeks of supplementation, the high dose of MPC further stimulated the endogenous expression of SOD isoforms in Atlantic salmon skin, confirming previous findings in distinct organs in rodents, laying hens and broilers. In conclusion, MPC supplementation has the potential to support the antioxidant defence in Atlantic salmon not only under normal rearing conditions, but also when subjected to H_2O_2 treatment. Further studies are required to assess whether a targeted MPC feeding strategy would mitigate the adverse effects of H_2O_2 on Atlantic salmon skin and gill integrity. This offers a new perspective to better maintain Atlantic salmon health during the seawater phase of its growth cycle.

Fig 1: Skin SOD levels relative to pre-challenge levels (Control diet only)

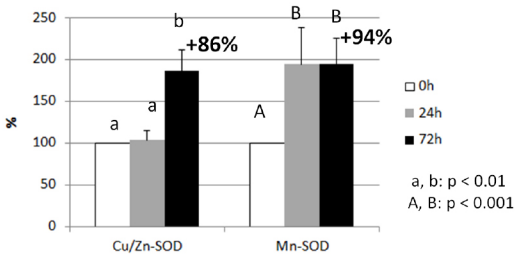
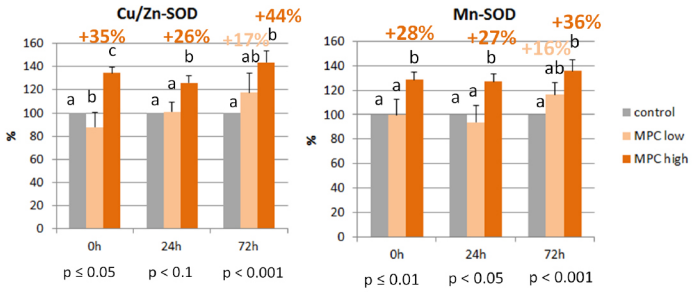


Fig 2: Skin (**a**) Cu/Zn SOD and (**b**) Mn-SOD levels in MPC groups relative to the control at each time-point



ASSESSMENT OF MICROBIOLOGICAL PATHOGENS ON SUGAR KELP *Saccharina latissima* FARMED IN COASTAL WATERS OF MAINE, U.S.A.

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Kelp aquaculture is a growing industry in Maine, U.S.A due to it being a low maintenance crop and a nutritious, versatile sea vegetable. The Food and Drug Administration (F.D.A), which monitors food and crop safety in the U.S.A, currently has no growing or harvesting restrictions on sea vegetable aquaculture. Bacterial pathogens that are potential contaminants of kelp blades may persist in waters where kelp farms are sited. These include pathogens of human and animal origin, such as intestinal bacteria from point or non-point anthropogenic or natural sources, and pathogens of aquatic origin, such as *Vibrio* which naturally inhabit coastal ocean waters. Since kelp is often consumed raw, the possibility of these bacteria present on algal tissue poses potential health concerns. The F.D.A regulations for shellfish aquaculture are considered for application in sea vegetable aquaculture, but the lack of filter feeding and suggested antibacterial properties of kelp question whether testing water is as applicable as testing kelp directly.

Little is known about the microbial communities associated with kelp in New England or the abundance of pathogens within these communities. This research aims to evaluate bacterial contamination of farmed kelp tissue and identify the relationship between water quality and kelp harvested from nearshore waters. Kelp, surface water, and water at 2 m depth were sampled from kelp farms in Saco and Casco Bays, Maine during late January-May 2018. Traditional microbiological methods of membrane filtration and plating on selective media were used to enumerate total coliforms, *Escherichia coli*, *Vibrio parahaemolyticus, V. alginolyticus*, and *V. cholera* from the kelp and water samples. qPCR analysis was utilized on enriched kelp samples to improve detection of *V. parahaemolyticus* in addition to enterohemorrhagic *E. coli* (EHEC) and *Salmonella enterica* Typhimurium, all microorganisms whose natural concentrations may be below the detection limit of plating techniques. Variations in bacterial abundance between kelp and water samples, throughout the growing season, and between farm locations were analyzed.

Significantly lower numbers of total coliforms and *Vibrio* were found in kelp compared to water sampled from Saco Bay during May-July 2017. For kelp, total coliform plate counts ranged from 0-2 colony forming units (CFU) per 100 mL and *Vibrio* ranged from 0-63 CFU/100 mL. The majority of plate counts from kelp sampled in February-March 2018 showed no coliform or *Vibrio* growth, with some having *Vibrio* presence (1-12 CFU/100 mL). Lower bacterial levels are expected during the winter-spring (time of kelp harvest) since cold temperatures and lower tourism activity reduce pathogen concentrations in the water. This research provides preliminary data needed for developing best management practices regarding food safety and siting of kelp aquaculture and mitigates public health concerns with consuming sea vegetables farmed in coastal waters.

PRELIMINARY RESULTS ON THE ESTIMATION OF THE MINIMUM INFECTIVE DOSE OF VIRAL NERVOUS NECROSIS VIRUS IN EUROPEAN SEA BASS *Dicentrarchus labrax*

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The EnviGuard project was developed to provide a response to the growing need for accurate real-time monitoring of the aquaculture industries and for a reliable and cost-effective risk management tool. It will include several detection units, including the automatic detection of the Betanodavirus in the water. Its sensitivity will be based on the results of this study.

Viral nervous necrosis virus (VNNv) belongs to the family of Betanodavirus and is one of the most problematic diseases in the aquaculture industry in the Mediterranean sea. European sea bass (*Dicentrarchus labrax*) is the most susceptible farmed species to this pathogen. Hydrographic models can be useful in developing pathogen dispersal models and assessing transmission risks, but robust pathogens may spread further. To understand the transmission of the disease, infectious dose and stability of VNNv are essential parameters to be understood.

Groups of 30 fish (in duplicate) were challenged by immersion at different concentrations of VNNv consisting in 10^3, 10^1, 10^{-1} and to 10^{-3} $TCID_{50}$ mL^{-1} for 6 hours. 2 additional groups were mock infected by immersion in PBS. In order to study the shedding rate of VNN, 2 more groups were injected intramuscularly (IM) with a dose of 10^6 $TCID_{50}$ mL^{-1}, whilst the stability of the virus was tested *in vitro* at 27°C.

Mortalities started on the 4th day post-challenge and continued until day 13. The IM group showed a 45% average accumulated mortality, whilst dilutions 10^3 $TCID_{50}$ mL^{-1} and 10^1 $TCID_{50}$ mL^{-1} had a 10 and 13% average accumulated mortality respectively (no mortalities occurred in the other groups). From the mortalities which occurred (41), a subsample of 14 fish was analysed by RT-PCR and all fish were found to be positive to VNNv. The same analysis on a 10% of the surviving fish (on the end of the experiment) showed that 100% of the IM and 10^3 $TCID_{50}$ mL^{-1} immersion were positive to VNNv, whilst only 50% of the fish from 10^1 $TCID_{50}$ mL^{-1} were positive, and the fish from other treatments were negative to the pathogen. The shedding rate of the virus could not be determined because water samples of the IM tanks were negative for VNNv. The virus could be recovered from water samples even 3 months after the challenge was performed.

From the information obtained in this study, the minimum dose of VNNv required to reliably induce infection in European sea bass juveniles by bath immersion is approximately 10^1 $TCID_{50}$ mL^{-1}. The study also showed that it is a highly stable virus. The low dose of virus required to induce infection in combination with the high resistance of the pathogen in the environment, facilitate the onset of periodical disease outbreaks. As a consequence, the farmer might not be able to eradicate it, but early warning by the EnviGuard system could reduce economic losses.

This study was carried out within the framework of the EnviGuard project, which has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 614057.

TAPAS CASE STUDY: NUTRIENT RETENTION OF FISH FARM EFFLUENT AND SITE ASSESSMENT FOR THE FEASIBILITY OF INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) IN MALTA

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Aquaculture is the fastest growing food production sector in the world, with average annual growth rates of 6-8% since 2000 (FAO, 2016). The EU self-sufficiency in terms of seafood is low, with nearly 70% of the seafood consumed being imported. To turn this situation, EU member states prepared national strategies for an ambitious growth with 300,000 tonnes extra in 2020 (e.g, plus 25%). The EU Horizon 2020 research project 'Tools for Assessment and Planning of Aquaculture Sustainability' (TAPAS) will produce scientific research data to support the sustainable expansion of coastal aquaculture systems.

AquaBioTech Group completed a case study to quantify nutrient retention of carbon and nitrogen within a coastal environment surrounding aquaculture pens in Malta. The samples collected include suspended sediments, *Posidonia oceanica*, seafloor sediment and biofilms and were sampled using sedimentation traps and a Remote Operated Vehicle (ROV). All samples were extracted from locations surrounding the coastal aquaculture site in order to assess the dispersion of nutrients. The samples were analysed for ^{13}C and ^{15}N isotopic composition to assess the dispersion of fish farm effluent and estimate which organisms or substrate retained the most nutrients. The ROV, equipped with a video recording system and a sediment sampler, was deployed to survey the benthic community and the presence and absence of *P. oceanica*, used as bioindicator in the Mediterranean Sea. A current profiler was deployed at three distinct locations around the fish farm to demonstrate the dominant water movement patterns. The current patterns were incorporated with the nutrient dispersion isotopic and benthic community data to validate the nutrient waster dispersion model. This model will evaluate the feasibility, design and optimal species for an Integrated Multi-Trophic Aquaculture (IMTA) system in Malta.

The second proposed stage of the project will be to establish an IMTA system off the coast of Malta, to utilize the fish farm effluents for further shellfish production and evaluate the site assessment method.

This research is supported by the EU H2020 research and innovation programme within the collaborative project "TAPAS – Tools for Assessment and Planning of Aquaculture Sustainability" (www.tapas-h2020.eu) under Grant Agreement No. 678396.

ASSESSING BIOFOULING GROWTH ON AQUACULTURE NETS

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Biofouling is the unwanted accumulation and growth of living organisms on a submerged surface. Causing serious problems for a wide range of marine industries and navies around the world, the impacts in the aquaculture sector cost between 5-10 % of production. Fouling restricts water exchange and causes deformation of cages and structures. As a result, nutrient exchange and waste removal are restricted and the risk of disease increases since fouling itself can act as a pathogen vector.

To prevent and combat fouling, the use of antifouling coatings is the most widely spread approach. In the present study, the efficacy of a commercial biocidal antifouling coating is assessed on nets and the results compared to untreated nets. Nets were submerged at 6 m depth, in Malta, central Mediterranean Sea and monthly checked. Different endpoints were used to assess biofouling growth on nets; The Percentage Net Occlusion (PNO) was monthly calculated using image analysis, chlorophyll a content, biomass and species richness were evaluated in the laboratory after 268 and 369 days of exposure.

After 369 days of exposure, treated nets presented a PNO rate considerably lower than the untreated control nets. Treated nets never exceeded 26 % of net occlusion even during the spring bloom, when untreated nets reached 94 % of occlusion. On the same note, the organic matter accumulated in the nets until Day 369 of immersion was of 6.99 g/cm^2 in the control nets, and significantly lower in the commercial nets, with 0,06 g/cm^2. In terms of chlorophyll a content, on Day 268 of immersion, the results showed 31,26 mg/m^3 in the treated nets and 0,91 mg/m^3 in the untreated nets. The total number of species observed was also lower in the treated nets than in the untreated control nets, the latter dominated by Hydrozoa, Bryozoa, Macroalgae and Porifera.

Overall, the application of a commercial biocidal antifouling coating with cuprous oxide successfully inhibited the settlement of Ascidiacea, Bivalvia and Porifera species on the nets up to one year after immersion. Also, the commercial coating reduced net occlusion by 68% during intense fouling pressure, maintaining adequate water flow through the netting throughout the exposure period.

The use of image analysis is a reliable and non-invasive method to monitor fouling growth over time on aquaculture nets while the assessment of species richness gives information about the spectrum of activity of antifouling coatings.

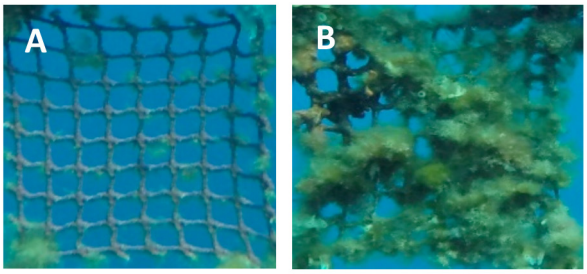

Figure 1. Nets after 211 days of immersion, at 6 m depth. **A)** Treated net with biocidal coating. **B)** Untreated net.

OFF-SHORE CULTIVATION FOR SMALL OYSTER-FARMING COMPANIES IN FRANCE: POTENTIAL AND CONSTRAINTS

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In Europe, oyster production has historically taken place in intertidal zones. Shellfish farms already occupy large extents of intertidal space, and sometimes have for more than a century. The possibility of expanding shellfish aquaculture in intertidal areas is therefore spatially limited, and is also constrained by carrying capacity, water quality, and ecological impacts issues. Moving offshore is considered to be a real possibility to overcome these problems. Finding new sites at which to expand oyster aquaculture off-shore relies on the combination of different requirements, but a basic prerequisite is to be able to obtain significant growth, survival, and quality of the product.

Bivalves are suspension-feeders that exploit the organic fraction of the suspended particulate matter (SPM), and phytoplankton is expected to be their main food source in off-shore environment. However, this resource is characterized by a strong temporal variability (seasonality, interannual variations) and spatial patchiness. Moreover, off-shore sites may still be under the influence of coastal turbidity in turbid plume regions, and SPM concentrations are known to affect the ecophysiological responses of bivalves. A combination of Earth Observation (EO) data and bivalve ecophysiological modeling was used to obtain spatial distribution maps of growth potential from near-shore to off-shore locations. Dynamic Energy Budget (DEB) modeling of the Pacific oyster was carried out, with satellite-retrieved Chlorophyll-a, SPM, and temperature time-series maps used as the driving variables. Finally, many other criteria must be taken into account for site selection and to develop spatial plans for off-shore oyster production, these related to the feasibility (e.g., spatial restrictions due to other activities taking place) and suitability (i.e., physical, environmental, economical variables that make a possible site more or less well-suited to production). This approach is currently being developed within the European project *TAPAS* (http://tapas-h2020.eu/), from the perspective of small oyster companies run by young farmers who are interested in off-shore bivalve aquaculture that would be compatible with their investment capacity.

This work is based in Bourgneuf Bay, south of the Loire estuary (France), where off-shore experiments have been carried out since 2008. The biological potential of off-shore oyster cultivation is undeniable and the simple technique using bottom-cages is not economically out of reach for small companies. Different production strategies could be envisioned, some of them combining off-shore and intertidal cultivation. However, ten years after off-shore testing first began, this approach remains experimental. As elsewhere in Europe, there are conflicts with fisheries and tourism, but the complexity of the administrative processes necessary to obtain an off-shore license is perceived as a stronger barrier by farmers owning small companies who would like to move off-shore.

INVESTIGATION INTO THE CAUSES OF AND POSSIBLE SOLUTIONS TO FAILED RAINBOW TROUT (*Oncorhynchus mykiss*) IN AQUACULTURE

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In aquaculture, the term failed fish (or stunts) is a synonym for a fish with impaired growth or substantially slower growth rate. This issue is observed throughout salmonid aquaculture, typically occurring at a rate of ~10%. However, this is greatly increased for rainbow trout (*Oncorhynchus mykiss*) reared in full salinity where failure levels of >30% are often encountered.

Although having been previously associated with osmolarity, we hypothesis that as the fish don't die, this is not the route cause. Our objective is to undertake for the first time a comprehensive assessment of failed fish by the incorporation of information on brood stock (strain, family), genetics, husbandry, life cycle, anatomy, physiology and biochemistry to isolate why these fish have impaired growth. We also aim to develop a solution through nutrition by testing the impact of various feeds. This holistic approach shall allow the complete investigation into the mechanisms involved in this phenomenon, indicating the causes and ultimately highlighting what preventative measures (i.e. selective breeding, nutrition) can prevent its occurrence.

Preliminary biochemistry results showed no significant differences in electrolytes (Table 1) indicating no osmoregulation imbalance in the failed fish. Significant decreases were observed in albumin, alkaline phosphatase, creatine kinase, creatinine, ammonia, urea, iron, phosphate and zinc in failed fish, with an increase in magnesium. This indicates malnutrition and lack of protein, proving that the fish are not eating. Further investigation on anatomy, physiology, genetic and proteomic endpoints shall be presented.

	Healthy	Failed
ALP	208.6 (20.54) U/L	27.2 (4.4) U/L*
ALT	12.9 (1.78) U/L	9.3 (1.32) U/L
TBIL	1.1 (0.14) µmol/l	4.8 (2.89) µmol/l
TP	36.2 (2.22) g/L	31.9 (2.22) g/L
ALBUM	17.8 (0.99)g/L	14.5 (0.79) g/L*
LDH	1741.8 (149.9) U/L	1723.9 (139.8) U/L
AMY	374.7 (56.67) U/L	473.1 (45.42) U/L
CK	36508.9 (6644.5) U/L	7056.4 (1626.6) U/L*
CREAT	19.6 (1.1) µmol/L	14.7 (0.87) µmol/L*
Ammonia	1406.2 (52.58) µmol/L	933.2 (51.29) µmol/L*
UREA	0.9 (0.05) mmol/L	0.7 (0.05) mmol/L*
SODIUM	130 (6.37) mmol/L	135.7 (5.64) mmol/L
POTASS	2.8 (0.24) mmol/L	3.8 (0.53) mmol/L
CHLORI	113.4 (3.87) mmol/L	124.3 (4.19) mmol/L
IRON	13 (0.97) mmol/L	10.1 (0.97) mmol/L*
CAL	2.5 (0.13) mmol/L	2.3 (0.08) mmol/L
PHOS	7.0 (0.55) µmol/L	3.4 (0.2) µmol/L*
MAG	1.4 (0.08) µmol/L	1.8 (0.76) µmol/L*
COPPER	13.9 (1.04) mmol/L	13 (0.76) mmol/L
ZINC	311.5 (19.63) mmol/L	155.5 (12.62) mmol/L*
Na/K	65.8 (8.73) mmol/L	56.9 (7.33) mmol/L
Globul	18.5 (1.26) g/L	17.4 (1.55) g/L

Table 1. Preliminary biochemistry of rainbow trout. n=30; * significant (<0.05).

MALE MONOSEX PRODUCTION IN TILAPIAS: A REVIEW

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Tilapia is now the second most produced group of fish in the world. With the exception of a few very specific culture conditions, the use of monosex male populations makes it possible to optimize the profitability of tilapia production, on the one hand by preventing reproduction, and on the other hand by taking advantage of the best growth rate of males. Two main approaches are used to produce these monosex populations: a genetic approach based on the use of YY males (or ZZ females in blue tilapia) or on hybridization between certain tilapia species, and a sexual inversion approach induced by hormonal or thermal treatments. The advantages and disadvantages of each of these methods are presented. The ease of implementation, efficiency and low cost of hormonal treatments make this approach the most widespread for the production of male monosex populations. However, at a worldwide scale, large amounts of hormones are consequently used, which raises debates on the sustainability of tilapia farms using such treatments, and on their consequences at least on water quality and biodiversity. Indeed, data on the accumulation of 17α-methyltestosterone (MT) in water and sediment are few and sometimes contradictory. In ponds, under very specific conditions, certain bacteria are capable of degrading MT. More sustainable alternative approaches are now proposed. These include the use of genetic markers linked to sex chromosomes or the thermosensitivity of sex differentiation.

TRANS-NATIONAL COMPARATIVE STUDY ON PHENOTYPIC ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *Vibrio vulnificus* AND *Vibrio anguillarum* ISOLATED FROM DISEASED FISH

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Antimicrobial resistance (AMR) is a major issue for both human and animal health, and requires a *One Health* approach. Aquaculture is growing fast, both in Europe and at a global scale. Antimicrobials are widely used in aquaculture for therapeutic or prophylactic purposes. However, antimicrobial usage is strictly regulated in only few countries. There is a growing concern on good practices and other measures to support the prudent use of antimicrobials throughout the food chain. Knowledge on AMR in aquaculture is poor compared to other animal species. AMR in aquaculture may develop in fish and shellfish bacteria as a result of antimicrobial therapy or by contamination of the aquatic environment by human or animal waste containing antimicrobials.

Vibriosis caused by various *Vibrio* species have a major economic impact in freshwater, brackish and marine fish and shellfish culture both in Europe and worldwide. Among different species, *Vibrio anguillarum* and *Vibrio vulnificus* are considered as severe fish pathogens. Data on AMR in *Vibrio* spp. from aquaculture are scarce, and, most of all, AMR testing methods are not harmonized so far.

The aim of this collaborative study was to harmonize methods for AMR testing of these two *Vibrio* species among four national veterinary public health institutes (members of CoVetLab and MedVetNet association) by sharing practices, protocols and collections in order to test the same panel of *V. anguillarum* and *V. vulnificus* strains.

A collection of strains of *V. vulnificus* (n=26) and *V. anguillarum* (n=30) from three partners was distributed to all partners. Susceptibility test to a panel of antimicrobial agents (oxolinic acid, florfenicol, ampicillin, sulphametoxazole-thrimethoprim and oxytetracycline) was performed at 28°C, by two methods : the agar diffusion and the broth microdilution. *Escherichia coli* ATCC 25922 and *Aeromonas salmonicida* ATCC 33658 were used as quality control strains. For microdilution, either commercial (VetMic Aquatic and EUVSEC) or homemade microplates were used. All isolates were tested in each lab using a shared protocol according to CLSI recommendations. Based on results, a provisional harmonized protocol for AMR testing of *Vibrio* spp. will be drafted, allowing to compare the performances of the agar diffusion and the broth microdilution methods. A larger trans-national study might be then required, including other pathogenic *Vibrio* species to further validate these findings.

Acknowledgment to Dr. Peter Smith (NUI Galway, Ireland) for his scientific advice.
This project was supported by a grant from CoVetLab consortium.

PREVALENCE OF INTEGRONS IN *Aeromonas* ISOLATES FROM FISH

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Antimicrobial resistance (AMR) is increasing worldwide in humans, animals and the environment. Integrons are genetic elements involved in the dissemination of AMR, mostly among Gram-negative bacteria. Integrons are able to capture and express antibiotic resistance genes embedded within mobile gene cassettes Prevalence of integrons is well documented in *Enterobacteriaceae*, but data are still relatively rare in *Aeromonas*. Due to its ecological and genetic capabilities, *Aeromonas* is now proposed as an indicator of AMR dissemination in aquatic environment, and is considered, as well as integrons, as a vehicle of dissemination of antibiotic resistance genes. The aim of this study was to evaluate the prevalence of integrons in *Aeromonas* isolates from fishes: wild freshwater fishes, trout (fish farming) and diseased fishes (fish farming).

A total of 310 *Aeromonas* were collected in France from wild freshwater fishes (n=109, in 2014), diseased fishes (n=124, 1985-2014), and from healthy trout coming from fish farming (n= 77, in 2014/2015). Class 1, class 2 and class 3 integrons were detected by a multiplex qPCR. Antimicrobial susceptibility of the isolates was assessed using the broth microdilution method.

Eighty-one isolates harbored integrons. No integron was detected in isolates from wild freshwater fishes. Integrons were present in 54.8 % of the isolates recovered from diseased fishes and in 16.9 % of those recovered from healthy trout.

Class 1 integrons were the most often detected (n=80), only one isolate recovered from trout harbored a class 2 integron. No class 3 integron was detected.

In this study, we observed the highest prevalence of integrons in *Aeromonas* from diseased fishes and a higher prevalence in trout (fish farming) compared to isolates from wild freshwater fishes. It is assumed that this trend could be linked to the antibiotic selection pressure in fish farms, but further evaluation is necessary to investigate this point. The susceptibility profiles of the isolates and the link with integrons are under characterization.

Table 1: Prevalence of integrons in *Aeromonas*

Origin	Number of strains	Integron-positive strains (%)
Diseased fish	124	68 (54.8%)
Wild freshwater fish	109	0 (0.0%)
Trout (Fish farming)	77	13 (16.9%)

CHEMICALS RELEASED BY PREDATION INCREASE THE GROWTH RATE OF YELLOW PERCH *Perca flavescens*

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Water-soluble factors associated with walleye (*Sander vitreus*) and hybrid walleye (female *S. vitreus* x male *Sander canadensis)* predation on yellow perch and fathead minnows (*Pimephales promelas*) significantly increased the growth rate of yellow perch.

Juvenile yellow perch (~250 per tank) were distributed into 12, 110-L tanks and fed to satiation 2-3 times daily. 30-L aquariums were placed above each tank and plumbed so that the water discharged into the tanks below. There were four treatments replicated three times: (a) no predator; and predators fed to satiation once daily on (b) yellow perch, (c) dry diet, or (d) fathead minnows (Fig. 1). The yellow perch were weighed and measured every two weeks.

On day 98, the fish in the yellow perch and fathead minnow treatment groups were ~40% heavier than the fish in the control and dry diet treatments (Fig. 2). There were no differences in fish condition or survival among the four treatments.

The ecological significance of predator-mediated growth enhancement is likely predation mitigation. We are currently working to uncover the source and chemical identify of the pheromone, and determine its mechanism of action. We hypothesize that the pheromone is associated with either the skin of the prey or the feces of the predator, and acts by stimulating olfactory pathways that stimulate the release of pituitary growth hormone. In addition, we are evaluating the possibility that predation odors can be used to enhance the production of yellow perch in commercial aquaculture.

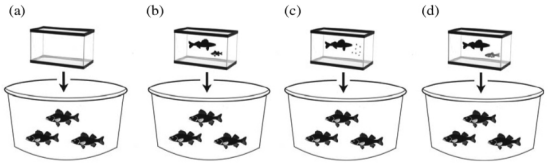

Fig. 1. Experimental design (see text for explanation).

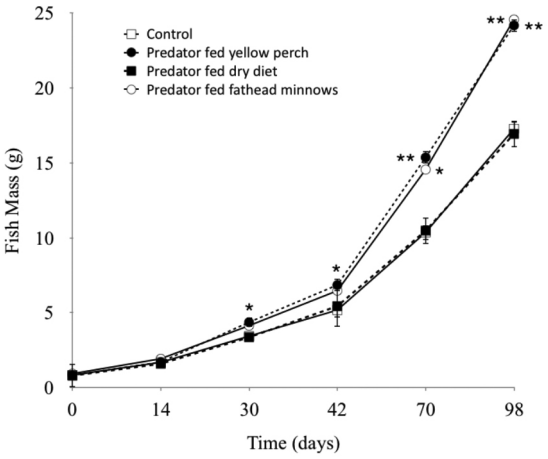

Fig. 2. Effects of predation odors on the growth of yellow perch at 21°C. *P<0.05 & **P<0.01 vs. control within sampling time.

PLANT-DERIVED COMPOUNDS IN AQUACULTURE HEALTH MANAGEMENT: FROM THE MODES OF ACTION TO PRACTICAL APPLICATION

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Plants synthesize a diverse array of specialized compounds as secondary metabolites. These compounds play vital roles in plant defense response to stress. Studies have shown that these plant-derived compounds cause a broad spectrum of beneficial activities in animal host, providing protection against attack by various abiotic/biotic stressors. The modes of action in mediating beneficial effects are manifold, and mostly considered to be due to their antioxidant, prooxidant, anti-microbial, immune-modulating activities. Owing to their multi-faceted properties, there is a growing interest in using these compounds as sources of health care products in fish and shellfish hatcheries, where significant mortality occurs due to stress. In recent years, rapid progress has been made in understanding the bioactivities and functional mechanisms of these compounds. This review brings this information together with contemporary advances in their practical application in aquaculture animal health.

MECHANISMS OF PATHOGEN VIRULENCE AND HOST SUSCEPTIBILITY IN VIRULENT *Aeromonas hydrophila* INFECTIONS OF CHANNEL CATFISH (*Ictalurus punctatus*)

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An emerging pathotype of *Aeromonas hydrophila* (vAh) has been responsible for widespread farm losses in the US catfish industry over the last decade. While our genetic and biochemical understanding of vAh has been greatly enhanced in this time frame, our ability to reliably induce the disease in the laboratory has remained limited. Taking cues from observed farm conditions associated with outbreaks, here we perturbed iron scavenging dynamics and catfish feeding status. Addition of a xenosiderophore, deferoxamine mesylate (DFO), to vAh cultures prior to immersion challenge significantly increased virulence in several vAh isolates but not in a non-epidemic strain. DFO addition did not impact vAh growth dynamics or perturb iron-sensitive gene pathways, but did significantly enhance hemolysis of catfish blood. Furthermore, hours between last feeding and immersion challenge (postprandial status), was observed to be a critical determinant of catfish susceptibility. Fish with a full gastrointestinal tract had significantly lower survival than those in a fasted state, and this effect was cumulative with that of DFO-enhanced vAh virulence. Taken together, our results not only provide a more robust challenge model, they offer actionable insights into pond level host-pathogen-environmental interactions potentially underlying vAh pathogenesis.

WHAT IS EGG QUALITY IN SPOTTED WOLFFISH (*Anarhichas minor*)? A KEY TO REPRODUCTIVE PERFORMANCE EVALUATION

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Spotted wolffish (*Anarhichas minor*) has been considered one of the most promising marine species for aquaculture diversification in Norway and Canada. However, the long egg incubation period is very labour-intensive and space consuming, lasts for up to five months, i.e. 800 to 1000 degree-days (dd). Furthermore, the egg survival, development and hatching are, at present, highly unpredictable. To succeed with this new species, it is important to develop better rearing protocols for production of high quality eggs that improve the species reproductive performance in captivity. Therefore, early indices of fertilization and hatching rates as well as of larvae survival rates are required.

Currently three institutions in the world hold an *A. minor* broodstock, AMINOR/Nord University and Akvaplan-niva in Norway and IML/DFO in Canada. In total, 38 egg batches from these three institutions were followed (14 from AMINOR/Nord University, 11 from Akvaplan-niva and 13 from IML/DFO) between 2017 and 2018. Each egg batch was fertilized with a pool of sperm and transferred to an incubation system. Egg batches were kept separate throughout the entire incubation phase. We analyzed different basic egg quality parameters (size, weight, colour, ovarian fluid pH and osmolality), that can be easily measured in the hatcheries from the time of stripping prior to fertilization (oocytes), or shortly after (fertilization rate, initial cell cleavage) as well as the biochemical composition of the stripped eggs (fatty acids, amino acids and carotenoids). These data were compared with egg development, hatching and larvae survival. Development rate was evaluated at 300dd and 600dd. At hatching, larvae were weighted and measured. Yolk conversion ratio and early survival were determined after the first week.

The comparison of the basic quality parameters and eggs development rate will help to build evaluation schemes that can be used to select rearing protocols, breeders and gametes for in vitro fertilization. Simultaneously, the results of the stripped eggs biochemical composition will also help to develop broodstock feeds for spotted wolffish. Presently wolffish are fed with diets developed for general marine fishes and do not fulfill wolffish nutritional needs.

This study was supported by the WOLFEGG (project number 269726) funded by the RFF-Nord from Norway.

EFFICIENCY OF MEMBRANE STABILIZERS AND ANTIOXIDANTS IN SPOTTED WOLFFISH (*Anarchichas minor*) SPERM CRYOPRESERVATION

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Spotted wolffish is considered a potential candidate for aquaculture diversification in Norway and Canada. Due to the lack of gametes synchronization it is important to have protocols for sperm storage. Cryopreservation is the preferred method since wolffish sperm is motile at stripping, and refrigeration is not an option. Protocols in use today are suboptimal giving a reduction in the sperm quality. Here we test the addition of membrane stabilizers and antioxidants to a cryopreservation extender.

Sperm samples were collected from October 2017 to June 2018. Samples with more than 50% motility were pooled together. Ten sperm pools were tested in total, five for the membrane stabilizers and five for the antioxidants. The cryopreservation protocol followed the results by Santana et al. (2017). The following membrane stabilizers were tested: bovine serum albumin (BSA) at 1 and 5% (w/v) and egg yolk at 5 and 10% (v/v). The following antioxidants were tested: reduced glutathione (1 and 5mM) and taurine (10 and 50mM). The treatments were evaluated for % of motile sperm and sperm velocity measured with a CASA system, sperm viability under a fluorescent microscope after dual stain with propidium iodide and SYBR-14, and, additionally for antioxidant treatments, for lipid peroxidation by determining the malondialdehyde (MDA) production. Fresh pre-cryopreservation sample, and a sample cryopreserved without addition of membrane stabilizers or antioxidants were used as controls.

The first results (Figs 1 and 2) indicate that adding 5% BSA has a positive effect on the % of motile cells after thawing. No improvement in the post-thawed sperm was obtained with the addition of antioxidants.

Work supported by the WOLFSTORE project (AF0078) funded by MABIT program Norway

J. Santana, B. Eggen and J. Beirão. 2017. Optimization of a sperm cryopreservation protocol for spotted wolffish (*Anarhichas minor* Olafsen, 1772). AE 2017, Dubrovnick, Croatia

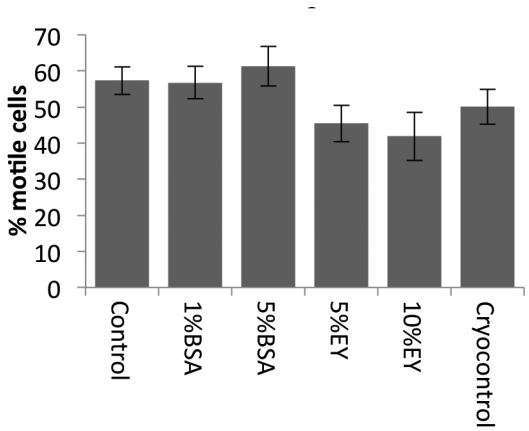

Fig 1 – Percentage of motile cells after cryopreservation with BSA and egg yolk (EY), n = 5.

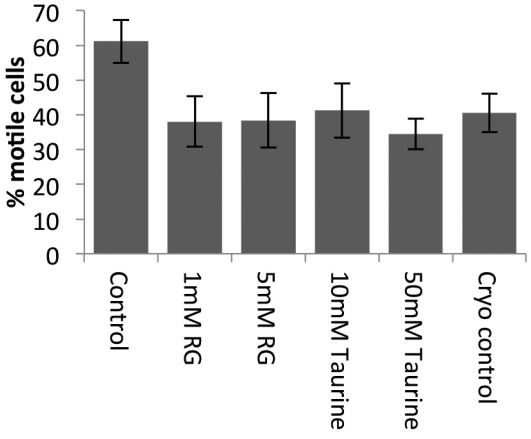

Fig 2 – Percentage of motile cells after cryopreservation with reduced glutathione (RG) and Taurine, n = 5.

THE QUIET REVOLUTION IN AQUACULTURE VALUE CHAINS

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This paper identifies seven key trends in the transformation of Asian aquaculture value chains. Together, these represent an urban domestic-market-driven "quiet revolution" that is one of the defining characteristics of Asian aquaculture development.

First, urbanisation has stimulated demand for fish as wages rise and diets diversify, during a period when capture fisheries landings have declined. Aquaculture has emerged in response to the opportunities created. Second, the growth of fish supply has been enabled by, and contributed to, a proliferation of non-farm enterprises providing inputs, logistics, trade, and other services (many, though by no means all of them, small and medium scale enterprises). Third, farms and supporting businesses have emerged in a largely 'immanent' or 'unplanned' way, supported by the provision of infrastructure (e.g. roads, waterways, irrigation, wholesale markets). Fourth, value chains have undergone rapid technological change in all segments as they have modernized. Fifth, structural changes have occurred throughout the chain as farms and related firms have proliferated and become more specialized, diversified and, (occasionally) more concentrated or vertically integrated. Sixth, product differentiation has occurred over time in line with the product cycle, as species become commoditized, prices have fallen, and new 'niche' species have been introduced as alternatives. This process has resulted in farmed fish becoming accessible to greater numbers of low income consumers. Seventh, development pathways are forged in the context of pre-existing relations of class, power and gender that structure access to productive resources (land, capital, technology), but have also contributed to the reworking of these relations, sometimes deepening existing inequalities, sometimes attenuating them

INTRAPERITONEAL INJECTION OF INACTIVATED VACCINE AGAINST *Streptococcus agalactiae* IMPROVES FISH IMMUNITY AND SURVIVAL OF NILE-TILAPIA (*Oreochromis niloticus*) UPON INFECTION

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Streptococciosis is a fish disease caused by *Streptococcus agalactiae*. Because of its resistance to different antibiotics, this bacteria causes an acute outbreak in fish farm in Brazil. A vaccine is needed to control this emergent disease and prevent the spread of antibiotic and drug-resistant bacteria. In this study, we used inactivated vaccine to provide protection against Streptococciosis disease in tilapia.

Three groups of tilapia (n=10) healthy and weighed 40 ± 3.0 g averagely were reared in a controlled system. Vaccine prime-injection and booster-injection were performed, in 15 days' time interval, with 0.05 mL of inactivated *S. agalactiae* (10^9 UFC mL^{-1}). Survival of the vaccinated fish (14 days' post vaccination (dpv)), were evaluated after challenge with virulent *S. agalactiae* (10^6 UFC mL^{-1}). Blood analysis was performed weekly (1, 2, 3 weeks) post challenge for hemogram.

Results showed that the relative percentage of survival (RPS) in the tilapia fish groups vaccinated was significantly high (RPS=100) after 21 dpv. Unvaccinated fish presented several clinical signs of Streptococciosis essentially alterations in behavior, erratic swimming, exophthalmia, skin hemorrhage. For control fish, the hemogram showed a significant decrease in hematocrit percentage, hemoglobin and erythrocyte number of 40%, 31.5% and 35.8%, respectively, one week post-challenge. For vaccinated fish, decreases of cited parameters ranged between 3.5% and 4.4% in the same period (Table 1). Comparison of RPS and hemogram of control and vaccinated fish demonstrated that the developed vaccine improves the immunity and survival of Nile-tilapia upon infection with *Streptococcus agalactiae*.

Acknowledgments
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Table 1. Hemogram of control and vaccinated fish, realized during the pre-challenge period and one week post challenge

	Ht (%)	Hb	Er 10^4 (mm^{-3})
Fish control			
Pré-challenge	26.2±3	5.11±0.6	175.4 ±28.52
7 days Post challenge	15.6± 3	3.5±0.5	112.5±13.5
Fish vaccinated			
Pré-challenge	28.3±2.2	5.01±0.29	181.6 ±21.6
7 days Post challenge	27±2.9	6.09±0.4	1901±33.5

Ht (%): Hematocrit; Hb: Hemoglobin; Er: Erythrocytes.

HEALTH AND FLESH QUALITY OF ATLANTIC SALMON (*Salmo salar*) FED A MODERN LOW FISHMEAL DIET SUPPLEMENTED WITH ANTARCTIC KRILL, *Euphausia superba*

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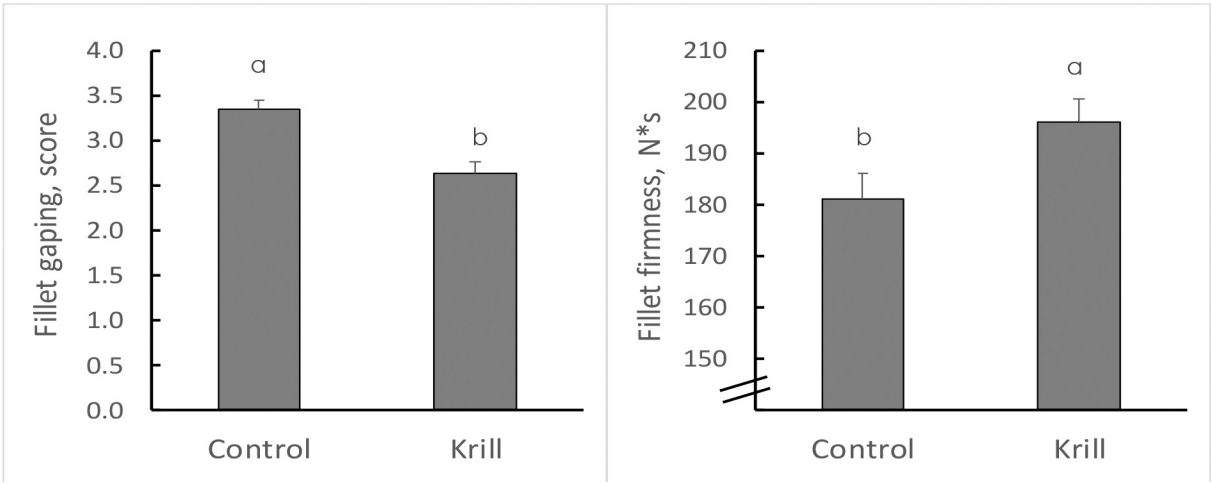
Atlantic salmon (*Salmo salar* L.) were fed a commercially relevant diet with 15% fishmeal (control group) or the same diet substituted by 12% Antarctic krill meal (krill group). The isoprotein (35%) and isolipid (35%) diets were fed to quadruplicate net pens (125m^3; average seawater temperature 11°C) during a 10-week period, autumn 2017. The body weight increased from 2.2 to 3.9kg during the experimental period, corresponding with a thermal growth coefficient of 3.88 and 3.92 for control and krill group, respectively. The average feed conversion ratio was 1.1. The krill group had a more voluminous body shape compared with the control group (higher condition factor). The hepatosomatic and cardiosomatic indices were similar for the dietary groups, but the livers of the krill group were darker and the amount of visible fat on the heart was significantly lower. Microarray analyses of liver tissue revealed that dietary treatment affected a large number of immune genes, and a panel of structural genes were upregulated in the livers of salmon fed the krill diet, including cadherin and connexin (tight junction proteins). Gene enrichment analyses revealed that sugar metabolism was stimulated in fatty hearts and a significant downregulation was observed for complement genes and the retinoid metabolism. Additionally, downregulation of extracellular matrix proteins, including collagens and glycan's, indicated weaker tissue structure of the control group. Fillets of salmon fed the krill diet had firmer texture, more intense red coloration, and the problem with fillet gaping was lower (i.e. improved muscle integrity). It is concluded that dietary inclusion of krill meal in modern low fishmeal diets has a positive effect on health and on fillet quality of Atlantic salmon.



OMEGA-3 FATTY ACIDS AND ZINC AFFECT ROBUSTNESS AND FUNCTION OF BARRIER TISSUES IN ATLANTIC SALMON *Salmo salar*

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We have studied the effects of different levels of omega-3 fatty acids (EPA and DHA) and Zinc (Zn) on function and robustness of skin, intestine and gills in Atlantic salmon post-smolt produced in recirculation units (RAS). These barrier tissues are the first line of defense, providing biochemical and physical protection against environmental stressors. Five different dietary groups were studied, through smoltification in freshwater, and 10 weeks into the seawater phase.

Results so far indicate significant effects of omega-3 and Zn on skin quality in Atlantic salmon. A high level of EPA and DHA provided fish with a thicker connective tissue and lipid layer, compared to fish given a low level of EPA and DHA. Fish given a high level of Zn had a smoother epidermis and higher number of mucus cells than the fish given a low level of Zn. These differences seemed to be important for fish robustness when the fish were transferred to seawater. A number of fish were inflicted with standardized wounds using a punch biopsy, and the wound healing process was followed for three weeks. The quickest wound healing was seen in fish given high dietary level of Zn, while the slowest wound healing process was seen in fish given low level of EPA and DHA. The results indicate that omega-3 and Zn strengthen the skin in different ways, thus different levels may be used in different periods to increase robustness and barrier functions.

Intestinal structure and function was also studied. Content of EPA and DHA in proximal intestine phospholipids was significantly increased with increasing dietary content. Intestinal barrier function was tested in an Ussing chamber, in both proximal and distal intestine. The lower level of EPA and DHA significantly reduced the transepithelial resistance (TER), as well as transport capacity (transepithelial potential difference, TEP), in the proximal intestine. The results indicate that low levels of dietary EPA and DHA may impair intestinal barrier function.

Results so far indicate that nutritional factors have a significant impact on both chemical composition, structure and function of barrier tissues in Atlantic salmon. Further studies are needed in order to find optimal nutrient levels for the different stages during smoltification and sea transfer.

RISK ASSESSMENT ON IMPORT OF AUSTRALIAN REDCLAW CRAYFISH *Cherax quadricarinatus* TO NORWAY

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The Australian red claw crayfish, commonly referred to as redclaw, is a relatively large freshwater crayfish, originating in tropical Australia and Papua New Guinea. This species holds biological characteristics that make it well suited for aquaculture. Redclaw has never been commercially farmed in Norway, although the species has recently been allowed into the country for ornamental purposes. Redclaw is widely translocated out of its native range globally, and is considered an invasive alien species. Hitchhiker organisms and infectious agents could potentially follow the import. In order to address the assignments for Norwegian authorities, we assessed risks from an environmental perspective and an animal health-specific perspective.

Being an exotic species to Norway, the redclaw was classified as a medium risk, based on the AS-ISK screening, justifying the necessity of a full ecological risk assessment. According to the model by the Norwegian Biodiversity Information Centre (NBIC), redclaw has a small invasion potential and minor ecological effect on local biodiversity.

However, redclaw may introduce a number of pathogenic agents that would have detrimental consequences on local fauna, including several redlisted species. Four disease-causing agents were identified as hazards based on requirements of the EEA agreement and guidelines from the World
Organisation for Animal Health (OIE).:

1. Bacilloform Virus (CqBV),
2. White Spot Syndrome Virus (WSSV).
3. the oomycete *Aphanomyces astaci*.
4. the fungus *Batracochytrium dendrobaditis* (*BD*).

Redclaw or related crayfish species can be infected or act as carriers of these pathogens. For a number of other microbial agents and hitchhiker organisms that might follow redclaw, scientific knowledge gaps prevents conclusions regarding hazard status.

Based on the analysis from the NBIC model, we conclude that the combination of small invasion potential and large ecological effect (arising from introducing pathogens) puts redclaw as a “potentially high risk”, under the current climate. The invasion potential of redclaw will increase from small to limited, if future climate changes with elevations in temperature. In this case, redclaw will be classified as a “high risk”.

Crayfish plague is already established in Norway in a few areas, after introduction of infected signal crayfish. Since *Aphanomyces astaci* (the agent that causes crayfish plague) may be transferred to areas that are plague-free, the risk of spreading crayfish plague through import of redclaw is considered as high in the absence of specific measures. As amphibians are very susceptible to *BD*, the risk of triggering disease outbreaks is considered as high.

IMPACTS OF CLIMATE CHANGE ON NORTH EUROPEAN AQUACULTURE

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As a consequence of global warming, optimal farming conditions for the species common in European and Norwegian aquaculture today will gradually move northwards in a parallel shift. Fish farms could have to be relocated and farming technology and/or species modified in order to reduce the undesirable effects of higher temperatures. Temperatures presently found in Southern Norwegian coastal waters will be common along the coast of Northern Norway in a 50-100y perspective. This change in the mean temperature is not a major obstacle for farming salmon in Southern Norway. On the contrary, culture of cold-blooded animals would in principle benefit from higher temperatures up to the point where they exceed or compromise the biological limits of the organisms. In a biological context, increased length and occurrence of periods with extreme temperatures may be more important, especially considering the impact of suboptimal temperatures on the immune system and the proliferation of pathogenic agents.

Studies of the future climate show that air temperatures will rise during the 21st century. In the seas off the coast of Norway, the temperature will raise by 1.5-2.0°.

Other major effects of the expected climatic changes along the Norwegian and North European coast the next century will be increased intensity and frequency of storms and increased rain, plus a moderate increase of high tidal level. Such effects will cause a need for improved technological solutions, and significant investments may be needed. However, they do not constitute “impossible” biological or technological problems. Intense storms may, however cause damages to fish farms, and increase the with high numbers of escaped fish, which itself is considered a major environmental problem.

Exposure to temperatures outside the ecological niche will normally affect the immune system negatively. Many diseases may occur more frequently in warmer weather, particularly bacterial infections with bacteria or parasites adapted to relatively high temperatures. Just as important as the temperature range of bacteria and parasites, is the temperature range of the cultured fish. Rearing a species at too high or low temperatures inevitably may compromise the immune system, leading to increased disease problems. For instance, important diseases such as francisellosis, vibriosis, furunculosis, as well as several parasites are typically associated with high water temperatures. On the other hand, winter ulcers and cold water vibriosis are typical examples of diseases occurring in cold waters. In this context, the extreme temperatures are much more important than the middle temperatures.

Reproduction of parasites like salmon lice is temperature dependent, thus shorter generation times, and thereby increased infestation rates may be expected with higher average temperatures.

In addition, spreading of salmon lice may be altered by increased freshwater along the coast, particularly in the fjords. Improved modelling of water movements in fjords and coastal environment in various climatic scenarios may thus be a useful tool for predicting changes, as is the case for predicting salmon lice spreading today.

GIS-ANALYSIS OF SPATIAL CONFLICTS WITH LARGE SCALE SALMON AQUACULTURE IN A NORWEGIAN FJORD ECOSYSTEM

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The project ECOAST - *New methodologies for an ecosystem approach to spatial and temporal management of fisheries and aquaculture in coastal areas* – is co-funded by the COFASP – *Cooperation in Fisheries, Aquaculture and Seafood processing* – ERA-net.

We aim to identify, develop and test new methodologies for spatial and temporal management of fisheries and aquaculture in coastal areas. In this approach, we use GIS-based analysis to assess the impact of large scale industrial salmon aquaculture on fisheries and other activities in a Norwegian fjord system.

The Hardangerfjord is Norway´s second largest fjord, 179 km long, located in the SW of Norway. It is a deep, steep sloped valley both over and below the sea level. The largest depth is 893 m. Two major glaciers are present in the area (Folgefonni and Hardangerjøkelen), both draining to the fjord. Large freshwater supplies create a hydrography typical of fjords, with a freshwater or brackish layer at the top of the water column.

A total of 3357 MW hydroelectric power is installed in 298 power stations within the case study area. This has also been the fundament for metallurgical industries (aluminium, ferrosilica, titanium, zinc) at several sites by the fjord. The area surrounding the fjord is an old cultural landscape dating back to the first colonization of the country after the deglaciation.

There is a large salmon-farming industry in the fjord, with 169 sites, typically with a max. licenced biomass of ~2500 tonnes per farm. Salmon *Salmo salar* is the only predominant species in cage farms in the sea. In addition, there are several hatcheries for salmon and trout *Salmo trutta* in the rivers adjacent to the fjords.

The main environmental impacts of the aquaculture are salmon lice, pharmaceutical compounds used against lice, escapees and nutrient releases.

The anti-lice compounds generate ecological pressure against wild salmonids, and crustaceans, mainly shrimps whereas excess fish feed affect the saithe and its fisheries.

GRID *GeoReference Interactions Database* was developed within the COEXIST project (www.coexistproject.eu), funded by the EUs 7th Framework programme. It aims to analyse and locate conflicts as well as synergies among various coastal activities. GRID calculates conflict scores and provides interaction matrices and maps.

ANTIVIRAL SUBSTANCES OBTAINED FROM ALGAE USED TO COMBAT AGAINST THE KOI HERPESVIRUS DISEASE (KHVD)

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The koi herpesvirus (KHV) disease (KHVD) is induced by an aquatic herpesvirus belonging to the family *Alloherpesviridae*. The disease may cause up to 100% losses inside a carp or koi (*Cyprinus carpio*) population. Within 10 years, the virus was spread all over the world mainly by trade of latent or persistent healthy appearing fish. The disease have been notifiable by OIE and EU in 2006. With this listing by EU it is forbidden to immunize carp or koi in virus free areas.

To provide an alternative to the farms and to protect the populations for losses after infection, a search for antiviral substances were conducted. Our goal was to used algea and their products from different origins and locations for this purpose.

First investigation onto cell culture systems went very promising. Different algea products were able to reduce or to avoid the infectivity to common carp brain cells (CCB cells) which are commonly used for KHV replication. Variable concentration of different algea compounds avoided the replication of KHV onto CCB cells. Our hypothesis were that on one hand the algea product damage or cover the viral envelope and the virus is not able to dock at the receptor of the cells. On the other hand, it is also possible that the algae substrates cover the cell receptors and the virus is also not able to dock on the cells. More investigations are necessary to clarify this phenomenon.

Based on the cell cultures experiments were planned and proceeded an experiment using SPF (specific pathogen free) carp, free of any cyprinid herpesvirus tested by virological and serological methods.

Two compounds were found to be effective against the virus infection: i) the lyophilized algae biomass and ii) the exopolysaccharide (EPS). The latter represents waste during the production of algae but it was most effective against the initial infection but also as a metaphylactic measurement, when the disease had already broken out. In both scenario the delivery of EPS was successful: i) EPS was presents for 2 to 6 weeks before the fish were exposed to the virus and ii) EPS was add the day after the symptoms induced by KHV were visible.

In both cases, the virus induced mortality was turned down below 10%, in some cases below 5% of the population in contrast to the not protected population.

Recently there are investigation to use EPS also against other virosis of fish like VHSV in Olive flounder (*Paralichthys olivaceus*) but also for Pacific oysters (*Crassostrea gigas*) against the Ostreid herpesvirus (OsHV-1).

PERSPECTIVES OF SPERM CRYOPRESERVATION IN PERCID FISH . . . *PAST-PRESENT-FUTURE*"

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Introduction

Both pikeperch (*Sander lucioperca*) and Volga pikeperch (*Sander volgensis*) are economically important fish species because of the high boneless meat quality and the angling utilization as well (Bokor et al. 2007). Eurasian perch (*Perca fluviatilis*) is a promising species among those that were recently introduced into European freshwater aquaculture. The SZIU Department of Aquaculture started to investigate sperm cryopreservation in the three species since 12 years ago. The main goals of the experiments were to develop cryopreservation protocols applicable for large amount of sperm. The aim of our studies was also to establish sperm banks available for the hatchery practice all year long.

Materials and Methods

A freezing method was developed for pikeperch and Volga pikeperch using a simple glucose extender (350 mM glucose and 30 mM Tris) at a ratio 1:1. Samples were freezed for 3 minutes at 3 cm of the liquid nitrogen (Bokor et al. 2007 and 2008). For the cryopreservation of Eurasian perch sperm an extender composed of 137 mM NaCl and 76.2 mM $NaHCO_3$ (Szabó et al 2005) at ratio 1:10 was used. Freezing was carried out using a controlled-rate freezer (from 7.5°C to –160°C, cooling rate: 56°C/min) (Bernáth et al. 2016).

Results

In pikeperch, a very high hatching rate (87%) was recorded with cryopreserved sperm during in hatchery conditions (50g of eggs). Our freezing protocol was successfully used for cryopreservation in Volga pikeperch (hatching rate: 60%). A large amount of sperm was efficiently cryopreserved with our method in perch and was used for fertilization (72%).

Discussion

Our established cryopreservation protocols resulted successful fertilization and numerous hatched larvae in all 3 species. However the improvement of repeatability in the methods is necessary to provide constant results. Our future aim in Percid sperm cryopreservation also to test the optimized protocols continuously at hatchery conditions and to integrate it in the artificial propagation.

Acknowledgement

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DIETARY SOY ISOFLAVONE SUPPLEMENTATION IN RAINBOW TROUT: INFLUENCE ON GROWTH PERFORMANCE AND FILLET QUALITY

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Fish meal are partially replaced by soybean meal, containing isoflavones: natural plant compounds that act as phytoestrogens and may affect fish growth performance, proximate composition of muscle and fillet quality. The potential effects of isoflavones on flesh quality and time of conservation may affect the utilization of soybean meal as an alternative protein source in aquaculture feed.

Rainbow trout were fed three experimental diets containing different concentrations of isoflavones (0, 500, 1500 ppm) for 70 days. Then trout were slaughtered and fillets stored at 4 °C during 7 d. The dietary isoflavone levels did not affect trout growth performance neither biometric indexes, skin and fillet colour, rheological characteristics, proximate composition, fatty acids profile of fillet. Otherwise, the time of storage influenced some parameters such as fillet yield, skin lightness (59.2 to 51.5 from 1 to 7 d of storage, $p < 0.01$) and yellow index decreased (6.99 vs. 5.07, $p < 0.01$). Fillet pH (6.22 vs. 6.34, $p < 0.01$) and lightness increased (38 vs. 43.6, $p < 0.01$), whereas yellow index (6.20 vs. 4.52, $p < 0.01$) and shear force decreased (0.94 vs. 0.80 g kg-1, $p < 0.01$). According to storage time, trout fillet showed increased water, decreased crude protein and increased total volatile basic nitrogen contents (19.3 vs. 21.2 mg 100 g-1, $p < 0.01$) (Table 1). As a result of PUFAs omega-6 decrease (13.1% vs. 12.7 %; $p < 0.05$), omega-3/omega-6 ratio increased (1.05 vs. 1.20; $p < 0.05$) during storage. Lipid peroxidation expressed as thiobarbituric acid reactive substances (TBARS) levels in fillet on day 1 of refrigeration was significantly higher than that at day 7 ($p < 0.05$) but no difference resulted among groups fed different isoflavone levels, evidencing no effect of feed soy isoflavones on fillet lipid oxidation (Figure 1).

In the present study, the dietary isoflavone levels did not modify the growth performance and the quality of reared fish as well as the biometric and physicochemical traits. Instead more substantial have been the variation on the whole fish and fillet after 7 days of storage at 4°C. The observed variations were maintained in a range that did not compromise the product acceptability.

Table 1. Proximate composition (%) and level of total volatile basic nitrogen of the rainbow trout fillets.

	Diets (isoflavone content, ppm)			Storage time (T)		Probability			RSD
	0	500	1500	1d	7d	D	T	DxT	
Rainbow trout (No.)	18	18	18	27	27				
Water (%)	76.8	76.4	76.5	76.0	77.1	0.55	<0.001	0.04	0.92
Crude protein (%)	19.0	19.2	19.2	19.4	18.9	0.38	<0.001	0.06	0.41
Ether extract (%)	2.31	2.56	2.52	2.65	2.28	0.57	0.06	0.12	0.69
(TVB-N) mg N/ 100 g	20.0	20.3	20.5	19.3	21.2	0.73	<0.001	0.86	1.46

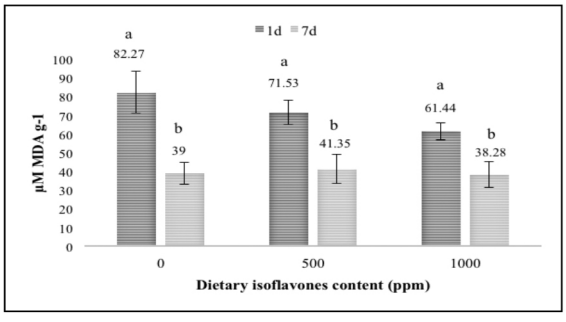

Figure 1. TBARS levels in grounded rainbow trout fillets.

SELECTIVE BREEDING ON TOLERANCE TO FASTING IMPROVES FEED CONVERSION RATIO OF SEA BASS

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Improving feed conversion ratio (FCR) is crucial to enhance the sustainability of fish farming. Still, FCR is difficult to improve by selective breeding as individual feed intake is difficult to measure in groups of fish.

Therefore, we tested if selection on tolerance to fasting (an easy trait to measure) over several generations could affect the FCR of the fish.

In a first time, 764 fish were phenotyped for their tolerance to fasting calculated as the average (negative) daily growth coefficient over two consecutive feed deprivation periods (F). F+ fish lose more weight than F- fish during fasting. The pedigree of these fish is known back to their wild grand-parents. These fish are the 3rd generation of divergent selection on F. F-/F- are fish with both parents F-, F+/F+ are fish with both parents F+, while F+/F- or F-/F+ are fish with one parent of each line. In a second time, the 764 fish were arranged in 16 tanks of 1.5m^3 for 2 periods of 3 weeks. Fish were fed *ad libitum* using automatic feeders. Uneaten pellets were counted daily to estimate the feed intake of each tank, and all fish were weighed to obtain the weight gain of each tank. These were used to calculate the FCR of the 16 tanks for the 2 periods. 566 of these fish as well as their parents and their grand-parents were genotyped for 1,923 SNPs markers in order to estimate the genomic heritability and the genomic estimated breeding value (GEBV) of F for all fish in all generations using single-step GBLUP in BLUPF90.

Tolerance to fasting was heritable (h^2 = 0.17 ±0.03) and GEBV of F diverges in generations G2 and G3 (figure 1). In G3, fish from the F-/F- line have the highest GEBV for F. The differences between extreme lines (F-/F- and F+/F+) are all significant.

In addition, the FCR of a given tank depends on the average GEBV of F of the fish in the tank (figure 2). This negative relationship is significant (P = 0.01) using a one-way repeated measures ANOVA in R. Thus, the tanks with the best FCR are the tanks with the highest GEBV for F. Thus, selecting fish for lower weight loss during fasting generates an improvement of FCR.

These results are important for fish farming as they show that FCR could be improved by selecting on tolerance to fasting, a trait both easy to measure and heritable.

This work was funded by the European Union H2020, grant agreement 654008 EMBRIC.

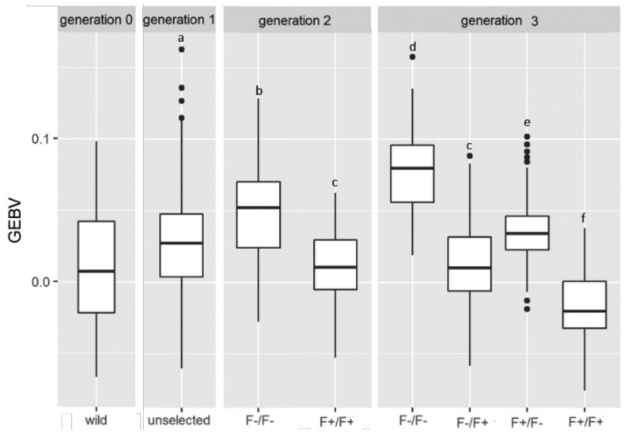

Figure 1: Boxplot of GEBV of F for the different lines in the different generations.

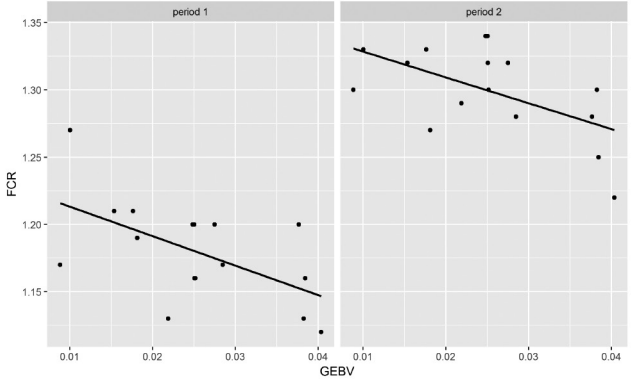

Figure 2: Regression of the FCR of each tank on the average GEBV of F of the fish.

VERY HIGH GENETIC CORRELATION FOR EGG PRODUCTION TRAITS BETWEEN TWO SUCCESSIVE SPAWNING IN RAINBOW TROUT

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Since the mid-90s, French commercial hatcheries of rainbow trout (*Oncorhynchus mykiss*) initiated selective breeding to improve growth performances, focusing on carcass traits thereafter. Now more than 800 million eggs derived from these programs are sold worldwide. It is common practice to reproduce the same female up to 3 times. According to previous publications, there is no result documenting the genetic parameters of successive spawning of the same individual. This is what this work focused on through the rearing of a commercial batch originating from Viviers de Sarrance (Pisciculture Labedan, Sarrance, France). The study is part of the BestOv project partly supported by the European Maritime and Fisheries Fund (EMFF) and by the French Government through the FranceAgriMer national body (n°: 2016-0152).

The broodstock originated from 8 successive generations of selective breeding. 30 sires and 38 dams were crossed in a partly full factorial mating design which created 254 full and half sib families being reared in common environment (spring water, 14°C during pre-growing phase, 9°C afterwards). A sub-batch of 835 pit-tagged and DNA-sampled females was kept under natural photoperiod and phenotyped at their first spawning (2-year-old, 2kg mean BW): individual length (L), body weight after spawning (BW), drained spawn weight (SW) and size of the eggs (ES). The same females were kept under natural photoperiod until their 2nd spawn at 3-year-old and the same measurements were recorded (only 526 individuals due to mortality, 3.6kg mean BW). The gonado-somatic index (GSI) was calculated. Traits followed by '_1' and '_2' were recorded at 1st and 2nd spawn. Heritabilities were estimated using VCE software, computing univariate animal mixed linear models, where *animal* is a random effect, and the *week of spawning* being a fixed effect. Genetic correlations between two traits were estimated with bivariate models. Accuracy of breeding values was estimated as the square root of the coefficient of determination (CD).

All traits are heritable: *e.g.* from 0.32 ± 0.12 for GSI_1 to 0.55 ± 0.10 for SW_1. Heritabilities tended to decrease at second spawn: *e.g.* 0.24 ± 0.10 for ES_2 or 0.38 ± 0.10 for SW_2. Either at first or second spawn, the genetic correlations between ES and SW were positive (between 0.53 ± 0.14 and 0.58 ± 0.18). Genetic correlations between BW or BL and GSI were negative. The SW is tightly correlated with GSI (0.95 ± 0.02 and 0.97 ± 0.02). All traits at first spawn were highly correlated with the same traits at second spawn (0.81 for ES, 0.84 for SW, 0.89 for GSI). Accuracies were rather high in this design: *e.g.* up to 0.72 for ES_1 and 0.63 for GSI_1. They decreased at second spawn, e.g. 0.56 for ES_2 or 0.67 for SW_2.

Spawning traits are heritable at first and second spawn. In this population, ES is not correlated with BW and BL, and is positively correlated with other spawning traits. However, selecting for big size trout would deteriorate their GSI. The accuracies of breeding values are higher at first spawn, and genetic correlations between traits at first and second spawn are high and positive. This means that since the first spawn it is possible to rank correctly the best females which will outcompete their relatives even for the next spawning events. Choosing to reproduce these females evaluated on their first spawn performances would decrease the generation interval, and thus improve the genetic gain on these traits especially if males are evaluated on their sib breeding values.

THE APPLICATION OF A LAB SCALE PHOTOBIOREACTOR FOR LABORATORY CULTIVATION OF *Osmundea pinnatifida*

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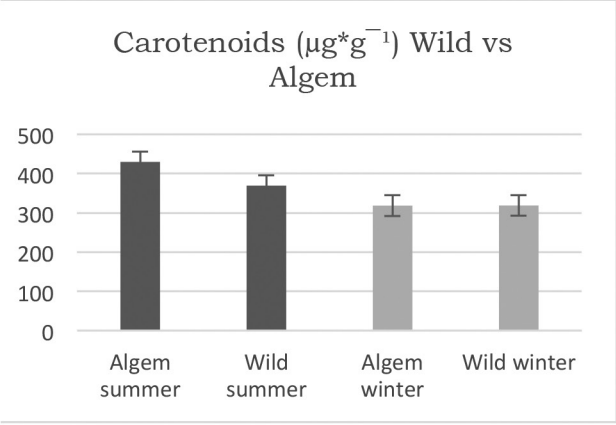
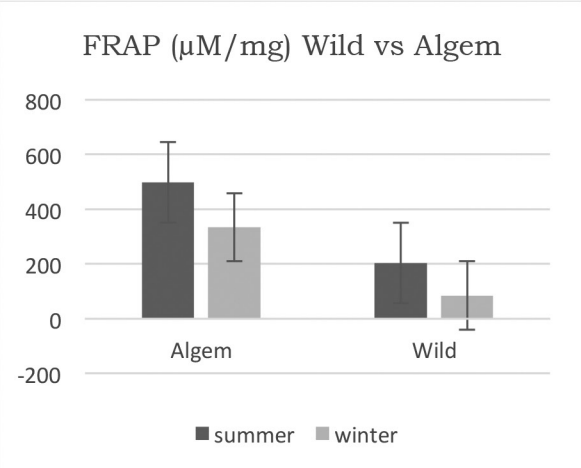
Currently the Algem® PBR system has only been used for microalgae cultivation. It offers the possibility to reproduce in a controlled situation, environmental parameters of a specific geographically region. There are links between seaweeds biochemical properties and abiotic factors (such as temperature, light's regime, photoperiod, etc.). The Algem allows this relationship to be explored in more detail.

The coordinates set in the Algem® bioreactor for the experiment were 56.4547° N, 5.4374° W (Dunstaffnage bay). Two different conditions were simulated: one representing winter (December) and one spring-summer (May). Temperature was kept constant at 10°C. Five grams (FW) of wild material were placed into 1 l flasks with 500ml of sterilized F/2. Every condition was set in triplicate. During the experiment the pH, light, growth and QY of the cultures were monitored. Weight of samples and SGR were assessed weekly on blotted-dry sample and the medium F/2 changed weekly. The length of the experiment was 4 weeks. Chl-*a* and carotenoids were estimated (Torres et al., 2014) as well as phycobiliproteins (Pereira et al., 2012); phenol content (Folin and Ciocalteu, 1927) and FRAP (Benzie and Strain, 1996).

The Algem bioreactor proved to be an efficient method of improving the production of antioxidant compounds in *O. pinnatifida* ($p<0.05$). These preliminary results show that cultivation conditions can be used to manipulate a species in order to exploit it as a high-value resource in either a pharmaceutical or nutraceutical field.





ASC-MSC SEAWEED CERTIFICATION: ADDRESSING ENVIRONMENTAL AND SOCIAL ISSUES ON SEAWEED CULTIVATION

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Building on each other's expertise in standard setting and seafood certification, the ASC and MSC have developed the environmentally sustainable and socially responsible Seaweed Standard. The ASC-MSC Seaweed (Algae) Standard applies globally to all locations and scales of seaweed operations, including both harvesting of wild population and cultivation (farms).

The ASC-MSC Standard also covers both marine and fresh water algae, and both macroalgae and microalgae. The ASC-MSC Seaweed Standard is the first third party certification scheme to address a full set of environmental and social issues related to wild harvest and cultured production of seaweed.

The current draft seaweed standard has been developed from MSC and ASC's existing standards, in consultation with seaweed experts, that involved 2 rounds of public consultation, face to face workshops in Bali, China and Japan, and pilots in Asia and Europe. The standard comprises five core principles: Principle 1, Harvesting and farming of seaweeds are conducted in a manner that does not lead to depletion of the exploited wild populations and, for those populations that are depleted, harvesting operations are conducted in a manner that demonstrably leads to their recovery; Principle 2, Harvesting and farming activities allow for the maintenance of the structure, productivity, function and diversity of the ecosystem (including habitat and associated dependent and ecologically related species) on which the activity depends; Principle 3: Harvesting and farming activities are subject to an effective management system that respects local, national and international laws and standards and incorporates institutional and operational frameworks that require use of the resource to be responsible and sustainable; Principle 4, Harvesting and farming activities operate in a socially responsible manner; and Principle 5, Harvesting and farming activities operate in a manner that minimizes impacts on other farms, activities and communities. The performance of the harvesting system or farm is scored against thirty-three Performance Indicators (PIs), each of which has one or more Scoring Issues (seventy in total).

The presentation will describe some of the details of these indicators, in addition to the assessment processes that will be required to achieve certification. The ASC-MSC standard enables producers to verify their production unit meets the environmental sustainability and socially responsible management of their resources by using a credible, independent third-party assessment process. Certified harvesters and farms can be recognised and rewarded in the marketplace, with an assurance to consumers that their products come from well-managed and sustainable sources.

KEY CHALLENGES TO SALMON FARMING AT EXPOSED SITES IN NORWAY

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Several innovations and new salmon farming concepts have been presented in the past years, driven in part by constraints to open cage farming in limited sheltered coastal areas and the persistent challenges of sea lice. In addition, the opportunity to apply for development licences in Norway resulted in 104 submitted applications for novel concepts, a significant number of which aim at farming in exposed locations. The on-site installation of SalMar's Ocean Farm 1 in the autumn of 2017 marked a paradigm shift for production of salmon at sea. There are still challenges associated with conducting fish farming and marine operations at conventional Norwegian salmon sites using state-of-the-art technology. Both current exposed farming operations and novel farm concepts underline these operational challenges, and the need for research and innovation in this area.

EXPOSED Aquaculture Operations is a Centre for Research based Innovation (SFI) funded by the Norwegian Research Council's Division for Innovation and the industry. The main objective of an SFI is to enhance the capability of the business sector to innovate based on results from long-term research through creating close alliances between research-intensive enterprises and prominent research groups. The EXPOSED Centre thus brings together global salmon farmers, key service and technology providers, and leading research groups to develop knowledge and technology for robust, safe and efficient fish farming at exposed sites. Partners in EXPOSED contribute with significant R&D to Ocean Farm 1 and several of the other novel fish farm concepts.

With its broad and competent consortium of partners, the EXPOSED centre is well positioned to identify both challenges and limitations to current fish farming operations, and knowledge gaps related to new farm concepts. Key challenges that increase with increased exposure are:

1) More demanding operations – Vessels must currently berth alongside the floating collars, and thus experience large relative motions with the structure while conducting complex handling of structures and nets during fish crowding. Operations such as de-licing are often postponed due to unpredictable weather and sea states. Non-contact operations need to be developed.
2) Demanding management and daily operations – Even routine operations and inspections become challenging in bad weather. Fish farming already poses a demanding working environment. This is amplified by harsh conditions. Some farms have prolonged periods with ocean swells and there are examples of farms that have been abandoned for this reason. Use of robotics will be an important part of the operational tools.
3) Fish welfare under rough conditions – Treating fish in short operational weather windows and under rough environmental conditions inflict additional stress, with a potential for higher mortality. A more holistic and knowledge-based management system built on: observation – interpretation – decision – action, is crucial.
4) Fish escapes – 1 of 3 escaped salmon escapes under bad weather. Extreme conditions can cause structural failures and increase the escape risk during demanding operations.

This presentation will highlight main results from R&D activities performed under the EXPOSED Centre's six research areas in 2017-2018.

THE EFFECT OF TEMPERATURE ON THE LIFE SPAN AND REPRODUCTION OF THE COPEPOD *Parvocalanus crassirostris*

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Copepods, thanks to their small size and high nutritional value, are critical to successful initial feeding of species of fish that have very small larvae such as grouper, snapper, and many marine ornamentals. However, large-scale copepod production is variable and inefficient due in part to insufficient knowledge of production trait responses to environmental parameters such as temperature. *Parvocalanus crassirostris* is an attractive candidate for culture due to its small size, high fecundity, and relative tolerance for high density culture compared to other calanoid species. In this study, we investigated the effect of temperature on the lifespan and reproductive characteristics of *P. crassirostris*.

P. crassirostris cultures were reared in triplicate containers at five temperatures (20, 22.5, 25, 27.5, 29.5°C). Sex ratio, egg production, hatch rate, and mortality rate were monitored. Copepods were grown in three 3L containers stocked with a cohort of eggs (1 egg mL^{-1}) laid within a 1 hour time span and fed a diet of *Tisochrysis lutea* at saturation density (>1000 µg C ml^{-1}) from time of hatching. Upon the first molt to the adult stage, trios of 1♀ and 2♂ were transferred into petri dishes containing 15mL of seawater and *T. lutea*. There were 4 replicate dishes per replicate culture container for each of the 5 temperature treatments. New algae at 50% of the original density was fed 12 hours post stocking. At 24 hours, copepods were pipetted to clean dishes with new water and algae. Eggs left behind were incubated for 12-16 hours (temperature dependent) before nauplii and unhatched eggs were counted. Daily transfers were conducted until all individuals died or the female failed to lay eggs for two consecutive days.

Sex determination was not influenced by temperature. The sex ratio was on average 1:1 at the time of maturity. Life span for both sexes was inversely proportional to temperature. Males lived about half as long as females, with male mean lifespan ranging from 9 (29.5°C) to 18 (20°C) days post hatch. Egg production rate was directly related to temperature, ranging from 1.63 (20°C) to 2.33 (29.5°C) $eggs^{-hour}$; but, the reproductive period of the females was inversely related to temperature, ranging from 14 (29.5°C) to 35 (20C) days. Egg production and hatch rate differed among individuals but was consistent over time within an individual during the first week of egg production. As females age, individual egg production decreases and hatch rate variability increases, particularly toward the end of the reproductive period after the males have died. Because of the strong effect of moderate variation in temperature on egg production and hatch rate, strict control of temperature during culture is necessary to achieve predictable production while minimizing variability in these parameters.

SWIM BLADDER INFLATION FAILURE IN POND-CULTURED PIKEPERCH *Sander lucioperca* LARVAE

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When the culture conditions in recirculating systems are poorly developed, the incidence of swim bladder (SB) non-inflation in pikeperch larvae can reach 70-100%. However, there is very limited knowledge about this phenomenon in pikeperch populations in ponds or lakes. The aim of this survey was to assess whether non-inflation of SB occurs in pikeperch larvae under pond conditions and to evaluate the effect of environmental factors such as surface area, pond depth, bottom structure, and presence or absence of littoral or submerged vegetation on the rate of SB non-inflation.

This study was carried out in 19 traditional production ponds with varied physical characteristics. To estimate the percentage of larvae without inflated SB, 300 larvae were randomly taken from each pond at 16 days post hatching. The potential influence of the pond characteristics on swim bladder inflation (SBI) was investigated.

Of the 5700 pikeperch larvae examined for SBI, 243 (4.2%) lacked SBI. The maximum rate of non-inflated SB in an individual pond was 17.3%. Three ponds yielded no fish with non-inflated SB. The number of larvae with inflated SB was significantly influenced by a two-way interaction of surface area and presence of littoral vegetation. The number of larvae with inflated SB ranged from 247 to 300 individuals in ponds without littoral vegetation compared to 279-299 individuals in ponds with littoral vegetation. Swim bladder inflation was also significantly influenced by interaction of pond depth and presence of littoral vegetation. Ponds without littoral vegetation and mean water depth < 0.9 m produced significantly higher proportions of larvae without initial SB inflation (11-53 of 300 compared to 1-21 of 300 in ponds with littoral vegetation and mean water depth over 0.9 m).

Based on the results of this study, we conclude, that: 1) ponds without littoral vegetation are likely to produce more pikeperch with non-inflated SB; 2) the inability to inflate SB could be a source of annually unstable production of pond-cultured pikeperch juveniles; 3) SB non-inflation might not be a problem restricted to intensive pikeperch larviculture only. To increase the quality and quantity of pikeperch larvae and fingerlings reared in ponds, stocking of larvae in water bodies with littoral vegetation and an average water depth > 0.9 m is recommended.

The study was supported by following projects: CENAKVA n. CZ.1.05/2.1.00/01.0024; CENAKVA II n. LO1205 under the NPU I program, project n. CZ.02.1.01./0.0/0.0/16_025/0007370; GAJU 060/2016/Z; NAZV QK1710310 and QK1820354.

ASSESSMENT OF NOVEL ANTIFOULING COATINGS FOR SALMON FARMING

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Biofouling on nets and sensor equipment
Biofouling is a challenge in salmon aquaculture worldwide, and large amounts of time and money are invested in the prevention of growth on the pen nets. One of the main strategies employed in farming regions such as Norway and Scotland is the use of copper-based antifouling coatings. Unfortunately, these coatings are rarely able to prevent growth for extended periods of time and, in addition, release approximately 90 % of their copper into the sea. This evokes environmental concerns regarding the poisoning of non-target species in the area, as well as the accumulation of copper in surrounding sediments. Finally, consumer support for more sustainable aquaculture production gives further incentive for the development of alternative net coatings.

Similar to nets, also sensor equipment (e.g., cameras or current sensors) submerged at farm sites suffers from extensive biofouling, which may impair the function of the device. Current strategies against biofouling rely on frequent cleaning of equipment or the application of copper shim tape designed to prevent the attachment of organisms. The search for alternative solutions that inhibit biofouling of sensor equipment is mainly driven by the desire for improved performance and cost reduction, with environmental pollution playing a minor role due to the comparatively small surface area of the devices.

Testing novel antifouling coatings for nets
The performance of six novel net coatings with either strongly reduced copper content or without copper was compared to a commercial copper coating and uncoated nets. Two field trials were conducted to coincide with the bi-annual immersion times of nets in Norwegian salmon farming. The results indicate that the success of a coating not only depends on the contents of a coating, but may also differ with the biofouling species present.

Testing novel antifouling solutions for sensor equipment
The biofouling resistance of experimental stick-on films cold-sprayed with copper particles of two different loadings was assessed in a 10-month field trial. The performance of these films taped to PVC panels was compared to commercial copper shim tape and blank PVC surfaces. The results indicate that copper cold spray technology may present a potential alternative to copper shim tape.

COMPERATIVE MORPHOLOGY OF FARMED AND WILD GILTHEAD SEA BREAM (*Sparus aurata* L. 1753) POPULATIONS IN AEGEAN AND LEVANTINE SEA

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In this study, it is aimed to understand morphological discrimination of gilthead sea bream (*Sparus aurata,* L, 1758) from four wild populations (WENZ, WDGN, WKYC and WYMR) and two farmed populations (FRMA and FRMB) which were collected along Mediterranean coast of Turkey. After photographing each specimen, 10 landmarks are determined in order to understand morphological structure of all populations by tpsDig 232 software programme. With all landmark measurements, PCA and DFA were used to see the discrimination of wild and farmed gilthead sea bream populations.

Canonical discriminant functions of the discriminant analysis of wild and farmed populations supported the predicted classification correction as both farmed populations are significantly unlike to the wild populations. Loy et al (1999) found the evidence of discrimination between S. aurata from two different hatcheries and explained this differentiation because of different larval and post-larval rearing condition. Current study has similar results as two hatchery origin samples are morphologically well discriminated. This might also be due to the broodstock management techniques of different hatcheries since it has been known that they sometime mix different wild origin populations.

As a result, even though wild population separated to four different region in scatter plots of DF, it is observed that no significant differences were found between wild populations (P<0.05), however farmed populations had significantly different body shape than all wild populations (Fig 1). This might show that phenotype of wild gilthead sea bream populations has not been affected by farmed fish escapees in the main aquaculture region of Turkey yet.

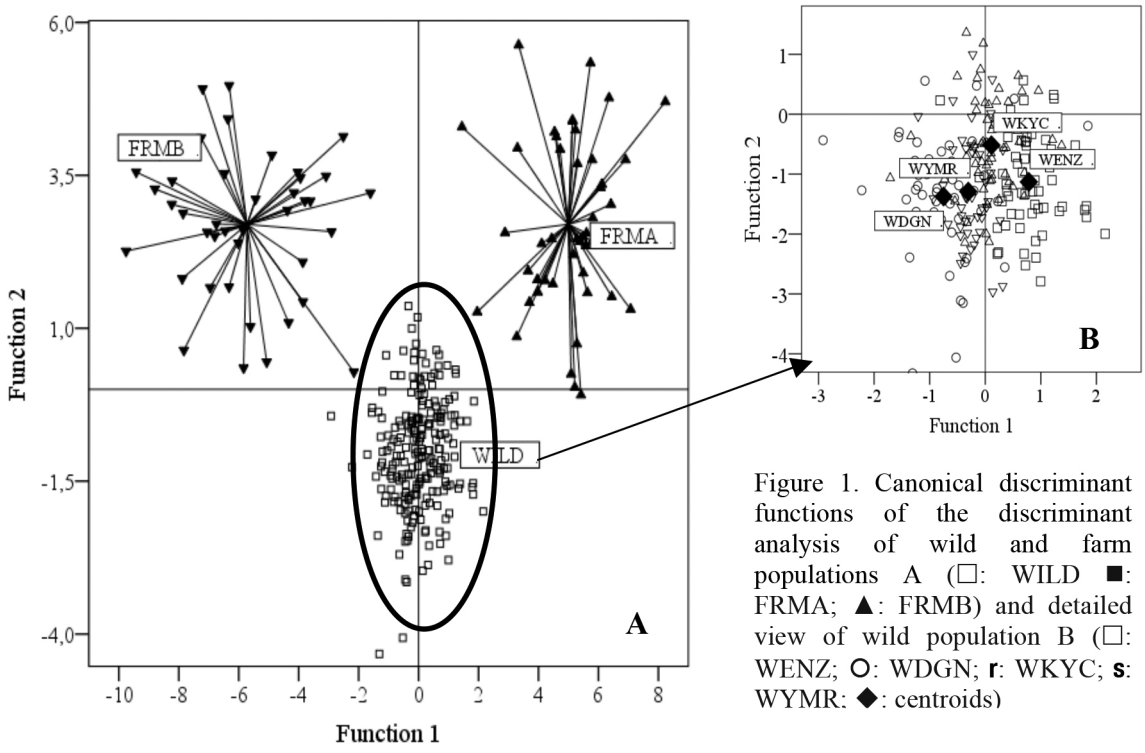

Figure 1. Canonical discriminant functions of the discriminant analysis of wild and farm populations A (□: WILD ■: FRMA; ▲: FRMB) and detailed view of wild population B (□: WENZ; ○: WDGN; **r**: WKYC; **s**: WYMR; ◆: centroids)

COMPARISON OF GROWTH RATES OF SOME PLANTS IN HYDROPONICS AND AQUAPONICS

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Aquaponics is an integrated system which combines aquaculture and hydroponics and aquaponics and hydroponics production of plants has been becoming more popular in the markets. Therefore, in the current study, it is aimed to understand growing performance of hydroponically and aquaponically grown of some plants. In this study, two variety of lettuces (*Lactuca sativa* var. cospirina and var. caipira) and parsley (*Petroselinum crispum* var Italian giant) were selected to see the leafy production performance in Mediterranean climate. The study was carried out in 250 m^2 plastic greenhouse in Fisheries Faculty in Akdeniz University, Antalya, Turkey. Common (*Cyprinus carpio*) (average weight 155 g) and Tilapia (*Oreochromis niloticus*) (average weight 127 g) were stocked in three cylindrical fish tanks (2.5 m diameter, 1m depth) in the fish unit of aquaponics (fed with 3% of their daily body weight (38% crude protein). In total 180 seedlings (60 piece for each plants) for each cultivation type were planted in plastic pots (5 cm diameter, 5 cm depth) which have vertical slots at the bottom and side surfaces to let the water through in and out. Seedlings were placed onto styrofoam rafts (120cmx60cm) by 20 piece/m^2. Hoagland nutrient solution was used in hydroponic system.

As a results, hydroponically produced lettuce were showed better performance than aquaponically produced (Table 1). However, their visual looks were very similar. Since no artificial fertilizer used in aquaponics, producer might be consider to choose this production method in other to supply more healthy vegetables to the market. Contrary to lettuce, parsley production was showed better growing performance in aquaponics, which the producer might be consider to produce low cost production with healthy food. As a conclusion, it can be said aquaponics is a new and promising production method in Mediterranean country.

Table 1. Some growing parameters of different plants in hydroponics and aquaponics

Parameters	Lettuce var (cospirina)		Lettuce (var caipira)		Parsley (Italian giant)	
	Hydroponics	Aquaponics	Hydroponics	Aquaponics	Hydroponics	Aquaponics
Number of Leaf	54	45	36	33	-	-
Length without root (cm)	409,21	254,98	338,35	206,13	48,54	102,66
Leaf Length (cm)	27,72	23,12	21,37	17,29	5,22	5,56
Dry weight of leaf (g)	0,88	0,50	0,48	0,39	18,96	30,74
Total production (g/m2)	7546,57	4173,65	5960,02	3365,04	676,44	1784,46

UP-SCALING OZONE TREATED SLUDGE AS CARBON SOURCE FOR DENITRIFICATION: FROM THE LAB TO RAS WORKING AT COMMERCIAL SCALE

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Recirculation Aquaculture Systems (RAS) have low water demands and efficient production based on the use of filtration/ sedimentation units and recycling. Denitrification filters lead to costs for acquisition, correct storage and manipulation of commercial carbon sources. Recycling implies that sludge (feed rests and faecal debris) must be also eliminated from the system.

In the present study we perform a batch experiment at lab scale comparing 60 min ozone-activated sludge and acetol as carbon source for denitrification. Sources were tested at different proportions with respect to the final volume of the reactors. Sludge was treated 24 h before use with an ozone dosage of 5 mg O_3 L^{-1} min^{-1}. We used four reactors per treatment in which 10%, 25% and 50% of the reactor volume was filled with ozone-treated sludge compared to reactors totally filled with denitrification water and acetol as the carbon source. Over 9 days, filter bodies were mixed and reallocated to the reactors with an adjusted initial nitrate concentration of 50 mg L^{-1} on a daily basis.

Use of ozone was then upscaled to commercial scales in RAS. We equipped a system rearing European seabass (30 kg fish/m^3) with a contactor device for the treatment of sludge with ozone. Fish were fed *ad libitum* with a commercial feed (Supreme-22, 4.5 mm) Coppens International GmbH). Ozone treatment was performed until a prefixed pH level of 7.8 was achieved with a dosage of 2 mg O_3 L^{-1} min^{-1}. We assessed the denitrification performance via nitrate and carbon depletion and characterized the process by controlling physicochemical parameters and the formation of organic and inorganic nitrogen species in the water column of the reactors (batch experiment) and all compartments of the RAS. We analysed system performance with and without ozone contactor and the savings in term of commercial carbon source needed. We fitted a model to the budgeting data in order to forecast system performance and limitations under the variables used. Our results verify that ozone-treated sludge can be used for denitrification in RAS, combined with lower concentration of commercial carbon sources. This allows the recycling of solid wastes of the system while permitting the acclimation of the bacterial population to the poorer carbon availability of sludge. This procedure also lowers the turbidity of the make-up water and costs due to water discharge. Details on system performance and nutrient budgeting of the different compartments of the system will be presented.

AMBIENT LIGHT INTENSITY INFLUENCES GROWTH PERFORMANCE AND DIURNAL STRESS RESPONSE OF JUVENILE STARRY FLOUNDER (*Platichthys stellatus*) IN RECIRCULATING AQUACULTURE SYSTEMS

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Understanding light requirements of important commercial species is essential, particularly for species such as starry flounder, *Platichthys stellatus*, held exclusively in land-based aquaculture systems where building and tank conditions interfere with natural light. In this study we determine the influence of different ambient light intensity on growth and stress markers of starry flounder reared in a recirculating aquaculture system.

Juvenile *P. stellatus* were held in triplicate at different light intensities (Low, 15 lx, Medium, 500 lx, High 2500 lx) to determine effects on growth and stress parameters.

Starry Flounder held at low ambient light intensities exhibited significantly higher weight gain after 63 days than those held at high light intensity. Feed conversion rate and condition factor did not differ between the treatments. Fish from the high light treatment exhibited significant decreases in daily feed intake and specific growth rate in comparison to fish reared under low light intensities (Tab. 1). With respect to stress markers, juvenile *P. stellatus* held for 33 days at low, medium, and high ambient light intensities exhibited no differences in diurnal plasma cortisol response, while blood plasma glucose level was significantly higher in fish held at low light levels.

The present study indicates that improper high light intensity markedly reduces the growth of juvenile starry flounder in land-based recirculating aquaculture systems without causing detectable or significant stress responses, as indicated by concentrations and daily patterns of plasma cortisol and glucose levels. Considering growth performance, economic constraints and pertinent animal welfare, adjustment of light conditions to maintain optimal intensities below 500 lx is recommended for this species in artificial rearing units.

AN OVERVIEW OF APPLIED AQUACULTURE RESEARCH ON JUVENILE LUMPSUCKER *Cyclopterus lumpus* PRODUCTION IN IRELAND

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One of the most significant threats to salmonid farming in Europe and elsewhere is sea lice. Infestations can lead to substantial reductions in growth and survival of the salmon, additional costs to the industry for treatments and potential risk to the environment. A reduction in the efficacy of chemical treatments (caused by growing resistance of lice to chemicals) has resulted in a concerted effort to evaluate, develop and utilise more sustainable methods of sea lice control.

The use of cleaner fish as a biological control is a viable alternative to chemical treatments. A number of species are known to remove the sea lice off the host, namely lumpsucker, cunner and wrasse (several species) are currently being used for this purpose in a number countries including Ireland, Norway, Scotland, Faroe Islands and Canada.

Between 2015 and 2018, BIM (Bord Iascaigh Mhara, Irish Sea Fisheries Board) funded research and production of lumpsucker at Carna Research Station (Ryan Institute, NUIG) with >350,000 juveniles transferred from the facility for use in an integrated pest management strategy by various salmon producers. Part of this initiative was to conduct applied research through a number of strategic experiments. The work presented here is an overview of the production of lumpsucker in Ireland with particular emphasis on experimental work examining feeding ration, temperature, use of hides, feeding frequency and stocking density on lumpsucker growth and survival.

EFFECTS OF INCREASING LEVEL OF DIETARY ORGANIC ACIDS AND NATURE-IDENTICAL COMPOUNDS ON GROWTH, INTESTINAL IMMUNE PARAMETERS AND GUT BACTERIAL COMMUNITIES OF EUROPEAN SEA BASS *Dicentrarchus labrax* JUVENILES

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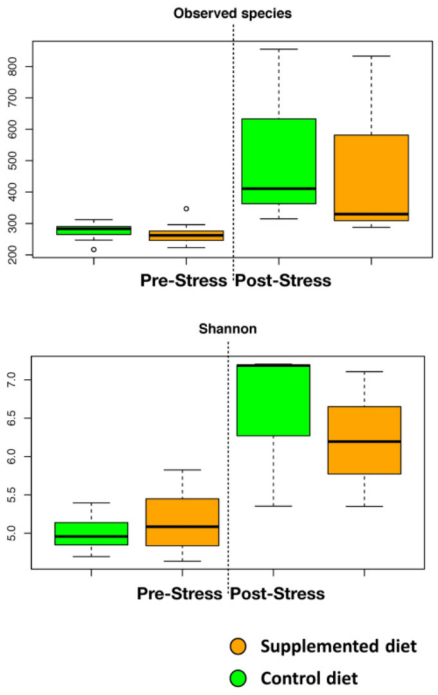
Despite the known beneficial effects of dietary organic acids (OA) and plant derivates in improving the performance and health of terrestrial livestock, limited comprehensive research has been conducted to evaluate their efficacy in aquatic animals and few data are available in European sea bass (*Dicentrarchus labrax*). A study was undertaken to assess the effects of increasing dietary levels of a microencapsulated mixture of citric acid, sorbic acid and nature-identical compounds (NIC - thymol and vanillin), on growth, intestinal immune response and gut bacterial communities of sea bass juveniles.

Four isonitrogenous and isolipidic extruded commercial diets containing the mixture of OA and NIC (MIX) at 0.0, 0.25, 0.50 and 1.0 g kg^{-1} were fed to triplicate fish groups of 60 individuals (initial weight 13 g) to apparent satiation over 71 days. At the end of the trial fish were also exposed to sub-optimal environmental conditions for 7 days.

No significant differences ($P > 0.05$) among diets for final body weight, specific growth rate, feed intake and feed conversion rate were detected as well as for viscerosomatic and hepatosomatic indexes. No significant differences among treatments were detected in the general pattern of cytokines expression from the distal intestine (IL-1b, IL-8, IL-6, IL-10, TGFb and TNFa) before and after the environmental changes, even if a slight increment of some transcripts at increasing dietary level of the MIX was observed. Gut bacterial community was performed by next generation sequencing. The sea bass gut bacterial ecosystem was dominated by *Lactobacillaceae* (24%), *Leuconostocacea*e (20%), *Streptococcaceae* (16%) *Bacillaceae* (8%), *Streptophyta* (8%) and *Corynebacteriaceae* (7%). Even if no significant differences among these groups were detected, PCoA based on the Euclidean distances between samples showed at the end of the trial a significant ($P < 0.05$) separation among the treatments. Gut bacterial community of fish fed with the MIX after the environmental changes showed a tendency in reducing biodiversity indices increase when comparing to control diet.



In conclusion, although growth and intestinal immune-system parameters were not significantly affected at this stage, dietary inclusion of the MIX slightly modulated the gut bacterial community indicating a possible ability to contain the bloom of opportunistic bacterial species that can proliferate under conditions of environmental stress.

Acknowledgements: This research was funded by Vetagro S.p.A. and POR-FESR 2014-2020 Emilia Romagna grant.

OREGANO ESSENTIAL OIL IN DIETS FOR SILVER CATFISH *Rhamdia* sp. LARVAE AND JUVENILES

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Agroecology-based aquaculture emerges as a particularly interesting production alternative for rural family farmers. In such systems, it is desirable to use means for enhancing the zootechnical performance and improving fish health from natural products. Thus, the present study aimed to evaluate the effect of organic diet supplementation with oregano essential oil, *Origanum vulgare*, on growth and resistance to the ectoparasite *Ichthyophthirius multifiliis* (ich) in silver catfish *Rhamdia* sp larvae and juveniles. Two experiments were carried out, where a completely randomized design with five treatments (concentrations 0, 0.5, 1.0, 1.5, and 2.0 g oregano essential oil kg^{-1} diet) and four replicates was adopted. In the first study, 1,200 larvae (15 days old; 20 ± 5 mg and 12.97 ± 1.30 mm of initial weight and length, respectively) were equally divided into 20 polyethylene tanks (65 L) connected to a recirculation system (26 °C) with individual aeration. The diets were prepared with certified organic ingredients and offered for 20 days, in four feeds daily. In the second experiment, groups of 20 silver catfish juveniles (initial weight 12.5 ± 0.5g) were stocked in the same recirculation system previously used. The organic diets were offered for 89 days in two meals a day. At the end of the feeding period, survival, performance, and fish body composition were evaluated, and an experimental challenge with ich infection was performed.

Dietary concentrations of oregano essential oil had no effects on the parameters evaluated in silver catfish larvae and juveniles (Table 1). Neither was there any influence on the accumulated mortality of the fish under challenge with ich (Fig. 1). Oregano essential oil did not prove to be an effective dietary additive in improving the performance or increasing resistance against ich in silver catfish.

Table 1. Zootechnical performance and body composition (wet basis) of silver catfish fed diets containing different concentrations of oregano essential oil. [1]Larvae; [2]Juvenile; [3]Non-significant (p>0.05).

Zootechnical performance		Oregano essential oil (g kg^{-1})				
		0.0	0.5	1.0	1.5	2.0
Survival (%)	L[1]	84.58±14.2	82.50±21.1	87.50±5.2	90.83±2.2	93.33±5.8
	J[2]	98.75±0.5	93.75±6.3	95.00±7.1	98.75±2.5	100.0±0.0
Weight gain (g)	L	0.204±0.01	0.205±0.02	0.202± 0.03	0.176± 0.01	0.200± 0.02
	J	53.22±6.6	50.82±4.9	53.13±5.2	50.21±9.9	48.87±1.6
Specific growth rate (%)	L	11.60±0.29	11.66±0.63	11.58±0.64	10.98±0.33	11.58±0.37
	J	1.89±0.11	1.85±0.09	1.69±0.08	1.63±0.16	1.62±0.03
Food conversion	L	1.86±0.16	1.73±0.17	1.88±0.24	2.04±0.09	1.81±0.15
	J	1.50±0.12	1.51±0.06	1.40±0.09	1.48±0.06	1.56±0.06
Body composition of juveniles (%)						
Moisture		68.35±0.69	68.31±0.66	69.73±1.16	69.31±0.23	68.98±0.73
Protein		17.22±0.51	17.53±0.36	17.91±0.66	17.61±0.96	18.03±0.47
Lipid		11.75±0.81	11.78±0.92	10.96±1.10	11.50±0.37	11.12±1.30
Ash		3.01±0.27	3.08±0.13	2.95±0.22	2.95±0.34	2.94±0.17
ANOVA *P*		ns[3]	ns	ns	ns	ns
Regression *P*		ns	ns	ns	ns	ns

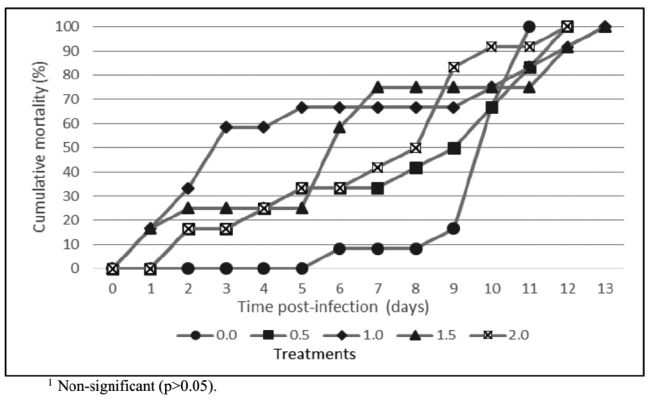

Figure 1. Cumulative mortality[1] of silver catfish juveniles fed diets containing different concentrations of oregano essential oil for 89 days and challenged with *Ichthyophthirius multifiliis*.

[1] Non-significant (p>0.05).

LINKING FLOC CHARACTERISTICS TO OPERATIONAL PARAMETERS

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Biofloc-based systems have become popular in aquaculture, both from a scientific and industrial perspective. Bioflocs in general can serve 2 functions. They can modify environmental parameters under which the target organisms are cultured and flocs can also serve as a source of microbial feed.

Bioflocs for these purposes should have a set of quality characteristics. In relation of environmental parameters for instance they should have a certain nitrogen immobilisation rate and/or nitrification rate but also a desired floc volume index (in ml/g flocs) and a minimal nitrous oxide generation rate. In relation for their nutritional value, they should have a certain, floc size, protein content, fat content, fatty acid profile, ash content, amino acid profile, polyhydroxybutyric acid content, to name just a few. This characteristics are often not determined, at the maximum only a few of them. Besides their putative nutritional value, the presence of microble associated molecular patterns (so called MAMPs) must have a strong influence on the immunological status of the target species.

These quality parameters must be determined by biofloc operational parameters. Here carbon nature, C/N/P ratio, floc loading rate, VSS (turbidity), sheer stress (oxygenation) must all influence these floc characteristics. One can even imagine that the aquaculture target species might have an influence on floc characteristics. In this respect, species and species density might be important “operational parameters”.

Despite the increasing importance of biofloc systems in aquaculture unsufficient attention is given to these operational parameters and their link with biofloc quality. The paper will present some of the limited information available on the link between floc operational parameters and biofloc quality.

Some attentian has already been given to substrate nature and C/N ratio’s and their influence of floc quality.

Other operational parameters are much harder to control such as floc loading rate (in mg C per g biomass C per hour). Yet it is known from for instance activated sludge plants for waste water purification that this parameters has a very important impact on sludge characteristics such as settlement.

Finally the paper wants to discuss the desirability to report a minimal amount of operational parameters in aquaculture reports , so that its link with biofloc quality would be revealed much faster relative to the chaotic progress that is experienced now.

SKILL DEVELOPMENT AND ACCREDITATION FOR CAGE-BASED AQUACULTURE: THE ERASMUS+ BlueEDU SECTOR SKILLS ALLIANCE

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The Erasmus+ funded BlueEDU Sector Skills Alliance for aquaculture project (Lot 1: Dec 2016 -Nov 2018) was catalysed by anecdotal evidence of knowledge and skills gaps within the cage-based marine fish farming sector. This appeared to be the result of a combination of:

- The deployment of increasingly technologically sophisticated equipment within an automated, industrial scale salmon farming system,
- an influx of staff to the workforce from different backgrounds that lacked formal aquaculture training and qualifications, and
- specialisation within the workforce, which was placing greater demands on the in-company staff development programme.

The project is led by NTNU (Norway) and is designed to establish the current and future skills, education and training needs of the cage farming sector in 12 countries; 6 in the north and 6 in the south of Europe. The intention is to use the findings to inform a Lot 2 bid for a project that will develop innovative vocational education and training (VET) solutions through a collaboration between VET providers and industry.

The partners carried out background research on current VET structures and provision across the 12 target countries and are now engaged with more in-depth stakeholder dialogue. The 2017 Federation of European Aquaculture Producers' (FEAP) General Assembly endorsed the importance of aquaculture VET and confirmed that there were knowledge and skills gaps in both the north and south of Europe to address. There are many in the European aquaculture workforce, who lack a Nationally Recognised Qualification (NRQ) and are dependent on non-formal and informal VET. Whilst mature aquaculture VET systems are to be found in some countries, most notably in Norway, Scotland and France, they are relatively undeveloped in others. Most Mediterranean countries appear more dependent on degree level graduates as well as knowledge transfer from feed and veterinary supply companies. Husbandry operatives are trained on the farms as very few pre-entry NRQs exist. European funded projects with a training element have been of some assistance, but have not led to the development of a sustainable formal aquaculture VET system. Although there are well established VET systems for school leavers in Norway, a mature entrant can have difficulty accessing and completing an appropriate NRQ. Conversely, Scotland has lost its full time VET provision and is entirely reliant on work-based NQs which are available to those individuals who wish to put themselves forward. For various reasons including the pace of technological development in cage-based aquaculture, there is increasing reliance on in-company non-formal VET as the main mechanism for knowledge transfer and skills development. The partners are exploring how the workforce can become professionalised through the development of more innovative, flexible and accessible VET delivery systems and resources, and the creation of an improved partnership between the aquaculture VET sector and industry that can be supported by relevant EU and National agencies.

MIXED-FAMILY ESTIMATION OF GENETIC PARAMETERS OF GROWTH, MORPHOLOGIC, IMMUNE AND BEHAVIORAL TRAITS IN THE EUROPEAN ABALONE *Haliotis tuberculata*

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In response to a declining supply from abalone fisheries, aquaculture production has greatly increased in recent decades. Selective breeding has been proposed to improve growth, the main trait constraining the increase of aquaculture production. Among abalone species, *Haliotis tuberculata* is in an early stage of domestication and no estimates of heritability or genetic correlations were yet available for growth-related traits. In this context, weight, length morphology, immunity and behaviour traits were recorded on 945 offspring from a cohort generated by the full factorial mating of 24 sires and 16 dams reared under standard conditions. SNPs were used to assign parentage of this cohort (Harney et al., Aquaculture 491 (2018): 105-113). BLUP was used to estimate additive genetic components and genetic correlations. Shell length exhibited moderate to high heritability estimates, from 0.13 at 14 months old to 0.49 at 42 months old (figure 1). Variation of foot colour and bled meat yield were also proven to be significantly heritable, unlike behaviour and immunity traits. Novel phenotyping methods such as ultrasound to estimate meat yield were developed and evaluated as potential tools for selection. A strong positive genetic correlation was observed between live weight and gonad yields, suggesting a trade-off between reproductive effort and meat yield. Sex ratio variation was measured between paternal half-sib families. Females were heavier at 32 months old but not at 42 months old, suggesting earlier reproductive allocation in males. As a result, a low but significant heritability of sex ratio (0.25±0.11) was observed and moderate positive genetic correlations were estimated between sex ratio of families and weight at 32 and 42 months old. These observations raise the questions about sex determinism in *H. tuberculata* and the potential interest of selecting for high female-to-male ratios high sex ratios. Overall, our results provide essential information to initiate selective breeding to improve growth-related traits in the European abalone. More precise heritability estimates would however be needed to better predict the expected genetic gains.

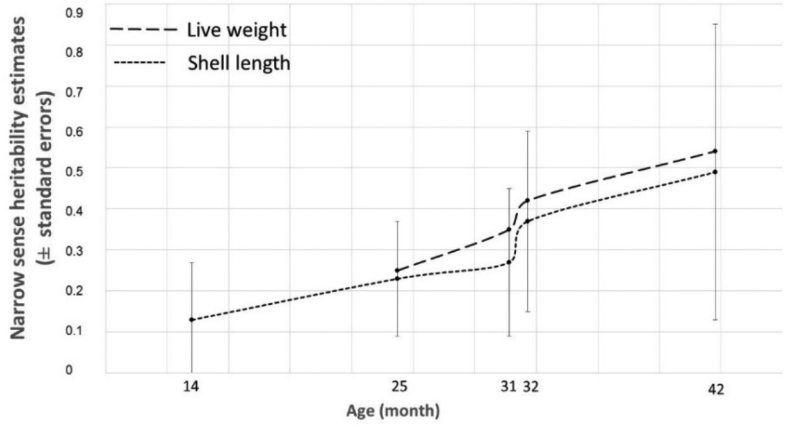

Figure 1. Narrow sense heritability estimates of live weight and shell length in 14- to 42-month-old European abalone (*Haliotis tuberculata*).

AQUACULTURE STANDARDS AND CERTIFICATION: SOCIAL CERTIFICATION AND LABOR WELFARE

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Environmental issues are widely addressed in current governance through standards, certification schemes and guides of best practices. But social issues are trickier to assess, measure and monitor. To foster improvement in social, labor and human rights compliance within the seafood supply chain, social benchmarking should be developed to fit aquaculture farms and boats' needs. This means that relevant indicators must be identified and accordingly, new tools for social monitoring would be designed.

The Social Expert Group (SMEG) is a group of experts in human rights, human-trafficking, child labor, fisheries and aquaculture sustainability from credible organizations and companies in the Asia-Pacific region. These experts have developed a set of metrics divided into 12 categories (see figure 1).

INTEGRATED SOCIO-ECONOMIC APPROACH

The digital social monitoring expert group aims at exploring digital social monitoring solutions to foster social and labor improvement in the seafood supply chain. The expert group is dedicated towards doing that by (1) scoping for business implementation and civil society relevance, (2) identify social performance indicators and matching metrics, (3) deploying on-site digital monitoring systems as information source, (4) continuous risk assessment to debug the digital monitoring system and (5) co-creation process involving synergy and support.

The metrics have been integrated to an online platform (Verifik8) that links environmental monitoring and social risk, triangulating and benchmarking among different sustainability standards. The social metrics also create room for corporate social responsibility that engenders an in-depth evaluation of issues with surrounding communities concerning resource use.

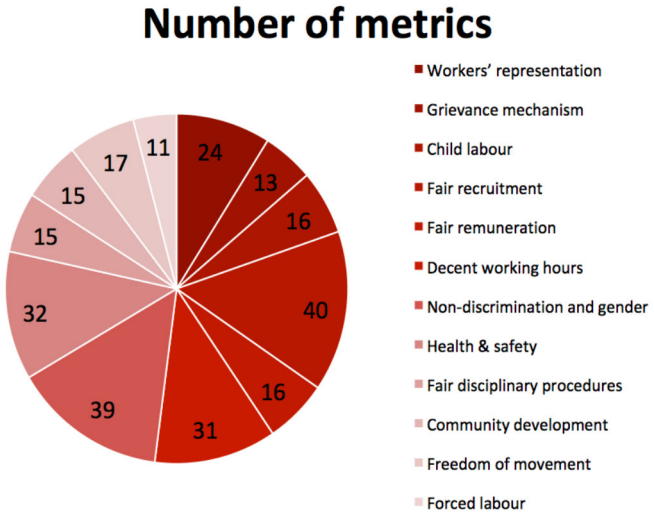

Figure 1. 12 categories of metrics and the metric distribution.

AQUACULTURE STANDARD AND CERTIFICATION: MEASURING SUSTAINABILITY IN A CHANGING WORLD

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Sustainable aquaculture production is powered by the surge in consumer base as well as the drive for products that deliver quality and leave little impact on the environment as a result of its production. Regulations are being tightened hence the need to ensure that ingredient source as well as production process are truly sustainable. The need for a seamless flow between raw material sourcing, the production process and the marketing of products while still ensuring compliance with little effort is indeed the next level in terms of compliance monitoring. VerifiK8 helps seafood buyers to make safer and more responsible procurements through a credible monitoring and verification software platform to de-risk operations in their supply chain.

VerifiK8 software uses mobile devices to collect and digitalize data directly from farms and cooperatives. It triangulates information, compares and crosschecks information with other data streams to provide solid analytics and benchmarking of each farm against the relevant sustainability standard. The system makes all aggregated data easily accessible on a single dashboard, from the farms to the buyers' desktop. Our unique technology allows a cost and time effective analytics and verification process to make certification more inclusive to medium and small-scale farms. The inclusive platform allows data inputs from farmers, cooperatives, processors, suppliers and buyers to validate each and every transaction. It secures aquaculture farms market outreach by proving their level of social and environmental compliance. It reduces labor and environmental risks, while increasing food safety and productivity.

In order to do these, Verifik8 works through three channels: Data collection, Data analytics and on-Farm verification.

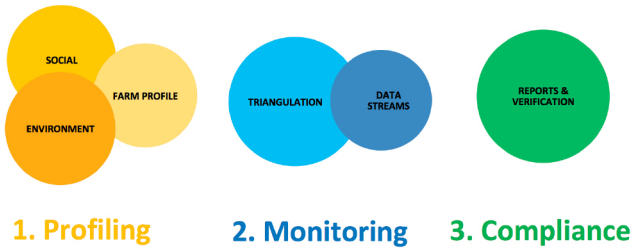

Figure 1: Verifik8 process flow

Figure 2: Incentives and benefits for farmers, processors and buyers

COMPLIANCE WITH SUSTAINABLE STANDARDS: FOCUS ON SHRIMP FARMS IN THAILAND

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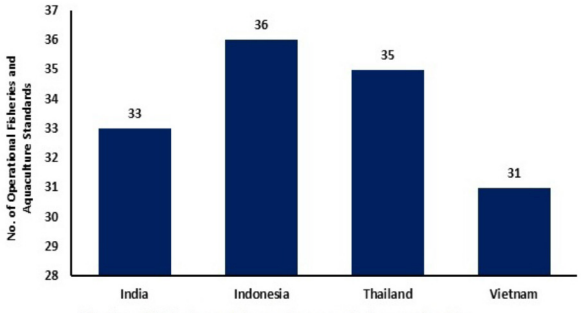
The contribution of aquaculture to the world food supply has been growing steadily over the past two decades. Currently, Asia produces almost 89% of the world aquaculture output of which shrimp farming is a major component. In 2017 the major shrimp producers and exporters were China, India, Ecuador, Vietnam, Indonesia, and Thailand. The whiteleg shrimp *Penaeus (Litopenaeus) vannamei* accounts for over 70% of Asia's shrimp aquaculture production effectively dwarfing *P. monodon* and *Macrobrachium rosenbergii*. There are several challenges to sustainable shrimp farming in the Asia Pacific region with the intensive scale of operation that is the norm in most places.

Shrimp is a highly traded commodity. Quality standards and certification are currently key determinants for the sustenance of this trade internationally. While standards designate some useful reference points for an aquaculture enterprise, certification reinforces compliance of the production system to these standards and would help ensure the sustainability of aquaculture systems. Food safety, social responsibility, animal welfare and environmental sustainability are the key components of an aquaculture certification scheme. Responsible trading also involves traceability of the certified products with accurate records of product movement. Here we review the aquaculture certification domain of Thailand.

A majority of the Thai farmers assessed, share common constraints that slow down their progress towards sustainable practices. Over the past 40 years, new farms developed in coastal sites of Thailand have resulted in the decline of mangrove areas by more than 50%. Farmers often lack the technical and financial capacities to fulfill the mandatory requirement of providing environmental risk assessment reports for the farms located in sensitive habitats. Excessive abstraction of groundwater for farming and inappropriate discharge of pond effluents are other shared issues that hinder farmers to reach compliance. Apart from environmental issues, the social aspects related to worker conditions, particularly those of seasonal workers such as harvesting personnel are difficult to assess because of their number and the short working periods in farm sites. The lack of evidence to demonstrate migrant working conditions is one of the key issues for social compliance. There are also several issues to be addressed beyond certification. The need to make improvements, assess risks, open new markets and expand the distribution and acceptability of shrimps from Asia demand greater effort to keep product quality and appeal to high-value international markets.



Number of Fisheries and Aquaculture standards operational in the four countries under review. Source: http://standardsmap.org/identify

DIRECT GENETIC INTERACTIONS BETWEEN WILD AND ESCAPED FARMED ATLANTIC SALMON FOLLOWING A SINGLE ESCAPE EVENT IN SOUTHERN NEWFOUNDLAND, CANADA

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Hybridization of escaped farmed Atlantic salmon (*Salmo salar*) with wild populations occurs throughout their native range, and can threaten population stability and persistence. Nonetheless, an understanding of the consequences of single escape events on wild salmon populations remains limited. We apply SNP-based screening following a single large aquaculture escape in the Northwest Atlantic and report evidence of wide-spread interbreeding through the broad landscape-scale detection in rivers of hybrid and feral salmon (i.e. 27.1% of individuals, 17/18 rivers). The proportion of hybrids varied significantly across and within rivers. Binomial mixed models and logistic regression showed increased proportions of hybrid or feral offspring within smaller rivers ($p<0.0001$). Within-river distribution of individuals of farmed descent was strongly associated with the migration effort required to reach a given spawning site; farmed and hybrid proportion decreased significantly ($p<0.05$) with increased elevation, geographic distance and the presence of obstructions. This supports previous observations that escaped farmed Atlantic salmon are less capable than wild individuals when faced with migratory challenges, which results in reductions of farmed and hybrid individuals in upstream spawning sites relative to downstream locations.

Repeated annual sampling from this cohort revealed a clear temporal signal of the escape event, with decreases in the presence of domestic alleles over time consistent with selection against domestic genotypes in the wild. Our research demonstrates that interbreeding with escapees following a single escape event can be wide spread, and levels of hybridization vary spatially and are associated with recipient population size and landscape features. Consideration of spatial variation in levels of hybridization and the estimates of the strength of selection against hybrids will be essential for the evaluation of impacts that escaped farmed salmon impose on wild Atlantic salmon populations.

DO DIFFERENCES IN COPING STYLES INFLUENCE STRESS RESPONSE AFTER VACCINATION IN GILTHEAD SEA BREAM?

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In aquaculture production, fish are exposed to stressful stimuli, including those related to industry intrinsic processes, such as handling and vaccination. These stressful situations can affect animal welfare, having potential effects on production. Previous studies have shown that fish present variability in behavioral responses when exposed to environmental pressure, describing two opposing stress-coping styles: proactive (adrenaline based, active coping or 'fight-flight') and reactive (cortisol based, passive coping or 'conservation-withdrawal'). Due to the differential/different characteristics of these responses, both in the behavioral as in the physiological aspects, some authors suggest that proactive fish will fare better in stable, plentiful, high-density environments, while reactive individuals will best thrive in environments with sparse, unpredictable resources, and low animal densities. In recent years, there has been a growing interest in this subject, since differences in coping styles could influence crucial aspects, such as growth performance, disease resistance and fish welfare.

The present study aims to evaluate how fish with different coping styles respond to the stress of vaccination over time. For that purpose, fish were firstly screened for coping styles and posteriorly subjected to the stress of a bath vaccination against vibriosis (*Vibrio anguillarum*). Sampling was divided in four time-related experimental groups: control (T0), 1 day post-vaccine (T1), 3 days post-vaccines (T3) and 7 days post-vaccine (T7). Molecular, endocrine and biochemical endpoints were evaluated in plasma, gills and liver of the tested animals. Cortisol and glucose were measured in plasma, while gene expression of immune, oxidative stress and lipid metabolism related genes was assessed in liver and gills. Finally, biochemical parameters related to oxidative stress (TOS, TAC, EA) were also measured in liver and gills.

Results show that one day after bath vaccination (T1), significantly higher plasma cortisol and glucose levels were observed in the reactive group of fish, when compared with its respective control group (T0) (Fig. 1). A significant difference in glucose levels was observed between proactive and reactive groups at day 1 post-vaccination. Differences in gene expression were found between groups in immune, oxidative stress and metabolism related genes.

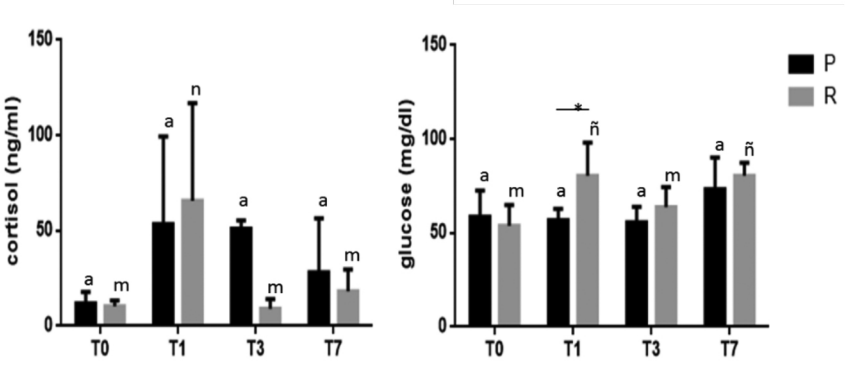

Figure 1. Cortisol and glucose levels in plasma of juvenile gilthead sea bream. Levels were measured in Control (T0) and at three different times post-vaccine (T1, T3, T7). Significant differences from control are indicated by different letters. Significant differences between copying styles are marked with an asterisk (*). P-Proactive; R-Reactive.

TUNA OCEAN RESTOCKING (TOR) PILOT STUDY - SEA-BASED HATCHING AND RELEASE OF ATLANTIC BLUEFIN TUNA LARVAE – THEORY AND PRACTICE

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Introduction

Since the early 1970s it has become clear that the overfishing of the Atlantic bluefin tuna in the Mediterranean has led to a large depletion of the biomass present for spawning. The International Commission for the Conservation of Atlantic Tunas ICCAT came up with a recovery plan which was based on strict yearly quotas starting with 12,750 t in 2008 and progressing with limited increases yearly until a predicted quota of 32,000 t in 2020. Whether the indicative , increasing biomass observed in the last three years will continue is unknown. The aim of the TOR project is to provide millions of DNA tagged larvae for release into the sea, thus thwarting egg predators before they hatch and assisting the spawning ABFT. The use of molecular markers thus overcoming the problems of previous mark and recapture experiments in terms of numbers and assessment of success rates.

Material and Methods

- 48 ABFT (average weight 90-150 kg) in a sea cage (Ø 30 m, depth 20m) as a broodstock
- Low stocking density to support animal welfare
- Broodstock Food will be only out of MSC-certified sustainable sources
- Broodstock management and supplementary feeding will be according to proven methods during the decade of research carried out in the EU DOTT projects.
- Egg collection after spawning (natural or induced) and incubation of eggs until hatching on a sea based platform.
- Restocking Events: Larvae release (millions of larvae each year) into natural spawning grounds (Each female can produce up to 3 million eggs per spawning event – Multiple-Spawners)
- Traceability of the offspring - using molecular biological techniques of DNA parental markers
- No antibiotics used for the captive broodstock and no chemical treatments of the larvae
- Use of both inshore and offshore aquaculture facilities together with newly developed hatchery techniques
- Pilot program is planned over a 3 year trial period which could be extended and broodstock can be released for scientific purposes at the end of the project.

Results and Discussion

The fertilized eggs that will be collected daily in large quantities from the offshore broodstock cage will be incubated in special floating incubators so that they can hatch on site in a contained environment. Upon hatching, they will be released at a site a few kilometers further out in the open sea, away from the cages so as to improve their survival opportunities. This method will thus improve larval survival as it will remove the added threat of depletion by early predators and will most probably lead to higher survival rates than those that occur in the natural situation if the spawned eggs are not collected and released away from the cages site. Since all broodstock animals will be DNA "fin-printed" individual parents can be identified and spawning success estimated for each broodstock whether under natural spawning, or induced spawning. These DNA "fin-prints" are passed on to the millions of offspring which are produced during each spawning event and modern Molecular Techniques allow us to tag millions of fish for release and later re-identification of their DNA in adult tuna at the market in 3- 5 years' time. The pilot project can be used over the next 3 years to provide evidence that Stock Assessment and Recovery can be supported by Aquaculture based technologies within the Mediterranean.

MEDITERRANEAN MUSSEL GROWTH UNDER CLIMATE CHANGE: A MODELLING STUDY CARRIED OUT IN THE NORTHERN ADRIATIC SEA

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Introduction

Forecasted long-term trends in environmental parameters, induced by climate changes, need to be included in the ongoing and future management of shellfish farming, in order to attempt a sound adaptation to changes that are likely to affect these activities (Cochrane et al., 2009; FAO, 2015). The present work, carried out within the H2020 project Climefish, focuses on the assessment of Mediterranean mussel *Mytilus galloprovincialis* growth potential along the Western Northern Adriatic coast, Italy, an area in which mussel farming has traditionally been practiced on longline farms. The work was carried out by means of individual based mathematical models of the mussel.

Materials and methods

Different individual models, based on Scope for Growth and Dynamic Energy Budget formulations, available for the Mediterranean mussel *M. galloprovincialis* were used in this work. Core model forcings are water temperature and chlorophyll-a concentration; additional forcings include Particulate Organic Carbon and Total Suspended Matter concentrations. The growth-potential was mapped by performing model simulations under current conditions and under future scenarios of climate change. This was carried out by considering 2 Representative Concentration Pathways (RCP) scenarios, RCP 4.5 and 8.5. Downscaling for forcing variables were taken both from the EURO-CORDEX, and biogeochemical model outputs (ERSEM) recently produced in the framework of the CERES H2020 project. Uncertainty was assessed by means of a Monte-Carlo approach, which considered farming practices and environmental conditions as input factors of the analysis. Uncertainty in mussel growth trajectories with respect to different factors was evaluated by means of a Monte-Carlo approach. Explored factors included: 1) mussel adsorption efficiency, which changes linked to changes in the average composition of particulate organic matter, in this coastal area highly influenced by river discharges; 2) mussel seedling and harvesting time schedule, presenting variability within the late spring-summer time window; 3) water temperatures in the investigated scenarios RCP4.5 and RCP8.5: for each scenario 14 different downscalings were made available within the EURO-CORDEX experiment; 4) different mussel model formulations and parameterization.

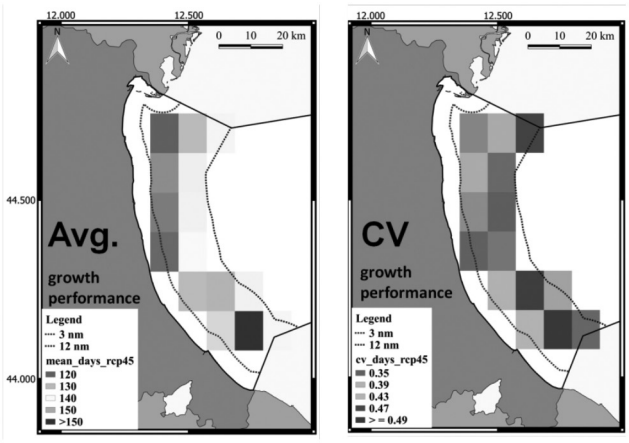

Figure 1. Model estimations, growth performance was assessed as days required for reaching the market size of 50 mm from seeding. Avg=average; CV= coefficient of variation.

(Continued on next page)

Results and discussion

The analysis performed allowed to map the expected long term changes in growth performance in the area, to estimate their uncertainty, and to identify further research needs. An indicator of mussel growth performance, the "Time required to Reach the Commercial Size of 5 cm" (TRCS), expressed in days, was taken into account. Statistics were estimated for each cluster of simulation and in each decade (from 2006-2015 until 2045-2055). The average TRCS is expected to increase of 10 days under RCP4.5, while maximum TRCS is predicted to increase of almost 4 weeks (25 days) under the same scenario. The minimum TRCS does not present any change under RCP 4.5. Overall, increase/decrease of TRCS present differences within average and maximum values predicted for RCP4.5, and between RCP4.5 and RCP8.5. Simulation results present high variability, due to uncertainty in input data. Sources of this uncertainty must be further investigated. With respect to our simulations, very relevant were: a) variability in downscaling, for water temperatures, Particulate Organic Carbon, and Chlorophyll-a; b) future changes in farming practices.

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THE INTERREG ITALY-SLOVENIA PROJECT BLUEGRASS: SUSTAINING AQUAPONICS DEVELOPMENT IN THE AREA

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Introduction

Aquaponics is an integrated technique that supports aquaculture and vegetable production by combining recirculating aquaculture systems (RAS) and hydroponics. Many fish and vegetable species are potentially suitable to be farmed in aquaponics and in these last decades many different combinations have been proposed (Goddek et al., 2015; Forchino et al., 2017). This contribution is aimed at presenting the key objectives and first results of BLUEGRASS, a project founded in the framework of Interreg V-A Italia-Slovenija Programme with a duration of 30 months, which started in October 2017. The project overall goal is to present the aquaponics technique, and sustain its development in the region. In particular, we will focus here on the results of a market analysis and their utilization to support the designing of two pilot aquaponic systems.

Materials and methods

Based on the experience gathered in previous analyses (Milicic, 2017). An online dynamic questionnaire was published in the Internet from December 2017 until March 2018. The questionnaire included numerical answers, closed questions, semi-closed and open questions and Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). Printed versions of the questionnaires were also distributed during local events in Italy and Slovenia (e.g. traditional fests, farmers markets, Aquafarm 2018 aquaculture fair). Consumers were invited to compile the questionnaire through different social media, healthy food blogs, and by networking activities (i.e. involving university students, local buyer groups, etc.). Based on the results of the market analysis, preferences of consumers interested in aquaponics products freshwater fish and vegetables were assessed, and different combination of fish and vegetables species to be grown in aquaponics were identified and ranked.

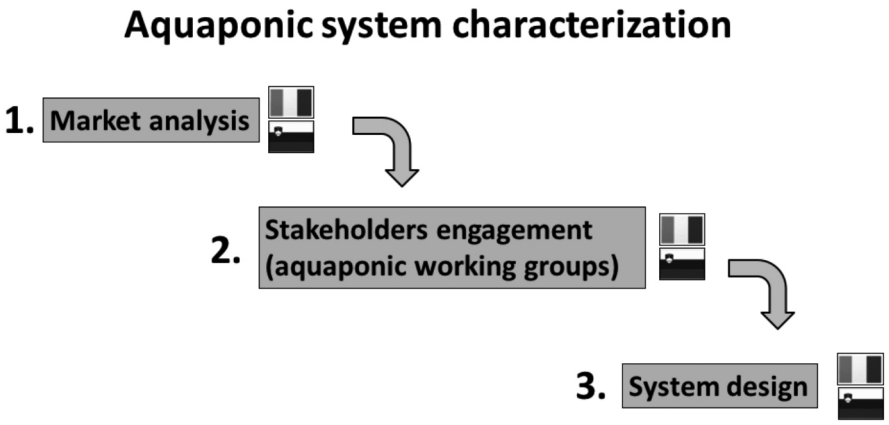

Figure 1. Layout of the methodology used for characterizing aquaponic productions in the Bluegrass project.

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Results and discussion

Almost 300 questionnaires were collected among consumers in Italy and Slovenia. Questionnaires allowed to assess consumers awareness about aquaponics, and their acceptance of aquaponics products. In general, products with local origin resulted to be appealing for consumers. Results of the market analysis were used to identify priorities in terms of species production to be tested in the two aquaponics pilot systems, which are going to be deployed in Italy (Porcia) and in Slovenija (Koper). Trout and perch were identified as the most interesting fish species in both areas. In terms of vegetables, lettuce was the most interesting crop for both Italian and Slovenian consumers. Italian consumers reported high interest also on strawberries and tomatoes, while Slovenian reported remarkable interest in rampion.

Systems are going to be used in the remaining 18 months of duration of the project as operational tools for the involvement of local stakeholders, such as potential farmers, product customers, perspective workers. During this time frame, different combinations of the proposed crops will be tested, in order to assess technological challenges and market opportunities in the area.

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ANTIOXIDANT POTENTIAL OF *Gracilaria* sp AGAR EXTRACTION WASTE

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Gracilaria sp. (GRA) is used for agar extraction, generating solid residues (extraction waste). The use extraction wastes to be reintroduced into the production cycle has generated great interest as a mean to improve the sustainability of industries. Solid residues from agar industries may contain several antioxidants compounds. To confirm this assumption, we analysed indicators of antioxidant capacity on the waste generated from GRA extraction and compared with GRA ethanolic extracts. Synthetic antioxidants [butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), vitamin C (Vit C) and vitamin E (Vit E)] were tested in extracts and wastes. Agar extraction was carried at 95 ºC (EW) or 25 ºC (AEW) for 1 hour. The extraction wastes were reextracted with ethanol (purity: 96 %) at 60 ºC for 3 h. For control group, GRA were directly extracted with ethanol at 25 ºC (SW/25) or 60 ºC (SW/60), for 3 or 6 hours.

Total phenolic content (TPC) of both SW/25/3 (33.07 ± 4.04) and EW (31.76 ± 5.16) were significantly higher (4 fold-change) than those in the SW/60/3 (7.52 ± 3.32; P=0.00) and SW/60/6 (7.08 ± 3.20; P=0.00) and significantly higher (1.5 fold-change) than those in the SW/25/6 (25.02 ± 7.21; P=0.00 and P=0.028, respectively) and AEW (20.53 ± 1.47; P=0.00). TPC expressed as mg phloroglucinol (PGE) equivalent g^{-1} dry extract. The α, α-diphenyl-β-picrylhydrazyl (DPPH) radical assay was performed as indicator of free radical scavenging. Results were expressed as efficient concentration (EC_{50}), which represents the concentration required to reduce 50% of free radicals. Lower EC_{50} represents higher free radical scavenging ability. Lower EC_{50} was detected on AEW (838. 49 ± 95.57) and SW/25/3 (1212. 52 ± 114.42). Instead, SW/60/6 (2497.22 ± 486. 57) and SW/60/3 (2920.66 ± 837) had the higher EC_{50} for the all samples tested. In addition, experimental extracts presented higher EC_{50} than those in the Vit C (5.08 ± 0.34), Vit E (10.11 ± 0.92) and BHA (13.10 ± 5.73). Interestingly, EC_{50} of AEW and SW/25/3 was similar with that in the BHT (822. 51 ± 104.49). EC_{50} expressed as μg ml^{-1}. Thiobarbituric Acid Reactive Substances (TBARS) is an indicator of lipid peroxidation. The TBARS content in homogenate of fish liver was quantified to analyse the lipid peroxidation inhibitory potential of experimental extracts. Units were expressed as mM TBARS g^{-1} tissue. The highest inhibition of TBARS was observed on the EW (6.98 ± 0.22) and AEW (7,01 ± 0.18) at 50 mg ml^{-1} (P<0.000). Similar results were observed for Vit E (6.99 ± 0.22) at 0.75 mg ml^{-1}. On the other hand SW/60/6 (8.03 ± 0.27), SW/60/3 (8. 06 ± 0.17), SW/25/6 (8. 53 ± 0.11) and, SW/25/3 (8.10 ± 0.21) showed the lowest inhibition of TBARS at 50 mg ml^{-1} which were not significantly different of that in the Vit C (8.46 ± 0.22) at 0.75 mg ml^{-1} (P>0.05). Instead, BHA (1.84 ± 0.22) and BHT (2.29 ± 0.41) exhibited inhibition of TBARS in all experimental extracts tested.

In conclusion, at high concentrations, agar extraction waste of GRA presented similar antioxidant capacities with those showed by synthetic antioxidants. Considering extraction conditions of GRA, our work indicated that high extraction temperature (60 ºC) and high extraction time (6h) can affect negatively the TPC as well as the free radical scavenging ability of SW extracts. This study suggests that agar extraction waste of GRA represents a natural and sustainable source of compounds with antioxidant capacity with potential application to supplement animal feeds.

THE POTENTIAL FOR KELP (*Saccharina latissima*) CULTIVATION IN NORWAY

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In order to meet the increasing global demand for food, a greater part of the human food consumption will need to be based on mariculture at lower trophic levels, e.g. cultivation of macroalgae. Macroalgae are, by volume, the largest group of species in aquaculture, with a global production of 2.7×10^7 t yr^{-1}, of which 88.3% is produced in China, 6.6% in South Korea and 4.4% in North Korea (FAO 2016). Interest in macroalgal aquaculture in the Western hemisphere has soared lately, and it has been suggested that in Norway alone the value of the industry based on cultivated macroalgae may have a turnover of 4×10^9 euro yr^{-1} by 2050.

Today, macroalgae cultivation is limited to inshore waters, in competition with natural kelp forest areas. However, space is one of the factors limiting the expansion of the growing aquaculture industry, which is why areas outside the coastal region is evaluated for future kelp cultivation. For spatial planning and resource management of both inshore and offshore macroalgal cultivation, it is important to consider the temporal and spatial variability in the key environmental variables important for kelp growth, such as light and nutrients.

In the present talk, the 3D hydrodynamic-biogeochemical model system SINMOD coupled with a kelp growth model, is used to estimate the cultivation potential for sugar kelp (*Saccharina latissima*) in Norway. *S. latissima* is the most important kelp species currently being cultivated in Europe. The results are spatially explicit, and allows for comparing the suitability of different locations for kelp cultivation, both inshore and offshore (Fig. 1). Simulation results are compared with growth data from cultivation facilities. Furthermore, relationship between the dynamic simulation model and in situ observations on density of natural kelp communities in Southern Norway are explored. The simulation results are finally incorporated with management-relevant GIS information (existing aquaculture, fairways, protected areas, fisheries zones etc) in order to illustrate how dynamic kelp modelling can be applied in spatial planning.

This research was funded by the Research Council of Norway (grants no. 267536 "KELPPRO" and 267536 "MACROSEA") and the counties of Møre og Romsdal and Trøndelag. HPC resources were made available through NOTUR grant no. NN2967k.

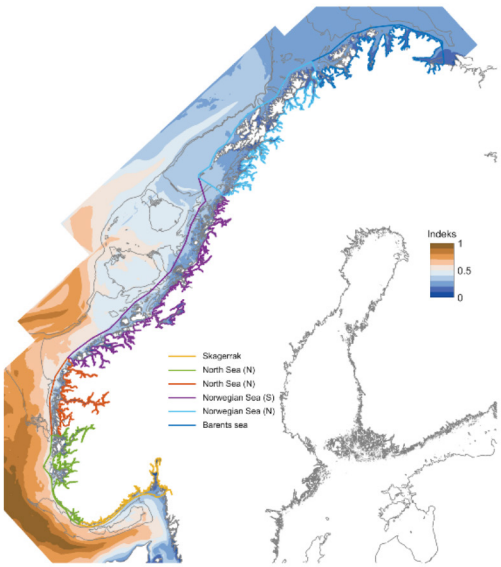

Figure 1. Model-based spatial index of the cultivation potential for *Saccharina latissima* in Norway.

RESTORATION OF THE ADRIATIC STURGEON (*Acipenser naccarii*): 30 YEARS OF ACTION

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The Adriatic sturgeon, endemic of Po River basin and Northern Adriatic Sea area, is the only one still surviving of the three species once present in Italy, thanks to a variety of conservation actions conducted in the last almost 30 years by different public and private entities. The report traces the whole history of the conservation of the Adriatic sturgeon, trying to combine the main actions carried out into a single collage, pointing out their successes, but also emphasizing the need for a national coordinated activity, in which different high-level skills can be combined in a synergistic and more effective conservation effort. The different activities carried out, in fact, have been crucial for the preservation of the species, but managed without any global coordination led to a suboptimal management of the residual population at different levels: i) choice of individuals to reproduce to maximize genetic diversity, ii) establishment of training and releasing protocols to maximize the fitness for survival of the reintroduced animals, iii) organization of an effective and coordinated monitoring activity.

STATUS UPDATE ON GLOBAL STURGEON AND CAVIAR PRODUCTIONS TO THE END OF 2016

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The data presented are based on official data as well as on information obtained from other scientists, farmers and traders. Even though not complete and sometimes integrated with indirect information, the scenario shown seems to be quite reliable. During the last 35 years, high demand and good perspective for caviar markets together with the drastic decline of the natural populations and the consequent protection measures, made the production of farmed caviar very profitable and has led most of the farmers to rear sturgeons mainly for caviar production. Looking at this profitability, several new entities entered in this business, both in the natural range of sturgeons and also in countries out of the natural range. At present about 56 countries with more than 2150 plants are involved in sturgeon farming, both for meat production and for caviar, with estimated global productions respectively of more than 130,000 t and 330 t in 2016. This development met almost unprepared markets. Due to the increasing of availability of the product and the not yet analogous increasing of the market, the prices have decreased and the profitability of this activity has becoming less interesting and the first signs of trouble have begun to appear, even if some signs of a new market segment, alongside with the traditional rich one, seem to appear.

USING MOLECULAR TOOLS TO DIFFERENTIATE BETWEEN LIVE AND DEAD AQUATIC PATHOGENS

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PCR is one of the most commonly used diagnostic tests for pathogens of importance to the aquaculture industry. While PCR has many benefits including sensitivity, specificity and efficiency, it provides no information regarding the viability of the pathogen detected.

Culture is the gold standard to establish viability of any pathogens detected. However, culture can however yield false negative results due to poor sample quality, the target pathogen being outcompeted, inappropriate use of nutrient media or cell line or the pathogen being in a viable but non-culturable state. In addition, culture can be time consuming with delays affecting management decisions for farming activities, responses or export consignments.

As RNA degrades more rapidly than DNA, RNA may be used as a marker for cell viability. However associating a positive RNA signal with viability is problematic. For example, due to its instability RNA is difficult to extract in high quality and is prone to contamination with RNA-degrading enzymes.

The use of nucleic acid intercalating dyes coupled with PCR, termed "viability PCR" (vPCR) has been proposed as a reliable method to assess viability. These dyes work by passing through cell walls of pathogens with compromised membranes (i.e. dead cells) and binding with the nucleic acid. This nucleic acid/dye molecule is then exposed to light of a certain wavelength which crosslinks the nucleic acid thereby inhibiting or significantly suppressing its amplification by PCR (Figure 1).

This technology is highly applicable in the aquaculture industry and has the potential to be used to detect live pathogens in fallowed sites, to limit or avoid cross reactivity of PCR tested autogenously vaccinated fish, or to be used as a pre-step to next generation sequencing, hence limiting background reads from contaminants or dead pathogens.

New Zealand's Animal Health Laboratory is undertaking a project to apply vPCR for testing for aquatic animal pathogens. The aim of this project is to optimise and validate vPCR for aquatic animal pathogens and to transfer this method to a high-throughput platform. This project uses model bacterial and viral pathogens for experimental trials. To date, vPCR is showing promise as a tool to be incorporated into the diagnostic laboratory toolbox.

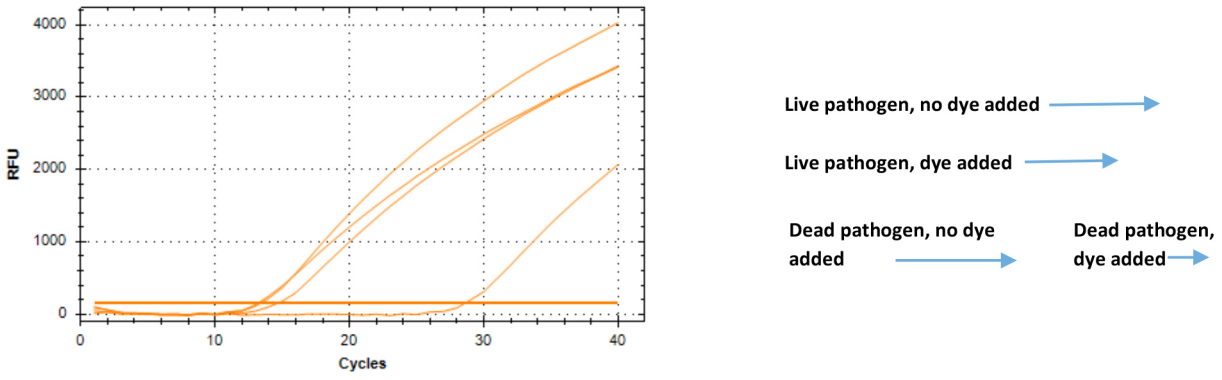

Figure 1. Real-time PCR plot showing suppression of amplification in sample treated with dye.

WHAT MOTIVATES FISH FARMERS' CHOICE FOR FISH FEED IMPROVED WITH NON-CONVENTIONAL INGREDIENTS? INSIGHTS FROM THE THEORY OF PLANNED BEHAVIOUR, WITH AN APPLICATION TO POND FARMERS IN INDIA

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The Omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential dietary nutrients for human health. With finite supplies and a growing global population, availability is well below the minimum recommended intake, particularly in low income countries. One option to improve access is based on the fact, while some seaweeds can contain small amounts of EPA and occasionally DHA, the metabolic precursor alpha-linolenic acid (ALA) can be abundant in many terrestrial plants and seaweeds. As some fish including carp and tilapia can convert dietary ALA to EPA and DHA, and these are two of the main cultured species in India and sub-Saharan Africa respectively, there is potential to exploit this endogenous pathway by supplementing ALA-rich non-conventional ingredients (e.g. freshwater plants, seaweeds, some microalgae, and microbes) to enhance the EPA and DHA levels in consumed fish. However, in addition to the nutritional properties of these ingredients, their potential acceptability by fish farmers must be elicited to ensure utilisation and long-term adoption as part of improved feeding practices.

The Theory of Planned Behaviour (TPB) is a well-used theory in agricultural studies to establish the motives behind farmers' decisions about technology adoption but it has so far found few applications in aquaculture. Its relevance to aquaculture development and the adoption of improved fish feed is examined here through a case study of Indian carp farmers' perceptions of the attributes of fish feed containing non-conventional ingredients, and factors influencing their intention to use these feeds.

Results show that farmers familiar with the use of manufactured feed display a more positive attitude to the inclusion of non-conventional ingredients in fish feed than those who aren't. Compatibility with current farming practices, incentives, peer pressure, perceived relative advantage of the innovative feed compared to current feed, risk minimisation and convenience are key factors behind farmers' intention to use fish feed containing non-conventional ingredients. By eliciting ex-ante fish farmers' intrinsic motivations for changing their feeding practices, we identify the behavioural factors that condition the long-term adoption of technological innovations enhancing both animal and human welfare. This study underscores the valuable insights that can be gained from the application of the TPB to aquaculture development, and its relevance in designing the interventions promoting technological innovations among fish farmers.

This research was undertaken as part the SNIPH (Sustainable New Ingredients to Promote Health) project led by the University of Stirling, UK and funded by the Newton Fund Global Research Partnership in Aquaculture of the UK's Biotechnology and Biological Sciences Research Council (BBSRC), the UK Department for International Development (DFID) and the Indian Department of Biotechnology (DBT).

WE R… WOMEN IN AQUACULTURE: OVERCOMING WOMEN'S INVISIBILITY AND GENDER INEQUALITIES IN AQUACULTURE

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Women's participation in all sections of the aquaculture value chain is huge but their opportunities for further development have not kept pace with the rapid growth of the sector. Good sex-disaggregated statistics are missing for aquaculture, in all spheres – from industry, academia, producers in all corners of the world, and most aquaculture policies are gender-blind. Women are the bulk of workers in post-harvest and product transformation activities. On small-scale farms, women and men frequently work together, carrying out different but complementary activities. In medium and industrial scale aquaculture enterprises, women are at the lower end of responsibilities and pay scales. As production intensifies, women's engagement drops. Few are found in managerial positions. This situation is mirrored in aquaculture research. Despite a growing number of female students undertaking aquaculture courses and high-level degrees, and more women entering highly skilled employment, the contribution of this growing pool of talents remains in the shadow.

There are signs that momentum is growing to redress this situation. Aquaculture can empower women, increase their economic freedom and lead to better household food security and nutrition, but these benefits are not automatic. We stress that in aquaculture – as in many other economic sectors - gender equality is in the detail and must be advocated for rather than expected to arrive from some sense of natural justice. Examining gender issues in aquaculture requires delving into the specificities of both the sector and women's involvement in it. Attention needs to be paid to the species grown, the type of equipment used, the organization of work, the predominant socio-economic and cultural contexts and their influence on men and women's attitudes, access to knowledge and assets, empowerment and control over production. This needs to happen at all stages of the development cycle of aquaculture projects and enterprises.

In support of this, collection and dissemination of sex-disaggregated and other data relevant to gender equality should be mandated in every jurisdiction and systematically used in aquaculture planning and development. Gender should be mainstreamed in policies, toolkits and guidance on aquaculture development. More research is needed on what makes women lose (or retain) control over their activities as the scale, intensity and profitability of aquaculture production grows. Gender equality targets should become commonly integrated in all aquaculture certification, accreditation, labour policies and practices. Aquaculture practitioners need good technical instruments for gender-responsive planning, indicators of progress, training and communications.

Promoting gender equality in aquaculture requires challenging both conscious and unconscious bias against women's participation and progress, and this should be everyone's responsibility in the sector.

THE ECOSYSTEM APPROACH TO AQUACULTURE TEN YEARS ON: STOCKTAKING AND WAY FORWARD

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In 2007, the ecosystem approach to aquaculture (EAA) emerged from discussions between the Food and Agriculture Organization of the United Nations (FAO) and international aquaculture experts on how to move aquaculture development towards greater sustainability. Ten years since its inception, it is timely to critically examine the use and mainstreaming of the EAA in aquaculture development to date, and consider its possible evolution in the next decade.

We systematically searched citations of key EAA publications in the academic and related literature for the period 2007 to 2016 and analysed all relevant FAO publications and project documents. We assessed the lessons learnt from the past decade of EAA experiences, the opportunities the EAA has created, and the links between the EAA and the new development agenda.

Our findings suggest that mainstreaming the EAA in planning processes has raised awareness of the usefulness of holistic and participatory approaches in aquaculture and helped to steer the sector towards greater sustainability. However, the approach has had varying degrees of resonance and uptake with different user groups. The emphasis on spatial planning that has developed as part of the EAA implementation efforts, and close links between the EAA and initiatives such as "Blue growth", constitute significant opportunities for the future of the approach, although its ability to tackle increasingly complex governance issues may be limited.

As part of the updating and reconsideration of the EAA's *raison d'être*, we recommend that climate change adaptation and visibility of small-scale producers gain more prominence, and that ongoing developments within and outside the aquaculture sector (e.g. SDGs, value chain developments, other planning approaches) be closely taken into account. Reinvigorating the EAA through wide stakeholder consultations and discussions is now needed to ensure its continued relevance in the next decade, and we call upon the FAO to lead this process.

SEA POWER: IMPROVED SEAWEED FARMING TECHNOLOGY FOR WOMEN'S EMPOWERMENT, LIVELIHOODS AND ENVIRONMENTAL PROTECTION

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In the Western Indian Ocean, seaweed farming is a small-scale activity carried out mainly by women. Introduced 30 years ago in Zanzibar, seaweed farming is very important for their livelihoods. Women producers are, however, confronted with many challenges: inadequate technology, climatic variations, low yields, economic inefficiencies and social and cultural constraints. Tubular nets have recently been piloted and are showing promise over the traditional 'off-bottom' peg and rope technology to improve seaweed productivity and local ecosystem conditions. However, tubular nets are more suited to deeper water, and thus, require either swimming or boat handling skills that most women do not have. Moreover, little is known about the economic profitability of the technology, its potential cultural acceptance, as well as its impact on livelihoods and women's empowerment. These economic and cultural factors are pivotal in the sustained adoption of the technology. Whilst the trials of *Kappaphycus* (cottonii), a higher-value seaweed, grown in tubular nets are showing encouraging results that must be capitalised upon, it must also be shown that this innovation benefits women by acting as a vector for their emancipation before it is scaled out to the wider region. The promotion of the use of tubular nets therefore requires a gender-sensitive approach that gives women producers full control over all farming tasks and contributes to their empowerment and better work conditions.

The goal of Sea PoWer, a winner of the Blue Economy Challenge award (http://theblueeconomychallenge.com), is to achieve an adapted seaweed technology that is ready for scaling out and wide adoption among women producers in the Western Indian Ocean. We will show how the results of the growth trials in Zanzibar are validating the effectiveness of tubular nets for enhanced seaweed productivity, seaweed quality and coastal ecosystem services. Using insights from the producers themselves, elicited through an enquiry embedded in the Theory of Planned Behaviour and gender analysis, we will share how our gender-sensitive approach to the introduction of seaweed farming innovation is contributing to both the adoption of tubular nets and women's empowerment. We will also highlight the remaining challenges that need to be lifted to ensure the sustained use of tubular nets and their transformative impact on the lives of women producers.

Sea PoWer and its team are grateful for the funding received from the Government of Australia's Department of Foreign Affairs and Trade (DFAT) and from SwedBio, a programme of the Stockholm Resilience Centre, Sweden.

Seaweed seedlings in the tubular nets.
Photo credit: C. Brugère.

RESTOCKING OF SEA URCHIN: LABORATORY-RAISED INDIVIDUALS OF *Paracentrotus lividus* KNOW THE WAY

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The effectiveness of sea urchin restocking activities is dependent on the locomotor behaviour of released individuals, and it is important given that animals must quickly find food and shelter to avoid predation. In this study, we compared locomotor performance of laboratory-raised (Reared) juvenile *Paracentrotus lividus* (1.2-1.8 cm) with wild-harvest conspecifics (Wild) of similar size.

Locomotor performance was evaluated in absence (Indoor), partial (Closed) and total (Open) exposure to external cues. Indoor was conducted under laboratory conditions using a cylindrical tank coated on the inner side with a white plastic cloth. Closed was set up in the sea by using a white circular plastic cloth to cover the bottom and enclosing it with a white plastic ring. Open was set up in the sea without cloth or enclosure-ring. For each exposure, sea urchins were individually placed in the centre of a rough cylindrical stone and movement was recorded with a time-lapse camera (5 sec intervals). Video images were analyzed to calculate latency of locomotion, average speed, straightness-of-path and locomotor direction.

In Indoor, Reared resulted in a 87% lower latency and a 45% higher speed than Wild, while similar locomotion was obtained in Closed and Open. Closed and Open showed different results in comparison with Indoor for the latency of locomotion (35% and 92% lower in Closed and Open, respectively), the average speed (46% and 38% higher in Closed and Open, respectively) and the trajectory (26% and 29% more linear in Closed and Open, respectively) (Two-way ANOVA). Locomotion was oriented to north-western direction only for Reared-Open and Wild-Open (Rayleigh's test).

Locomotor behavior of *P. lividus* is influenced by the availability of external cues. The absence of cues (Indoor) resulted in a higher latency time, lower speed, tortuous trajectory and random directionality compared with exposure to external cues (Closed and Open). Even though Reared had never been exposed to external stimuli or wild conditions, they displayed the same behaviour as Wild, suggesting the locomotion in sea urchins is an innate behavioral adaptation. Similar behavior displayed by Reared and Wild gives support to the potential feasibility of sea urchin restocking, specifically in terms of motion pattern in wild growing conditions.

COMPARISON OF NUTRIENT UPTAKE AND GROWTH IN *Ulva lactuca* CULTIVATED IN GREY MULLET *Mugil cephalus* WASTEWATER AND NATURAL ESTUARINE WATER

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This study investigated the potential cultivation of *Ulva lactuca* in aquaculture wastewater systems with the aim of decreasing ecological and environmental impacts of wastewater by decreasing dissolved inorganic nutrients.

Nutrient uptake and growth of *U. lactuca* were evaluated in *Mugil cephalus* wastewater and compared with *U. lactuca* cultivated in estuarine water. Fresh thalli were cultivated in outdoor aquaria for 10 days in 5 L transparent cylindrical tanks, three replicates. Gentle aeration was provided to the tanks. The uptake of dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorous (DIP), biomass yield (dry weight) and specific growth rate were assessed every two days.

On completion of the experiment, *U. lactuca* showed higher assimilation of DIN in estuarine water (95.7 ± 0.3%, mean ± SE) than in wastewater (68.7 ± 1.0%), while no significant difference was observed in DIP assimilation (> 80% at day 10 for both culture media) (two-way ANOVA). Regardless of the culture medium, biomass yield was higher on days 2, 4, 6 and 8 than on day 10. No differences between culture media were found in biomass yield or specific growth rate (repeated-measures ANOVA).

Due to the high biofiltering efficiency for DIN and DIP removal, our results show *U. lactuca* is a promising macroalga species for water treatment purposes in integrated aquaculture systems. Our findings have important environmental implications for the potential use of *U. lactuca* to reduce the amount of dissolved inorganic nutrients released from aquaculture facilities into the natural environment.

ENABLING OFFSHORE AQUACULTURE – TRANSLATING POTENTIAL TO PRODUCTION

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With the convergence of ecological, social licence, and aesthetic concerns, aquaculture, which has already competed with other established and accepted uses for space, has increasing difficulty expanding into coastal waters. Given the constraints on the expansion of current production methods, it is clear that alternative approaches are needed to enable the marine aquaculture sector to make a meaningful contribution to global seafood supply.

Farming of aquatic species in offshore marine waters has been identified as one potential option for increasing seafood production and has been a focus of international attention for more than a decade. Although there are technical challenges for farming in the often hostile environment of the open ocean, there are sufficient reasons to promote the development of offshore farming. Benefits of open ocean waters include; large space for expansion, enormous carrying and assimilative capacity, fewer conflicts with many stakeholders, lower exposure to human sources of pollution, the potential to reduce some of the negative environmental impacts of coastal fish farming, and mostly optimal environmental conditions for a wide range of marine species.

The drivers for open ocean aquaculture (OOA) are not only linked to food, trade, and the technology industry. Additionally, there are powerful social and ethical concerns afloat as many Western nations import most of the seafood they eat, and export most of what they catch or produce. This “food insanity” ensures that these nations are to date far too dependent on imports from aquaculture from other nations. Unfortunately, in many production countries aquaculture is threatened by coastal urbanization, industrialization, water pollution, and overall environmental degradation. Hence, aquaculture dependent countries need to endorse, to a greater extent moral and ethical responsibility to develop large scale OOA to feed their own people and not take these valuable foods from undernourished, food scarce nations.

While there is a great deal of global interest in the development of offshore aquaculture, for commercial scale food production, the transition from concept to reality has yet to come to fruition. Much is known about the economics, environmental, political and societal effects of individual production sectors. However, what still needs to be learned is that there are still many unknowns and challenges with regard to OOA economics, engineering of adequate system design, O&M as well as liability aspects including insurance and ownership (IMTA) and finally social views and stakeholder acceptance. Mutually agreed upon principles, such as those articulated in the Bremerhaven Declaration on OOA, directives of the EU and others, as well as grant funding opportunities to advance research and development indicate that progress, although measured, is being made. The development of true commercial-scale sustainable offshore aquaculture will require investment in more demonstration projects and multi-national cooperation and collaboration across public and private sectors.

FORMULATED PACIFIC BLUEFIN TUNA DIETS IMPROVE FLESH QUALITY AND INCREASE SHELFLIFE OF SASHIMI-GRADE PRODUCT

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The tuna ranching industry is a global, high-value aquaculture activity. Current feeding practices are impractical, unsustainable and pose serious ecological risks. The daily feeding of copious quantities of fresh/frozen fish results in extremely high feed conversion rates (~28 : 1). Seasonal availability and quality variation of locally sourced baitfish highlight the need of a balanced feed. Objectives for the present experiment were to compare performance and quality indicators of Pacific bluefin tuna (PBFT, *Thunnus orientalis*) fed sardines or a compound feed.

A seventeen-week feeding trial was conducted in two oceanic net pens moored east of Coronado Island (BC, Mexico, 32º24'48.80" N; 117º13'70.10' W). Each cage held ~440 fish, weighing approximately 50 kg (average initial individual weight, assessed by AQ1's AM 100 fish sizing system). Feed consumption and condition factor were recorded. A first sampling effort was conducted at week 6. Dorsal loins were collected and color, mercury, proximate composition, scombrotoxin levels, oxidative stability index and peroxide values were evaluated in the resulting steaks. In addition, a blind sensory evaluation and a commercial taste assessment were performed on the main sashimi cuts obtained from fish fed either diet.

Steak samples from formula-fed fish had lower lipid contents but improved color, texture and had increased oxidative stability and reduced histamine. Results from sensory and organoleptic evaluations (professional sashimi chefs) indicated that sashimi slices from formula fed fish were similar in flavor but more stable on the counter and superior in color.

In addition, the formulated diet offered feed management options that were quite compatible with the present operation and equipment available at commercial tuna farms. A preliminary economic evaluation (2017 prices) indicated that tuna feed is more cost-effective than frozen baitfish but more expensive than fresh sardine. Given the current forecasts for the Pacific sardine fishery in Southern California, the formulated diet may offer a viable alternative to baitfish feeding. In conclusion, these results indicate that the balanced feed provided suitable nutrition for adult tuna, enhanced the quality and shelf life of the final product and may enable management strategies to optimize tuna performance, thus increasing farm efficiency and reducing the environmental impact of commercial tuna ranching.

DEVELOPMENT OF EUROPEAN PERCH (*Perca fluviatils* L.) BROODSTOCKS: COMPARISON OF TWO PERCH STOCKS FROM DIFFERENT GEOGRAPHIC ORIGINS

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Introduction:
The European perch (Perca fluviatilis L.) is a popular food fish in Europe. Due to a high sensitivity of larvae a high mortality rate in the first weeks of larval rearing can appear. There are different ways to improve the fitness of larvae and fingerlings. On way to boost larval fitness is to use a high quality broodstock for reproduction. In studies differences in weight and length between wild stocks of different geographic origin were found for the European perch (*Perca fluviatilis* L.) and the Yellow perch (*Perca flavescens* M.) (Mandiki *et al*., 2004; Rosauer *et. al*., 2011). The purpose of this experiment was to investigate possible differences between two regional wild stocks of European perch with the aim to possibly initiate a selective breeding program in the future.

Materials and Methods:
Two different wild perch stocks were compared with each other (Lake Hohen Sprenz (LHS) and Strelasund (S)). Mature perch were caught and brought into 3 m^3 raceways in March 2016. These fish were reproduced in April 2016. The eggs were incubated. The first larvae hatched on the 29.04.2016. Then the perch larvae were reared in six 300 l raceways. After 12 weeks they were transferred into six 3 m^3 round tanks. The fish were kept divided by their origin and were regularly graded into three size classes. Every two weeks 50 fish of every tank were weighted and measured. The trial ended when the most fish reached the marked size of 150 to 250 g (April 2017). All fish were reared in the same RAR-systems at all times. Both populations were compared in survival, weight, length and fillet yield.

Results & Discussion:
The perch from the LHS grew faster and reached a significantly higher mean body weight of 246.35 ± 66.80 g (N=100) than perch originated from the S (137.05 ± 44.6 g (N=100)). Nevertheless the fillet yield showed no significant differences. In Fish from LHS a mean fillet yield of 47.35 ± 4.03 % (N=30) was detected, while perch from S showed a mean fillet yield of 46.35 ± 3.9 % (N=30).

The results show that the compared perch stocks varied significantly in some parameters. Perch from the LHS showed higher growth than perch from the S and reached marked size earlier. Further comparison of the descendants of both stocks will give more data for breeding purposes.

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Mandiki, S.N., Blanchard, M., Melard, G., Koskela, C., Kucharczyk, J., Fontaine, D.,Kestemont, P., 2004.Effects of geographic origin on growth and food intake in Eurasian perch (Perca fluviatilis L.) juveniles under intensive culture conditions. Aquaculture 229, 117–128.

GROWTH OF LOCAL SEAWEED SPECIES OF WESTERN AUSTRALIA IN POTASSIUM-FORTIFIED AND NUTRIENT ENRICHED INLAND SALINE WATER

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Growing seaweed in inland saline water (ISW) can provide an additional product for the seaweed industry, with a lower capital investment than farming in the sea. This study is the first attempt to grow two local seaweed species, a red seaweed, *Fushitsunagia catenata* and a brown seaweed, *Sargassum podacanthum*, in ISW of Western Australia by focusing on the effects of fortifying ISW with [K^+], as past research has shown that K^+ deficiency in ISW can adversely affect the survival and growth of marine species. Three fortified levels of 33%, 66% and 100% of [K^+] equivalent to [K^+] in ocean water (OW) at a salinity similar to OW were evaluated. K^+ fortification was performed by adding KCl. The results showed that K^+ fortification of 66% equivalent to [K^+] in OW at 30ppt and 100% of the [K^+] equivalent to OW at 35ppt provided positive growth of *F. catenata* and *S. podacanthum* in ISW, respectively.

The study then attempted to improve the productivity of these two species by supplementing nutrients to K^+-fortified ISW (K^+ISW). *F. catenata* culture in 66%K^+ISW at 30ppt was supplemented weekly by three levels of ammonium:phosphate at a ratio of 75:7.5, 150:15 and 300:30 μM, whereas *S. podacanthum* growth in 100%K^+ISW at 35 ppt was investigated at five weekly supplemented levels of 80:8, 120:12, 160:16, 200:20 and 240:24 μM of ammonium:phosphate. At the end of 25 days, the *F. catenata* did not show any supplementary nutrient requirement for its normal growth in 66%K^+ISW, whereas, *S. podacanthum* could not grow without nutrient enrichment, and the enriched level of 160:16 μM ammonium:phosphate in 100%K^+ISW resulted in a highest specific growth rate of *S. podacanthum* in 84 days.

Finally, it can be concluded that K^+ fortification is necessitated for seaweed growth in ISW and the requirements for nutrient supplementations are species-dependent.

Table 1. The SGR (% d^{-1}) of seaweeds culturing in OW and K^+ ISW

Time	Species	OW	ISW0	ISW33	ISW66	ISW100
Day	*SP*	-0.26±0.41				$_1$-0.17±0.24
1–56	*FC*	0.07±0.16^{ab}	-0.23±0.24^{a}	0.23±0.12^{ab}	0.30±0.07^{b}	$_2$0.14±0.15^{ab}

SP - S. podacanthum; *FC - F. catenata* Values (mean±SE) within a row sharing a common superscript are not significantly different (LSD test; P>0.05; n=4). Values (mean±SE) within a column sharing a common subscript are not significantly different (t-test; P>0.05; n=4)

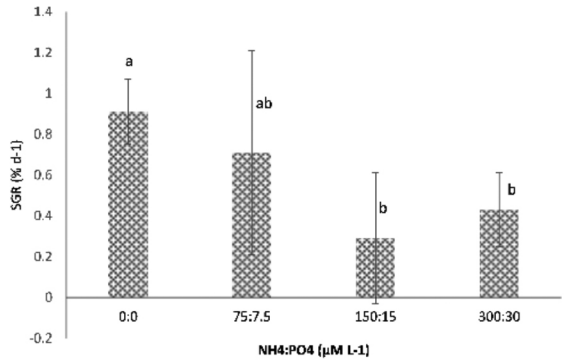

Figure 1. The SGR of *F. catenata* in nutrien enriched 66%K^+ISW at 30ppt

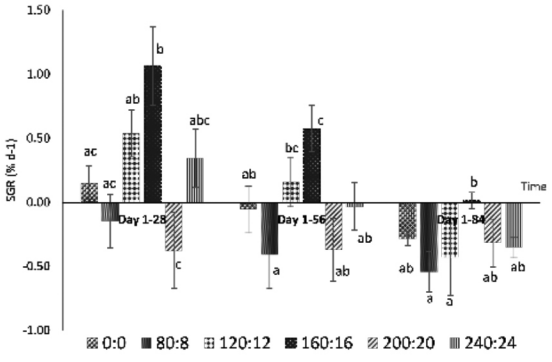

Figure 2. The SGR of *S. podacanthum* in nutrient enriched 100%K^+ISW at 35ppt

(Values (mean±SE) in bars sharing a common letter in a time are not significantly different (LSD test; P>0.05)

EMERGING TRENDS IN AQUACULTURE VALUE CHAIN RESEARCH

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This paper presents an overview of a recently published special issue in the journal *Aquaculture* that brought together the largest collection of research on aquaculture value chains compiled to date, comprising 19 individual papers. In doing so five key themes of value chain research emerge, namely: multi-polarity, diversity and scale, dynamics of transformation, performance and equity, and technical and institutional innovation. Contrary to much research to date, this state-of-the-art review shows how the expansion of aquaculture has resulted highly diverse configurations of production for consumption in the global South. Two conclusions emerge. First, there is a clear need for research on neglected value chain segments and categories of actor, modes of production, regulation, and innovation, and access to both market and non-market benefits. Second, there is a need for more rigorous and diverse research on value chains to better understand the diversity and impact of aquaculture in order to contribute to the sustainable expansion of the sector as a fundamental component of the global food system. Based on these conclusions an agenda for the future of aquaculture value chain research is set out for discussion.

SUBSTITUTION OF CORN BY STALE BREAD IN FEED FOR GREY MULLET *Liza ramada*

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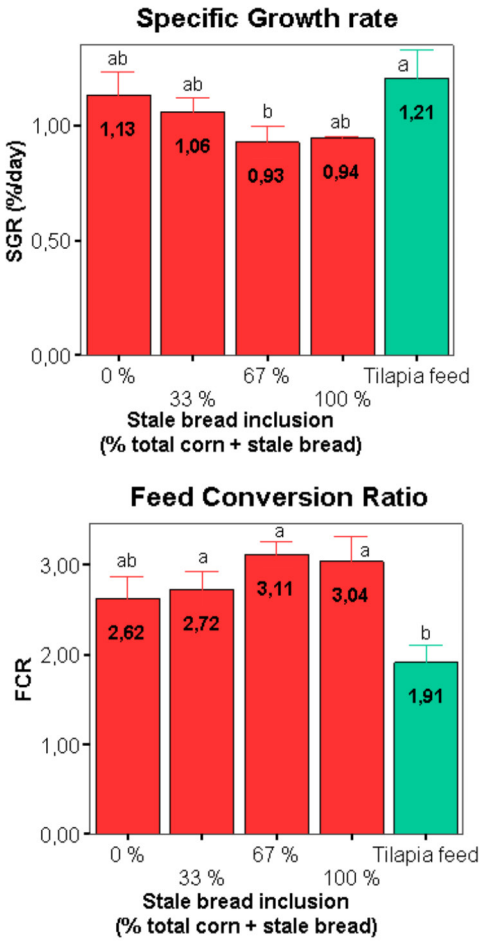
This experiment was done in the frame of a project towards the reduction of the food waste. Bakery waste is about 300,000 tons per year in France, i.e. 10% of the national consumption. Stale bread might be recycled through aquaculture feed as source of starch especially for omnivorous species. Among them, grey mullets might be relevant thanks to none bony flesh and high tolerance to water quality (salinity, temperature, oxygen and ammonia). Yet few quantitative data are available hence we performed an experiment to evaluate the interest of replacing corn by stale bread in compounded pellets. The test was done with wild juveniles of thin lip grey mullet *Liza ramada*; this species is the most abundant among the five grey mullet species in the Mediterranean lagoons in France.

Four diets have been prepared with four levels of corn flour substitution by stale bread flour (0, 33, 66 and 100%); both ingredients together made 35% of the total ingredients. The other ingredients were soybean cake and oil, sardine meal and oil, and vitamin and mineral premix. The mixtures were processed into pellets through a small extruder at about 120°C temperature. The mean proteins, lipids and energy contents were 32.9%, 7.3% and 3.8 kcal/g in dry diets. A fifth diet was tested as a kind of control, a commercial tilapia feed (Le Gouessant™) with similar proximate composition.

Each diet was tested in three tanks stocked with 35 fishes per tank (70 L per tank). Tanks were set up in a recycling aquaculture system including the sea lettuce *Ulva rigida* as biofilter. Initial mean body weight and fork length were 42.2 g and 15.5 cm. The experiment lasted for 23 days at 27.2°C.



DESIGN OF AUTONOMOUS ROBOTS FOR SEA BASED AQUACULTURE USING THE SEATONOMY METHOD

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The ARTIFEX project aims at developing robots for remote and/or autonomous inspection, maintenance and repair (IMR) operations at fish farms. An unmanned surface vehicle (USV) is used as a platform for carrying a remotely operated vehicle (ROV) for underwater operations and a remotely piloted aircraft systems (RPAS) for airborne inspections (**Error! Reference source not found.**). The robotic platform shall execute inspection operations (e.g. nets and mooring lines), and light intervention tasks (e.g. net repair, cleaning of mooring lines, dead fish removal).

The level of autonomy, i.e. the ability of a robot to make decisions and carry out tasks on its own, is a crucial design specification for a complex robotic system. Interaction and coordination between the parent and peripheral vehicles as well as operational planning in an unstructured environment and the handling of unforeseen events, like loss of communication, are also significant challenges that complex robotic systems such as ARTIFEX must overcome. The SEATONOMY methodology provides the tools required to tackle such problems. It was established by SINTEF for design and development of maritime mobile autonomous systems and operations, and it provides a set of methods, guidelines, principles and best practices for designing robotic systems. One of these is Autonomous Job Analysis (AJA) which is a structured way of breaking down an operation into sub-operations and helps the design team reveal challenges, needs and limitations regarding autonomous behaviour. The following table shows the IMR operations that have been investigated via the AJA method in ARTIFEX:

This presentation will show how the AJA method has been applied when designing the ARTIFEX robotic platform. After the main goal of each operation had been defined, the operation was broken down into sub-goals and sub-operations to reduce the complexity of the analysis, and questions related to the AJA categories were answered for each sub-operation. These categories include key aspects for autonomous systems such as human machine interaction, success criteria, safe states, perception, communication, failure modes and safety barriers. Through an iterative workflow, where complexity was added incrementally, the required tasks for each unit to achieve the overall inspection / intervention goals were identified. Solutions for coordination between the different vehicles were obtained as well, together with the technical specifications of the necessary equipment for sensing and communication.

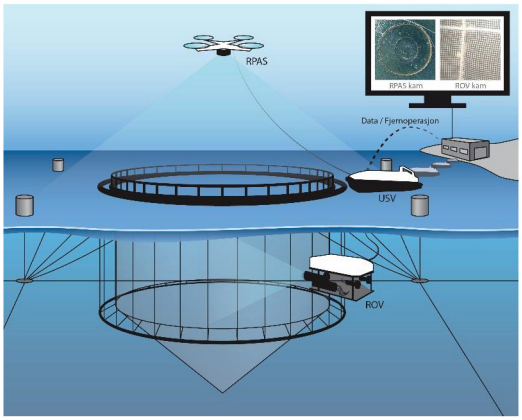

Figure 1 The ARTIFEX concept

DEVELOPMENT AND VALIDATION OF A MODULAR INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM FOR MARINE AND BRACKISH WATER SPECIES (AQUAMMIN)

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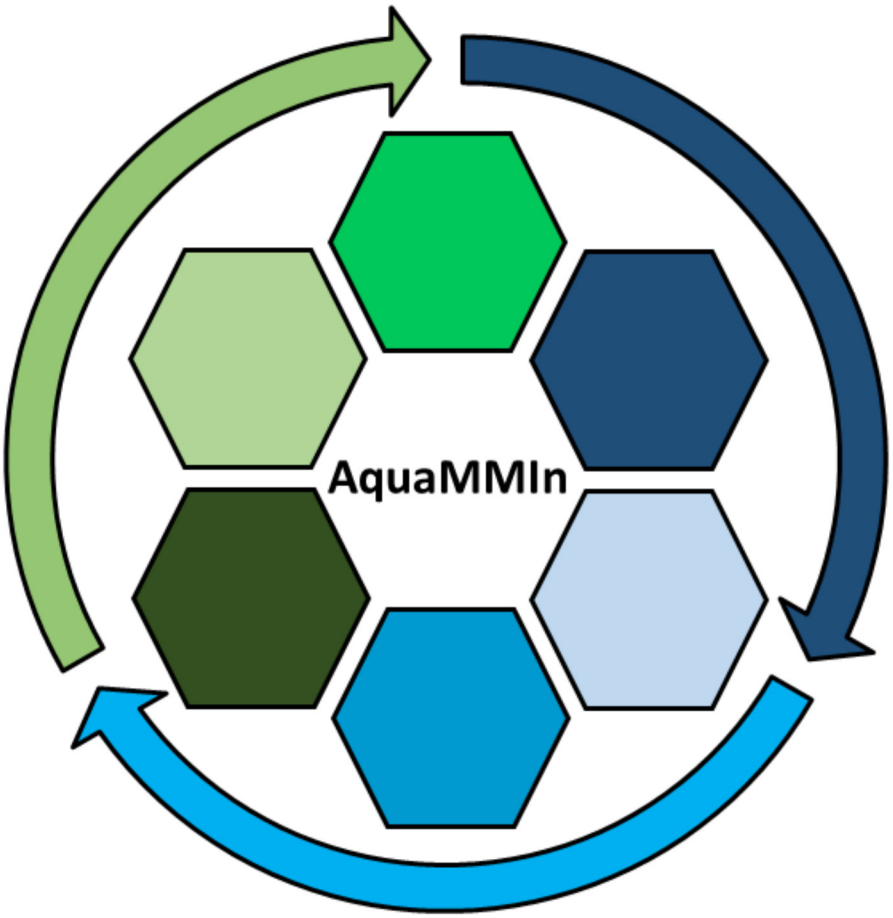
Project AquaMMIn project aims to design, implement and validate a modular integrated multitrophic aquaculture system (IMTA) for the production of marine and brackish water species, with emphasis on sea bass (*Dicentrarchus labrax*), oyster (*Crassostrea gigas*), ragworms (*Hediste diversicolor*), sea asparagus (*Salicornia ramosissima*) and sea lettuce (*Ulva* sp.). The different combinations of IMTA modular systems to be evaluated will be fully equipped with *in situ* and *ex situ* real-time monitoring systems, thus allowing their survey through the cloud, as well as the correction of physico-chemical parameters through automation solutions (which will also be implemented). The life support systems to be implemented will also test the viability to operate on a 100% reuse water regime (zero water exchange).



CAPTURE OF INFECTIOUS OSTREID HERPESVIRUS FROM *Crassostrea gigas* USING ANIONIC POLYMER-COATED MAGNETIC BEADS

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Ostreid herpesvirus-1 (OsHV-1) has been involved in massive mortality outbreaks of Pacific *Crassostrea gigas* (Pacific oysters) throughout the world, causing important economic losses to aquaculture. Therefore, rapid virus isolation/purification and detection systems are highly needed to provide early warnings as well as for research activities. In this work, magnetic beads (MBs) coated with an anionic polymer were used to efficiently capture infectious OsHV-1 through electrostatic interactions (Fig 1). MBs were incubated with two types of matrix (oyster homogenate and seawater) prepared using naturally infected oysters collected from Fangar Bay (NW Mediterranean Sea). MB-virus conjugates were then thoroughly washed by the application of a magnetic field. Quantitative PCR (qPCR) of the conjugates demonstrated the adsorption of the virus on the MBs from both oyster homogenate and seawater. Calibration curves were constructed using virus and MB dilutions.

To study the infectivity ability of the virus captured by the MBs, MB-virus conjugates (from both oyster homogenate and seawater) were injected into naïve spat oysters, using oyster homogenate and seawater with no MBs as positive controls, as well as MBs with no virus and only water as negative controls. Mortalities were observed when MB-virus conjugates were injected as well as in the positive controls, and no mortalities were recorded in the negative controls. Furthermore, subsequent OsHV-1 DNA and RNA analysis of the oysters by qPCR and RT-qPCR, respectively, confirmed the ability of the beads to capture infectious virus, and the virus being the responsible for the mortality event. The capture of infectious OsHV-1 using MBs is a promising tool, which can be applied as a rapid virus isolation/purification system or, combined with qPCR, as a method for virus detection in aquaculture facilities.

Acknowledgments: The research leading to these results has received funding from the European Union's H2020 Framework Programme under grant agreement no 678589 (VIVALDI project).

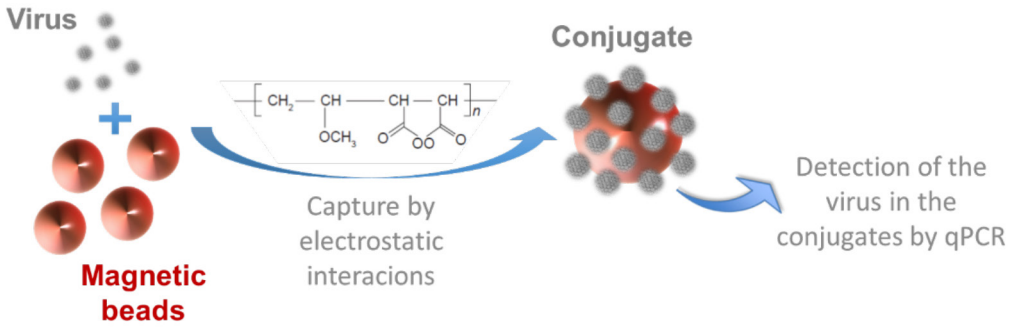

Fig.1. Capture of OsHV-1 by MBs and subsequent detection by qPCR.

NUTRIENT RESURGENCE IN SOBRADINHO LAKE AFTER DROUGHT PERIOD: MASSIVE FINANCIAL LOSS FOR TILAPIA CAGE SMALL FARMERS

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Sobradinho Lake, in the San Francisco River-Brazil, is the second largest artificial lake in the world. In the study area, there are approximately 16 tilapia cage farms with 10.000 m^3 total area. Annual production is around 1.3 ton, but very important for families involved. In January, the water level in the lake starts to increase due to summer rain falling in the southeast of Brazil, where the river begins. Along with the water, organic matter comes, increasing the level of nutrients. The aim of this study was to evaluate water nutrients during spring and summer to verify resurgence phenomenon, as well as relate it to massive tilapia mortality during this season. The study area was a small farm managed by a woman. Production cycle started in August/17 with 19.000 tilapia fingerling (1.5g initial weight). Monthly, samples of water were collected from August through February/18, for ammonia nitrite, nitrate, orthophosphate and chlorophyll levels (all in $mg.l^{-1}$). Rainy season in the upper river basin started in late November/17. Lake total volume value was recorded. The farmer recorded daily mortality. To calculate financial loss, price (live fish in the farm) considered was the expectation for Easter (US$2.4/kg), due to seasonal demand.

Results (Figure 1) shows lake volume increases and tilapia montly mortality. Concomitant, the level of nutrients in the water increased and fish mortality started massively. Financial loss due mortality was estimated in US$24.637,00.

As a conclusion, resurgence phenomenon caused massive tilapia mortality and, consequently, financial loss for local small farmers.

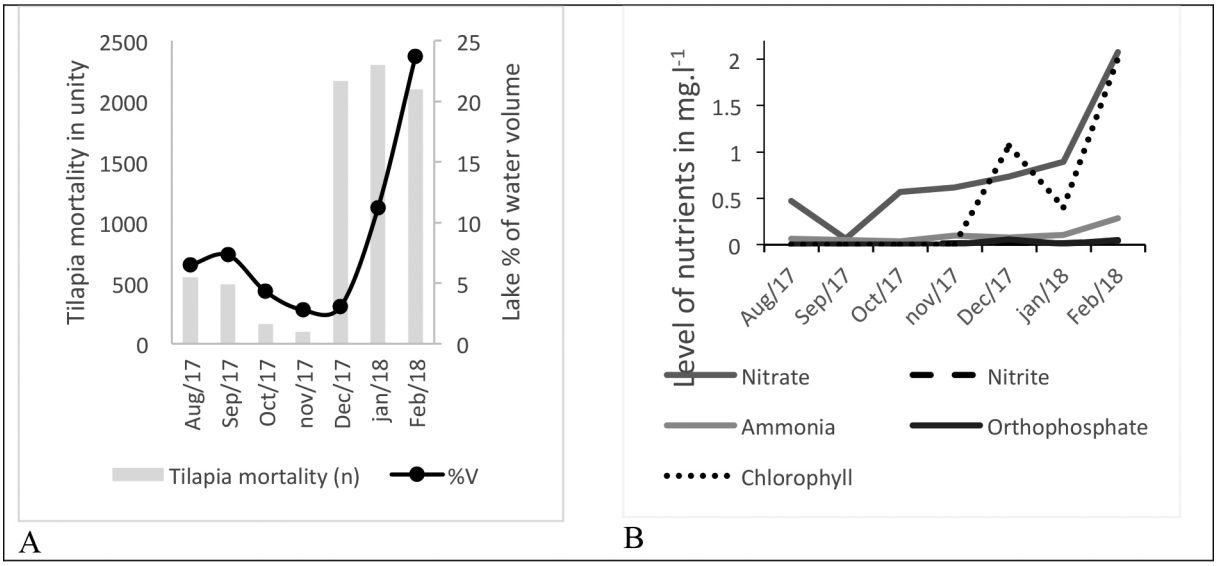

Figure 1: A. Total cages tilapia mortality and lake level; B. Water nutrients levels.

FEEDING MANAGEMENT AND GROWTH IN TILAPIA CAGE SMALL FARMS: DIFFERENCES AMONG NEIGHBORS

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Most of the small fish farmers in the Brazilian semi-arid do not check neighbor´s production management to observe what he is doing that should be followed. The aim of this research was to monitor fish growth and management throughout one entire growing cycle among tilapia small farmers from an association. Results showed different management strategies

A 200 days productive cycle in 4 cages tilapia areas from an association was followed at Sobradinho Lake, Brazil. Each farmer (man and woman) is responsible for his or her own cages. All cycles started on the same day and tilapia juvenile (19g) proceeded from the same farm. Every 15 days the area was visit to observe the management and ask questions. A survey was applied to design process flow. Results showed that feeding management, in all cases, were different. Also, the density chosen by farmers and process flow. Results, at the end of the production cycle (Figure 1), showed different process flow, feeding management strategy (Table 1) and tilapia growth and, consequently, fish growth.

Table 1: Difference in feeding management

Farmer	Fish size (g)	Times.day^{-1}	Feed crude protein %
1	17-30	6	42
	30-100	4	36
	200-300	3	36
	300-1000	2	32
2	17-60	4	35
	60-1000	3	32
3	17-32	4	45
	32-160	4	36
	160-190	4	32
	190-800	3	32
4	17-45	4	45
	45-100	4	32
	100-550		32

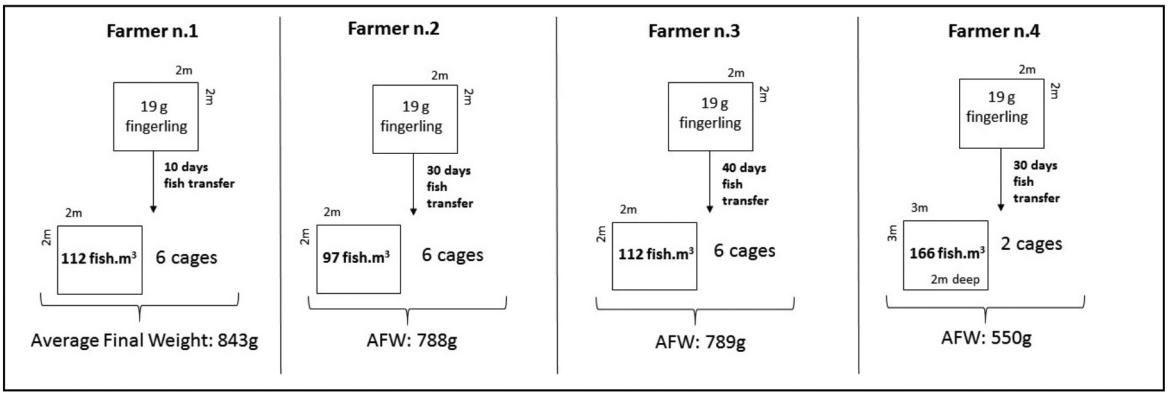

Figure 1: Farmer´s production process flow, cage dimension, fish density practiced and final weight

DEVELOPEMENT OF A NEW MULTIPLEX PCR ASSAY FOR THE CONCOMITENT DETECTION OF ENDEMIC LISTED PATHOGENS OF FLAT OYSTERS: *Marteilia refringens* AND *Bonamia* SP.

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Bonamia ostreae, *Bonamia exitiosa* and *Marteilia refringens* are protozoan parasites of the flat oysters *Ostrea edulis* responsible for the bonamiosis and the marteiliosis, two diseases which have caused significant mortalities in European population of flat oysters. *Marteilia refringens* also infect mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) although the induced disease is less severe than in flat oysters. Those parasites are endemic in Europe; however some countries/ zones are still free of *Bonamia* and/or *Marteilia*. Bonamiosis and marteiliosis are both listed as notifiable diseases at the European and the International level.

A wide range of PCR assays, including conventional and real-time PCRs, are currently available for the detection of either *Marteilia refringens* or *Bonamia sp*. parasites, with heterogeneous level of validation. In order to facilitate the diagnosis of these two listed diseases, the EU reference laboratory for mollusc diseases has developed and validated a new multiplex Taqman® PCR assay allowing the detection of both parasites in one step. This study describes the validation process and analytical performances of the new multiplex assay.

Analytical specificity of the assay was determined by testing samples infected with closely related parasites. **Analytical sensitivity** was established by testing serial dilutions of a known amount of plasmidic DNA (artificial DNA containing the PCR target sequence). **Diagnostic performances** of the assay were assessed on a panel of more than 600 field samples coming from several flat oyster farming areas in France, and included samples infected with *M. refringens*, *Bonamia ostreae* and *Bonamia exitiosa* as well as uninfected samples. Results were compared with currently recommended conventional PCR methods for the detection of *M. refringens* and for the detection of *Bonamia* sp. Finally, the new PCR assay was evaluated in the context of an **Inter Laboratory Comparison test (ILC)**. A total of 17 laboratories, all across Europe, tested a set of 24 samples of flat oyster tissues (including negatives, *Bonamia* sp. and *M. refringens* infected samples) with the new PCR assay and with their own routine PCR assays.

Results showed that the new PCR assay have equivalent or better diagnostic performances than currently recommended PCR assays in term of sensitivity, specificity, repeatability and reproducibility. All the laboratories that participated in the comparison test (ILC) obtained similar or better results with the new PCR assay than with their own routine PCR methods, demonstrating that this new diagnostic tools is easily transferable to other laboratory settings. This is the first assay designed to detect both *Marteilia refringens* and *Bonamia* sp. in a single step and it should allow reducing the number of analysis to perform to demonstrate freedom from these two pathogens.

RESOURCE PROVISIONING FOR MUSSEL CULTURE IN THE NETHERLANDS: THE CHANGE FROM MUSSEL SEED FISHERY TO SEED MUSSEL COLLECTORS

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The Dutch bottom culture of mussels is based on mussel seed fished from natural beds. For reasons of nature conservation it was agreed this seed fishery is replaced by alternative means of seed collection in such a way that a profitable mussel culture is maintained. This was laid down in a covenant, signed in 2008 by the involved nature organizations, the industry and the ministry. As a result of this covenant, the production of mussel seed from seed mussel collectors (SMCs), steadily increased since 2009 in both the Wadden Sea, Oosterschelde, and near shore on the North Sea. Statistics on SMC harvest and efficiency are reported annually. In more than a decade of experiences and developments by the industry several lessons have been learned. SMCs proved a suitable alternative to obtain part of the resource requirements. The introduction of SMCs required a substantial investment of the mussel industry. It resulted in a more reliable seed supply, providing mussel seed also in years without natural seed beds. Various SMC systems with ropes or nets are used or have been used. However, variation in production seems to be more related to location than to system design. Locations also differ with regards to the abundance and timing in number of mussel larvae. However, no clear relation between number of larvae in the water column and spatfall success on settlement substrate is found. Several problems were experienced that affected production and increased effort, primarily fouling, starfish predation and storm damage. Yield from SMC seed after relay on the bottom is currently comparable to seed from bottom fisheries. However, production costs of MSC-seed are substantially higher. This stresses the need for innovations that obtain a higher control on each production step in the chain from spat to consumption mussels and not at last the need for culture area of sufficient quality for the grow-out of SMC seed to market sized mussels.

ECONOMIC COMPARISON OF SALMON MONOCULTURE VERSUS A THREE-COMPONENT INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) SYSTEM: A DISCOUNTED CASH-FLOW ANALYSIS OF AQUACULTURE OPERATIONS IN EASTERN CANADA

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Integrated Multi-Trophic Aquaculture (IMTA) has been proposed as a sustainable aquaculture technology that can help offset some of the environmental impacts of fed finfish aquaculture. It improves on conventional production by integrating extractive components (inorganic absorbers such as seaweeds, and organic suspension- and deposit-feeders such as invertebrates) that make use of various nutrients, not considered as wastes or by-products but as co-products, in a circular economy approach.

Improving on previous financial analyses, our study presents a more detailed and complete assessment of the financial performance of Atlantic salmon (*Salmo salar*) monoculture versus a three-component IMTA system [combination of Atlantic salmon, blue mussels (*Mytilus edulis*), and kelps (*Saccharina latissima*)]. Using discounted cash-flow (DCF) analysis, we assessed profitability in each case over a 10-year period in New Brunswick, Canada. The three-component IMTA operation was more profitable than the Atlantic salmon monoculture, with and without the inclusion of a price premium. Adding a 10% price premium on IMTA salmon and mussels resulted in a substantially higher net present value (NPV) for the three-component IMTA operation.

Despite the positive results for IMTA in our study and other IMTA-related financial analyses, the ongoing uncertainty related to IMTA financial and environmental performance, as well as IMTA increased technological and managerial complexity, may act as barriers to IMTA adoption in Canada at this time. Further, our analysis suggests that the disproportionally small revenue contribution of extractive species in our initial IMTA configuration may have a negative influence on IMTA adoption by the Atlantic Canada salmon-dominated aquaculture industry.

However, as salmon production has declined in recent years in that region, crop diversification could provide economic stability and incent industry development, rendering IMTA a more attractive practice in the future.

Vibrio aestuarianus ASSOCIATED TO PACIFIC OYSTER *Crassostrea gigas* MORTALITY IN THE EBRO DELTA, IN THE CATALAN MEDITERRANEAN COAST

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The presence of *Vibrio aestuarianus* has been associated to mortality events in pre-commercial and commercial *Crassostrea gigas* Pacific Oyster in recent years in France and Ireland. However, no mortality events associated to this pathogen has been detected in the Mediterranean. In May 2017, when temperatures were around 20 ºC, *C. gigas* cultures in the Ebro bays (Alfacs and Fangar) experimented for the first time a massive mortality event of commercial size oyster. Different analyses for detecting the presence of pathogens (qPCR Saulnier *et al*. 2009 *V. aestuarianus*/ *V. splendidus*; qPCR Webb *et al*. 2007 herpesvirus OsHv-1), as well as presence of contaminants, were performed. Retrospective samples from 2016 were also studied.

Results regarding presence of pathogens pointed out the detection of 22+/30 *V. aestuarianus* positive individuals for oysters with 50% mortality collected in April 2016 (EFCgAbr16), as well as 9+/30 positive individuals in May 2017 adult oyster mass mortality (FmCgMa17). Furthermore, in this last sample the presence of *V. splendidus* clade was also observed, however similar results were also observed in oyster with no mortality. The *V. aestuarinus* presence was corroborated by the Spanish National Reference Laboratory (CSIC-Vigo) on samples with mortalities collected from the same bay one month later. Results on contaminant analysis revealed the presence of metolachlor (organitrogen pesticide), bentazone (insecticide), methylparaben (endocrine disruptors), with levels ranging from less than 1 ng/g dry weight (dw) to 10 ng/g dw for both oysters experiencing mortality and with no mortality. Furthermore, esterase activities indicative of OP pesticide exposure did not suggest neurotoxicity signs caused by these compounds. Those results do not support association of contaminants with the studied mortality events and the cause seems of pathogenic aetiology.

Present results indicate the first detection of *Vibrio aestuarianus* associated to commercial *Crassostrea gigas* mortality in the Ebro Delta shellfish culture site, at the Catalan Mediterranean coast, as well as for Mediterranean oyster culture in general. First sample detected positive dates from April 2016 and had been immersed in the Ebro Delta two weeks earlier, coming from French Atlantic waters. Those samples are now being further studied by histology to discard other potential pathogens involved to the mortality episodes. In the case of commercial adult oyster mortality in May 2017 (FmCgMa17), the potential influence of a pathogenic *V. splendidus* strain being also involved cannot be discarded.

Acknowledgments: The research leading to these results has received funding from the European Union's H2020 Framework Programme under grant agreement no 678589 (VIVALDI project) and from the Spanish Government with the INIA E-RTA2015-00004-00-00 project (EMERGER). As well with the project XENOMETABOLOMIC (CTM2015-73179-JIN) (AEI/FEDER/UE) and AimCost project (CGL2016-76332-R MINECO/FEDER, UE).

TABLE 1. Results regarding recent pre-commercial and commercial *C. gigas* oyster mortalities in Fangar Bay in the Ebro Delta, Catalonia. Legend: Va, *Vibrio aestuarianus*; Vs, *Vibrio splendidus*; METO, metolachlor; BEN, bentazone; MP, methylparaben.

LOT	☐ SIZE	% MORT	qPCR Va	qPCR Vs	qPCR OsHv1	Contaminants
EFCgAbr16	50 mm	50%	22+/30	1+/30	0+/30	No analyzed
FmCgMa17	107 mm	85%	9+/30	24+/30	0+/30	METO, BEN
FmCgOct17	108 mm	30%	0+/30	0+/30	0+/30	BEN, MP

COMBINED EFFECTS OF DAILY THERMOCYCLES AND LIGHT SPECTRUM DURING EARLY DEVELOPMENT OF FISH

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In the wild, the environment is not constant but cycles daily: water warms up after sunrise (thermophase) and cools down after sunset (cryophase). In addition, the water column acts as a potent chromatic filter, modifying the sun's spectral profile: wavelengths below violet (λ<390 nm) and beyond red (λ>600 nm) are quickly absorbed with depth. Blue wavelengths (λ~450 nm) penetrate the deepest, reaching depths of up to 150m in the clearest ocean waters. However, under artificial rearing conditions in fish farming, the environment is kept constant and the natural fluctuations are rarely considered.

The aim of this research was to investigate the combined effect of daily thermocycles and light spectrum during early development in fish, looking at hatching rate, larval growth, feeding activity and mortality.

The model fish species selected for the study was the zebrafish (*Danio rerio*). Six different groups were set combining three different wavelengths (blue, red and white) under a 12h light-12h dark (LD) cycle; and two temperature regimes (constant temperature 26±0.5ºC vs a daily thermocycle of 28:24±0.5ºC, TC, where the thermophase coincided with the light phase and the cryophase with the night). Embryos and larvae were kept under these different conditions from 0 to 30 days post fertilization (dpf). During the experimental period the hatching rate was calculated, as well as growth in terms of total length (TL) every 5 days. In addition, at 10 dph the feeding activity was determined as an index of the proportion of the digestive tube (DT) of the larvae filled with food in relation to the total length of the body.

The results revealed growth and TL were significantly affected by both the light wavelength and temperature regime. At 25 dpf, the greatest TL were found in the larvae reared in TC under blue or white light (ANOVA, $p<0.05$). No significant differences were found when comparing the percentage of hatched larvae at 3 dpf, obtaining hatching rates between 85 and 95%. On the other hand, constant temperature combined with red light led to reduced feeding activity and the lowest survival rates (81% mortality).

In summary, the best performance was observed in the combination of blue wavelength and TC, which are the most similar to the environmental conditions present in the wild. In contrast, constant temperature and red lighting conditions negatively affected larval development. These finding should be taken into account to optimise growth and welfare of fish in hatcheries.

ECOLOGICAL INTENSIFICATION OF GIANT GOURAMI PONDS IN INDONESIA: BIOLOGICAL EFFECTS OF *Azolla filiculoides* IN TOTAL OR PARTIAL REPLACEMENT OF COMMERCIAL FEED ON JUVENILES OF GIANT GOURAMI *Osphronemus goramy*

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The use of floating macrophytes such as *Azolla filiculoides* could represent an important alternative for partial replacement of commercial fish feed in small scale aquaculture; particularly for omnivorous/vegetarian fish as the giant gourami *Osphronemus goramy*. In order to evaluate biological impacts of macrophytes as feed on gourami, 480 juveniles (12.4±0.6 cm; 32.4±4.5 g) divided in four groups were placed in floating cages (three cages per group) and fed for six week using four levels of *A. filiculoides* in replacing commercial feed (A=100% B=56% C=29% and D=0%). Commercial f eed were distributed at 5% of the biomass 3 times a day whilst *A. filiculoides* was distributed *ad libitum*. At the end of the experiment, zootechnical parameters, biochemical, somatic (HIS, SSI), and immunological indicators and histological modifications of gut have been quantified. Additionally, effects of transportation stress and experimental infection with *Aeromonas hydrophila* have been evaluated.

Results shown that *A. filiculoides* depressed significantly the growth of gourami and that can be estimated through a polynomial regression (R^2=0.99). Compared to the control fish (100% commercial feed group D), the reduction of biomass gain in the others groups decreased of 18, 45 and 100% respectively according the level inclusion of *A. filiculoides* into the diet. Total protein, albumin and globulin in control fish were significantly lower compared to the others. Serum glutamic pyruvic transaminase was not modified by feeding regimes (P=0.760). Significant reduction of lymphocytes was observed in groups A and B (P <0.001). In opposite, fish from these groups had significant higher level of monocytes (P<0.001). Neutrophils were significantly increased only in group A (P=0. 012). Plasmatic lysozyme, was significantly different (P = <0.001) with an increase depending of the doses of *A. filiculoides* replacing commercial feed. Bactericidal activity of serum was also significantly increased according the level of *A. filiculoides* included into the feeding diet (P<0.001, n=15 for each treatment). Before stress, blood glycaemia was significantly lower in fish of group A fed with 100 % of *A. filiculoides* (P<0.001); and after transport, the glycaemia increased significantly in all groups, excepted for group A. Fifteen days after transportation, the log rank survival analysis was different and mortality rate was significantly reduced in group A. One week after intramuscular injection of 0.1 ml $1x10^7$ CFU/ml of *A. hydrophila*, the survival curves between groups were different (P = <0.001) and survival times of groups A and B were significantly higher than the others two groups.

A. filiculoides showed positive effects on immunological indicators and resistance to stress and disease however, fish may require a longer time to obtain the commercial size. Under this point of view, further economic analysis should be undertaken; but results suggested that the replacement level of commercial fed by *A. filiculoides* shall probably not be over 30%.

ETHNOBOTANIC USES OF PLANTS FOR THE HERBAL THERAPY IN SMALL SCALE AQUACULTURE IN NORTH VIETNAM

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Aquaculture provides nowadays half of the world's seafood consumption and it is expected to expand since wild fisheries will remain stable at best for next decades. Diseases are a severe threat to the sustainability of aquaculture and often jeopardize the efforts of millions of small fish farmers throughout the world. However, the spread of resistant bacteria to antibiotics that are released in the environment pose a threat to both human and animal health, "the most urgent global risk" that needs to be tackled at global level (UN General Assembly 2016). Considering the importance of aquaculture for contributing to food security over the world, therefore, producers will have to meet challenging goals to make aquaculture more sustainable and productive. In this regard, a growing interest has arisen towards herbal therapy for reducing the use of antibiotics, enhancing fish resistance to diseases and improving growth and feed efficiency in order to strengthen the sustainability of aquaculture.

As part of a regional project in South East Asia (BioAsia programme), an ethnobotanical study was conducted in different districts of the northern region of Vietnam. Knowledge Attitude Practices (KAP) surveys were conducted through semi-structured questionnaires involving 280 farmers. Fish farmers that use plants represented 66% ± 5.5, identifying 24 plant species. Plants are used either for the treatment (53%), or prevention (22%) of diseases, or for both (25%). The major reasons for use expressed by the farmers are tradition > efficiency > lost cost. Plants are mainly used against bacterial and fungal diseases (80%), and in less extent against parasites (21%). They are directly dipped into the water (83%) or administered through the feed supplementation (14%). Farmers used them alone (80% of cases) or in combination with drugs (20%). This research shows that ethnobotany is a relevant approach for the preservation of the traditional knowledge related to the use of plants in aquaculture. It is essential for identifying levers and believes that might trigger the use of plants as alternatives to chemicals (e.g.antibiotics) at more global level.

EFFECT OF DIFFERENT LEVELS OF PROTEIN AND CELLULOSE INCLUSION ON GROWTH, SURVIVAL AND BODY COMPOSITION OF JUVENILE FRESHWATER PRAWN *Macrobrachium borellii*

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Macrobrachium borellii is a small prawn endemic to South America inhabiting the water bodies of the La Plata basin. The natural diet includes animal and vegetable items but the effective use of cellulose and protein requirements is still unknown. We compare the growth performance (weight gain-WG, specific growth rate-SGR, condition factor-CF), survival, proximate composition and amino acid profile of prawns fed foods with different levels of fish meal (60%, 45%, 30%) and cellulose (0%, 15%, 30%) inclusion. We related the amino acid quality of foods and prawn muscle through the essential amino acid ratio (A/E) and essential amino acid index (EAAI).

The diets were fed (10% of total biomass) per triplicated to juvenile prawns during 64 days of controlled conditions essay at a prawn density of 0.4 prawn/liter. The growth performance in terms of weight gain were found significantly higher ($p<0.05$) in prawns fed with 15% of cellulose and 45% of fishmeal while showed a greater and positive trend ($p>0.05$) in the other growth parameters. Survival was not significantly different ($p>0.05$) between the diets at the end of the experiment and decreased over time in all cases. Although the proximal composition of the food was significantly different, that difference was not reflected in the proximal composition of the muscle (Table 1). We identified 16 amino acids in the prawn's muscle of which nine were essentials with the following importance order: Lysine > Leucine > Arginine > Fenilalanine > Isoleucine > Valine > Threonine > Methionine > Histidine. Regarding the three diets, they all had an EAAI greater than 1. The limiting EAA in the diets were Arginine, Methionine and Lysine. According to these results, a food elaborated with an inclusion of 45% of fishmeal (around 12% of crude protein) and 15% of cellulose could sustain the growth of the prawn with a performance similar of the diet with more fishmeal inclusion. This result would indicate that the prawn could be using the cellulose for energy and that 12% of crude protein in the feed is sufficient to maintain the growth. In addition, the diets used showed a good quality in terms of EAA. This study provides an initial approach to the development of artificial foods specifically designed for the native prawn *M. borellii*. Future studies should focus the efforts in the digestibility of nutrients and in the replacement of fishmeal by more sustainable protein sources.

TABLE 1: Production responses of juvenile prawn *Macrobrachium borellii* fed diets with different levels of protein and cellulose.

	60/0	45/15	30/30
Initial length	1.01 ± 0.11	0.98 ± 0.11	1.03 ± 0.10
Final length	1.01 ± 0.97	1.03 ± 1.32	1.04 ± 1.38
Initial weight	0.14 ± 0.04	0.13 ± 0.04	0.15 ± 0.04
Final weight	0.16 ± 0.07[a]	0.16 ± 0.07[b]	0.16 ± 0.05[a]
Initial CF	13.20 ± 0.40	14,17 ± 1.48	13.79 ± 1.25
Final CF	15,49 ± 1.62	14.48 ± 1.12	14.26 ± 0.69
Survival	83.3 ± 9	77.8 ± 10	83.3 ± 9
WG	0.03 ± 0.02	0.03 ± 0.01	0.01 ± 0.01
SRG	0.11 ± 0.11	0.12 ± 0.05	0.04 ± 0.04
Protein	20.04 ± 4.21	21.87 ± 4.29	21.37 ± 2.90
Carbohydrates	0.60 ± 0.16	0.60 ± 0.22	0.45 ± 0.13
Lipids	5.85 ± 1.99	6.53 ± 1.10	5.95 ± 0.85
Wet	79.74 ± 0.98	80.63 ± 0.34	80.45 ± 1.39
EAAI	1.03	1.08	1.01

THERMAL STABILITY OF ASTAXANTHIN IN OILS

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Food rations for red rainbow trout incorporate from 30 to 100 mg of astaxanthin per kilogram. Consequently, astaxanthin is one of the inputs that most influences final prices of these salmonids on the market. During food processing, astaxanthin is unstable due to its sensitivity to various environmental factors, such as light, oxygen, acidity and temperature, which cause the loss of its bioactivity. In this context, it is necessary to find new sources of astaxanthin or to achieve a reduction in pigment loss during manufacturing processes. *Dilocarcinus pagei* is a native crab that is commonly found in fish culture ponds and presents an attractive reddish-brown coloration.

The present research aims to study the concentration of astaxanthin present in this crab, and the stability of this carotenoid dissolved in sunflower, soy, and fish oils within temperatures important for fish food processing technology (extraction, cooking and extrusion temperatures), transporting and stoking.

Individuals of *D. pagei* were collected and homogenized. Carotenes were extracted from different samples using acetone. The extractions were analyzed using HPLC-spectrophotometer and astaxanthin was quantified. Astaxanthin oil solutions were distributed into several closed test tubes and incubated at different temperatures between 25 and 180 °C. Samples were taken from each tube at different intervals of time and astaxanthin was quantified.

The quantification method of astaxanthin was validated for sunflower and soybean oil but not for fish oil. Due to the variance between replicates and the consequent impossibility to estimate astaxanthin concentrations, the fish oil was excluded from the study of stability of astaxanthin at different temperatures.

Astaxanthin dissolved in soy and sunflower oil showed a great stability in the studied range, from 25 to 180 °C, and astaxanthin was more stable in commercial soy oil than in sunflower oil. The information obtained in this study allows to estimate astaxanthin loss during fish food processing.

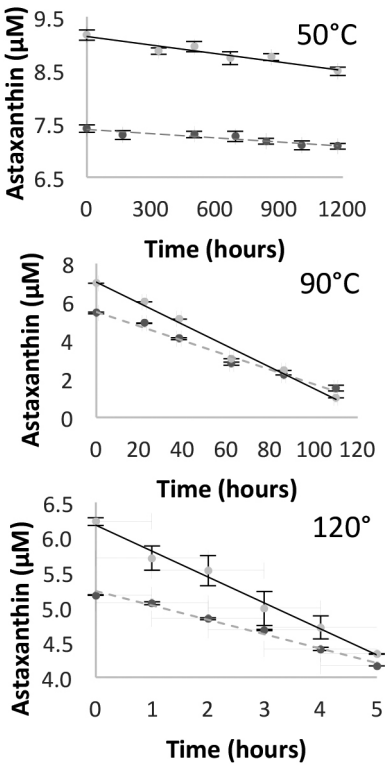

Fig.1 Astaxanthin content in soy oil (black) and sunflower oil (grey) incubated at different temperatures

EVALUATION OF THE NUTRITIONAL VALUE OF DRIED MACROPHYTES AS AN ALTERNATIVE DIET FOR ADULTS OF THE REARED SEA URCHIN *Paracentrotus lividus*

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In order to promote shellfish aquaculture diversification in the northwest French coast by echinoculture, finding alternative sources of food is needed. In the present work, the objective was to test dried macroalgae in the nutrition of *Paracentrotus lividus*. Adults of 18 months old were fed *ad libitum* with six different feeding treatments: (1) fresh *Palmaria palmata*, (2) fresh *Saccharina latissima*, (3) dry *P. palmata*, (4) dry *S. latissima*, (5) dry *Laminaria digitata* and (6) dry *Grateloupia turuturu*. Survival, ingestion, absorption and growth (diameter and biomass) rates were assessed during 8 weeks. Total water-soluble proteins and carbohydrates and total lipids contents were analysed in the six diets.

Fresh *P. palmata* and *S. latissima* allowed the best growth performance but dried macroalgae induced an efficient somatic growth (Fig. 1). Significant differences between fresh and dried diets appeared only after 4 weeks. These differences could be explained by the loss of water-soluble compounds observed in dried diets due to the rehydration carried out. Thus, dry macroalgae food could be used by farmers during short periods as a substitute to fresh algae without detriments to the production. This could be done when algal biomass is less abundant in the environment. Moreover, dried macroalgae presents a lower cost compared to the formulated diets usually used as alternative food to fresh macroalgae, allowing small producers to diversify their activities.

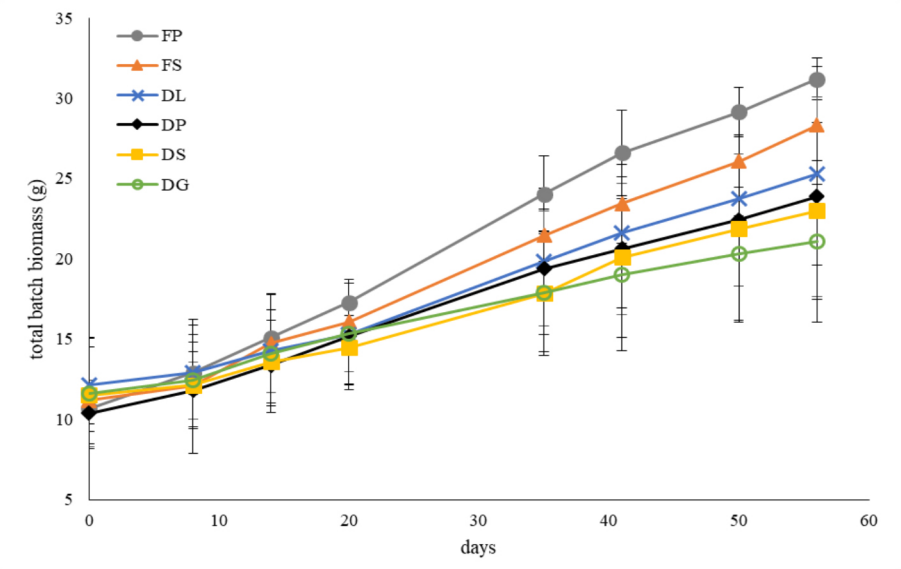

Fig. 1 Mean biomass of sea urchins *P. lividus* batches fed with six different fresh or dry macroalgae diets. FP = fresh *P. palmata*; FS = fresh *S. latissima*; DL = dry *L. digitata*; DP = dry *P. palmata*; DS = dry *S. latissima* and DG = dry *G. turuturu*. Error bars represent confidence intervals (95%).

REVIEWING PUBLIC CONSULTATION MECHANISMS ENHANCING SOCIAL ACCEPTABILITY OF AQUACULTURE IN THE MEDITERRANEAN SEA

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Nowadays aquaculture represents one of the fastest growing sources of food worldwide accounting for half of the fish supply, and the demand of farmed fish is expected to rise. This sector plays a major role in the Blue Growth strategy both at European and international level. However, the aquaculture of the future should be more environmental, economic and socially sustainable and comply with the requirements of existing legislation. In 2010, FAO introduced the concept of Ecosystem Approach to Aquaculture (EAA) to promote a more integrated and sustainable development, taking into consideration the socio-economic systems. This approach should be applied especially in the selection of Allocated Zones for Aquaculture (AZA) that need to be established on the basis of a participatory process which encompasses the consultation and exchange of information between the promoter, the stakeholders, elected representatives and the citizens to enhance social acceptability. Social acceptability is linked to local specificity related to the territorial dimension, thus the conditions acceptable in one situation will not necessarily be acceptable in another. Nonetheless, through a literature review, we have identified the most relevant factors that can enhance social acceptability in aquaculture. The aim of this review is to investigate whether and how the governance mechanisms of public consultation established to meet the obligation of international and European marine legislation are addressing those factors (Table 1).

This study shows that if properly implemented, existing legislation adequately support sustainable aquaculture development where social, economic, governance and environmental factors enhancing social acceptability are taken into consideration.

Table 1. Some of the factors and actions enhancing social acceptability of aquaculture development and the related pieces of legislation.

Factors	Actions	Legislation
Socio-economic benefits of local communities	Bring socio-economic benefits for local communities (e.g. employment) and good labour conditions	-
More open, transparent and participatory consultation	key communications messages and activities to disprove negative stereotypes of aquaculture industry	EU level: Common Fisheries Policy Environmental Impact Assessment, Maritime Spatial Planning
	communities and stakeholders are kept well informed about the industry's environmental impacts	International level: FAO - Code of Conduct for Responsible Fisheries Århus Convention
Conservation of habitats and of species and maintenance of biodiversity	Governments should demonstrate their commitment and ability to prevent or lessen negative environmental impacts.	EU level: Environmental Impact Assessment, Natura 2000 Directive, Marine Strategy Framework Directive
		International level: UN Convention on Biological Diversity

THE DIETARY TAURINE ADDITION IN FISH FEED; ITS PROTECTIVE ROLE TO THE REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN THE EUROPEAN SEABASS (*Dicentrarchus labrax*) DURING A FORCED SWIMMING TEST

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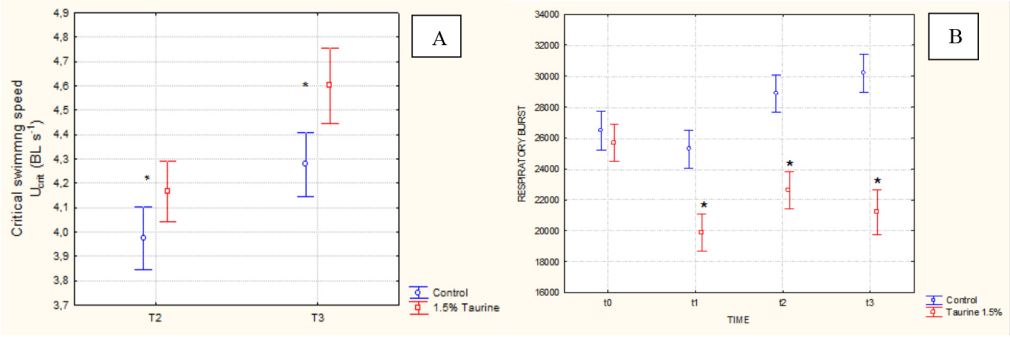
Taurine (Tau) is a non-essential amino acid, but extremely required, as nutritional feed additive, for the carnivorous fish species, that may exhibit low growth performances. The lacking performances are due to the vegetable meal (VM) introduction as alternative to fishmeal (FM), and the consequent amino acid deficiencies, as well for the methionine (Met) and the lysine (Lys). Thus, Tau could assume a nutraceutic role to mitigate poorer fish performances. Besides such Tau role, others properties were observed as anti-oxidizing agent, promoting the cell and tissue protection from toxicity injury, stabilizing the cell membranes, reducing the membrane permeability, and scavenging the reactive oxygen species (ROS). Particularly, ROS are the response to oxidative stress generated by invading microorganisms such as viruses, bacteria and fungi, but also derived by an intensive physical activity where an increase oxygen demand is required. Our research has been undertaken to afford the Tau effects study on increase of ROS production due to and its consequences on swimming performance of European sea bass.

28 sub-adult seabass (*Dicentrarchus labrax*) of an average weight 92.57±3.842 g were equally divided in two dietary groups: a control (Ctrl) group and a 1.5% Tau-group. Three time intervals (t0, t1, t2, t3) were chosen to perform the swimming test and the respiratory burst (RB) assay. The swimming respirometry was performed in a Loligo system swim-tunnel, where oxygen consumption (MO_2) and the critical swimming speed (U_{crit}) were measured. The RB activity was directly monitored from heparinised blood, calculating the luminescence signal as relative light units (RLU).

Both at t2 and t3 time intervals, the Tau feeding group reached highly significant U_{crit} respect to the Ctrl group. In figure 1A, at t3 the Tau group recorded a higher U_{crit} 4.60±0.12 BL s^{-1} than the U_{crit} 4.27±0.07 BL s^{-1} recorded by Ctrl group ($p<0.01$). Similarly, in figure 1B the RB activity of lymphocytes, after PMA stimulation, at t_2 and t_3 was lower in Tau group than Ctrl group ($p<0.05$), after the swimming test. In our experiment, the 1.5% Tau addition had the ability to mitigate the ROS production, even in forced swimming conditions.

Figure 1 (A): Critical swimming speed (U_{crit}) expressed in mean ± s.d. (N=14). Stars mean the significance ($p<0.05$). Figure 1 (B): The graphic shows the respiratory burst activity after PMA stimulation of lymphocytes in control group and 1.5% taurine group. Stars mean statistically different for $p<0.05$.



AN OVERVIEW OF DESERT AQUACULTURE IN ALGERIA

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Aquaculture in Algeria is relatively recent, but is growing steadily under the PNDPA program (national plan for the development of fisheries and aquaculture). Following the positive results recorded in marine aquaculture distributed along the coastline (10 marine fish farms and 15 shellfish farms), beside inland aquaculture mainly in dams, interest has been focused recently on Saharan aquaculture in the south, taking into account the successes recorded in other countries in this field. Furthermore, this trend is clearly recommended by FAO in various reports.

In the case of Algeria, the promotion of aquaculture in the Desert will certainly contribute to the sedentarization of the populations, to the creation of new jobs through the diversification of agricultural and aquacole production, thus contributing to the guarantee of food security, which constitutes one of the goals of the first national priority. In this area, several actions have been carried out: (1) water from extensive Nile tilapia culture is used to irrigate small-scale agriculture production, (2) intensive Nile tilapia and North African catfish culture are practiced using Flow-Through and Recirculation Aquaculture Systems, (3) whiteleg marine shrimp is produced using biofloc technology.

In this project, we will attempt to carry out an audit of Saharan aquaculture in Algeria, before targeting some problems that hinder or delay the achievement of the objectives outlined by the Ministry of Agriculture, Rural Development and Fisheries. In particular, issues relating to the diversification of farmed species, livestock feed and pathology. The impact of integration on agricultural yields will be also considered.

A survey was conducted in 2017 in the 7 provences of southeastern Algeria, considering the characteristics of agricultural farms, the typology of irrigation ponds (concret or earthen ponds) and especially aquaculture practices, type of fish, density and distributed feeding. Different farming methods are practiced: (1) the intensive system wich represented by 12 farms distinated to raise red tilapia, Nile tilapia and catfish, (2) Shrimps are produced at the high-tech Shrimp Cultivation Research Center, a project between South Korea and Algeria, (3) the extensive system is represented by integrated fish farming. More than 600 farmers cultivate fish in ponds or tanks destinated for irrigation, using nutrient-rich water to irrigate agricultural crops.

THE PRIMEFISH PROJECT: AN OUTLOOK OF THE COMPETIVENESS AND ECONOMIC SUSTAINABILITY OF THE AQUACULTURE SECTOR

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The European Union is considered as the largest market of the world for seafood, but what are the future trends of this demand? The PrimeFish project has developed a set of market studies focused on some of the most popular aquaculture species, i.e. salmon, trout, seabass, seabream and pangasius. The analyses focuses on the willingness to pay for some attributes of seafood, the different seafood niches in the most relevant markets of EU and how the negative media information impacts on consumers. These studies embraces more than 10,275 consumers from France, Germany, Italy, Spain and United Kingdom.

On one hand, the concept of consumer segmentation for aquaculture products is analysed in-depth. Clear and distinctive segment profiles for the single countries and for total Europe were identified which give actionable insight for the aquaculture firms' new product development/marketing decisions. Additionally, it also identifies segments that cut across the different nations as well as groups of consumers that are idiosyncratic to just one or a few countries. The findings thus may support the existence of similarities across the European fish market that would allow the fish industry to target the so-called "pan-European segments" with an almost standardized marketing program. In addition, the willingness to pay (WTP) for nutritional and health claims is varied among countries. The results show higher premiums for pangasius and salmon. In particular, price premiums above 20% were found in Spain for pangasius (68%), trout (37%) and salmon (20%), in Germany for pangasius (44%), seabream (30%) and salmon (24%), in Italy for seabream (27%) and salmon (21%), and in the UK for pangasius (26%).

On the other hand, the public's perception of aquaculture was explored through an experimental study that involved salmon consumers. Their attitudes towards salmon consumption were assessed before and after reading a negative message issued by an official and non-official source. Results quantify how determinant is a negative message in the attitudes related to salmon consumption.

While already the aquaculture consumers' profiles are highly informative, if the key attributes of the aquaculture products are included in the equation, the communication and promotion of seafood will be most efficient, and therefore, the competitiveness and economic sustainability of the involved stakeholders will be improved.

Acknowledgment: this project has been developed thanks to the support of the European Commission's Horizon 2020 Research and Innovation programme. Only the authors can be held responsible for this content.

COMBINATION OF FARM, HYDRODYNAMIC AND PARTICLE TRACKING MODELS TO PLAN THE DEVELOPMENT OF ENVIRONMENTALLY SUSTAINABLE AQUACULTURE PROJECTS IN MAYOTTE ISLAND

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Waste management of marine aquaculture for regulatory purpose is a main concern. In Europe, the authorization are delivered according to an environmental impact assessment, in which solid emissions (faeces and uneaten feed) play a significant role. Providing a decision support system to the aquaculture industry with respect to the best locations and optimal size of finfish aquaculture sites can help the authorization process and participate to a sustainable development of the sector.

Our project aims developing a decision support methodology which coupled (1) an integrated farm management and solid emissions model, (2) a hydrodynamic model (ROMS) (3) a waste-particle tracking model (NewDEPOMOD), in order to estimate the solids accumulation on seabed and associated benthic effects. This modelling framework is applied on the context of red drum (*Sciaenops ocellatus*) farming development in Mayotte Island. The parametrization of the farm management and solids depositional models required the acquisition of species-specific data (feed digestibility, feed and feces settling velocities) and systems-specific data (cage layout, cultural practices).

Farming scenarios (production scale, cultural practices) were constructed from field surveys on existing commercial farms and study of potential projects on the territory. Aquaculture sites scenarios (bathymetry, currents flows and distance from the shore) in the North East Mayotte lagoon were constructed from a clustering method analysis on current velocities simulated using ROMS model, previously computed for the studied area.

The application of the modelling framework offer a powerful method to define a priori the scale and type of culture systems adapted to each site at regional scale, by screening the different possible farm/site combinations. This approach is particularly adapted for poorly documented aquaculture systems, such as new production systems or species in small islands and it is relevant for an integrated ecosystem-based management to the aquaculture sector as it considers ecosystems functions and services and involve local stakeholders in the process.

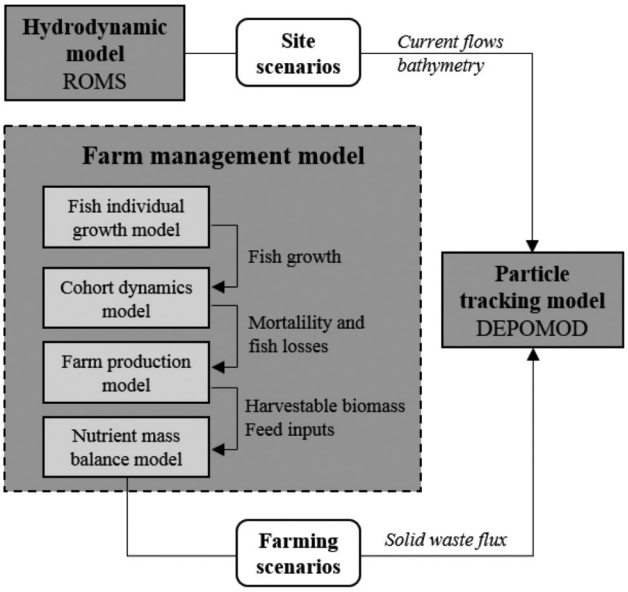

Figure 1 : Modelling framework for predicting the waste dispersal of various farming and site scenarios.

COMBINED EFFECTS OF HYDROGENATED SOYBEAN OIL AND DHA ON GROWTH AND FATTY ACID PROFILE OF MARINE GROUPER *Epinephelus coioides*

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The dietary effects of hydrogenated soybean oil (HSO) and DHA in combination on the growth performance and DHA deposition of the grouper were studied. A lipid comparison experiment (8 weeks), followed by a washing out experiment (4 weeks), was carried out. The lipid comparison experiment was of 3x2 factorial design, in which regular soybean oil was replaced by HSO at 0, 50, or 100%, and DHA was supplemented at 3.8 (low) or 10.8 (high) g/kg. Juvenile groupers with an initial mean body weight of 64.3 g were stocked in flow-through tanks at a density of 20 fish/tank. Each dietary treatment was randomly assigned to four replicate tanks (N=4). The fish were hand-fed twice daily to apparent satiation. The results of the lipid comparison experiment showed that weight gain of the fish fed with the high DHA diets was significantly higher than that of the fish fed with the low DHA diets. No difference in weight gain was detected among the HSO groups. The results of the washing-out experiment showed that feed intake, growth, and feed conversion ratio were increased significantly with the increasing levels of HSO in the diets fed previously in the comparison experiment. The carryover effects of HSO supplementation on growth and fatty acid profile, especially DHA deposition, will be discussed.

CHARACTERIZATIONS OF TYPE I INTERFERON RECEPTOR (IFNR1) FOR IMMUNE-STIMULANTS-INDUCED RESPONSES IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Grouper aquaculture was among known high economic profit sector in aquaculture industry, raising interests in fish farmers. However, easily outbreak of virus diseases caused huge losses to fish farmers. The mechanism of first line of defense against virus diseases in teleost fish is activated through production of antiviral protein through signaling of secreted cytokine (type I IFN) to neighboring cells. Previous research proved up-regulation of gene expression level of grouper IFN and downstream Mx protein in response to virus infection.

To investigate relationship between type 1 IFN receptor system with virus infection in grouper, full length orange-spotted grouper IFNR1 was firstly cloned and identified as 1444 nucleotides sequence including untranslated region with ORF encoding 433 amino acids. Phylogenetic analysis indicated osgIFNR1 shared high homology similarities with other teleosts. Downstream relationship of receptor from ligand was characterized through ligand treatment, relative expression of osgIFNR1 was measured with real-time qPCR after ligand treatment in immune organs. Gene expression of *osgIFNR1* was up-regulated during early hours of rIFN treatment while downstream *osgMx* showed increased expression level during similar early hours post-stimulation. The reported up-regulation of IFNR1 mRNA expression and downstream Mx protein gene indicated transcriptional expression response of interferon system through up-regulation of gene expression level during 4 hours of ligand treatment. This study reported insights of orange-spotted grouper IFNR role in type 1 interferon system and contributed in understanding of first line defense system against disease infection in grouper species.

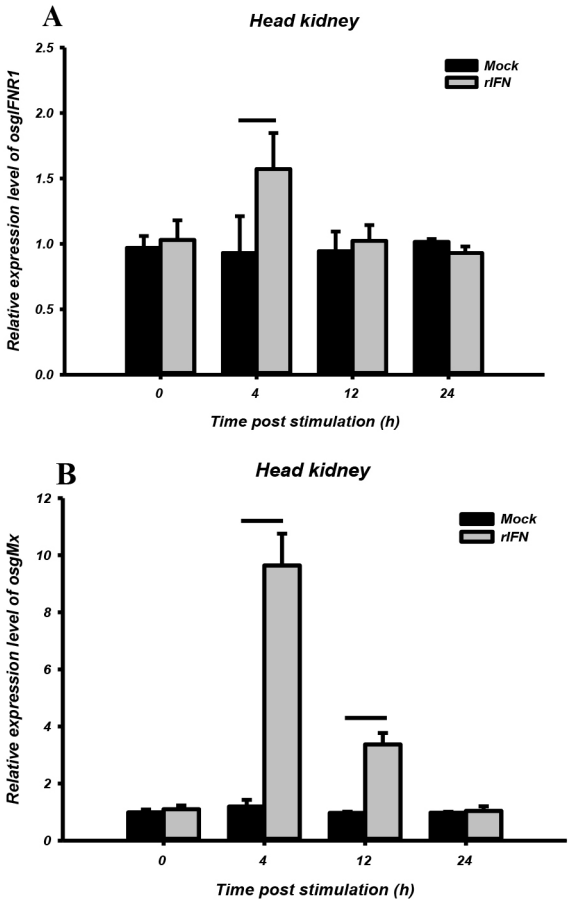

FIGURE 1. Gene expression analysis of IFNR1 in immune organ of orange-spotted grouper. Using -actin as internal control, mRNA expression in ligand stimulant treatment on orange-spotted grouper in (A) *osgIFNR1* and (B) *osgMx*.

OOCYTE ATRESIA IN CULTURED COMMON COCKLE *Cerastoderma edule*

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A firm knowledge of biological processes, particularly reproduction, is needed for good management of exploited animal resources. The histological characteristics and quantitative impact of oocyte atresia were examined in Common cockle *Cerastoderma edule*, on a cultured site (Croisic Traict) and on a fished and unfished site in Bourgneuf Bay, on the French Atlantic coast.

Oocyte atresia characteristics were documented using microscopic observations of modified Masson's trichrome and alcian blue stained tissues. Stereological study was done monthly on samples from fished and unfished sites of the Bourgneuf Bay over a 26 – month period. Five volume fractions were calculated inside the acini: atresics oocytes (AO), matures oocytes (MO), immatures oocytes (IO), intra-acinal tissue (IAT) and acid mucopolysaccharide sheath (S). Each of these categories were present throughout the sampling period, which indicates that atresia is a phenomenon observed before and after spawning.

Despite some species specificities, the nuclear, membrane and cytoplasmic characteristics were comparable to those found in other bivalves. The stereological results (Table 1) indicate that 30% of all oocytes were atresic, and that atresic oocytes outnumbered mature oocytes over the sampling period in Bourgneuf Bay. Similar quantification is underway for the farmed Croisic Traict site. The causes of oocyte atresia in cockles are currently unknown, underscoring the importance of studying this under-reported aspect of reproduction.

TABLE 1. C*erastoderma edule.* Mean intra-acinal cell volume fractions over the sampling period at the two Bourgneuf Bay sites.

Bourgneuf Bay Sites	AO	MO	IO	IAT	S
Fished	8.75	9.12	22.18	24.62	26.45
Unfished	11.61	8.25	29.07	27.45	32.50
Global average	**10.18**	**8.68**	**25.63**	**26.03**	**29.47**

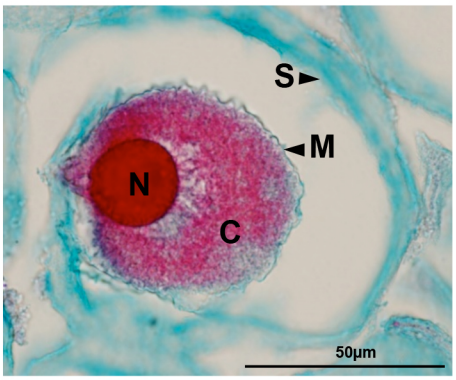

Figure 1. Oocyte showing some characteristics of atresia: absence of chromatin structure, irregular cell membrane (M). Nucleus (N), cytoplasm (C),

OXYGEN BALANCE IN PHOTOBIOREACTOR CULTURE OF *Picochlorum sp*: RISKS OF HYPOXIA AND HYPEROXIA

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Microalgae culture in outdoors ponds may be subject to two paradoxical constraints related to dissolved oxygen (DO). From one side, during the night, DO could become too low to maintain the aerobic metabolism and cells growth. From other side, during the day, the oxygen from photosynthesis could reach high concentrations and induces oxidative stress. Indeed, DO at high concentrations in the cultures would pose a severe threat to microalgae as: light-energy dissipation through photorespiration, enzyme inhibition of the photosynthetic pathways, and might cause damage to the photosynthetic apparatus, membrane structures, and cellular components (i.e. DNA).

The objective of this study is to access the influence of the day/night cycles on the oxygen balance of a microalga from *Picochlorum* genus experimentally grown in photobioreactors (PBRs) and to highlight a possible relationship between hypoxia, normoxia and hyperoxia with the productivity and biochemical composition of microalgae.

The oxygen balance can be summarized by the following equation: B = P + R + EA; where B represents the Balance, P oxygen production by photosynthesis, R oxygen consumption by microalgae, and EA water-air diffusions. P and R were determined in a close respirometer respectively under illumination and in the dark. The study has been carried out by comparing two treatments: 24h/24 and 12h/24 photoperiod (24hPP vs 12hPP)

During night, the growth stopped and the cells were characterized by a change of pigmentation and lower carbon content. Treatment 12h PP compared to treatment 24h PP exhibited 34% lower growth rate and 36 hours delay to reach stationary phase. These differences are the result of lower growth of the 12hPP treatment the first 72h of culture, causing an initial shift.

The respiration and photosynthesis rates evolved with the growth rate and cell density and were different according to the treatment: the first 3 days, the photosynthesis and respiration rate are at the same level for both treatments, then the metabolism rate in 12hPP treatment becomes much higher compared to 24h PP treatment. Our study showed also a positive relationship between respiration rate and O_2 concentration in culture. The question is therefore whether if there is a control mechanism of the cell metabolic rate by the oxygen level in the medium. Such a mechanism would allow microalgae to slow down their metabolic rate during the night in order to reduce the risk of hypoxia or anoxia. However, there are situations, characterized by a high density of rapidly growing cells, which lead, during the day, to an excess of oxygen and oxidative stress of the culture. In our study, oxidative stress leads to an exacerbated metabolic rate with excessive consumption of oxygen of microalgae at the following night causing anoxia in culture medium.

Our study highlights the need of monitoring the oxygen in microalgae cultures in order to prevent problems from hyperoxia and/or from hypoxia that could negatively impact the growth and the productivity.

This study has been carried out in the frame of AMICAL R&D program (Aquaculture of MIcroalgae in New CALedonia) conducted in partnership between IFREMER and ADECAL Technopole.

THE SEASONAL AND INTERANNUAL VARIATIONS OF ENVIRONMENTAL CHARACTERISTICS IN THE MARINE AQUACULTURE AREA

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To understand the variability of the oceanic and environmental conditions around the abalone marine aquaculture area, seasonal hydrographic survey were carried out and time series datasets of hydrodynamic were collected in the study area simultaneously. Marine aquaculture cage systems are used to increase productivity by concentrating energy supply much higher than natural ecosystems. Massive culture system and intensive food supply (and excretion) disturb the oceanic and environmental conditions around the aquaculture area. This study focused on the assessment and discussion of the seasonal and interannual variations of oceanic conditions as well as environmental effects on the marine aquaculture area.

The observed hydrodynamic data showed that the seawater movement was dominated by tidal current with a semi-diurnal period in the study area. Depending on the water depth, the current speeds at surface and bottom layer decreased rapidly with range of from 80cm.s.$^{-1}$ to 5cm.s.$^{-1}$. Particularly, due to the abalone aquaculture facilities(cages), the current speeds at surface layer decreased markedly. Preliminary results from the water quality parameters (dissolved oxygen, COD, nutrients, SPM, etc) indicated that the changes of environmental conditions are closely related to the current speed. The weaken tidal current at the surface by the dense and massive abalone aquaculture facilities should seem the main cause of environmental degradation in the study area due to restriction of water-mass exchange.

The seasonal and interannual variation of the sea surface temperature (SST) in the study area suggested that the increased SST in summer is associated with the weakened cold water in the southwestern coast (JCW). The JCW is strongly correlated with the temperature of bottom water affected by the Yellow Sea Bottom Cold Water (YSBCW). The more extended YSBCW to the south is bound to affect the temperature of bottom water in the southwestern coast of Korea. Southern limit of the YSBCW in summer is strongly correlated with the SST of the northern YS in previous winter. Further research is essential to understand the mechanism of the southward movement and southern(western) limit or area(volume) of the YSBCW.

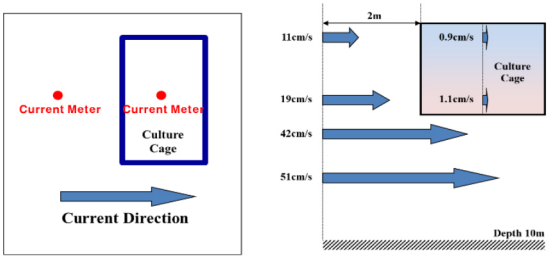

Fig. 1. Schematic drawing of the current meter deployment and the vertical structure of tidal current speed in the vicinity of marine aquaculture cage.

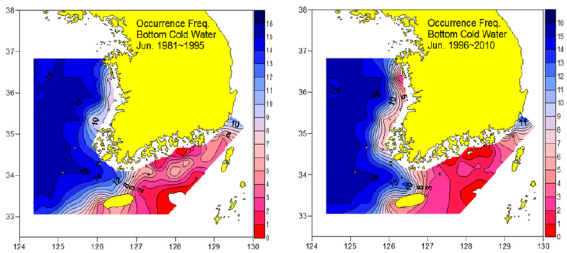

Fig. 1. The comparison of southern and western limit of YSBCW with the cold water occurrence frequency between the period of 1981~1995 and 1996~2010.

BEYOND LINEAR PROTEIN SOURCING: BRINGING ENVIRONMENTAL SUSTAINABILITY, ECONOMIC STABILITY THROUGH CROP DIVERSIFICATION AND SOCIETAL ACCEPTABILITY WITH INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) IN A CIRCULAR ECONOMY FRAMEWORK

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Integrated Multi-Trophic Aquaculture (IMTA) is much more than looking at protein sourcing. Moreover, aquaculture should not be only about seafood security, but also about nutritious seafood security. A nutritious diet comes from diversified sources of food; so, we have to grow and eat more than fish, but also seaweeds and invertebrates.

Examination of landing and value data from the four Atlantic provinces of Canada between 1986 and 2016 shows that, though salmon aquaculture in Atlantic Canada developed strongly from 1986 to 2000, annual landing numbers have fluctuated between 2001 and 2016, and have remained relatively stagnant. The production in New Brunswick increased steadily from 1986 to 2002; since, it has decreased. How can we, then, still talk about "aquaculture development" in this region when the driving sector (salmon) is showing obvious signs of decline? The development will not come from fish aquaculture, so the only hope is diversification by growing invertebrates and seaweeds. At a time when some finfish sites have been inoperative for several years, it would make sense to reevaluate them and determine their appropriateness for cultivating extractive species.

IMTA fits very well within circular economy thinking. We have to stop seeing nutrients as wastes or by-products, and consider them as co-products, useful for the cultivation of other crops in more efficient and responsible food production systems, while bioremediation of coastal nutrification takes place. Because different nutrients (small particulate organic nutrients, large particulate organic nutrients, dissolved inorganic nutrients) need to be recaptured, different spatial and temporal strategies should be designed. This means that entire bays/regions can be units of IMTA management, within an Integrated Coastal Area Management (ICAM) strategy, hence challenging traditional aquaculture regulations/policies.

Moving IMTA along the Research & Development & Commercialization continuum will require profound regulatory changes. The apathy for changes can be frustrating. We are still in the infancy of western IMTA (after all, we have been improving agriculture for centuries and it is still not perfect). Science and society need time to think and evolve. The adoption of IMTA will not happen overnight, especially in the western world, which presently prefers monocultures, linear processes and short-term profits. We will need patience, determination and persistence to get people to see the advantages of growing complementary species together, creating circular economy processes and seeking sustainability in the long term.

To calculate IMTA's full value, extractive species need to be valued for not only their biomass and food trading values, but also for the ecosystem services they carry out, along with the increase in consumer trust and societal/political license to operate they provide the aquaculture industry. The value of the ecosystem services should be used as financial and regulatory incentive tools (e.g. nutrient trading credits). Moreover, the IMTA multi-crop diversification approach (fish, seaweeds and invertebrates) could be an economic risk mitigation and management option to address pending climate change and coastal acidification impacts.

THE IMPACT OF POST-OVULATORY AGEING ON THE DEVELOPMENT OF DIPLOID AND TRIPLOID ATLANTIC SALMON *Salmo salar*

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Introduction
A common observation in domesticated triploid salmon populations is the larger spread of growth in fry and parr, and increased mortality in early egg (i.e. pre-eyeing) stages compared to diploid siblings. Many of the suggestions that stem from earlier results point to early life stage developments which have yet to be explored. Good egg quality is the fundamental biological requirement for the adequate development of an individual. One factor that may influence egg quality is post-ovulatory ageing i.e. the length of time between ovulation and manual stripping of the broodstock. Post-ovulatory ageing can contribute to decreased egg quality which could be a likely factor contributing to this phenotypic difference. Ageing of the eggs post-ovulation may alter the composition to a "sub-optimal" status.

Under routine commercial practice in Atlantic salmon aquaculture, females may be stripped up to 21 days post-ovulation with no deleterious effects on offspring in "normal" diploid production protocols. However, egg-ageing effects need to be explored to determine what impact this has on egg quality and development in diploid and triploid Atlantic salmon and whether different "ageing thresholds" can / should be defined for commercial practice.

Methods
Females (n = 5) will be partially stripped over 5 day increments to generate populations of eggs with varying ages post-ovulation (0, 5, 10, 15, and 20 days). Egg quality indicators will be investigated in all groups. To assess the maternal influence on egg quality, all populations will be fertilization from the same male. Diploid (2N) and triploid (3N) Atlantic salmon eggs will be incubated in parallel and grown to ~5g. At this final stage, a final assessment will be conducted to assess the response of ageing on development in both ploidies.

Results and Discussion
A suite of ovarian fluid and egg quality indicators including; fatty acid composition, lipid class, proximate composition, carotenoid content, vitamin and mineral content, and lipid peroxidation assessment will be presented. Differences of compositions and quality between different ages post-ovulation will be compared.

Conclusion
Understanding the impact of post-ovulatory ageing may suggest refinements to the current stripping protocols for production of diploid and triploid Atlantic salmon. Identification of ploidy specific egg ageing thresholds may help prevent negative impacts on survival and growth. Investigating a range of egg quality parameters may highlight particular biomarkers that could be used as real-time indicators to determine the potential of egg populations.

HIGH PERFORMANCE MICRODIETS FOR FISH LARVAE: PROGRESS & DIFFICULTIES

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Major efforts by academia and industry have targeting high performance microdiets for fish larvae since the 1990´s. Progress has been considerable, with good weaning results being currently delivered by several commercial microdiets, for major cultivated species. Over recent years, significant progress on weaning has also been achieved for some candidate species for the expansion of the Aquaculture industry. Still, there is room for substantial improvements in microdiets for marine fish larvae, in particular for the very early stages. Even if we know that fish larvae require diets with high levels of protein, essential fatty acids, and micronutrients, and that these are provided by highly digestible ingredients, the exact nutritional requirements are poorly understood. It is also clear that early nutrition has consequences during the larval, but also later in the juveniles stage, in terms of health status, survival, skeletal deformities and growth performance. Fish species have different nutritional requirements, and high quality ingredients that work very well in a species, may be not suitable for other species. Very early weaning is becoming a reality in several species, and its long-term effects seem to be very positive. Novel ingredients such as microalgae and organic forms of minerals may bring either positive or negative effects on larval quality. Nutrition is always a matter of balance. Moreover, the success of a high-tech microdiet depends also on the feeding techniques used, and needs to meet some specifications in addition to nutritional adequacy to the target species (Fig. 1): it must ensure a high ingestion, allow easy digestion, prevent nutrient loss to the surrounding water by leaching and disaggregation; and present optimal physical properties such as floatability, sinking speed, dispersion both in tank surface and water column.

Leaching losses may compromise larval quality by nutrient loss, as well by reduction of water quality. Microencapsulation can reduce nutrient leaching, but also microdiet digestibility, so a good balance is required. As knowledge on larval nutrition progresses and newer technologies become available, better performing microdiets for fish will soon be available. This will mean faster growing larvae, with higher survival rates, that will lead to better quality juveniles. Live feed replacement will increasingly be a reality, leading to many species start feeding exclusively (or almost) on high quality inert microdiets.

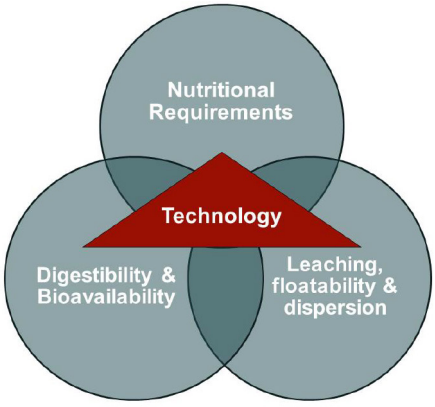

Figure 1. Main factors determining success of microdiet for fish larvae.

AQUACULTURE FOR CONSERVATION: THE CASE OF STURGEONS

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During the last few decades, most natural populations of sturgeons have experienced a dramatic collapse, and their persistence often depends on continuous restocking programs. Due to the scarcity of mature specimens in the wild, fingerlings to be released are often generated by controlled reproduction of captive breeders maintained in aquaculture plants. These hatcheries however are often standard facilities with a main interest in sturgeon production for commercial purposes and their culture process does not take into account important criteria that should be considered in designing rehabilitation programs.

Among the several technical aspects that should be taken into account for a proper establishment and management of captive broodstocks, the preservation of the genetic diversity should rank as a very high priority in the approach to built and maintain a broodstock. In addition, the choice and the pairing of individuals employed in controlled reproductions is crucial because when their offsprings are released into the natural habitat, these will have a direct effect on the transmission of genetic diversity to the following generations, and also on the genetic make up when retained in captivity as future brood stock. Possible effects of captive breeding are a high potential of decreasing the adaptive potential of released fish and such effects will be briefly presented and discussed. These include: (a) reduced size of the founder stock, (b) inbreeding and outbreeding depression, (c) initial acclimation but also long-term adaptation to captive conditions, (d) negative effects because of the lack of a long term breeding plan, (e) unknown relatedness among breeders will be overlooked with regard to some sturgeon features such as the long life cycle and the different degrees of ploidy.

ULTRAFILTRATION, A SOLUTION FOR SHELLFISH AQUACULTURE

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Some hatcheries can produce exotic shellfish (like polyploid oysters) with the risk that gametes and larvae reach the environment, threatening the local biodiversity. Therefore, effluents from farms must be treated but conventional processes are efficiency limited due to the quality of the water (UV) or produce by-products (oxidation). The objective of this work was to develop a new process to remove oyster gametes from shellfish hatchery effluents. The retention by ultrafiltration membrane was determined and the oyster gamete viability was evaluated after treatment.

Tests were carried out with a pilot able to treat 20 $m^3.d^{-1}$, continuously fed with seawater. This automated filtration unit included filtration, backwash and cleaning steps. First, several tests of seawater filtration were performed for different operating conditions. Sustainable conditions for which a modest degree of fouling occurs, providing a compromise between capital expenditure and operating expenses, have thus been determined. Then, two types of effluents were realized by addition of spermatozoa or oocytes from oyster *Crassostrea gigas*, to simulate (a) a chronic pollution: low concentrated effluents during a long time and (b) an accidental pollution: highly concentrated effluents during a short time to reproduce an accidental release of biological material during oyster maturation processes. Flow cytometry analyses were performed to determine gametes concentrations and assess their integrity before and after ultrafiltration.

As expected, the retention rate was 100 % whatever the gametes treated and their concentrations: spermatozoa concentration measured in permeate was lower than detection limit (Figure 1). More than 3 log removal is obtained whatever the inlet concentration for the oocytes and spermatozoa, demonstrating the relevancy of ultrafiltration for the treatment of these effluents. The hydraulic performances remained stable on the period of the tests (> 6 months) confirming that the process is adapted for this application. An impact on integrity of both oocytes and spermatozoa was observed in the case of backwash and more especially when membrane was first drained. In fact, in the case of spermatozoa, a drastic reduction of the number of these species was observed, from 50545 to 10340 $spz.mL^{-1}$, reflecting a loss of integrity (Figure 1).

This ultrafiltration process allowed the protection of the marine environment biodiversity with (i) the retention of oocytes and spermatozoa whatever the filtration conditions and gamete concentrations and (ii) the sustainability of the process facing this organic pollution on the duration. Finally, filtration of real effluents coming from shellfish tanks was performed in order to determine the impact of microalgae and feces on the performances.

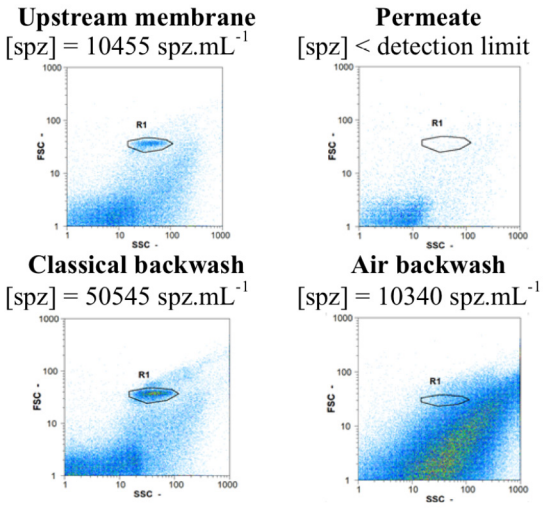

Figure 1 : Cytometry analyses – Treatment of spermatozoa

INFLUENCE OF FEEDING A *Saccharomyces cerevisiae* FERMENTATION PRODUCT, YEAST FRAGMENTS, or WHOLE YEAST CELLS ON THE IMMUNE STATUS, MICROBIOTA AND SURVIVAL RATE OF FISH AND SHRIMP

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Whole yeast cells and yeast fractions are commonly used to improve the health of food animals worldwide. However, recent research results demonstrated that feeding a *Saccharomyces cerevisiae* fermentation product (SCFP; Diamond V Original XP™) results in broader and stronger benefits than commonly observed with whole yeast cells or yeast fractions. In these studies, feeding SCFP improved the immune status of both fish and shrimp, but in addition, maintained these immunological benefits over a prolonged period of time (>3 months). The benefits observed included: increased lymphocytes counts, phagocytic activity, respiratory burst, enzyme secretion (Lysozyme, SOD, Phenol oxidase) and complement levels; and substantial improvements in the survival of shrimp (average +24 % over 7 tests) and fish (average + 60 % over challenge tests). When challenged with white spot syndrome virus, different pathogenic *Vibrio* species, *Streptococcus agalactiae* or *Edwardsiella tarda*, feeding SCFP to shrimp conferred robust protection against these diseases, even after months of use. In contrast, yeast cell wall fractions, such as β1,3-1.6 glucans, typically cause an immune overload reaction even after only 2 weeks of use.

Studies have also shown that feeding SCFP to poultry and swine reduces the load of pathogens, such as *Salmonella* sp., *E. Coli*. and *Campylobacter* by up to 96%. In fish and shrimp, SCFP reduces intestinal load of *Vibrio* sp., E. coli O20:H42, Bacilli MS030A1_FO2 like, *Pseudomonas fluorescens* YC0357-like, and increases numbers of beneficial bacteria as has been reported for probiotics and prebiotics.

In summary, the unique broad metabolite profile of SCFP confers enhanced protection against many diseases that reduce the profitability of food animal operations.

RECRUITMENT SUCCESS OF PACIFIC OYSTERS, *Crassostrea gigas*, IN RELATION TO TROPHIC QUALITY, LARVAL QUALITY AND METAMORPHOSIS DELAY

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In the Mediterranean Thau lagoon, while the shellfish industry is interested to develop spat collection activity, we observed an important fluctuation in recruitment success of pacific oysters (*Crassostrea gigas*) at the micro-geographical scale. Between 2012 and 2014, metamorphosis delays have been reported, with 40% variability of the size at metamorphosis between sites. We suggest that metamorphosis success is dependent on the sufficient amount of energy reserves accumulated by competent larvae (pediveliger) affected by the trophic resources quality. We test the hypotheses that 1) the larvae delay their metamorphosis when trophic condition is not optimal and 2) the sites with higher recruitment success was characterized by settlers showing higher quality in terms of energetic reserves. During spatfall event, collectors were installed in six experimental sites inside and outside the shellfish culture areas in the lagoon. Abundance and lipid content of young settlers (pediveligers and post-larvae) were determined two weeks after their installation and spat abundance four weeks later. Results showed heterogeneous recruitment rates between sites inducted by different settlement and recruitment processes which are under environmental factors (Fig. 1). A decrease in energy reserves of young settlers linked to metamorphosis delay may cause a failure of metamorphosis and a decrease in the recruitment success. Current lipid quantification analysis will attempt to establish a link between *C. gigas* recruitment, survival at metamorphosis, energy reserves of young settlers and lipid quality of the trophic resource. A better understanding of these interactions could allow the development of better spat collection management in lagoon environments, and contribute to the general understanding of the ecological and physiological mechanisms of settlement and recruitment in contrasted and changing environments.

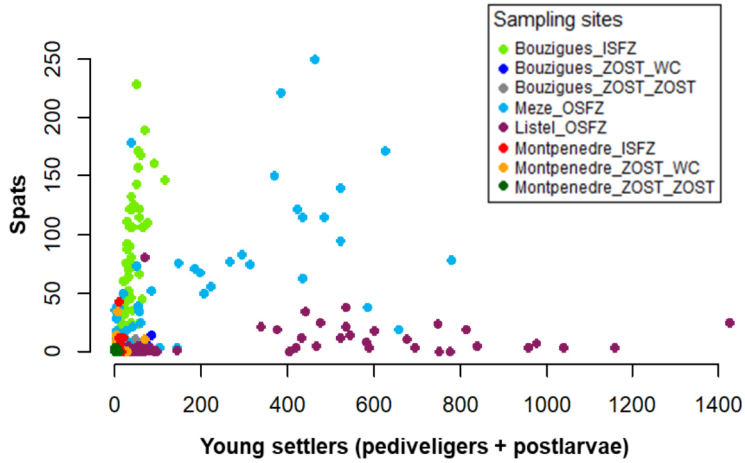

Fig.1. Mean oyster settlers abundance in relation to spat abundance per artificial collector observed at the different spatfall sampling sites in Thau lagoon.

OCEAN FOOD SYSTEMS: A TRANSDISCIPLINARY, ECOSYSTEMS ECOLOGY APPROACH TO MARINE AQUACULTURE

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In 1793 the French chemist Antoine Lavoisier wrote that science and the useful arts needed to develop a team approach to meld diverse contributions from different disciplines. Over 200 years later, Illman (2007) stated that, "History may look back on these last couple of decades as a time when science grew up and took on real-world problems instead of sticking to the safe and tidy world of the tractable, in which studies are undertaken because they are doable, even if not directly useful." Global organizations such as FAO and The World Bank agree that population growth to 2050+ will increase demands for aquatic foods some 40-60 MMT. However, global analyses of the status and needs for the expansion of ocean food production systems are inadequate to inform regional policy-makers who too often use aggregated global data to validate their perceptions that fisheries are an ancient profession of a dying generation destroying aquatic ecosystems everywhere, or that aquaculture is either the savior of seafood or the next tragedy of the commons. This presentation introduces an ecosystems ecology approach to regional planning for ocean food systems using a new rubric we call a "Taxonomy of Seafood Systems" (TSS) that identifies first the structure of regional ocean food production systems to reveal the full diversity of modern fisheries and aquaculture production systems and their local to global value chains. The TSS is then used to structure data acquisition and establish functional connections between systems. The TSS offers insights into the need for more comprehensive social-ecological approaches that can help identify top priorities in ocean food systems and marine conservation research. Evolving more sustainable aquaculture systems and obtaining an accelerated social contract for them requires these transdisciplinary, ecosystems ecology approaches, such as the use of social-ecological systems advances pioneered by Nobel Laureate Elinor Ostrom. We present an example of the current TSS in Maine, USA, and the development of a functional model of the current flows of finfish to/from Maine, the North Atlantic, and globally. Our methods can be used to develop a baseline to design transdisciplinary, social-ecological processes that will accelerate regional ocean foods production and trade that will have positive impacts on societies and environments. We are using these concepts in partnership developments and local-regional to global training of graduate students in twinned Master's Programs in Ocean Food Systems in Maine, USA, and in the Nordic Master Program of Sustainable Production and Utilization of Marine Bioresources coordinated by Gothenburg, Sweden; and we plan to extend them elsewhere in the EU. Our goal is to develop further the use of ecosystems ecology and design thinking to deliver working models of more sustainable aquaculture developments that are connected closely to fisheries systems and communities.

AVAILABILITY AND ENVIRONMENTAL EFFECTS OF COMMERCIAL FEEDS (FISH MEAL *VS* PLANT MEAL BASED) & FISH FEED WASTE IN AN IMTA SYSTEM OF FISH, FILTER- & DEPOSIT- FEEDERS: A MULTI-INDICATOR ASSESSMENT APPROACH

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A multi-indicator approach was applied to assess the potential utilization and environmental impact of commercial fish feeds [fish meal (FM) *vs* plant meal (PM) based] and fish feed particulate waste by co-cultivation of mussels (*Mutilus galloprovincialis)*, oysters (*Crasostera gigas)* and cucumbers (*Holothuria tubulosa)* in an European sea bass IMTA system, in the framework of a case study for the IMTA-EFFECT project (COFASP, EU program).

The experiment was performed at the installation of the Institute of Marine Biology, Biotechnology & Aquaculture located at Agios Kosmas, Athens, and lasted for three months. Two identical concrete tanks (IMTA) (15m^3 water capacity each) situated at the coast and equipped with an open flow seawater system (flow rate ~ 980 - 1000 l/h) were used for the co-cultivation of the species from the different trophic levels. In one tank the fish were fed on a FM based diet, while in the other tank the fish were fed with a PM based diet. Physiological [scope for growth (SFG), condition index (CI), immunological markers (IM)], biochemical [proximate composition (PC)] and molecular [fatty acids (FA)] levels of the co-cultured organisms were evaluated. Proximate composition, feed convention ratio (FCR) and toxicity as well as fatty acid analysis of feeds and fish feed waste (solid sediment) including seston loads and feces were also performed. Chlorophyl-a, total particulate matter (TPM), organic and inorganic fractions (POM & PIN) as well as particulate organic nitrogen (PON) and carbon (POC) were measured in the water column as well. Yet, screening changes in sediment bacterial community was evaluated. These indicators were compared to those measured at monoculture system (only fish).

Significant differences were found in most of the measured indicators included CI, IM markers, FA, FCR, toxicity, POM, PON, POC and sediment bacterial community.

We consider that a multi–indicator approach can provide a more holistic vision of the benefits and effectiveness of an IMTA system under different environmental conditions.

SUSTAINABLE AQUACULTURE PRODUCTION IN OUTERMOST REGIONS: FOCUS ON LOCAL AND HIGH-VALUE AQUACULTURE PRODUCTS

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European overseas regions and territories are in majority islands that have high biodiversity, limited resources, small economies and a undersized domestic market, mainly relying on primary sector products and tourism. Nevertheless, they have a huge potential for Blue Growth development considering their large marine surface which management can be integrated in the established and planned growth strategies. From this perspective, the Canary Islands (Spain), started to develop their aquaculture production in the early 90's based on fish production and, at present time, are acting towards improving public outreach about the benefits of aquaculture products as well as promoting the diversification of species under a ecosystem approach and through sustainable production techniques.

Sustainable development of seafood production suggests increased reliance on local resources while integrating associated development of economical activities with service and facility provision. Therefore, there is an increasing interest to orientate aquaculture development of these territories towards local, emblematic, environmentally respectful and regionally distinct species that offer high quality and high value products attractive to the consumers, enhancing at the same time local food production and associated processing sectors.

The aim of this contribution is to present a sustainable model of integrated production of a low trophic, endemic species in a multi-trophic system. This IMTA model illustrates the feasibility of such production and the benefits in terms of local food production, valorization of aquaculture activities and promotion of associated products.

RESEARCH PRIORITIES FOR A SUSTAINABLE MARINE FINFISH FARMING DEVELOPMENT IN SMALL ISLANDS TERRITORIES

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In addition to their small size, isolation, limited arable agricultural land and dependence on high-priced imports, island territories are also particularly vulnerable to both economic and social crisis, natural disasters and impacts of climate change. Nevertheless, because of their maritime surrounding, marine aquaculture has frequently been identified as a high potential sector that could bring a significant contribution to their blue economy, not only because of their large ZEE but also because of their often strong seafood demand.

Several keys steps have been identified during the course of development of an island fish-farming sector, from emergence to sustainable production. Importantly, the specific features of these remote areas create higher complexities for investors, especially because of local market specificities that shape the possible volume of production, the number and size of businesses, their economic model, as well as the value chain organisation from production to market. Moreover, each island's characteristics must also be considered within the global picture, among which governance schemes, regulatory frameworks, economic conditions, biodiversity etc.

During the preliminary steps to develop marine aquaculture in a given island territory, a certain number of pre-requisite must be filled. The first goal is to perfectly understand the market target (local and/or export) as well as the various stakeholders involved in governance and production, and also identify potential sites and project initiators. A tight coordination between the various components of a R&D continuum, from the research upstream to the producers downstream is mandatory.

Experience gained from several case studies in French overseas islands located in different geographic regions (red drum *Sciaenops ocellatus* farming in Martinique Guadeloupe, Mayotte and La Réunion, bat fish, *Platax orbicularis* in French Polynesia, Atlantic cod *Gadus morhua* in Saint Pierre & Miquelon) as well as outcomes obtained from a recent workshop organized by FAO on the Blue Growth Opportunities in Small Island Developing States allowed us to identify research priorities, especially with regards to the organization of research and prioritization of topics.

If research (stakeholders, agenda) has shown its capacity to promote and support marine fish farming development, it cannot alone ensure a successful sustainable development over the long term. In close consultation and concertation with the local fish farming stakeholders in priority but not exclusively, public policies have to create a supportive regulatory and financial framework which is essential to allow the emergence and continued growth of this new economic sector.

A GROWING INTEREST IN SEAWEEDS – INDUSTRY AND COASTAL COMMUNITY ENGAGEMENT TO SUPPORT COMMERCIALIZATION OPPORTUITIES IN COASTAL BRITISH COLUMBIA, CANADA

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Seaweed production continues to grow in the global arena, with a >5% increase annually. Yet despite a clear market demand for a broad range of seaweed products (food, alginates, bioethanol, antiviral agents, fertilizers, etc.), and a coastal environment that supports one of the greatest diversity of species in the world, very little movement has been realized with respect to the development of a seaweed aquaculture sector in western Canada – why? Integrated Multi-Trophic Aquaculture (IMTA) research has delimited the spatial extent of the wastes generated from fish farm operations, revealing that the use of extractive species such as seaweeds offers an opportunity to capitalize on these inorganic wastes and could generate a substantial revenue stream given the high demand and market value of such products. Furthermore, the inorganic fraction has a much broader spatial impact downstream of a fish farm, and these wastes represent a clear commercial opportunity for co-culture while offering important ecosystem services. The use of seaweeds in adapting to climate change offer additional ecosystem services for the coastal environment – the potential for mitigating ocean acidification impacts to shellfish, carbon sequestering, and/or habitat rejuvenation.

This presentation describes a 5-year applied research program we have initiated to facilitate commercialization of seaweed aquaculture in coastal British Columbia, and the unique industry and coastal community (aboriginal peoples) partnership that has come together in support of this effort. It provides some preliminary performance results and the options for business development.

THE STRUCTURE AND FUNCTION OF "THE SALMON FARM REEF" – IT'S NOT SIMPLY BIOFOULING

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The installed infrastructure comprising a fish farm represents a physical habitat that provides a new and unique substrate for colonization by marine invertebrates, kelps/seaweeds, and an associated assemblage of resident and transient demersal/pelagic fish species. Given the relative permanency of the farm structures, these habitats can, in essence, be considered floating "reefs". A 2-year study in coastal British Columba has documented the structure and function of the fish farm reef, given the nature of the physical infrastructure as well as the inputs of the farm itself that will inevitably stimulate or depress colonization processes.

The Salmon Farm Reef Project examined the sub-surface structures that comprise a salmon farm facility. Our inventory and sampling approach used a fixed focal length high-resolution camera system (Go-Pro) operated via relayed Wi-Fi signal to the surface operator, a sample scraper and material capture unit, and a new artificial substrate component to document surface recruitment related to exposure. Four farm sites were selected for seasonal monitoring of the farm's reef community – the farms were chosen based on differing oceanographic conditions, assuming these conditions would support different biological reef communities.

The results of the project revealed a very clear difference in the reef community depending upon oceanographic conditions (water quality and flow) and the exposure of each farms physical structures to the environment (orientation to flow, sunlight, depth, etc.). There is also a very high degree of internal variability among similar structures, suggesting the patchy nature of the community structure. "Function" of the Salmon Farm Reef assumed the average commercial farm comprises a 10-cage array (30-metre cages) with 14 x 28 metre feed/accommodation barge, anchored in 55 metres of water. The structures are anchored with 2" polysteel line in an average of 55 metres of water with a 3:1 scope. The entire subsurface structures was estimated at 1,119 m^2 - using our biomass data would suggest the system supports 19.2 MT of biological community.

Using species-specific filtration rates derived through the literature, and actual mussel density data from our sampling program, our functional estimate suggests that those sites where a high proportion of filter feeders exist revealed a significant filtration capacity across the farm. With 50% mussel (smalls) coverage and an individual filtration rate of 0.6 litres/hour, the entire farm would filter approximately 14.5 million litres of seawater daily.

The positive and negative aspects of the Salmon Farm Reef were discussed in terms of potential biosecurity, farm management practises, organic/inorganic waste mitigation, habitat creation, and interactions with wild species.

ROCK SCALLOP CULTURE: TO CEMENT OR NOT TO CEMENT?

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The purple-hinge rock scallop, *Crassadoma gigantea*, is a promising candidate for culture along the west coast of the United States. The juvenile cementing behavior -- where it goes from free swimming with temporary byssal attachment to permanently attached to hard substrates -- has proven problematic for culturists because cementation often damages both the product and culturing gear during harvest. Our earlier work found that upon reaching cementing size rock scallops are physiologically capable of cementing throughout life, but that they are less likely to cement at larger sizes. Further, it suggested that growth was better when artificially cemented (attached) to a substrate in comparison to ear hanging (unattached). Questions remained as to whether 1) other methods that inhibited attachment may result in adequate growth, and 2) manipulation of the cementing behavior would only be needed until scallops reached a certain size.

To address these questions we conducted two field experiments. First, we tested whether periodic disturbance of scallops in mesh bags inhibited attachment of scallops while still achieving good growth. Replicate bags of 50 scallops (~30 mm shell height (SH)) were deployed at sites in Washington State. The bags were shaken approximately every 100 days such that any scallops cementing to the gear were dislodged. After 25 months, survivorship and growth was high at most sites, averaging 73% and 78.9 mm SH across sites. Less than 1% of scallops continued to exhibit cementing behavior, with the majority of animals ceasing this behavior after reaching 50-55 mm SH. Second, we assessed the influence of continual attachment on scallop growth in California. Scallops (~30 mm SH) were artificially attached to either short (70 mm) or tall (140 mm) flat panels and grown in replicated shellfish culture trays. After nearly 25 months, scallop survivorship was high (89%). Shell growth was higher for scallops that continually cemented to panels (tall treatment, 101.6 mm mean SH) as compared to those that grew past the panels' edge and continued to grow without cementing to a substrate (short panel, 96.4 mm mean SH). Scallop adductor muscle weight did not differ significantly between the short and tall panel treatments; 19.7 g and 21.2 g respectively.

Our results support the hypothesis that cementing may not be an obligate behavior of *C. gigantea*. In some environments (e.g., high currents), attachment may be beneficial and thus exhibited, whereas in less dynamic conditions it may not be required thereby not displayed. For culture, promoting or inhibiting (when possible) attachment may result in good growth and survival, and it may be required for only a small window of time thereby reducing costs associated with such manipulation. Overall, the need to and the methods for manipulating the cementing stage will depend on the system and environmental conditions for grow-out.

UNDERSTANDING THE INDIVIDUAL ROLE OF FISH, OYSTER AND MACROALGAE IN THE ECOLOGY OF INTEGRATED PRODUTION IN EARTHEN PONDS

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Three different production treatments with distinct combinations of trophic level species were designed: (i) a combination of Fish+Oysters+Macroalgae (FOM), (ii) Fish-Oyster (FO) and (iii) Fish-Macroalgae (FM) to permit the evaluation of the role of each trophic level within the IMTA system-Each treatment was carried out in replicate, in a total of 6 earthen ponds of 500 m2 surface and depth of 1.5 m at Estação Piloto de Piscicultura em Olhão (EPPO) which is located at the Ria Formosa coastal lagoon, south Portugal.

The commercial species were a carnivore (meagre - *Argyrosomus regius*), an omnivore (white seabream - (*Diplodus sargus*) a detritivore (grey mullet - *Mugil cephalus*), a filter feeder (oyster triploids - *Crassotreas gigas*) and a primary producer (macroalgae – *Ulva* spp.). Since this was an open system that lasted for 8 months other organisms grow spontaneously such as phytoplankton and periphyton. Fish were fed daily and water renewal in the ponds, equipped with bottom air injectors, ranged 40 - 120% per day.

Results (Fig. 1) revealed better water quality in FOM treatment with higher mean dissolved oxygen (DO) and higher transparence resulting in the lowest use of air injection. The highest use of air injection was observed in the FM treatment with the highest turbidity and lowest chlorophyll, DO and transparency. Meagre grew better in ponds without macroalgae where the water was cleaner. Seabream and mullets grew better in FOM and FM treatments where there is more surface for periphyton and associated fauna to develop. Oysters were an additional source of dissolved nutrient in the ponds but also acted as controllers for phytoplankton proliferation. Macroalgae exerted a high level of competition for nutrients preventing phytoplankton to growth and consequently the oysters.

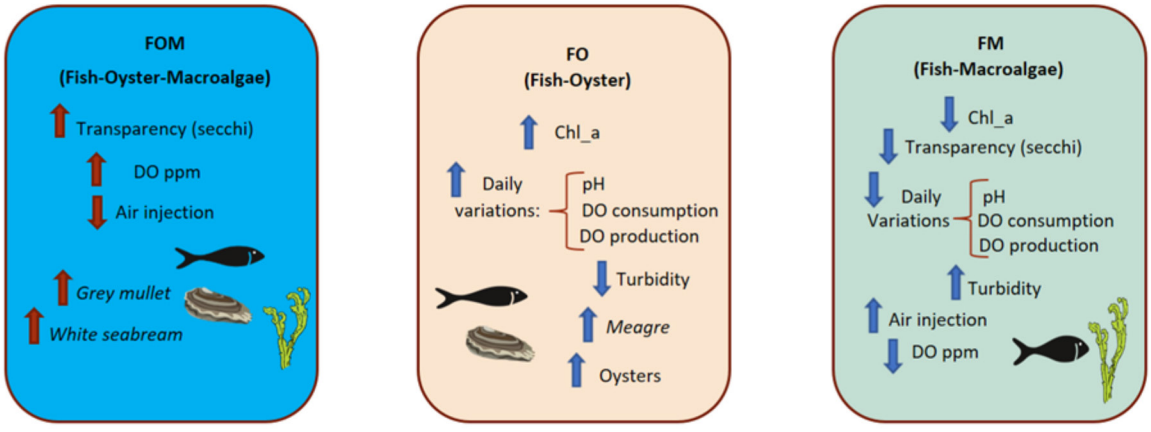

Figure 1. – Comparative levels of water quality parameters and animal weigh in the treatments

IMPROVING LARVAL PRODUCTION OF THE COMMERCIALLY IMPORTANT SEA URCHIN *Tripneustes gratilla*: SETTLEMENT, METAMORPHOSIS AND POST-SETTLEMENT SURVIVAL

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In many aquaculture operations, farm production is often limited by access to a steady supply of juveniles, due to the vulnerability and often poor survival rates of larvae. For *Tripneustes gratilla*, this is compounded by a lengthy pelagic larval stage and the subsequent successful metamorphoses of competent larvae into juveniles. Improving the survival and number of larvae that successfully complete metamorphosis will significantly improve commercial production of this species.

Successful larval settlement and metamorphosis have in many instances been linked to associations with the recognition of specific substrates or substratum-specific biochemical signals. In many intensive aquaculture systems, the required morphogenetic inducing substances are often absent. As a consequence, these aquaculture operations suffer from costly and extensive early post-larval mortality, which can be linked to abnormal development during metamorphosis. This study examined the effects of a range of inductive substrates and chemicals on larval settlement and metamorphosis as well as post-settlement survival and growth of juvenile *Tripneustes gratilla*. The effect of each settlement substrate or inducer was evaluated by quantifying the number of competent larvae that successfully completed metamorphosis within a 48h period. We tested a range of benthic microalgal species (*Amphora* sp.; *Cocconeis* sp.; *Navicula* sp.; *Nitzschia* sp.; and a tank diatom community (TD)), macroalgal species (*Ulva* sp., *Ulvella* sp., *Porphyra* sp.) and chemicals (histamine, dibromoethane, γ-aminobutyric acid (GABA), Ulva extracts). A significantly higher percentage of larvae successfully completed metamorphosis in association with the tank diatom community (98 ± 2.0%), *Ulvella lens* (72 ± 2.6%) and fresh seaweed *Ulva* (68±10.7%), when compared with the benthic microalgal species and certain chemicals. Further testing of Ulva extracts revealed that specific fractions of Ulva, rich in glycolipids (fractions #9) were responsible for the improved (>70%) settlement and metamorphosis of larvae. Settlement substrates also had an effect on post-settlement growth, with the macroalgal crust, *Ulvella lens*, producing the best juvenile growth over a 30 day period, compared with a range of benthic diatoms often used as industry standards. These findings will contribute towards more consistent and successful production of *T. gratilla* juveniles and the development of echinoculture globally and within South Africa.

USE OF DNA POOLING IN GENOMIC SELECTION FOR A DISEASE TRAIT IN ATLANTIC SALMON

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Genomic selection can increase genetic gain in aquaculture breeding for many traits, especially for traits which are not measured on the candidates themselves. However, its implementation in this industry has been hindered by staggering genotyping cost due to many individuals to genotype. DNA pooling is a strategy for reducing genotyping cost for different applications. In this study we demonstrated the potential of DNA pooling as a tool for genomic selection for a disease resistance trait using *in silico* pooling technique.

Data on 4115 postsmolt from SalmoBreed population were challenged for pancreases disease (PD) and 914 individuals with extreme phenotypes (435 alive and 479 dead) were genotyped with ~55k customized SNPchip. The data was divided into reference dataset (589 samples) and validation dataset (325 samples). The effect of number of pools were investigated by grouping individuals into 1, 2, 4, 10, …, 200 pools per phenotype group (the respective number of fish per pool is given in Table 1). Given the genotype of individuals in each pool, allele frequencies were calculated by sampling with replacement. Effect of sequence coverage on accuracy of selection was studied by varying the sampling times to 40X and 100X. SNP effects from the pool data were calculated based on allele frequencies estimated form the pools and compared with SNP effects estimated from individual genotypes. Genomic breeding values (GEBVs) were calculated for the validation individuals as sum of SNP effects. The accuracy of selection was calculated as the correlation between predicted GEBVs and phenotypes and weighted by the square root of the heritability (h^2).

The correlation between SNP effects estimated based on individual genotypes and pooled data increased from 0.3 to 0.898 and from 0.411 to 0.912 for the 40X and 100X respectively, when the number of pools increased from 1 to 200. The correlation between GEBV increased from 0.84, when only single pool is used per phenotype, to 0.976 when number of pools increased to 200. Similar trend also observed for the accuracy of prediction, it increased from 0.574 to 0.691 (Table 1). The accuracy of prediction for this dataset using individual genotypes was 0.712. Limited effect of sequence coverage on correlation of GEBVs and accuracy was observed (Table 1).

Results showed that large number of pools are required to achieve as good prediction as individual genotypes, however, alternative effective pooling strategies should be studied to reduce the number of pools without reducing prediction power. Nevertheless, it is demonstrated that pooling of reference population can be used as a tool to optimize between cost and accuracy of selection.

Table 1: Accuracy of prediction

# pools per phenotype group	# fish/pool	Accuracy 40X	Accuracy 100X
1	295	0.575	0.574
2	147	0.574	0.575
4	74	0.575	0.575
10	30	0.576	0.576
20	15	0.581	0.579
40	7	0.592	0.588
100	3	0.640	0.636
150	3	0.668	0.687
200	1.5	0.676	0.691

SYSTEMATIC MICROBIOTA PROFILING AND DEVELOPMENT OF DECISION SUPPORT TOOLS FOR NEXT GENERATION AQUACULTURE MANAGEMENT

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The microbial communities in aquaculture systems are composed of a large number of different species in different abundancies, representing a major factor for biological water quality. While the majority of microbes does not affect fish health directly, some species can have negative and others positive impact. The microbiota also affects the chemical water quality, and is a vital part of the water treatment in recirculating aquaculture systems (RAS). The water quality is determining for fish health and thus of high operational interest. Outbreaks of diseases cause significant loss in aquaculture systems. While the chemical water quality is typically well documented, not much is known about the normal composition and variation of the microbiota in closed and land-based systems, the dynamics over time during operation and the correlation to fish health and mortality.

The intention of the MonMic project is to monitor structures and dynamics in the microbiota at various sampling locations of five different commercial RAS for Atlantic salmon over a 15-month period. The project aims at profiling microbiota under normal operation, but also identifying known and new pathogens. Microbiota profiling is performed using next generation sequencing (NGS) technology (16S amplicon and shot gun sequencing). This will facilitate identification of known pathogens and new indicator species, but also provides information about dynamics in biological processes. Multivariate statistical analysis will be used to interlink microbiota, chemical water quality data and fish health. Novel data integration routines are used to develop models and tools for advanced aquaculture management systems.

The first results show that microbial communities were different between the sites, and between different sampling locations (biofilm and water). This confirms that NGS provides powerful tools for systematic and detailed microbiota profiling and monitoring to reliably assess biological water quality in closed and land-based production systems for Atlantic salmon.

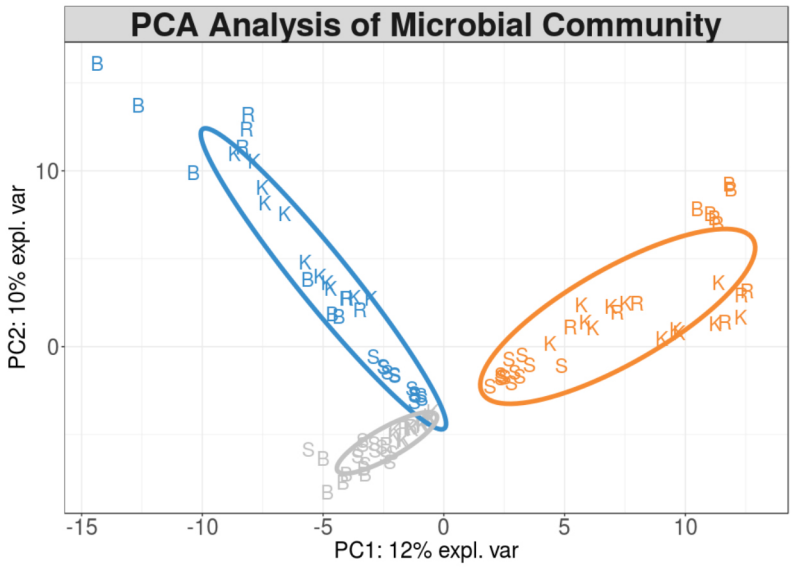

Fig. 1. PCA analysis of two first sampling dates in three different sites (blue, red and grey). with Atlantic salmon. K=tank water, R=intake water, B=biofilm biofilter, S=biofilm tank wall.

GENOMIC ESTIMATES OF DIVERSITY BETWEEN AND WITHIN FRENCH RAINBOW TROUT POPULATIONS

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Recent access to the genome sequence of rainbow trout and a medium-throughput genotyping chip of 57,501 Single Nucleotide Polymorphism (SNP) markers open up new perspectives for the design of efficient breeding programs. However, the genetic variability of farmed fish can decrease significantly due to high selection intensity, jeopardizing the long-term genetic progress as well as the adaptation capacities of populations at the onset of a change in the environment. The aim of the study was therefore to evaluate the impact of selection practices through molecular estimates of genetic diversity between or within population. The analysis of linkage disequilibrium (LD) was used to estimate effective population sizes (Ne) across the last 5-10 generations for each strain. Average inbreeding (F_{ROH}) was derived from runs of homozygosity measured in each individual within population. This work was funded by the European Maritime and Fisheries Fund (EMFF) and French Gouvernment via the national public body in charge of agriculture products and seafood FranceAgrimer (n°2015-0638).

Four commercial strains of French breeding companies, hereafter SA, SB, SC and SD, as well as two INRA experimental lines, SY and SU, were considered. The synthetic line SY was developed by intercrossing several domesticated strains during the 1980s to create a population with a large genetic variability. The line has since been managed without any selective breeding. The SY cohort from birth year 2006 was the founder generation (SY0) for a new strain (SU) selected its ability to survive and grow with a complete plant-based diet. The 5th generation of SU line was analyzed, jointly with the corresponding generation of the unselected SY line (SY5). The Axiom™ Trout Genotyping Array was used to genotype 290 females comprising 32 to 49 fish with low kinship coefficients within strain. After quality control, only 38,351 SNP were kept for the analysis of genetic diversity between strains. For the within strain analysis, we kept subsets of 31,191 to 34,724 SNP with minor allele frequency greater to 5% in each population.

Our results showed a moderate genetic differentiation between strains (Fig.1). F_{ST} values ranged from 0.02 (SY0-SU) to 0.15 (SA-SB). Within population, LD declined rapidly over the first 100 kb (mean r^2 decreased from 0.34 at 10 kb to 0.23 at 100 kb), but then stayed quite high at long distance compared to other livestock species (mean r^2 was still 0.16 at 1 Mb). Estimates of Ne in the last generation were low (< 25) for all strains. Average F_{ROH} ranged from 7% for SY0 to 8.5-12.5% for the selected strains (Fig.2). Molecular inbreeding was high compared to values in other livestock populations. Our study gave the breeding companies a better understanding of the genetic diversity of their strains to implement effective genomic breeding programs.

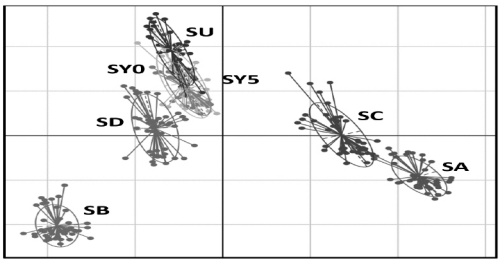

Fig 1. PCA of genetic diversity of French strains

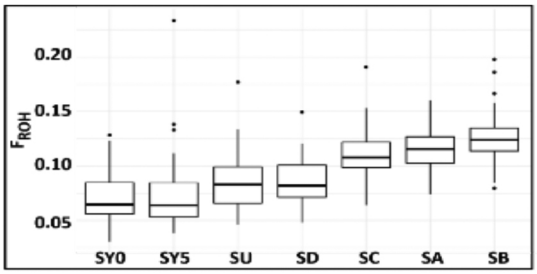

Fig 2. Average inbreeding (F_{ROH}) per strain

EFFECTS OF DIETARY SPIRULINA (*Spirulina maxima*) PECTIN NANOPARTICLES ON GASTROINTESTINAL METAGENOME OF ZEBRAFISH

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This study was conducted to investigate the effects of dietary Spirulina (*Spirulina maxima*) pectin (SP) and Spirulina pectin nanoparticles (SPNPs) on the gastrointestinal microbiota of zebrafish (*Danio rerio*) using a metagenomics approach. Zebrafish diet was prepared by adding 4% of SP or SPNPs to the commercial feed on a weight basis. After 2 weeks acclimatization, zebrafish were divided into three groups (n=30), namely control, SP, and SPNPs. Fish were fed three times per day at 4% of bodyweight. After 6 weeks feeding, zebrafish gut was collected and genomic DNA was isolated. Then 16S rRNA gene sequence based metagenomics sequencing was conducted to understand the change of microbiome profiles.

Total of 125, 130 and 111 operational taxonomic units (OTUs) were resulted for control, SP and SPNPs fed fish, respectively. Upon feeding of SP and SPNPs diet for 6 months, changes in the natural gut bacterial composition was observed. The phylum Proteobacteria and Fusobacteria were found to be the most abundant in zebrafish gut. Increased number of Fusobacteria were shown in SP (47.6%) compared to control (33.8%). Moreover, Proteobacteria was reduced in SP (36.8%) and SPNPs (38.5%) fed group compared to control (48.3%) fish. Overall results suggest that SP and SPNPs based feed supplementation may have beneficial modifications of gut microbiome to enhance the digestibility and immunity in fish.

ZEBRAFISH EMBRYOS DISPLAY EARLY HATCHING AND TRANSCRIPTIONAL UP REGULATION OF HATCHING ENZYME UPON EXPOSURE TO PECTIN ISOLATEDFROM POMELO (*Citrus maxima*) PEEL

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Pectin is non-toxic, biodegradable polysaccharide mainly consists of alpha (1-4)-linked D-polygalacturonic acid residues. Pectin shows gelling properties and it has been widely used in food and pharmaceutical industries as drug delivery agent to the gastrointestinal tract. We purified the pectin from Pomelo (*Citrus maxima*) using acid extraction method and then characterized. Extraction recovery (yield), degree of esterification, methoxyl content, equivalent weight and anhydrouronic acid % were estimated to be 14.25 %, 72.56 %, 7.82 %, 1245.56 and 68.27%, respectively. Aiming to use pomelo pectin as a health promotion agent in aquaculture, effects of pectin on zebrafish larvae were assessed on embryo toxicity and hatching rate. Moreover, effect of pectin on transcriptional responses of hatching related genes namely hatching enzyme 1 (HE1) and Krüppel-like factor 4 (KLF4) were investigated by quantitative RT- PCR and in-situ hybridization techniques. Exposure of pectin at lower concentrations (0-100 µg/mL) did not show any toxic effects in zebrafish embryos (0-120 hpf). Interestingly, pectin exposed (100 μg/mL) zebrafish embryos showed early hatching (96%) compared to control (3.3%) at 60 hpf. There were no any malformations in early hatched larvae under pectin exposure. Zebrafish HE1 exhibited significantly ($p<0.05$) higher expression of 4.3, 55.6 and 26.8-fold at 12, 24 and 48 hpf, respectively at 100 μg/mL concentration of pectin. In-situ hybridization results revealed remarkably strong signals of HE1 in pectin (50 and 100 μg/mL) exposed embryo compared to control. In addition, size of the hatching gland area was found greater in pectin exposed larvae (100 µg/mL) than that of non- exposed control larvae. KLF4 showed a slight induction (3-fold) in pectin (100 μg/mL) exposed group compared with the respective control group. Our results indicate that pomelo pectin has ability to induce the hatching of zebrafish embryos which may be associated with upregulation of HE1 and KLF4.

NOVEL SHELLFISH AQUACULTURE: EUROPEAN LOBSTER - ENVIRONMENTAL MONITORING TO INFORM REARING SUCCESS

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Sea-based on-growing of hatchery-reared European lobsters (*Homarus gammarus*) in oyster spat baskets has shown considerable promise as an extensive culture technique. Trials have established: 1) low energy requirements; 2) zero feed costs; 3) fixed unit cost of production (compared to an escalating cost against time in land-based culture), and; 4) good short-term survival and growth rates. The promise of these early trials prompted Lobster Grower, a multidisciplinary collaborative research project (led by the National Lobster Hatchery, UK - project partners University of Exeter, Westcountry Mussels of Fowey, Centre for Environment Fisheries and Aquaculture Science and Falmouth University) to develop a novel sea-based rearing container designed to overcome technical limitations and impracticalities of the oyster system. A follow on project, Lobster Grower 2, is now testing both designs on a semi-industrial scale at a UK offshore shellfish farm. Over 25,000 juveniles have been deployed to the site since 2016, while controls are reared in hatchery aquaria facilities. Data are being collected on a combination of biological, ecological, histological, pathological, environmental and oceanographic factors while considering practical, operational, engineering, regulatory, economic and social elements associated with establishing and operating a mariculture site.

On site, environmental data (water quality and hydrodynamics) are being monitored to understand suitable conditions for rearing for *Homarus gammarus*. Remote sensing data is being provided by collaborative links with ShellEye, a project applying satellite ocean colour data and biotoxin modelling to advance the creation of a tool to give aquaculture operations forewarning of potential water quality issues. Using both *in situ* and remote sensing data increases the likelihood of identifying environmental drivers affecting rearing success, monitored in this project through lobster growth, survival and health status.

Lobster Grower research is laying the foundations for expansive culture of juvenile lobsters at sea. The use of natural settlement to supply feed and the effects of an enriched culture environment on lobster development suggest that the method has considerable potential for (i) cost-effective and ecologically-conditioned on-growing to improve the effectiveness of releases to enhance capture fisheries, and (ii) the advent of sustainable aquaculture of this prized seafood species. With global seafood consumption expected to rise by 8% during the next decade, this novel approach could facilitate growth in the production of European lobster without threatening pressured natural stocks.

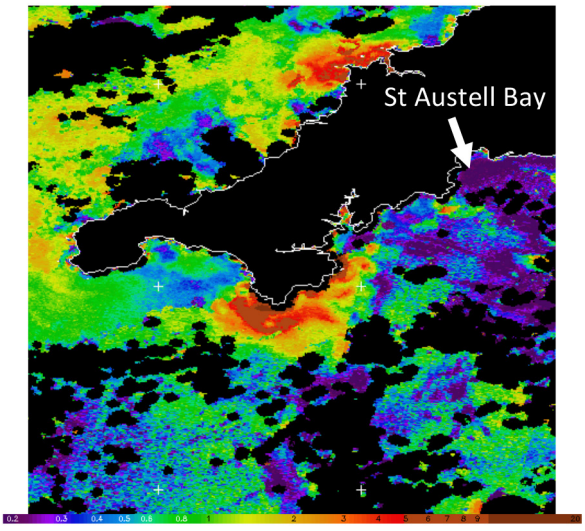

Figure 1 Satellite ocean colour observation of algal blooms off south Cornwall close to St Austell Bay lobster farm, produced by ShellEye project using latest European Sentinel-3A satellite with 300m resolution. Chlorophyll-a concentration (mg Chl m^{-3}) composite image for 27 Feb. to 05 Mar. 2018.

DESCRIPTION OF THE PROFILE OF FATTY ACIDS OF THE PATAGONIAN TOOTHFISH (*Dissostichus eleginoides* Smitt, 1898 (Perciformes: Nototheniidae): A SPECIES FOR THE DIVERSIFICATION OF THE CHILEAN AQUACULTURE AND THE SOUTHERN HEMISPHERE

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The Patagonian toothfish (*Dissostichus eleginoides*) is one of the most important resources of the industrial and artisanal fishery of Chile and the Southern Hemisphere. It is a white meat fish widely desired in international markets, which is why its natural populations show signs of depletion after 30 years of intense exploitation (Reyes *et al*., 2016). This has encouraged the state of Chile to promote research to develop a technology of cultivation within a period of 10 years. The objective of this work is to describe the composition of fatty acids in wild fish of this species, mainly in its contribution of omega 9, omega 3 and omega 6 fatty acids.

A total of 30 wild fish of different sizes were captured between September 23 and October 19, 2017 in the area of the Beagle Channel south of the Strait of Magellan between 55º and 56º South Latitude and between 70º and 68º West Longitude. Skinless muscle samples from three size ranges (7-10 kg, 11, 14-15 kg and 15-17kg) were taken immediately after capture and stored at -20ºC. The samples kept the cold chain from the capture zone until reaching the laboratory for analysis. The fatty acid composition was determined by gas chromatography.

The most abundant fatty acid is oleic acid (18: 1n-9), which represents between 33 and 36% of total fatty acids. In relation to omega 3, the concentration of EPA + DHA represents between 12 and 14%, with DHA being twice as abundant as EPA in all cases. Of the omega 6, arachidonic acid (ARA) represents between 4 and 5% of the total. No significant differences were found ($P > 0.05$) in the fatty acid profile between the different size ranges.

Samples of deep-seated muscle tissue show a fatty-acid profile of a fish with cold, deep waters and its composition does not seem to vary with the size of the fish. The high content of oleic acid (18: 1n-9), as well as the concentration of DHA (22: 6n-3), EPA (20: 5n-3) and arachidonic acid (20: 4n-6) in a species of white meat like this, they make it nutritionally attractive, which allows to account for its quality as a consumer fish and candidate for the diversification of aquaculture in Chile.

Reyes A., Kido R. & Moreno C.A. (2016) Captura y mantención de *Dissostichus eleginoides para conformar un plantel de reproductores*.Latin American Journal of Aquatic Research 40(4), 1066-1071.

GENE EXPRESSION PROFILE OF THE MAIN DIGESTIVE ENZYMES IN *Arapaima gigas* EARLY JUVENILES REARED IN A RECIRCULATION SYSTEM

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Arapaima gigas, native to the Amazon basin in South America, is one of the world's largest scaled freshwater fish species and shows the highest growth rate among Amazonian cultured fish. Primarily cultivated in Peru and Brazil, there is still little information about its biology during early development. The present study aimed to analyze the gene expression profile of the main digestive enzymes of early juveniles of *A. gigas*. The offspring were obtained from natural spawning of a pond-reared pair of *A. gigas* and transferred at 3 cm TL to 30 L tanks connected to a clear water recirculating system (n = 6; initial density 1 ind/l; 29.0 ± 0.03 ºC; 12L:12D photoperiod). Fish were sampled throughout 17 days of culture for molecular analyses and tissues were stored in RNAlater until analyzed. Results from quantitative PCR showed that the expression of amylase and trypsin was higher in the anterior intestine, followed by the liver and to a lesser extent in the middle intestine and the stomach. No expression of these genes was detected in the posterior intestine. Chymotrypsin was expressed mainly in the anterior intestine, followed by the liver, the posterior intestine, the middle intestine and the stomach. The expression of lipase was higher in the anterior intestine and the stomach, followed by the rest of the analyzed tissues. The highest level of phospholipase expression was found in the stomach and the posterior intestine, followed by the anterior intestine, the middle intestine and the liver. Pepsin was mostly expressed in the stomach, followed by the anterior intestine with a 15-fold lower level of expression. The expression of neuropeptide Y was mainly located in the posterior intestine, followed by the stomach, the middle and anterior intestine and the liver. The level of expression of amylase, trypsin, chymotrypsin and pepsin at day 1 of culture (D1) was 67, 60, 25 and 1000 times higher than at D3. From D3, the level of expression of these genes was stabilized, which suggests a response to the change in the diet when juveniles were transferred from the pond to the rearing tanks. The expression of amylase fluctuated throughout the period, being higher at D3 and D10 than at D5 and D15, while the expression of trypsin remained constant. The expression of chymotrypsin was highest at D3, followed by D10, D15 and D5. The expression of trypsin showed a similar pattern to that of chymotrypsin, although differences were not significant throughout development. The expression of lipase increased from D3 to D5 and then remained constant. The expression of pepsin increased significantly from D5 onwards. Both the expression of phospholipase and neuropeptide Y remained constant throughout the studied period. The gene expression profile of the digestive enzymes analyzed in different tissues as well as during development followed the common pattern of expression of carnivorous fish, characterized by a high level of expression of pepsin in the stomach, responsible for the acidic digestion of complex proteins. Taking into account these results, it can be concluded that the digestive system of *A. gigas* is functionally developed from D3 (3.3 cm TL), although the considerable increase in pepsin expression detected between D10 and D15 indicates an improvement in the efficiency of protein digestion during this time.

WASTE PRODUCTION IN AQUACULTURE: SOURCES, CONSTITUENTS AND MANAGEMENTS IN DIFFERENT CULTURE SYSTEMS

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Intensification of aquaculture has emerged as a viable alternative for increasing aquaculture production due to competition that arose from the use of natural resources such as land and water by other production and developmental sectors. However, intensification demands increase in the inputs such as fish and feed per unit culture area and hence, increase waste generation from the aquaculture production systems.

The impact of waste products from aquaculture has raised public concern and threatens the sustainability of aquaculture practices. The needs for increasing production of aquaculture products cannot be overemphasized, therefore there is a need to develop culture systems that will increase fish production with efficient waste management in order to limit environmental degradation resulting from aquaculture waste and hence, ensure its sustainability. This paper reviewed waste production from aquaculture, its sources, constituents and methods of management in different culture systems.

EPIGENETICS AND SEX CONTROL IN FISH FARMING

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We now know that early environmental conditions can influence an individual's future phenotype. As a mediator between environmental signals and phenotypic responses, epigenetics raises many hopes but also many questions in aquaculture, as in most agricultural production sectors. We will present the main principles regarding the Maternal & Paternal transmission of epigenetic patterns and the transgenerational epigenetic inheritance. Epigenetic mechanisms can be in the form of DNA methylation, Histone variants, chromatin remodelling and post-translational modifications with non-coding RNA. We will show from recent fish examples, how it should be quickly used and taken into account especially for sex control in aquaculture.

The first potential application of epigenetics in aquaculture is by environmental manipulation of the fry or bloodstock. It relies on studies carried out on 3 species. In sea bass, *Dicentrarchus labrax*, high temperatures applied at early stages of development induce hyper-methylation in the promoter region of the aromatase gene (*cyp19a1a*), which inhibits the expression of this gene and leads to fry masculinization (Navarro-Martin et al., 2011). In the half-smooth tongue sole, *Cynoglossus s emilaevis*, the pseudo-male testes induced by early treatment of genetic females at high temperatures exhibit methylation patterns comparable to those of genetic males, but different from those encountered in the ovaries of genetic females. The pseudomale phenotype and its methylation patterns are found in F1 resulting from crosses between pseudomales and normal females, suggesting a transgenerational effect of masculinization induced by high temperatures in this species (Shao et al., 2014). The third species studied has been the Nile tilapia where temperature treatments can induce masculinization of XX individuals resulting in higher levels of DNA methylation in both promoters and gene bodies (Sun et al., 2016).

The second approach, validated on oil content in oil palm, consists in integrating the presence or absence of epigenetic marks in epigenetic selection programmes.

Epigenetics is involved in the development and maintenance of the phenotype, including a sexual phenotype. A better understanding of epigenetic mechanisms will allow a functional annotation of genomes, but also to anticipate and probably to select (via epigenetic markers) the phenotypes induced by certain early environmental conditions. Epigenetics should be rapidly integrated into aquaculture practices and selection schemes, especially for sex control purposes.

WATER QUALITY MAPPING WITH DRONES FOR AQUACULTURE MANAGEMENT

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In outdore aquaculture systems, like ponds, nearshore or offshore aquaculture, the environment has an impact on the final yield and quality of the product. With the correct information you can monitor the environment and improve planning and management practices of the aquaculture site. Currently, in-situ instruments provide a weath of information but lack the spatial extent. This is where remote sensing can play a role by observing the area of interest from a distance. Remote sensing data includes images taken by satellite, airborne, drones or fixed cameras. This study shows the potential of drone-based camera systems for aquaculture monitoring and zooms in on a test-case of the Scheldt (Belgium) and Breskens (the Netherlands) where sediment concentration maps were derived using a low-cost RGB frame-camera on an Octocopter.

When trying to derive water quality information from drone imagery, there are some challenges that need to be considered regarding georeferencing, lens distortions and radiometric corrections. Algorithms and corrections valid over land are not necessary valid over lakes or oceans since water can have a dynamic behaviour, not allowing to use for example structure for motion techniques for georeferecing. An ideal set-up, makes use of a frame camera with an advanced GPS IMU system to enable a proper georeferencing. The frame camera has the advantage that it can monitor the dynamics in time, if the drone stays at the same spot, or obtain an overview of the site when stiching images taken at different locations.

After performing the aforementioned correction steps, it is possible to derive water quality information from the drone imagery. The test-case of Breskens and the Scheldt river illustrate that a low-cost RGB camera is adequate to obtain suspended sediment concentrations, see Figure 2. Since a lot of research has been performed on deriving water quality products (e.g. chlorphyll-a, primary production, cyanobacteria) from satellite images, this knowledge can be used to derive these products from drone imagery by selecting cameras with similar spectral characterics. Such specific camera systems that can be integrated on a drone are becoming affordable. The dynamics of the sediment concentration in the Scheldt river and the harbour of Zeebrugge monitored with a drone will be presented.

This research received funding from BELSPO Stereo III under grant agreement No SR/67/311 (DRONESED) and from the European Union's horizon 2020 research and innovation programme under grant agreement No 776480 (MONOCLE).

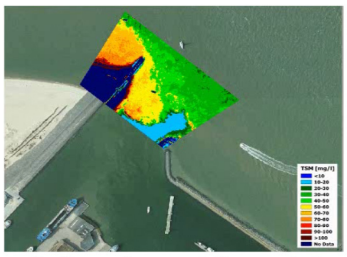

Figure 2: Georeferenced Suspended sediment map taken on 15/07/2016 in the harbour of Breskens (NL).

IN VITRO SCREENING OF PROTEIN HYDROLYSIS (DH, pH-stat) USING STAGE-SPECIFIC ENZYME EXTRACTS OF *L. vannamei* LARVAE AND POSTLARVAE

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Efficient feed formulation, processing and feeding practices are crucial for the timely delivery of accessible nutrients, and this needs to be combined with suitable absorption/utilization. In shrimp physiology, the use and incorporation of dietary dense nutrient fractions such as protein is significantly dependent on secreted digestive enzymes at simpler (larva) or "more preconditioned" (juvenile) digestive systems, with different transit times and ingestion rates.

The pH-stat assay was used as an *in vitro* method to evaluate protein digestibility of a series of ingredients used in shrimp diets, including different marine and terrestrial plant and animal derived protein sources. This assay indicates that the mysis II stage of *Litopenaeus vannamei* (MII) can digest protein sources more efficiently than the other stages (Fig. 1). Data obtained by the pH-stat method indicated that the potential to digest proteins via enzymes increases from zoea ZII to MII, followed by a decrease from MII to postlarva PL3 and a further slight decrease from PL3 to PL7. Only fish meal did not result in a decrease in DH from PL3 to PL7. Freeze-dried fish reached the highest DH, followed by squid meal and gelatin. Brewer´s yeast was highly digestible in early stages as opposed to soybean meal and wheat gluten.

The current results are expected to allow reliable and rapid screening of existing and novel ingredients for the production of highly digestible larval aquafeeds.

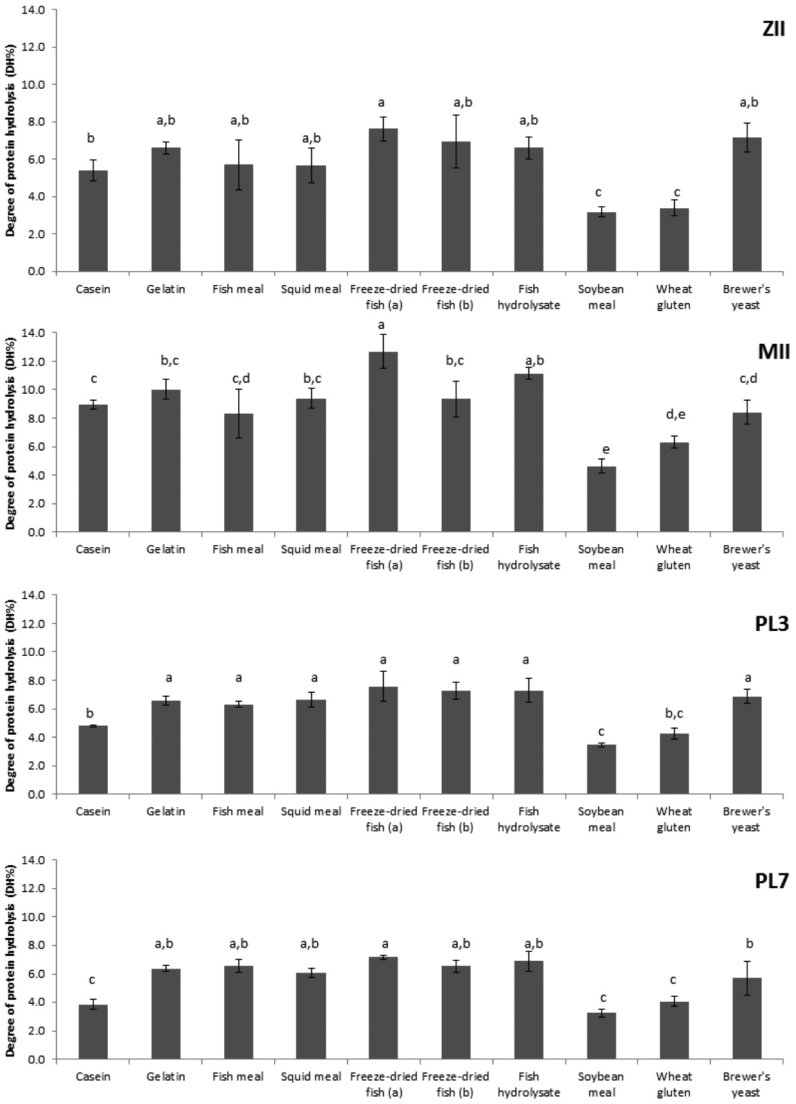

Figure 1: The *in vitro* pH-stat degree of protein hydrolysis (DH, %) of selected ingredients for the Z2, M2, PL3 and PL7 Pacific white shrimp (*Litopenaeus vannamei*). Samples were hydrolyzed for 60 min at 30 ± 1 °C. Different superscript letters indicate significant difference ($P<0.05$) between ingredients within a shrimp stage. Results shown as mean (vertical bar) and s.d. (error bar), n=4. Freeze-dried fish (a) from Thailand; (b) from Russia.

ADVANTAGES AND DISADVANTAGES OF ADDING CARBON IN INTENSIVE CULTURE OF *Litopenaeus vannamei* WITH BIOFLOCS

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Indoor shrimp farming has gained a lot of attention recently, because it creates the opportunity to supply high value fresh shrimp to the local market at premium prices. The climatic conditions in some countries makes it necessary to recycle water, even after the harvest.

CreveTec has developed a system for 100 % recycling, which is running for 3 consecutive years, using a modified biofloc system.

Bioflocs have been shown to increase shrimp growth while maintaining water quality. However, higher biofloc densities can create secondary problems, like accumulation of sludge resulting in anaerobic conditions and vibrio proliferation. Therefor, most biofloc farms include a system to remover surplus bioflocs and keep biofloc densities low.

The addition of carbon promotes biofloc growth but will at the same time increase the risk of a high biofloc density.

Therefore a trial was set up to evaluate and compare 2 strategies. 8 tanks of 400 liter were filled with culture water. 4 tanks only received feed and 4 tanks received feed + carbon in the form of sugar. The trial lasted for 4 weeks.

Results indicate that shrimp benefit from the addition of sugar, even though growth was slower. However, the biofloc density at the end of trial was higher in the tanks receiving sugar, which could lead to more water quality problems and lower survival eventually.

	Feed	Feed + sugar
Initial ABW	2,07 g	2,02 g
Final ABW	6,02 g	4,99 g
Survival	49 %	62 %
Final biomass	295 g	309 g

MEASURING INDIVIDUAL FEED EFFICIENCY AND ITS CORRELATIONS WITH PERFORMANCES AND AGONISTIC BEHAVIOURS IN JUVENILE NILE TILAPIA *Oreochromis niloticus* REARED IN GROUPS

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Estimating individual feed intake of fish held in groups has long been a challenge precluding precise knowledge of the individual variation in feed efficiency (FE).

In this study, we measured individual feed conversion ratio (FCR) of 100 juvenile Nile tilapia *Oreochromis niloticus* held in groups and estimated the correlations between FCR, performance and agonistic behavioural traits.

For this purpose, fish were video-recorded to estimate the occurrence of agonistic behaviour during a fasting and a refeeding period, occurring just before the individual feed intake (FI) measurement. To measure FI, we counted the number of feed pellets eaten by each individual tilapia using video recordings made over a period of 10 days where fish were fed twice daily.

Accumulated measures of FI over 11 meals were found to achieve 95% repeatability and a highly accurate FI estimate. Thus, with this approach, we can accurately investigate FI and FCR in fish.

During the FI measurement period, average fish growth was 12.0 ± 3.6 g and FCR was 0.86 ± 0.20. FCR was negatively correlated with body weight gain (BWG, r = -0.62 ± 0.06) but not with FI (r = -0.02 ± 0.10). Conversely, residual feed intake (RFI) was positively correlated with FI (r = 0.60 ± 0.06) but not with BWG (r = -0.03 ± 0.10). These findings highlight the complex relationships between the main feed efficiency traits, FCR and RFI.

Fish were 58% less aggressive during the fasting period compared to the refeeding period, but generally, an aggressive fish during the fasting period was also aggressive during the refeeding period.

The correlations between agonistic behaviour and growth and feed conversion ratio were low, and in most cases not significant. We conclude that the hierarchical rank and agonistic behaviour should not affect Nile tilapia growth and feed conversion ratio. Consequently, a dominant fish should not necessarily be more efficient than a subordinate fish.

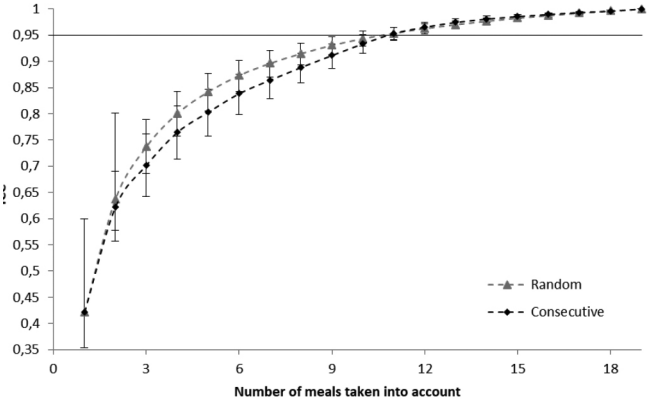

Figure 1- Estimation of the intra-class correlation (ICC) between FI (19 meals) and x consecutive (in black) or randomly chosen (in grey) meals (from 1 to 19).

RESISTANCE TO *Yersinia ruckeri* IN RAINBOW TROUT: QTL DETECTION AND GENETIC LINKS WITH STRESS RESPONSE

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Enteric red mouth disease (ERM), caused by the bacterium *Yersinia ruckeri,* is a major fish disease. In rainbow trout (*Oncorhynchus mykiss*), efficient vaccines have been developed but emerging resistant bacterial variants have been described. A few studies have investigated the genetic bases of resistance to ERM. Moderate values of heritability have been recorded (Henryon et al, 2005), and selection for resistance could be a promising alternative to manage the disease. In this study we detected quantitative trait loci (QTL) for resistance. The QTL families were previously used in the Aquafirst EU project to detect QTL for cortisol response to confinement (Quillet *et al*. 2014). Comparison of the QTL found in the two experiments offered insights into the possible genetic relationships between immune capacities and response to environmental stressors.

The experimental work was carried out under the UK Animals (Scientific procedures) Act 1986, Project Licences no. 40/2600 and 60/3333 F0 grand-parents belonged to two *O. mykiss* lines selected for divergent plasma cortisol responsiveness to confinement (high *vs* low responsive, Pottinger *et al*., 1999). Five F2 full sib QTL families were produced by single-pair mating of F1 parents. At 11 months, 215 fish per family were individually tagged and fin sampled for further DNA analysis. Individual plasma cortisol levels after a standardized confinement stress were measured in two successive trials four and five months later (see Quillet *et al,* 2014). When animals were 21 months old, 781 fish were injected with a pathogenic strain (MT3072) of *Y. ruckeri* (0.5x106 cfu per fish in phosphate buffer saline; 0.5ml per fish). Response to infection was assessed 48 h post injection using the relative spleen weight (SW) and the spleen bacterial burden (BB) measured by qPCR. All fish were genotyped for 268 genetic markers (mostly microsatellites). QTL detection was performed using QTLMap software after adjustment for fixed effects. Unitrait and multitraits analyses were performed, in a first step for resistance traits only (SW, BB) and then combining resistance traits and cortisol response.

Eight unitraits QTLs were identified on distinct chromosomes for resistance traits. There was no common QTL for BB and SW. One more QTL was found on another chromosome after a joint analysis (SW and BB). Several genes that may play a role in the antibacterial response and inflammatory response were found in QTL confidence intervals and could be further tested.

Thirteen QTL had been identified previously for response to confinement. Only four were common with resistance QTL. Multitrait analyses combining resistance traits and cortisol response were carried out to refine the comparison. Overall, twelve QTLs were found, with only three being common to cortisol response and resistance traits. Altogether, these results suggest that antibacterial response and response to confinement share limited genetic control.

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Pottinger TG, Carrick TR, 1999. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. *Gen Comp Endocrinol* 116: 122-132.

RESTORING EUROPEAN FLAT OYSTER *Ostrea edulis* BEDS IN BELGIAN WATERS: A FEASIBILITY STUDY

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European flat oyster Ostrea edulis beds used to be highly abundant in the Southern North Sea, ranging from Norway to Germany, France and the UK, including Belgian waters. Due to overexploitation, most oyster beds were destroyed and *O. edulis* populations were brought to near extinction, by the end of the 19th century. Nowadays, only a small number of relict populations are known in France, the Netherlands and the UK. Because of the major autogenic and allogenic bioengineering capacities and hence the important ecological value of oyster beds, interest in their restoration is ever growing. This project aimed at investigating the feasibility of oyster bed restoration in the Belgian part of the North Sea and developing possible restoration scenarios.

After collating a wide knowledge base on oyster ecology and restoration we analysed the environmental constraints for *O. edulis* as to be able to identify areas suitable for oyster bed restoration in Belgian waters. We also assessed the connectivity between relict and restored beds to evaluate the long-term viability of possible restoration measures. All information was integrated to develop restoration scenarios that were scored for feasibility and sustainability.

Full-coverage data on the distribution of substrate, currents and suspended particulate matter allowed delineating areas suitable for oyster settlement and growth. Connectivity modelling identified areas that possibly still receive substantial larval influxes from the oyster relict populations in the English Channel. Two dimensions were identified along which restoration scenarios could be adjusted, introduction of oysters and modulating habitat condition. We deducted 20 scenarios, which were eventually weighted based on five criteria: time to successful restoration, respecting existing biodiversity, active promotion of biodiversity, restoration cost and maintenance cost.

We conclude that oyster bed restoration is still feasible in Belgian waters. We identified the gravels in the Belgian fisheries management zones (inside a Special Area of Conservation) and in the planned offshore wind farm zones to offer opportunities for oyster bed restoration.

MR.GOODFISH, A SUSTAINABLE SEAFOOD CONSUMPTION PROGRAMME

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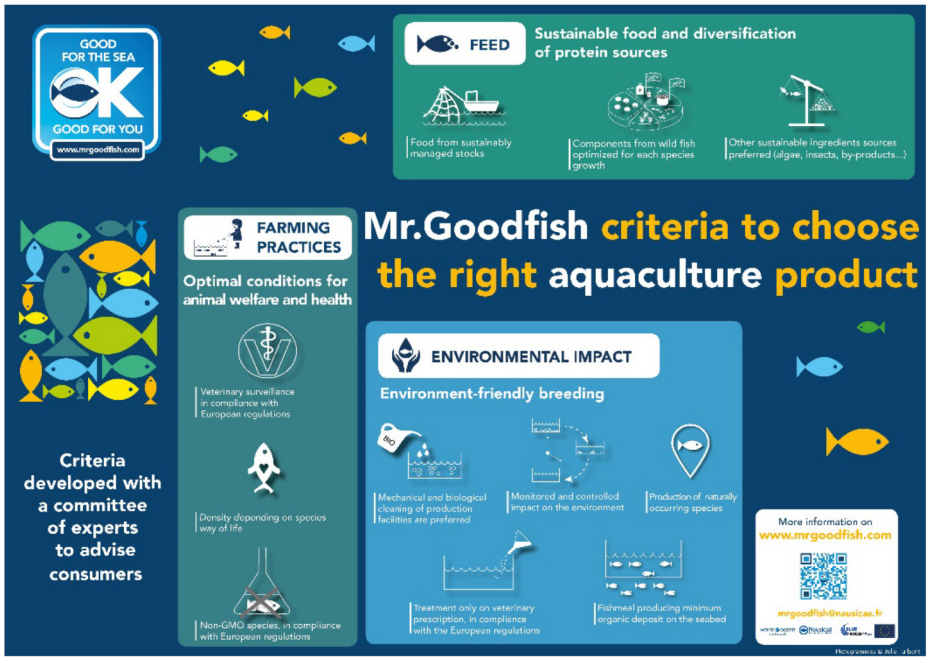
The Mr.Goodfish programme has been launched in France, Italy, and Spain in 2010. It aims to better manage seafood resources and to secure the sustainability of seafood stocks, by helping consumers to choose their seafood smartly. It conveys a positive message based on “choose the right fish”.

The Mr.Goodfish criteria for wild seafood products are: right season, right size, from healthy stock. The programme addresses the chain of custody as a whole, from fishermen, fishmongers, restaurants to school canteens and consumers. All of them are considered as possible vectors to disseminate the message to a larger audience through their own local networks. Every three months, a committee of experts meet to establish sustainable seafood lists that are widely published.

Since 2016, Mr.Goodfish has been working on aquaculture recommendations. It endorses an exclusively positive approach and promotes the aquaculture and all sustainable fish production process to encourage new consumption patterns. A committee of fish farmers, scientists, representatives of the seafood distribution sector, restaurant owners and association of consumers meet annually to establish a list of aquaculture species recommendations through easily understandable criteria for the public. By reassuring consumers, this list will help fish farmers to develop sustainable aquaculture in Europe.

Mr.Goodfish has received strong media attention including 770,000 web pages read on www.mrgoodfish.com, 531 newspaper articles, 136 TV/radio shows and Seafood Summit Nomination. The programme is expanding rapidly in Europe with new partnerships and members: aquariums, scientific institutions, professional organizations, etc.



Picture: Poster to general public about Mr.Goodfish’s aquaculture criteria

DISCOVERY OF GENES PROMOTING GROWTH OF *Aeromonas salmonicida* IN ATLANTIC SALMON MUCUS

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Aeromonas salmonicida is the pathogen responsible for furunculosis in salmonid and non-salmonid species, including Atlantic salmon (*Salmo salar*). Disease can manifest at any stage of the *S. salar* life cycle, with outbreaks compromising fish welfare and reducing farm productivity. Therefore, deciphering the molecular mechanisms underlying bacterial pathogenicity and virulence is of great interest. Infection by *A. salmonicida* occurs by various means, including via direct contact with the fish skin. Thus, to initiate infection *A. salmonicida* must be capable of surviving and growing in fish skin mucus, which is the first line of the host immune defences. Therefore, the objective of this present study was to identify genes facilitating survival and growth of *A. salmonicida* in *S. salar* skin mucus.

A *mariner*-based transposon mutant library was generated for the pathogenic *A. salmonicida* Hooke isolate. This library contained mutants where the transposon had inserted at random at a single site in the bacterial genome to knock out the function of just a single gene. Each transposon mutant was screened for growth in the presence of mucus that had been collected from the surface of *S. salar* skin. As the *A. salmonicida* Hooke parent isolate shows enhanced growth in this mucus, transposon mutants lacking this phenotype would be expected to contain a transposon insertion in a gene with a role in this growth enhancement in this milieu.

In total, 5% of transposon mutants screened for enhanced growth in mucus were confirmed to lack this phenotype. Subsequently, the transposon insertion site (and thus the gene disrupted) was determined for each of eight mutants by arbitrary polymerase chain reaction (PCR), sequencing of the PCR products and mapping back to the reference genome. Transposon insertion sites identified included an unnamed gene producing a hydrolase that functions in nutrient assimilation from lipid-rich environments. In another mutant, the transposon had inserted into the gene encoding the ABC-type transporter protein *abcA*, which is involved in the correct functioning of the well-characterised A-layer protein virulence factor.

This present study furthers our understanding of *A. salmonicida* virulence and may assist with the development of new approaches to reduce the burden of furunculosis outbreaks in aquaculture.

MASTERS-LEVEL SKILLS AND ATTRIBUTES VALUED BY EMPLOYERS IN BLUE BIOTECHNOLOGY AND AQUACULTURE

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Blue biotechnology and aquaculture are global growth industries in the blue economy with an increasing requirement for a highly skilled workforce. In recognition of this, and as an outcome of the *Blue Growth Strategy* launched by the European Commission, the Executive Agency for Small and Medium-sized Enterprises (EASME) launched the *Blue Careers in Europe* call as part of the European Maritime and Fisheries Fund Work Programme 2016 to develop educational programmes that address the skills needs of people already employed in or aiming to embark on a career in the blue economy. Prior to the development and introduction of a new *Applied Blue Biotechnology* Masters II programme at the University of La Rochelle, a survey of skills gaps perceived by blue biotechnology and aquaculture employers was performed by the *Blue Biotechnology Master for a Blue Career* consortium to inform programme structure and content.

An online *Google* survey form was designed and distributed to blue biotechnology and aquaculture enterprises across Europe (available at https://bit.ly/2HvqOn0). Data collected included the name, location, number of employees and the main focus of each company (e.g., food, energy, human health and wellbeing, environment, life science products, aquaculture). Participants were asked to complete the form as if they were intending to recruit a newly qualified technical member of staff with masters-level training. In the first section of the form, participants were asked to rank each of five broad skills and attributes for relative importance (least to most). In a second section, participants were asked to provide an opinion for how important each of a series of more specific skills and attributes was (divided into 'general' and 'scientific' or technical skills) on a scale from 'Not needed' to 'Desirable' to 'Highly desirable' to 'Essential'. A free text box allowed participants to state any other skills or attributes they felt would be important if they were about to employ a masters-level worker.

The survey revealed that the most important of five broad skills and attributes for a new masters-level employee were 'Project management skills', which was defined as the 'ability to plan and run a project on time and budget', followed by the 'ability to perform key methodologies and learn new techniques' (i.e., 'Technical competencies'). Amongst the most important more specific skills were ability to: 'use simple software packages', 'acquire new skills, knowledge and techniques', 'communicate effectively with colleagues', and 'work as part of a multidisciplinary team'. Notably, knowledge of 'experimental design and research methodologies' was also highly valued.

The structure and content of the *Applied Blue Biotechnology* Masters II programme was designed to incorporate the new insights provided by the relevant employers. The programme includes 6 months working at a blue biotechnology or aquaculture enterprise to develop key employability skills and to apply knowledge and skills obtained during a 4-month period of teaching to an industrially-relevant research project.

BACTERIOSTATIC ACTIVITY OF PHYTOGENIC FLAVONOIDS AND BENEFICIAL EFFECTS ON GROWTH AND SURVIVAL OF RED NILE TILAPIA DURING SEX REVERSAL

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Numerous studies have shown that polyphenol based feed additives, especially those rich in flavonoids, have beneficial effects on animals and humans. These plant extracts are known to have anti-bacterial properties and to improve the immune response of the animal as well as their health and performance. In critical phases of the production cycle, these positive effects are of particularly high importance. The sex reversal in tilapia farming is such a challenging period, during which farmers are especially concerned about survival of fry. Nevertheless, this is common practice as the use of exclusively male tilapia in the grow-out stages leads to a better output in terms of volume and thus to a higher profitability. The reason is that male tilapia are more energy efficient and grow bigger and faster compared to their female counterparts.

In this study a phytogenic feed additive (Anta®Ox by Dr. Eckel Animal Nutrition, Germany) was supplied to red tilapia during sex reversal in hapas in PE-lined ponds. The objective was to test whether this polyphenol and flavonoid based additive leads to beneficial effects on growth and survival of fry. Six hapas (5 m^2 each) were set up in a double PE-lined pond and stocked with 30,000 red tilapia swim-up fry. The animals were fed a fishmeal-based diet containing 60 ppm 17-α-methyltestosterone (MT) over 21 days. The feed allocated per day was divided into five feeds and fed at 8:00, 9:30, 11:30, 13:30 and 16:30 by broadcasting the powdered feed over the water surface. The test diet was prepared by mixing the product Anta®Ox (dosage 0.6 kg/Mt) into the fishmeal followed by addition of the ethanol/MT solution and thorough mixing in a blender-type mixer. Aeration was provided in each pond by 12 homemade diffusers consisting of PVC pipe and material. After 15 days the 5 m^2 hapas were replaced with 10 m^2 ones to supply more space for growth.

The addition of Anta®Ox resulted in a significantly higher survival rate. Total harvest of fish per hapa was enhanced by almost 1/3 from 22,712 individuals in the control to 30,000 in the treatment group. The outcome clearly shows the potential of this additive for the improvement of animal health and profitability.

One reason for the benefits is the bacteriostatic activity of the product against common bacteria in aquaculture (i.e. *Vibrio spp*.). This was demonstrated by a parallel study whose results suggest using Anta®Ox for the control of AHPND in the field.

TABLE 1. Performance results. Means in a row with different letters were significantly different (P<0.05)

	Diet	
	Control	Anta®Ox
No. Harvested	22,712a	30,000b
Survival (%)	76a	100b
FCR	2.85	2.49
SGR (%bw/day)	9.6	10.2

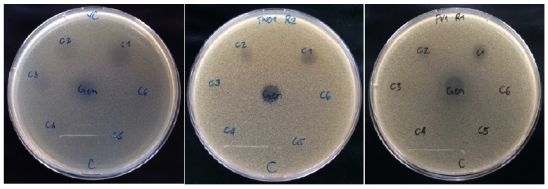

Figure 1 Antibacterial activity against three pathogens (agar diffusion).

NEW INSIGHTS ABOUT LIPID MODULATION ON PACIFIC WHITE SHRIMP FED DIFFERENT COMBINATIONS OF BROWN SEAWEEDS AND THEIR INVOLVEMENT IN WHITE SPOT DISEASE RESISTANCE

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In previous study, shrimp fed 4% of *Undaria pinnatifida* dry biomass showed an overall improvement of hemato-immunological parameters, lower *Vibrio* spp. count in the gut and higher survival after White Spot Disease (WSD) challenge (52% when compared to 28% of control group). On the other hand, the lowest level of *Sargassum filipendula* (0.5%) improved significantly shrimp gut physiology and their survival after thermal shock, while had a moderate effect on shrimp survival (44%) against WSD challenge. Thus, this study aimed to evaluate the combined effect of *U. pinnatifida* and *S. filipendula* on Pacific white shrimp resistance to WSSV challenge and bring new insights about the lipid modulation in shrimp hemocytes after challenge.

Shrimp (11.0 ± 0.2 g) were reared during 21 days in 400 L tanks (50 shrimps/tank), under constant aeration and temperature (±29 °C), 100% of daily water exchange and feeding four times day^{-1}. Experimental diets had different combinations levels of both seaweeds: 0.5%/1%, 0.5%/2% and 0.5%:4% of *S. filipendula* (S) and *U. pinnatifida* (U), respectively. Control group were fed the same diets without seaweed addition. At 22nd, shrimp were challenged with WSSV and kept for 96h to estimate the mortality. Hemocyte sampling were performed before and 24h after viral challenge, and the MALDI-TOF MS lipid analysis were done in positive and negative ion mode. MALDI MS/MS LIFT were performed for lipid identification, and http://www.lipidmaps.org database were used to confirm the assignment of lipid species.

Shrimp from 0.5S/2U and 0.5S/4U treatments showed significantly higher survival after 96 h of WSSV challenge (72 and 69%, respectively), when compared to 0.5S/1U and control (31 and 21%, respectively). After WSSV challenge, we identified some common aspects in lipid modulation inside the hemocytes of shrimp from 0.5S/2U and 0.5S/4U treatments, which were significantly different from 0.5S/1U and control. It included the increase of signal intensity of sulfatides (SphHex), lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), lysophosphatidylserines (LPS) and a lysophosphatidylinositol (LPI), allied with the decrease of a hexosylceramide (HexCer) and most of phosphatidylcholines (PC) and sphingosine-1-phosphates (S1P). In addition, these treatments showed an upregulation of two specific PC (PC 33:1 and 35:4), that were downregulated in 0.5S/1U and control. Therefore, our results suggest a connection between some lipid-associated cell mechanisms, such as early-induced cell death, lower supply of membrane lipids for viral assembly and immune signaling and stimulation with the higher survival of shrimp from 0.5S/2U and 0.5S/4U after WSSV challenge.

USING MARKET SYSTEMS APPROACHES TO LEVERAGE PRIVATE SECTOR AQUACULTURE INVESTMENT IN BANGLADESH

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Aquaculture production in Bangladesh reached around 2 million tons in 2015, forming a key source of animal protein and micronutrients and making Bangladesh the fifth largest global aquaculture producer of fish (FAO 2015). Domestic demand continues to exceed supply but in such a densely populated country, no more land is available for expansion, placing the priority on sustainable intensification. With USAID support, WorldFish implemented the Aquaculture for Income and Nutrition (AIN) project in south-west Bangladesh from 2012 to 2017. The project increased production, profits for smallholder aquaculture producers and access to fish for consumers as well as improving the quality of feeds and the development of nutrition-sensitive aquaculture approaches. Meanwhile, a series of projects in Bangladesh pioneered the use of market systems approaches (MSA) starting with Katalyst (2002-2017). These aimed to provide alternatives to 'direct delivery' projects where interventions often discontinue after the project life-time. Market systems projects facilitate market change by identifying and addressing the underlying issues causing poor performance in the market system, stimulating co-investment in sustainable solutions by the private sector and improving their ability to influence the enabling environment. Variations on this principle have emerged including Making Markets work for the Poor (M4P) and Inclusive Market Systems and since 2013 have been increasingly adopted by USAID such that MSA has become the cornerstone of its economic development strategy.

After USAID initiated a process to develop a MSA-based follow-up to the AIN project, the Feed the Future Bangladesh Aquaculture and Nutrition Activity (FTF-BANA) was awarded to WorldFish in February 2018. This is a five year, $24.5 million investment by USAID that aims to achieve inclusive aquaculture sector growth through a MSA. Specific objectives are to increase the productivity of aquaculture systems, strengthen aquaculture market systems and expand the adoption of nutrition-related behaviour. FTF-BANA launched calls for proposals in July 2018 from the private sector and local NGOs to address its critical intervention areas. Successful proposals will be supported through grants, initially relatively small and of short duration but larger and of longer duration when responsive development partners have been identified. One of the main features of successful market systems projects is to be demand-driven and responsive to market needs rather than setting pre-determined plans. This presents challenges, particularly for the project monitoring, evaluation and learning team. FTF-BANA is by far the largest donor investment in aquaculture using a market systems approach and as such, its progress is being keenly followed. This paper provides information on the objectives, structure and initial experiences while further updates and outputs will be made available through the WorldFish and USAID Bangladesh web-sites.

FACTORS EXPLAINING ENTRY OF ESCAPED FARM SALMON IN NORWEGIAN RIVERS AND GENETIC INTROGRESSION INTO WILD ATLANTIC SALMON POPULATIONS

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First, we looked at the relationship between the amount of escaped farmed salmon in Norwegian salmon rivers and local salmon farming activity, while accounting for other factors that can affect the probability of escaped farmed salmon entering a given river. Our results show that low farming activity near the river mouth gives wild salmon populations some protection against escaped farm salmon entering the rivers, although several studies show that escapees can migrate far from their farm of origin. We also found that rivers with a large water discharge attracts more escaped farm salmon; so do rivers with large populations of wild salmon spawners. Dense populations of wild salmon are also positive by diluting the significance of escapees, so that the proportion of escaped farm salmon among all spawners becomes smaller in rivers with large wild salmon populations.

It is not necessarily the presence of escaped farm salmon on the spawning grounds that is critical for wild salmon populations, but rather to what extent the escapees succeed in reproducing and crossbreeding with the wild population. Earlier studies have shown that a larger proportion of escaped farm salmon in a wild population results in a higher genetic introgression, but this relationship is not always that strong. The same proportion of escapees among spawners can lead to a large genetic introgression in one wild salmon population and a much smaller introgression in another. Our models show that the genetic introgression from escaped farm salmon into wild salmon populations (Figure 1) correlates positively with local farming activity. The models also show that large wild salmon populations have less introgression, and that water discharge and salmon management initiatives, like cultivation and liming of acidified rivers, also affect the genetic introgression from escaped farm salmon into wild salmon spawning populations.

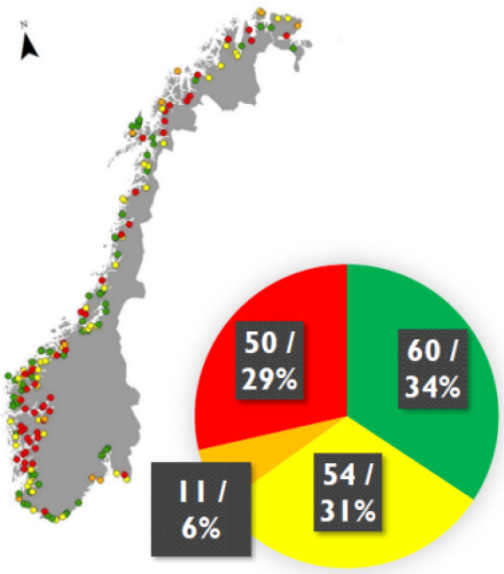

Figure 1. Genetic introgression has been estimated for 175 Norwegian wild salmon populations (Diserud et al. 2017). We observed no genetic change in 60 populations (green), a weak genetic change was indicated in 54 populations (yellow), a moderate genetic change was shown in 11 populations (orange), and a large genetic change was found in 50 populations (red).

EFFECT OF EGG STOCKING DENSITY ON GREATER AMBERJACK *Seriola dumerili* LARVAL PERFORMANCE

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Greater amberjack *Seriola dumerili* (Risso, 1810) is considered as a leading candidate for marine finfish diversification to enhance the European aquaculture due to its rapid growth, high demand and market price (Thompson *et al*., 1999), and excellent flesh quality (Nakada, 2000). Nevertheless, one of the major constraint on its culture development is larval rearing and the production of a sufficient number of fry (Papandroulakis *et al*., 2005). For the successful culture of any species, larval rearing is considered as the most critical step (Dhert *et al*., 1998) and, thus, greater amberjack larviculture protocol has to be improved to maximise the egg stocking density in order to achieve suitable larvae production.

Therefore, the present study aimed at examining the effects of different egg stocking densities on growth, larval survival and skeletal deformities of greater amberjack.

The eggs used in this experiment were obtained from natural spawning, from Grupo de Investigación en Acuicultura (GIA, ULPGC). Three different egg stocking densities were evaluated (25, 50 and 75 eggs/l) in triplicate tanks of 2000-l. Tanks were filled with filtered and UV-treated seawater. Larval rearing was conducted until 30 days post-hatching (dph), following the protocol of GIA, under a natural photoperiod, with a salinity of 37‰, average temperature at 24±0.2ºC and dissolved oxygen ranged between 7.0 and 8.0 mg.l^{-1}. Greenwater technique was used during the stage of live prey feeding. Rotifers and Artemia enriched with Ori-Green™ (Skretting, Spain) were progressively substituted with Gemma (Skretting, France) microdiets.

Larval growth was assessed in 30 larvae per tank sampled each 5 days of life. Growth was determined by measuring dry weight and total length using a profile projector (Mitutoyo, PJ-A3000, Japan). Larval survival was determined at the end of the trial (30 dph) counting all the remaining live larvae in each tank. To detect skeleton anomalies, a sample of 50 larvae at 30 dph was taken and processed, following the methodology of Vandewalle *et al*. (1998), modifed for *S. dumerili*. To describe the possible skeletal anomalies, the classification proposed by Boglione *et al*. (2001) has been followed.

A significantly higher growth, at 30 dph, in terms of total length and dry weight, was observed in larvae reared at 75eggs/l. This could be due to the massive mortality at 3 dph in the 75eggs/l tanks, and consequently reduction of feeding competition. In terms of survival rates, significant differences ($P<0.05$) were observed among all treatments being higher in 25eggs/l tanks and lower in 75eggs/l tanks. A significantly higher incidence of severe deformities in 75 eggs/l tanks. Most of these deformities affected the haemal and caudal zone. No significant differences between 25 eggs/l and 50 eggs/l treatments.

Table I. Growth, larval survival and severe deformities incidence of larvae from different treatments.

	Egg density (eggs/l)		
	25	**50**	**75**
Total length of larvae (mm)	12.00±2.05^{b}	11.88 ± 1.79^{b}	17.43±4.18^{a}
Dry weight of larvae (mg)	4.23±1.61^{b}	4.20±1.34^{b}	16.37±10.49^{a}
Larval survival (%)	11.25±4.92^{a}	6.74±0.65^{b}	1.06±0.06^{c}
Severe deformities (%)	15.33±6.42^{b}	17.33±9.45^{ab}	27.33±6.42^{a}

PACIFIC WHITE SHRIMP AND NILE TILAPIA INTEGRATION IN BIOFLOC SYSTEM UNDER DIFFERENT FISH-STOCKING DENSITIES

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This study aimed to evaluate the effect of different stocking densities of *Oreochromis niloticus* integrated with *Litopenaeus vannamei* reared in a biofloc system.

The performances of both species and the ecological efficient of the system were evaluated. Four levels of tilapia stocking density were evaluated: 0, 8, 16 and 24 fish per tank. The initial weight was 4.8 ± 0.1 g and 9.6 ± 0.1 g for shrimp and fish, respectively. The shrimp were fed according the feed table, and the fish were fed with 1% of the fish biomass, stimulating the tilapia to seek food in the biofloc. Results show no difference between average shrimp and fish weight and survival (Table 1). Total yield was higher based on the increase in fish density. The sludge produced per animal biomass (sludge:biomass rate) decreased as fish density increased ($y = - 0.0083x + 0.5995$ $r^2 = 0.87$). Nitrogen recovery rose linearly with the increase in fish density ($y = 0.036x + 0.2725$, $r^2 = 0.84$), as did phosphorus recovery ($y = 0.00711x + 0.1395$, $r^2 = 0.91$). Nitrogen and phosporou recovery were 27.9% and 223%, respectively, higher in the treatment with the higher fish stoking density in comparison to shrimp monoculture.

These results demonstrate the feasibility of increasing yield up to 31% by integrating *L. vannamei* and *O. niloticus* in a biofloc system. Also, the decrease in the sludge:biomass rate and the resultant increase in nitrogen and phosphorus retention made the cultivation of *L. vannamei* in a biofloc system more sustainable.

Table 1: *L. vannamei* and *O. niloticus* performance in an integrated biofloc culture system for 57 days.

	T0	T8	T16	T24	p ANOVA
Shrimp performance					
Mean final weight (g)	15.0 ± 0.2	14.9 ± 0.4	14.8 ± 1.1	14.9 ± 0.6	0.8354
Survival (%)	92.4 ± 1.7	93.6 ± 1.6	96.1 ± 8.8	92.7 ± 5.9	0.9313
FCR	1.8 ± 0.0	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	0.0680
Growth rate (g week $^{-1}$)	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	0.8730
Final biomass (kg)	3.1 ± 0.0	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	0.2895
Yield (kg m^{-3})	3.9 ± 0.0	3.9 ± 0.2	4.0 ± 0.1	3.9 ± 0.1	0.2895
Tilapia performance					
Mean final weight (g)	-	66.4 ± 11.7	59.7 ± 9.7	59.6 ± 6.7	0.5432
Survival (%)	-	84.5 ± 18.5	89.0 ± 3.1	100 ± 0.0	0.0826*
FCR	-	0.21 ± 0.1	0.24 ± 0.0	0.22 ± 0.0	0.4724*
SGR (% day $^{-1}$)	-	3.5 ± 0.4	3.2 ± 0.1	3.2 ± 0.2	0.3288
Final biomass (kg)	-	0.44 ± 0.1^{c}	0.67 ± 0.1^{b}	1.4 ± 0.1^{a}	0.0000
Yield (kg m^{-3})	-	4.9 ± 1.1^{c}	7.5 ± 1.1^{b}	15.9 ± 1.6^{a}	0.0000
Shrimp plus tilapia					
Total final biomass (kg)	3.1 ± 0.0^{d}	3.6 ± 0.2^{c}	4.0 ± 0.1^{b}	4.5 ± 0.1^{a}	0.0000
Total yield (kg m^{-3})	3.5 ± 0.0^{d}	4.1 ± 0.2^{c}	4.6 ± 0.1^{b}	5.1 ± 0.2^{a}	0.0000

Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the Tukey test ($p < 0.05$).* P value by the Kruskal-Wallis test.

PROBIOTIC BACTERIA MODULATION IN INTESTINE OF IN SOUTH AMERICAN CATFISH (*Rhamdia quelem*)

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The aim of this study was to evaluate the interaction of two probiotic strains *Lactococcus lactis* (autochthonous) and *Lactobacillus plantarum* (allochthonous) and the microbial community in *R. quelen* intestinal mucosa.

A total of 180 jundiás with 8.54 g of average weight were distributed homogeneously in 12 experimental units of 80 L (15 fish per tank), with constant aeration and heating system. The treatments were performed in quadruplicate and consisted of fish receiving supplemented diet with: (1) *Lactococcus lactis at* $1x10^7$ CFU.ml^{-1}, (2) *Lactobacillus plantarum* $4x10^7$ CFU.ml^{-1} and (3) non-supplemented diet. The diet was provided 4 times a day (6% total body biomass) during a total of 60 days. The water quality was monitored daily using multiparameter (model HI 9828 - Hanna Instruments) and colorimetric kits (Labcon Test). After the experiment, 6 fish from each treatment were anesthetized and terminated by concussion of the brain. A portion of the posterior was aseptically excised and the mucosa (intestinal tissue) was sampled and washed twice with PBS buffer. DNA was extracted using the protocol described by Falcinelli et al. (2015). DNA from all samples was used as template for PCRs and high-throughput sequencing (HTS) analysis was performed according Gajardo et al. (2016) after purification of the PCR products. Sequences were concatenated and sorted by sequence similarity into a single FASTA file, denoised and analyzed using QIIME 1.8.0 (Caporaso et al., 2010).

The water quality parameters were appropriate standards for the cultivation of the species: dissolved oxygen 8.00 ± 0,75 $mg.L^{-1}$, pH 7.2 ± 0.18; temperature 25.91 ± 1.40 °C; total ammonia 0.38 ± 0.28 $mg.L^{-1}$; toxic ammonia 0.005 ± 0.008 $mg.L^{-1}$ and alkalinity 34.00 ± 2.83 mg $CaCO3.L^{-1}$. HTS results reveal that relative abundance of *Cetobacterium* genus was high in all treatments (Figure 1A) and comprised 81.38%, 89.35% and 97.76% in control, allochthonous and autochthonous fed fish respectively. However, PCoA analysis reveals differences between treatments (Figure 1B).

This is the first report about intestinal microbiota modulation after HTS analysis in the intestine of jundiá after probiotic feeding. According Romero et al. (2014) bacteria from genus *Cetobacterium* is commonly found in fresh water fish intestine. Strains of *Cetobacterium somerae* are related with vitamin B_{12} production and peptides fermentation, highlighting its nutritional importance for carnivorous fish. The modulation of both probiotic bacteria was attested by in PCoA analysis. However, no difference in bacterial abundance was statically observed. The fact that both probiotic strains were not identified during HTS analysis lead to a conclusion that probiotics were not able to colonise fish intestinal mucosa. Nevertheless, HTS analysis is a new tool that should be best standardised for fish and aquatic environments. Further studies should be performed to evaluate if higher doses of these strains could improve colonization performance and higher abundance in mucosal tissue in *R. quelen* after probiotic feeding.

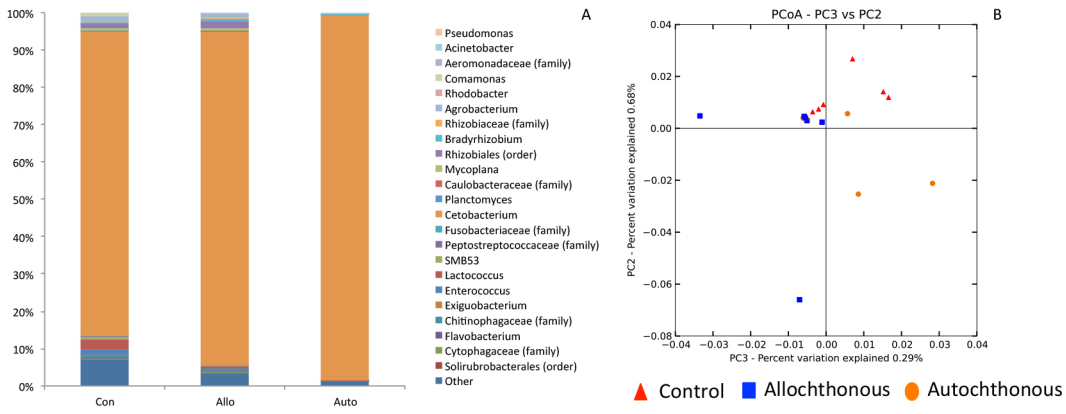

Figure 1: (A) Intestinal mucosa abundance of jundiá (*Rhamdia quelen*) after probiotic feeding: *Lactococcus lactis* (Auto) and *Lactobacillus plantarum* (Allo) and no-probiotic feeding (Con). (B) Principal Coordinated Analysis (PCoA).

EVIDENCE FOR HORIZONTAL TRANSFER OF pVA1-LIKE PLASMIDS FROM AHPND-CAUSING *Vibrio*

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Acute hepatopancreatic necrosis disease (AHPND) is a severe shrimp disease, impacting aquaculture production, originally shown to be caused by virulent strains of *Vibrio parahaemolyticus* (Vp_{AHPND})-harboured pVA1 plasmid. Whilst cases of AHPND caused by *Vibrio* species including *V. harveyi*, *V. campbellii*, and *V. owensii* other than *V. parahaemolyticus* have been reported, it is unclear the formation mechanism of diversity in AHPND-causing *Vibrio* pathogens. Here we showed that all of the virulent strains of *Vibrio* bacteria harbor the pVA1-like plasmids. Conjugation experiments indicated that pVA1-like plasmid could transfer from *V. parahaemolyticus* to *V. campbellii*. Horizontal transfer of pVA1-like plasmid in vivo showed that pVA1-like plasmid could transfer from *V. campbellii* to *V. owensii*. Moreover, type IV secretion system (T4SS), which is involved in conjugation, was found in all of the pVA1-like plasmids. This study provides timely information for explaining the diversity of AHPND-causing *Vibrio* bacteria and will form the basis of management strategies leading to the prevention and control of AHPND.

MANUFACTURE OF "FRESH / WET" AQUACULTURE FEEDS BASED ON BY-CATCHES, FISH BY-PRODUCTS AND ALGAE

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This work focuses on available bio resources transformation such as by-catches or fish by-products and marine plants (seaweed and microalgae) as raw material for short supply chain fish feed processing. The objective is to make this production as autonomous and sustainable as possible and fitted to the idea of circular economy. Thanks to previous projects, it seems that by-catches could be a plentiful source of proteins for fish. The challenge associated with this type of aquaculture formulations relies on the ability to incorporate abundant resources readily available and the significant reduction of certain energy-consuming steps (such as drying) in order to minimize the environmental impact of a fish feed production. The adequacy of this strategy (fresh raw material and wet feed) with a specification of a nutritional efficient fish feed is the first goal of this project.

Lot of studies indicate the potential benefits of a wet diet for fish (better feed conversion ratio, better palatability, increased survival). Otherwise, several studies suggested that seaweed and microalgae could be incorporated to fish diet with potential benefits such as antioxidant properties and promotion of immune responses.

Regarding the process, an extrusion technology has been selected. It allows the assembly and functionalization of various ingredients and the manufacture of feeds with specific buoyancy, good stability in water, various shapes and sizes. Highly digestible feed for fish culture, can be produced by extrusion from various raw materials. The central idea of this work is to exploit the cold gelling potential of the fresh fishes by a shear mixing phase obtained by the rotation of the screws of the extruder followed by a baking step.

The study of parameters allowing extrusion process control, the availability and possible assembly of the different raw materials according to nutritional requirements of targeted specie, the physical behavior of the feed (water stability, buoyancy) and its bioavailability (palatability, digestibility) as well as feed preservation and safety will be fully explored and exploited.

Economic and environmental analysis is also one of the pillar of this project. Indeed, the impact, both economically and environmentally to produce "fresh/wet" aquaculture feed made by transforming through extrusion unwanted catches and plants with a specification of an efficient aquaculture feed constitute the challenge to be met

Early trials indicate that textured fresh and wet feed can be made from fish muscles associated with algae by extrusion. Gelling of the fish is sufficient to obtain a feed of good texture.

INTEGRATED AQUACULTURE: AN ECO-INNOVATIVE SOLUTION TO FOSTER SUSTAINABILITY IN THE ATLANTIC AREA

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Despite being a key driver of the maritime economy in the European Atlantic Area, Atlantic aquaculture faces significant challenges: environmental management at farming sites, the European north-south divide, regulatory hurdles, long licensing processes, low levels of consumer knowledge and acceptance, competition for space with other maritime activities, etc. INTEGRATE www.integrate-imta.eu contributes to overcoming these challenges by facilitating the transition towards commercial Atlantic Integrated Multi-Trophic Aquaculture (IMTA), thus unlocking green growth within the European aquaculture sector.

INTEGRATE tackles the development of Atlantic IMTA through a wide range of actions. The work plan addresses the capitalisation of existing and new knowledge of IMTA (WP 3), the definition of Atlantic IMTA best practices (WP 4), environmental performance of Atlantic IMTA systems (WP 5) and the definition of a mid and long-term Atlantic IMTA strategy (WP 6). Dissemination of project outputs and outreach to end-users and the wider public are key to the project's success (WP 2). Specific INTEGRATE actions include the following:

Support of meaningful cooperation between academia, the corporate sector and relevant authorities through online discussion groups, thematic workshops, expert roundtables and open access to the project's pilot IMTA facilities:
Pilot 1: Testing new IMTA technologies attached to Atlantic cage farming (value added organic and inorganic extractive components, including the development of IMTA's benthic component).
Pilot 2: Near-shore eco-friendly IMTA developments attached to Atlantic oyster farming (Porphyra-oyster associations).
Pilot 3: Development of an IMTA eco-friendly standard model for Atlantic land-based semi-extensive aquaculture.

Assessment of national regulatory frameworks governing the development of commercial IMTA in the Atlantic Area.

Ecopath modelling, life cycle assessment and Emergy accounting of Atlantic IMTA pilots.
Direct consultation with aquaculture producers and key stakeholders to identify specific regional levers and hurdles in order to define the INTEGRATE strategy for the development of commercial Atlantic IMTA.

A knowledge compilation of IMTA in the Atlantic Area will be made available in the shape of best practice guidelines.

The INTEGRATE partnership comprises eight organisations from across the European Atlantic Area: CTAQUA (Spain), Irish Seaweed Consultancy (Ireland), Agrocampus Ouest (France), Scottish Association for Marine Science (United Kingdom), Portuguese Institute for Sea and Atmosphere (Portugal), National University of Ireland Galway (Ireland), ALGAplus Lda. (Portugal) and the Centre for Study and Promotion of Algae (France).

PRODUCTION OF A HIGH-PROTEIN INGREDIENT FROM WOOD: ARBIOM SYLPRO® IS A NUTRITIONAL, ECONOMICAL, TRACEABLE AND SUSTAINABLE ALTERNATIVE PROTEIN SOURCE FOR USE IN AQUAFEEDS

Amelie Drouault, Partnership Manager*
Ricardo Ekmay PhD, Animal Nutrition Director
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Arbiom produces a high-protein yeast product for use in aquaculture and animal feeds with proprietary processing technology that converts wood into highly fermentable hydrolysates which then serve as growth medium for single cell protein (SCP). Arbiom offers the next generation of technology for global aquaculture and livestock industries, with science-backed performance across several dimensions of a novel protein ingredient: nutritional value, economic value, traceability value, and sustainability value.

Arbiom SylPro® is a high-protein ingredient (60%+ crude protein) with enhanced amino acid content, particularly lysine, methionine, and threonine, high digestibility (92%), and rich beta glucan content. SylPro therefore offers additional gut health benefits, along with price stability and a completely traceable production process from forest to feed.

The product specifications of SylPro® are such that it retains the flexibility to be used by multiple species, starting with carnivorous fish. SylPro® is a proprietary strain of torula yeast that is devoid of endogenous allergens that are common in milk, egg, wheat, and soy proteins. SylPro® (enhanced torula) is already a globally approved feed ingredient/additive, therefore has a shorter route to commercial adoption than competing novel proteins that face regulatory hurdles.

Arbiom has developed the bioconversion processing technology to produce SylPro with the goal of creating maximum value from wood - a renewable, non-food, readily available biomass feedstock. Arbiom uses wood to produce protein because it is natural, not genetically modified and available in the volume required to match or exceed the supply of substrates from petroleum and food material.

Arbiom's wood to food process integrates proprietary and off-the-shelf technologies in an economical, waste-free process. Arbiom's technology encompasses the following stages researched and optimized over the course of Arbiom's development:

- Materials (wood sourcing from biomass suppliers)
- Biomass Pre-treatment
- Enzymatic Hydrolysis
- Fermentation
- Downstream processing

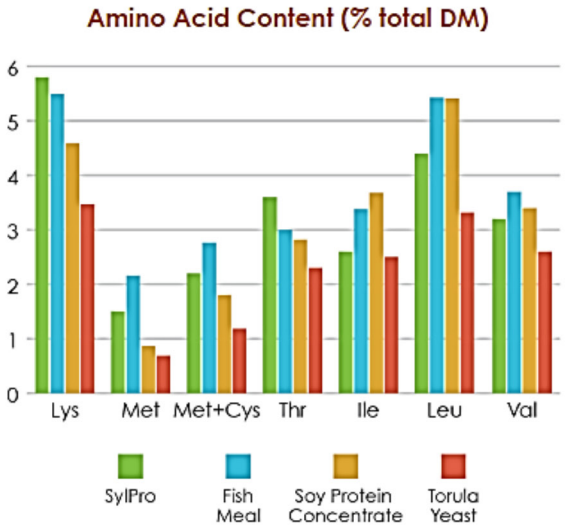

Figure 1. Comparison of target amino acid profile of SylPro with other protein concentrates on a total dry matter basis. Digestible amino acid content will be available for salmon, hybrid striped bass, and weanling pigs.

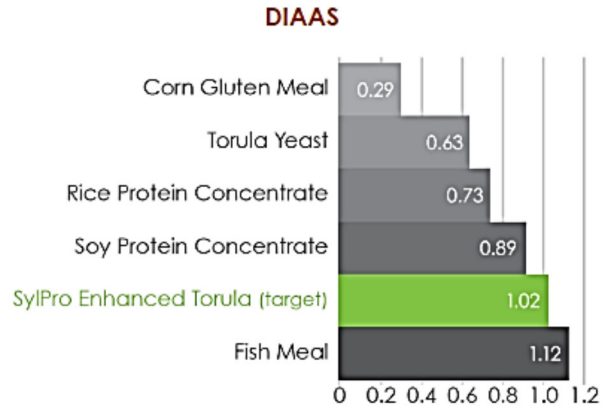

Figure 2. Digestible indispensable amino acid score (DIAAS) of common high protein feedstuffs. SylPro Enhanced Torula target value based on 92% digestibility.

THE USE OF MICRO- AND MACROALGAE AS NUTRACEUTICALS, WITH SPECIAL EMPHASIS ON NEUROPROTECTIVE PROPERTIES OF ALGAL METABOLITES - PROJECT OUTLINE

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Micro- and macroalgae are rich in health promoting ingredients. Thus in many parts of the world, macro- and micro-algae are used as a dietary supplement with health-promoting effects. So far, the health-promoting effects of certain substance classes such as vitamins, polyunsaturated fatty acids and antioxidants have been the scientific focal point of attention.

More recently, it was shown, that micro- and macro-algae-derived ingredients may also have neuro-protective properties. Based on this background, the University of Bremen (Marine Chemistry and Neurobiochemistry) together with the AWI recently started a new research-project (RecAl), in close cooperation with two algae-producing companies (MIAL and CRM). The main objective of RecAl is to screen algal ingredients as scavengers for various noxious chemical agents. Here we will present the outline and first results of this project.

The present project consists of four Work-packages (WP):

WP 1 aims to identify interesting macro- and micro-algae, which have chemical compounds with a high potential for neuro-protective application (AWI, MIAL and CRM). In **WP 2**, we test their cultivation abilities and optimize culture conditions regarding growth rates and production of the target substances (e.g. Fig. 1).

In **WP 3,** the effects of algal-products are screened on brain cell cultures (Fig. 2, Neurobiochemistry, Univ. Bremen).

Based on the data obtained in WP 3, the Marine Chemistry Group (Univ. Bremen) will chemically characterize the most important bioactive ingredients (**WP 4**).

Here we show first results of the project and discuss the potential of the application of algal nutraceuticals to mitigate adverse effects on neuronal structure and function.

Figure 01: Cultivation of Saccharina lactissima in the Baltic (left, CRM) and Nannochloropsis limnetica (right, AWI)

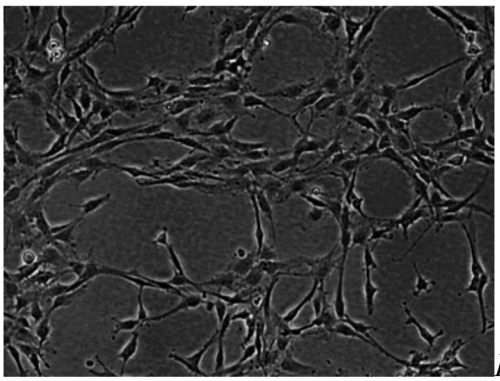

Figure 02: C6 glioma cells after 24 hours of cultivation (Phase contrast, Univ. Bremen)

THE INFLUENCE OF SURROUNDING SPECIES ON THE DISEASE RISK OF THE PACIFIC OYSTER *Crassostrea gigas*

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Disease risk in marine ecosystems rely on complex host-pathogen-environment interactions. In this context, abiotic factors such as temperature, salinity and acidification are already known as drivers of disease emergence. However, the effect of biotic factors such as biodiversity have received little attention so far.

Our study focus on the pacific oyster *Crassostrea gigas* and the ostreid herpes virus OsHV-1 which currently affects early stages and causes important mortalities. Our hypothesis is that surrounding living communities influence disease risk in oysters. More precisely, we hypothesize that non-susceptible filter feeders reduce disease risk of oysters by removing viral particles from the seawater (Burge *et al*., 2016). To test this hypothesis, a viral suspension was injected in specific pathogen-free oysters (SPF) and the surrounding seawater was used as source of infection (SI) in the laboratory. The SI transited into tanks containing filter feeders (or empty control) before being distributed in tanks containing recipient SPF. The recipients were either acclimated for cohabitation during 2 weeks with the tested species (acclimated) or directly added to the tank alongside with the SI (not acclimated). The tested filter feeders were the blue mussel (*Mytilus edulis*), the flat oyster (*Ostrea edulis*), the pacific oyster (adult and spat *C. gigas*), and the white tunicate (*Phallusia mamillata*).

Filter-feeders improved the survival of acclimated oysters only (Figure 1). Therefore, filter-feeders bring protection to the SPF oysters but they have not removed viral particles from the seawater. There may have been a microbiota sharing between the filter-feeders placed upstream and the SPF oysters. We will now compare the microbiota of acclimated/not acclimated oysters. Also, we will test the effect of non-filtering organisms on oyster mortality.

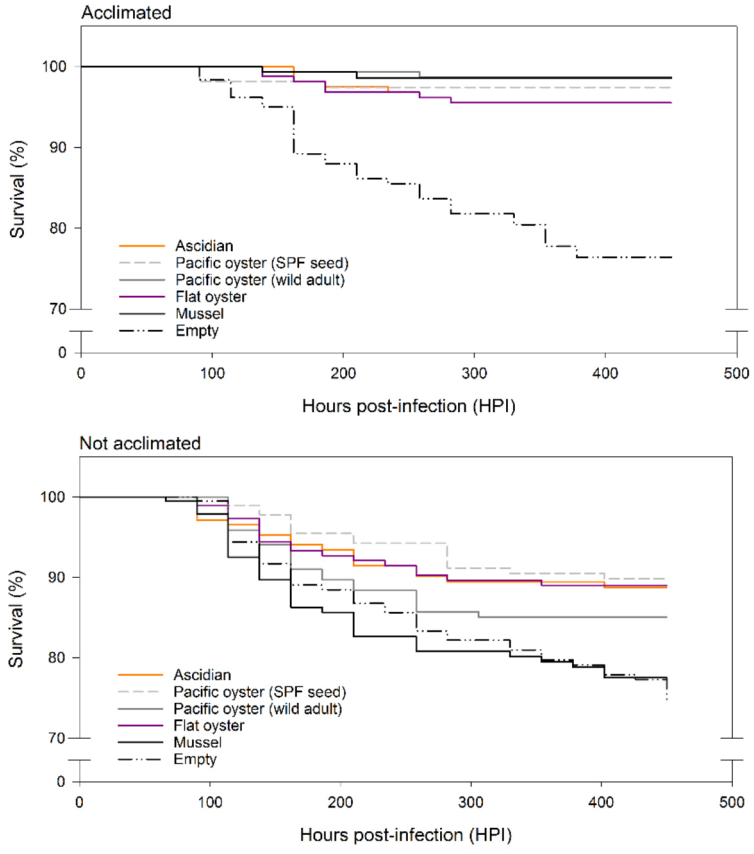

Figure 1. Survival curves of oysters exposed to OsHV-1 as a function of the placed upstream filter-feeders presence and acclimation.

TEMPERATURE-MEDIATED PLASTICITY IN GROWTH, SPRINT SWIMMING PERFORMANCE, AND BODY SHAPE IN RAINBOW TROUT *Oncorhynchus mykiss*

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While salmonids have high economic value as aquaculture species, their narrow thermal tolerance currently limits their utility in much of the world. Additionally, deeper-bodied salmonids result in less wastage and higher yield per fish, due to reduced loss from the fillet process. Considerable evidence suggests that salmonid populations vary in their response to temperature of phenotypic traits, including overall body shape. This likely influences several other traits with strong fitness implications in wild populations, particularly sprint swimming speed, vital for prey capture, predator evasion, and migration. Our research uses two clonal strains of Rainbow Trout (*Oncorhynchus mykiss)* from both cold-adapted and warm-adapted populations to understand how temperature mediates phenotypic plasticity in growth, body shape, and sprint swimming speed.

Clones for the study are produced using androgenesis, a method of propagation resulting in all-paternal line clones. The process involves irradiating an egg with gamma radiation to destroy the maternal DNA, then allowing a normal sperm to fertilize the egg. The then-haploid egg is subjected to heat or pressure shock interrupt mitosis after replication, thereby producing doubled-haploid individuals develop as fully-homozygous diploids. Fish were individually housed at 165 days post-fertilization at 10° C and 18° C, and fed *ad libitum* with commercial fish food pellets with a 10/14 dark-light cycle. Data collection occurred every two weeks beginning at 179 days post-fertilization, consisting of laser-arrayed swim tunnel sprints, body measurements, and digital photos for shape analysis.

Unexpectedly, the cold-adapted Swanson (AK origin) strain exhibiting significantly greater growth at both temperatures than the cold-adapted Whale Rock (CA origin) strain (Figure 1). Initial PCA has also shown differential response of body shape to temperature in several other clonal strains, including Swanson (Figure 2). These results indicate underlying genetic mechanisms for temperature response, and future QTL word will elucidate loci involved in phenotypic responses to temperature in rainbow trout., informing genomic selection for improved aquaculture utility and yield for the species and salomonids globally.

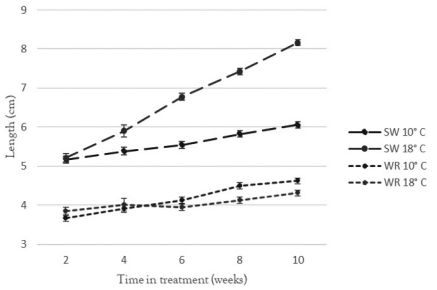

Figure 1. Length in response to rearing temperature in Swanson (AK) and Whale Rock (CA) rainbow trout strains at 10° C & 18°

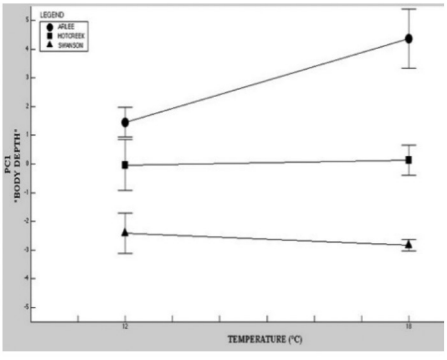

Figure 2. Preliminary principal component analysis showing variation among clonal rainbow trout lines in body shape at different rearing temperatures.

RAINBOW TROUT (*Oncorhynchus Mykiss*) FRESHWATER RECIRCULATING AQUACULTURE SYSTEM: NUTRIENT FLOW MANAGEMENT WITH AQUAPONICS

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Nutrient accumulation, solid or dissolved, in recirculating aquaculture systems (RAS) are a concern for the optimal rearing of fish. Since 2009, the research deployed at PEIMA on a semi-industrial rainbow trout outdoor RAS has focused on the study of nutrient flows inside RAS systems. To improve our control of nutrient flows, an aquaponic module was added and mass balance experiments of 50 days were carried out. The focus was to follow quantitatively total suspended solids, nitrogen and phosphorus. These experiments were conducted to determine the efficiency of specific treatment loops on nutrient flows and their suitability to RAS systems.

A main concern in RAS is the accumulation of total suspended solids (TSS) in the system. A treatment loop was implemented with a rotofilter with a 60μm mesh, a radial flow settler, a flocculation/filtration unit and a succession of vertical and horizontal flow filters of limestone gravel. This loop made it possible to go from 100mg/L in the backwash water of the rotofilter to <2mg/L of TSS in the outflow water of horizontal filters. Thus increasing the quality of recirculating water and reducing the release of TSS in the environment.

Another concern is the accumulation of dissolved components such as nitrogen and phosphorus. Much harder to extract than the solids components, it involved a treatment loop with a combination of biological transformation and extraction through an aquaponic system. Nutrient rich water coming from the fish was sent to a hydroponic system, which extracted the dissolved nutrients (see figure). The cleaned water was then sent back to the fish.

Overall the TSS treatment loop was able to minimize the impact of fish production on the environment and the effect of plants on dissolved components was minimal but it may be enhanced by working on the g of feed/m^2/day ratio and the variety used : in this case, *Lactuca Sativa*. Furthermore the perspective of a combination of RAS technology, extensive epuration technics such as vertical and horizontal flow filters and hydroponics may be the solution to reduce the impact of landbased fish production.

Funding: This study was carried out within the APIVA program funded by the European Union and CASDAR (Compte d'Affectation Spéciale pour le Développment Agricole et Rural).

Inputs	Total Nitrogen	Total Phosphorus
- Feed	100%	100%
Outputs		
-Fish	38%	39%
-Salads	3%	4%
-Sludge	3%	13%
-Media Filters	5%	0%
-Effluent	41%	39%
-Loss	10%	5%

EXCIPIENTS IN AQUACULTURE MEDICATION: SURVEY, MECHANISMS OF ACTIONS AND PERSPECTIVES

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Aquaculture requires to pursue its sustainable development of modern farming management and health practices. Intensification of fish farming and concentration of animals leads to the development of diseases resulting in severe outbreaks with high mortality rates. This is a threat for the farmers that have unstable and uncontrolled revenues, as well as for the population with respect to the quality and safety of the meat.

The development of new drugs or antigens have a lengthy development process and traditional pharmaceutical actives are commonly used to answer to urgent needs. A way to improve the efficacy of various drugs is the formulation with excipients. Excipients have no pharmacological effect but can modulate the role of active pharmaceutical ingredients (antibiotics, antiparasitics, antigens, hormones). It is thus possible to improve and control the delivery of pharmaceutical ingredients to the animals, while using less product, and improving handling safety. In this survey of commercially available medication for the aquafarming industry, we will present and explain the roles of different excipients (table 1).

Perspectives of new, modern delivery systems, their modes of actions and the advantage they can bring to the global health status of the farmed fish will be presented.

EXCIPIENTS	Chemical description	ROLE	Commercial product	Possible evolution
Polysorbates	Non ionic surfactants esters of fatty acids and sugar with PEG	Solubilisers, dispersants, stabilisers	Anaesthetics, antibiotics Antiparasitic	Biodegradable vegetable surfactants
Oil adjuvants for vaccines	Pharmaceutical oils and surfactants	Form an emulsion to give a depot effect	Bacterial injectable vaccines	Multivalents vaccines, safer formulations,
Solvants	Ethanol	Induce a fine dispersion when diluted in water or sprayed on feed	Anaesthetics, hormones	New safer vectors for easy handling
Matrix	Methyl ethyl cellulose	Sustained release for the oral route	Oral vaccine	Encapsulation, gastroresistant vectors, taste masking
Vegetable gum	Prosopis alba exudate	excipient for improving fish oil stability in alginate–chitosan beads	Oral drug administration	Simplified micro encapsulation

Table 1 : commercial aqua medications and their excipients.

A reliable and sustainable protection along the fish life cycle can only be obtained through vaccination. Nevertheless, vaccination of newly hatched, fingerlings or breeders require tailor made formulations. Microemulsions, water in oil emulsions and gastroresistant matrixes are essential excipient tools for modern vaccination programs.

HEALTH AND ECONOMIC BENEFITS OF COPPER ALLOY MESH (CAM) USE IN AQUACULTURE

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Biofouling is a major problem in marine aquaculture with significant production impacts. Nets made from nylon are susceptible to biofouling and require frequent maintenance with high costs. The use of copper alloy mesh (CAM) in aquaculture brings a promising solution to this problem. In the present study, that was conducted for the first time in Greece, we demonstrate the comparative results between fish's microbial flora, length, weight and mortality being cultured in conventional nylon net cage and those being raised in copper alloy UR30® net cage. In addition, we estimated major financial parameters such as operational costs and production profits in each case. Thus, we approached the study from an overall point of view.

As part of the research process, two fish cages were used in Kefalonia Fisheries where the species *Sparus aurata* (Linnaeus, 1758) (sea bream) was cultured. The cage A-249 was made of copper UR30® mesh whereas the cage B-228 was constructed of nylon mesh. Seven samplings of 10 random fish from each cage took place between December 2014 and November 2015. No biofouling was detected in the copper alloy cage in contrast to the nylon cage. So, the fish health is promoted, and the costs associated with net cleaning are decreasing. The fish from the CAM were of greater length and weight compared to those raised in the nylon cage. This potentially can reduce the harvest time, leading to multiple economic benefits for aquaculture. The mortality of fish was greater in the nylon net cage. The microbiological examination showed that the microbiological flora (*Aeromonas* spp, *Vibrio* spp, *Pantoea* spp., *Brevudimonas* spp., *Sphingomonas* spp., etc.) of fish from both cages ranged in normal levels with higher values in those being raised in the nylon net cage (Figure 1).

The preliminary results showed, that in the long term, profits from CAM use outweigh their initial relatively high installation cost. Therefore, the use of CAM can contribute to a sustainable aquaculture.

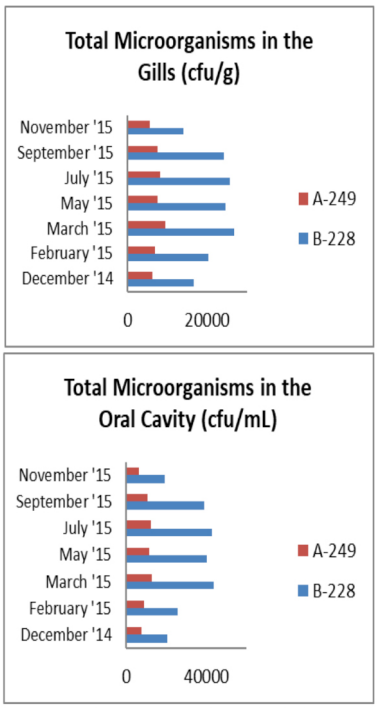

Figure 1 The microbiological flora of fish from nylon cage (B-228) and from copper cage (A-249).

EFFECT OF DIETARY POLYUNSATURATED FATTY ACIDS ON *Pinctada margaritifera* LARVAL GROWTH

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This study assessed the effects of partially supplementation of the larval diet with emulsions of PUFAs on *Pinctada margaritifera* D-shape and umbo larvae. PUFAs emulsions improved the survival and growth of D-shape and umbo larvae when compared with those that kept unfed, however, had no benefit when fed as a supplementary diet with flagellate *Isochrysis galbana* (T. Iso.)

In other experiments, correlation between microalgae composition and growth of *P. margaritifera* larvae, when was fed mono, binary and ternary combination of microalgae, were studied. Results have shown that single-species diet of TISO is sufficient to provide maximal growth performance of D-shape larvae and there was no statistically demonstrated benefit in using the various binary and ternary algal diets tested. Use of single-algal diets during hatchery production could greatly facilitate routine management. However, maximum growth of umbo larvae was not attained by any mono and binary combination of microalgae and required ternary combination of flagellate T. Iso and diatoms *Chaetoceros muelleri* and *C. calcitrans*. Results of this study indicated a positive correlation between majority of 18 C fatty acids (18:1n-9, 18:2n-6, 18:3n-3 and 18:4n-3) with D-shape larvae growth. All of these mono unsaturated fatty acids (MFAs) and poly unsaturated fatty acids (PUFAs) with 18 C had greater contents in flagellate T. Iso compared to diatoms *C. muelleri* and *C. calcitrans*. Correlations between the nutritional components of the microalgae and larval growth were determined. These results will assist to better understanding of nutritional requirements of *P. margaritifera* larvae and will contribute to the improvement of feeding strategy of this species.

EFFECT OF FEEDING RATE ON SURVIVAL, GROWTH PERFORMANCE AND FEED UTILIZATION OF GILTHEAD SEA BREAM *Sparus urata LARVAE DURING THE WEANING PERIOD*

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This study was carried out in the Marine Governmental Hatchery of the General Authority of Fish Resources Development (GAFRD), in Alexandria EGYPT in cooperation with the Faculty of Agriculture (Saba Basha) Alexandria University. The work aimed to study the effect of feeding rates (FR) in several experiments on survival, growth performance and feed utilization of gilthead sea bream (G. sea bream; *Sparus urata*) larvae during the weaning period. Fertilized eggs were collected from the spawning tanks and kept at an initial density of 50 egg / L (50000 egg / M^3) in 4 M^3 circle tanks filled with 3.6 M^3 salt water. The hatched larvae were fed live food (Rotifer and Artemia) started from 3 DPH according to the hatchery feeding schedule. Micro algae started at 3 DPH, rotifer (*Brachionus plicatilis*) were introduced to the larvae rearing water at the rate of 5 individual/ml and increased gradually to 15 at 22 DPH then decreased to 0 at 33 DPH. Artemia were also added to feed larvae started at 20 DPH using the rate of 0.2 individual/ml increased to 1.6 at 40 DPH and decreased to 0 after the larvae were arrived at the age of 55 DPH. Four weaning experiments were designed in 18 plastic aquaria using the micro weaning diet of El-Dahhar, 2016 to feed G. sea bream larvae at 6 feeding rates (10, 20, 30, 40, 50 and 60 % of the larval BW in four periods. Fourteen days were used for each period of the four experiments. An experiment was designed for each period; the 1st 20-34 DPH, 2nd 30-44, 3rd 40-54 and 4th 50-64 DPH. In each experiment larvae were fed several times by the amount adjusted for each experiment depending on the larval initial body weight. Survival, FBW, SGR, offered feed and growth performance were calculated.

Survival rate of the larvae increased with increasing FR (Figure1). In the 1st period survival % increased significantly from 23 ± 1.96 % to 48.1 ± 4.1 % with increasing FR from 10 to 60 % of the larval BW daily ($P < 0.01$). Also, in the 2nd period the increase of survival was found significant from 43.8 ± 2.1% to 74.1 ± 0.9% when FR increased from 10 to 40 % of BW ($P < 0.01$). Then it decreased insignificantly with increasing FR above 40% of BW ($P > 0.05$). In the 3rd and the 4th periods survival % increased with increasing FR to 40 % of BW without a significant effect ($P > 0.05$). Then it decreased significantly when FR increased to 60 % of BW. The same trend was happened with FBW. In the 1st period FBW did not affect with increasing FR. The FBW ranged from 3.6 to 48 mg / larvae without any significant effect of FR ($P > 0.05$). From the 2nd period increasing the amount of feed introduced to the larvae by increasing FR from 10 to 40% of BW increased FBW significantly from 5.6 ± 0.3 to 9.5 ± 0.1 mg ($P < 0.01$). Also, FBW in the 3rd period increased significantly from 13.85 ± 0.4 to 19.85 ± 0.1 mg when FR increased from 10 to 50 % of BW ($P < 0.01$). The same trend was found in the 4th periods. It was found to increase from 58.66 ± 3.3 to 152.37 ± 8.6 mg with increasing FR from 10 to 60 % of BW ($P < 0.01$). The larval gain and SGR have the same increase as FBW with increasing FR from 10 to 60 % of BW daily.

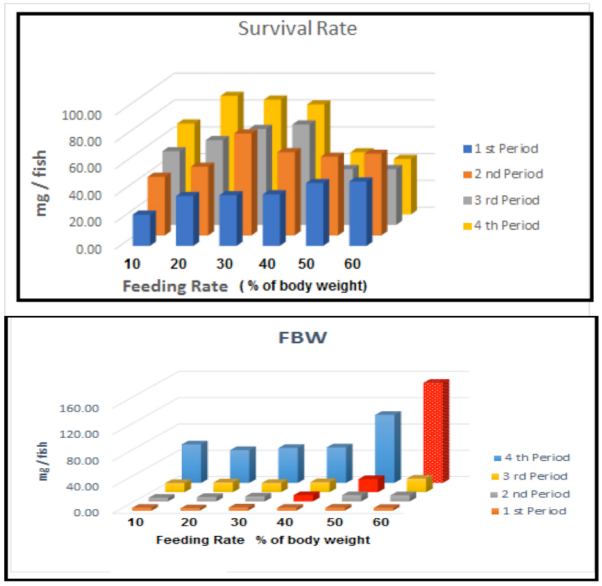

Figure 1: ***Effect of feeding rate (FR) in different periods on survival % and final body weight (FBW) of G. sea bream; Sparus urata larvae during the weaning period***

In the conclusion survival rate increased with increasing FR with amount could increase hatcheries profitability. Also, FBW and the other growth performance increased too much with increasing FR. With this concept FR should be adjusted with the larval ability to eat for best survival and growth performance.

THE USE OF ENVIRONMENTAL ENRICHMENT TO REDUCE FIN DAMAGE IN FARMED ATLANTIC SALMON *Salmo salar*

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Growing public demands for aquaculture sustainability and environmental concerns have led to an increased interest in fish welfare. Fin damage is a well-known problem in many species of farmed fish, detrimentally affecting fish health and farm productivity. In Atlantic salmon (*Salmo salar*) damage to the dorsal fin is the most common form of external injury and is perceived as a welfare issue. Fish fins are innervated living tissue and therefore fin damage can potentially cause pain. It also provides a route of entry for infections and affect swimming ability. Damaged fins are perceived as occurring in a poor welfare environment therefore has potential as a non-invasive operational welfare indicator (OWI) under practical farming conditions. The causes of fin damage are multifactorial however there is some evidence that fin biting by other fish is a major cause and is exacerbated by periods of food restriction. The use of structural enrichment in experimental tanks has been shown to reduce fin damage, however there are no studies of the effects of enrichment at the farm level. The focus of this study is to evaluate the effect of enrichment in salmon rearing tanks as an on-farm method for reducing fin damage.

This research was conducted at a commercial Atlantic salmon hatchery in the North West of Scotland, under standard production husbandry conditions. Atlantic salmon parr were randomly assigned to standard or enriched tanks where artificial kelp (18 plastic strips 150mm x 1200mm) was used as structural enrichment. Initial stocking density was 8 kg m^{-3} increasing to 14 kg m^{-3} after vaccination and grading, with a final average stocking density of 21.5 kg m^{-3} before transportation to sea. A 2 x 2 factorial design was implemented prior to vaccination where tanks were assigned to two different food restriction groups (24hr or 48hr) in combination with enriched and standard tanks. Twenty fish were sampled from each tank on six separate occasions: transfer to on-growing tanks, 1 week prior to vaccination and four time points post vaccination, up to fish being transported to sea. Samples were weighed and photographed for future morphometrics evaluation, using image analysis software. Dorsal fin condition was scored on a 5-point scale.

Data was analysed using multilevel linear models. Pre-vaccination, the main effect was a reduction in fin damage in the enriched tanks ($p = 0.016$); there was a trend for heavier and longer fish in enriched tanks but this was not highly significant ($p = 0.07$). Post vaccination there were complex interactions between food restriction and enrichment with 48hours food restriction associated with more fin damage ($p = 0.013$). Fish in enriched tanks seemed to recover better from an extreme stressful event such as vaccination and grading. The addition of this environmental enrichment may have, through facilitating more natural behaviours, improved fish welfare and consequently reduced the incidence of fin biting.

ASSESSMENT OF THE EFFECTIVENESS AND COST BENEFIT OF PROPHYLACTIC HEALTH PRODUCTS IN SHRIMP

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Increasingly stringent regulation of antibiotic use in aquaculture due to food safety and antimicrobial resistance concerns and the lack of commercially viable vaccines in these markets has contributed to a booming market for prophylactic health products (PHP); probiotics, prebiotics and immunomodulators. The IMAQulate project has, to date recorded over 276 PHPs being marketed in Andhra Pradesh, 40% of them probiotics, mainly targeting the shrimp sector. Although many studies have evaluated the efficacy of PHPs, few have been independently substantiated under 'on-farm conditions' to account for genotype environment interactions. Juvenile production stages are identified as key intervention points for the following reasons (i) being the life-stage that typically experiences highest mortality rates, (ii) poor quality juveniles are also likely to compromise grow-out performance, (iii) juvenile PHP treatment costs are relatively low compared to grow-out phases i.e. due to lower stock biomass and culture system containment volumes, Finally (iv) shrimp farm health management survey results indicate a growing trend toward extended nursing of shrimp juveniles; typically to around 2g in more biosecure lined/ covered ponds.

The aim of this study was to assess the effectiveness and cost benefit of PHPs on shrimp (*L. vannamei*) health and production performance during (i) extended nursing and (ii) post-treatment, during pond grow-out to harvest. For this trial, PHPs were selected based on 'pedigree analysis' on quality indicators and the credibility of label mode of action claims.

Five individual and combination PHP treatments (in-feed probiotic, in-feed prebiotic, in-feed synbiotic, water probiotic and combined in-feed prebiotic and water probiotic) were evaluated against a 'no-treatment' control and a biofloc system as a potentially low-cost alternative. In the covered nursing phase each of the 7 treatments was randomly allocated 5 replicates in $8m^3$ rectangular cement tanks. Tanks were stocked with PL12 at $1500/m^2$ and cultured for 32 days. Commercial probiotics and prebiotics were applied according to the manufacturer instructions. Biofloc tanks received additional carbon sources of dried molasses and sugar to maintain a C/N ratio of 15:1.

Preliminary findings indicate significantly enhanced growth outcomes for the biofloc and water probiotic treatments ($p < 0.05$) which could significantly reduce grow-out time and disease exposure. Results from a challenge test with *V. harveyi* indicated that, in-feed probiotic and water probiotic have higher survival rate than other treatments and the control. The same treatments will continue to be assessed for legacy effects in a second post-treatment grow-out phase in earthen ponds. Results will be used to determine low-cost prophylactic options with greatest potential for on-farm adoption by small-scale farmers and improved regulatory advice for policy-makers.

The study was part of the Immuno-modulators in Aquaculture (IMAQulate) research project funded by BBSRC and DFID in the UK and the Department of Biotechnology (DBT) in India. Ananda Group, Bhimavaram, Andhra Pradesh, India collaborated on implementation of the trials as well as providing generous financial support.

ASSESSING THE IMPACT OF *Bacillus* STRAINS MIXTURE PROBIOTIC ON WATER QUALITY, GROWTH PERFORMANCE, BLOOD PROFILE AND INTESTINAL MORPHOLOGY OF NILE TILAPIA, *Oreochromis niloticus*

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Egypt is the second largest tilapia producer globally providing affordable animal source protein for millions of low income people. Aquaculture production in Egypt is mainly based on low cost earthen pond systems to the north of the delta and around the natural lakes. In the last few years, due to intensifications and poor water quality and farm management, the industry was facing high mortalities of unknown or specific reasons. The aim of this study was to assess the impact of a commercial probiotic, Sanolife PRO-F, on water quality, growth performance, blood profiles and intestinal morphometry of monosex Nile tilapia. A field trial was conducted for 10 weeks in which tilapia fingerlings (20 ± 1.26 g) were randomly allocated three earthen ponds, each pond was sub-divided into three pens. A control group and 2 treatment groups, received Sanolife PRO-F at 0 (B0), 0.1 (B1) and 0.2 (B2) g kg^{-1} diet, respectively. The results showed a significant improvement in growth, performance, feed conversion ratio and blood profiles in tilapia fed on treated diets. The whole intestinal lengths, anterior and terminal intestinal villi heights and anterior goblet cells count were greater in tilapia fed on treated diets. There were no noticeable differences in growth and intestinal morphology between tilapia fed on B1 and B2 diets. The ammonia concentration in water was lower with B1 diet while electric conductivity, salinity and total dissolved solids were higher with the B2 diet. The level of pH was enhanced by both diets, B1 and B2. In conclusion, application of Sanolife PRO-F at 0.1-0.2 g kg^{-1} diet might have beneficial effects on growth, immunity, stress responses, gut health and function and water quality of farmed Nile tilapia. To our knowledge, this is the first field trial for assessing the impact of this probiotic on performance of Nile tilapia in Egypt.

ASSESSMENT OF THE EFFECTIVENESS AND COST BENEFITS OF PROPHYLACTIC HEALTH PRODUCTS IN AQUACULTURE: A SYSTEMATIC REVIEW

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In recent years, prophylactic health products (PHPs) such as probiotics, prebiotics and immunomodulators have witnessed growing application as alternative health management options for aquaculture. This is due in part to stringent regulations of antibiotic use in farming due to food safety and antimicrobial resistance concerns combined with lack of commercially viable vaccines for warm-water species. Although many studies have evaluated the effectiveness and the cost benefit of PHPs in aquaculture, estimates are inconsistent and often conflicting. The aim of this study was to systematically review and assess the quality of such studies and their underlying designs. PubMed and CAB Direct were searched for articles relevant to the research questions using different combinations of keywords. Original articles that assess the effectiveness and/cost benefit of any PHPs applied for aquaculture were selected for inclusion. There were no language, date or geographical area restrictions. All titles and abstracts were screened for relevance by two independent technical reviewers. The reference lists of relevant articles were also searched for further articles. The full text of articles determined to be relevant were then assessed using quality appraising forms adapted from Sargeant et al., 2005[1]. Intractable disagreements between reviewers were resolved by a third independent reviewer. Of 8,006 retrieved articles, abstracts of 2909 articles were screened for relevancy of which 272 articles were selected for full quality appraisal.

Preliminary results indicate that a significant proportion of published studies lacked proper study design. Furthermore, few have been independently substantiated under farm-conditions, effectively omitting critical key genotype-environment challenges from most of this body of work. We anticipate that this systematic review will help researchers better design experimental and field trials for the proper assessment of the effectiveness and cost benefit of PHPs. It will also support decision making for the use of PHPs in aquaculture.

https://www.researchgate.net/profile/Lisa_Waddell2/publication/242079481_A_Guide_to_Conducting_Systematic_Reviews_in_Agri-Food_Public_Health/links/0deec537f50fce499b000000/A-Guide-to-Conducting-Systematic-Reviews-in-Agri-Food-Public-Health.pdf)

PARTICIPATORY HEALTH MANAGEMENT STRATEGY DEVELOPMENT FOR AQUACULTURE IN EGYPT: A NOVEL APPROACH FOR POND DESIGN

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Egypt is the world's third largest tilapia producer providing affordable animal source protein for millions of low income Egyptians. Aquaculture production in Egypt is mainly based in irrigated earthen ponds located to the North of the Nile delta. In the last few years, significant intensification has occurred based around the increased availability of formulated diets. This appears related to farm management challenges, especially elevated mortalities of unknown cause. Experimental studies have shown that tilapia challenged by common pathogens can improve their immune response and survival if they have the opportunity to choose an optimal temperature. This allows them to express a fever response, which is known as behavioural fever. The aim of this study was to apply this concept to commercial earth pond systems and assess the impact on performance. This paper describes the approach towards the development of modified pond designs in participation with farmers in Kafrelsheikh governate.

Three participatory workshops, each with 15-25 participants, were conducted with farm owners, workers and local aquaculture researchers in three areas of Kafrelsheikh governate. The concept of behavioural prophylaxis was explained, and participants brainstormed how ponds could be modified to ensure thermal gradients. Based on the feasibility and logistics, three types of modifications were prioritised (dead bank, raised platforms and greenhouse). Each type of modification was implemented in 15 commercial earthen ponds making up a total of 45 trial ponds and their controls distributed in three geographical regions in Kafrelsheikh governorate. Data for water and feed management, water quality and tilapia performance were collected daily, weekly and monthly, respectively along the production cycle.

Preliminary results indicated that pond modifications have a positive impact on water quality and tilapia performance. Outcomes of this study have the potential to improve health and management of tilapia farms in the study area and beyond. This can also have obvious beneficial implication for disease management in pond aquaculture much wider field. To our knowledge, this is the first field trial for the implementation and assessment of the concept of behavioural prophylaxis in earthen ponds.

CURRENT AND PAST STATUS OF TILAPIA FARMERS IN KAFR EL SHEIK GOVERNORATE, EGYPT, WITH SPECIAL REFERENCE TO FARM PERFORMANCE AND FISH HEALTH ISSUES

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Egypt has developed its aquaculture sector such that by 2016 it had become the world's third largest global producer of farmed tilapia behind China and Indonesia (FAO, 2016). In recent years significant intensification of production systems appears to have taken place. In 2010 - 2011 a so called "summer mortality" syndrome emerged associated with mortality levels of up to 25% per production cycle causing significant adverse financial effects. Under these circumstances, a baseline survey was conducted with 119 randomly sampled farm enterprises across Kafr El Sheik governorate, the region which produces over 70% of national farmed tilapia production. The survey considered socio-economic, production performance, water quality and fish health related issues. Preliminary findings from the survey were then presented back in two workshops to key stakeholders including fish farmers, input suppliers, researchers and government officials for their feedback and validation.

In this paper we highlight ten key findings including farm productivity and performance related to: ownership or lease, water sources and frequency of water exchange, use of chemical treatments, mono or polyculture of species, fingerling stocking size and strategies, and training and education status of fish farmers. These are presented and discussed, with a view towards recommendations which can improve the sustainability of this hugely important aquaculture sector producing affordable, fresh fish protein sources for millions of peri-urban and rural Egyptians.

EFFICIENCY OF WITHIN-GROUP MASS SELECTION FOR A THRESHOLD TRAIT AS DISEASE RESISTANCE TO MANAGE INBREEDING AND ENSURE GENETIC GAIN: A SIMULATION STUDY

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Mass selection is a low cost method to improve performances for traits that can be measures on live candidates as production traits or survival when traits heritability is above 0.20. Rotational crossing between groups or cohort within a generation is a simple way to avoid immediate increase of inbreeding at the next generation. This study tries to quantify genetic gain and loss of variability by simulation for different breeding programs combining within-group mass selection on threshold traits and rotational crossing between groups. Selection for resistance is a direct application of this work.

Factorial mating plans were generated with a variable number of parents distributed in groups. 4000 progenies were simulated and distributed in all groups. Threshold trait is considered as polygenic. Genetic value was half of sire and half of dam genetic value, including meiosis hazard. Individual phenotypic value for the trait is the weighted addition of the genetic value, that the weight is the heritability, the maternal non-genetic effect, the micro-environmental effect and residual hazard effect. Threshold is defined as the quantile of Gaussian distribution for survival value. Individuals may be selected if their phenotypic value is upper than the threshold. Selected individuals are randomly sampled in selectable individuals. A rotation mating system is applied to cross groups and produce the next generation. Different rotational mating designs are considered. Ten generations are simulated. Effects of type of by group on consanguinity, genetic and phenotypic gains are estimated.

Genetic and phenotypic values improve after a few generations. To vary rotation system, number of founders, number of groups and selection pressure don't have significant different impact on performance. From 2 groups, within-group mass selection is better to limit consanguinity than mass selection, but it doesn't seem necessary to multiply the number of groups. Around sixty founders, and eight males and eight females selected by group is sufficient to limit inbreeding in a reference situation with 8 groups.

The main result of this work is that that within-group mass selection (without information on pedigree) and rotational crossing associated with factorial mating designs should allow to manage inbreeding and achieved important genetic progress for threshold traits as resistance to a disease.

MASS SELECTION WITH FACTORIAL MATING DESIGNS AND DNA-PARENTAGE ASSIGNMENT IS USABLE TO IMPROVE SURVIVAL TO SUMMER MORTALITY IN PACIFIC OYSTER *Crassostrea gigas*

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Previous result estimated intermediate heritability for survival to summer mortality in Pacific oyster *C. gigas* (Dégremont et al., 2015). Out of 384 SNPs, 206 have been selected to optimize a panel designed in a previous program (Lapègue et al., 2014). It uses for the first time at commercial scale to estimate parentage assignment, risk of inbreeding and evaluate performance in summer mortalities of *C. gigas*. Four French hatcheries (Marinove, Novostrea, SATMAR and Vendée Naissain) produced cohorts in spring 2014 based on different factorial mating designs involving 41 to 169 parents. Each spawn was equi-represented after fertilization. Cohorts were challenged expectedly to OsHV1 on sea-shore during summer 2014. For each hatchery, 752 DNA samples, including parents, were withdrawn on surviving oysters at the end of 2014 for parentage assignment, and some of them being tagged for two hatcheries to record their mortality until end of the second summer.

Achieved assignment rates varied from 72% to 94%. Almost all parents were represented when the number of genotyped offspring was coherent with the number of parents. Except in two cases, neither one dam nor one sire produced the majority of offspring from one factorial. Factorial mating plan was efficient to produce a large number of families in oyster *C. gigas*. 35% of effective population is maintained when the variance of offspring number per family is included in computation. Mass selection is able to be performed in breeding populations without risk on consanguinity, and parentage assignment could be a useful tool to control it.

Summer mortalities in the first summer ranged from 18% to 66% and from 9 to 35% for the second summer. Heritability for survival during the second summer is 0.16 ± 0.09 for the two hatcheries where individual mortality was recorded. Allele frequencies from SNP panel could explain a part of variability in resistance for the first and the second summer mortality.

Dégremont L., Nourry M., Maurouard E., 2015c. Mass selection for survival and resistance to OsHV-1 infection in Crassostrea gigas spat in field conditions: response to selection after four generations. Aquaculture 446, 111–121. doi:10.1016/j.aquaculture.2015.04.029

Lapègue S., Harrang E., Heurtebise S., Flahauw E., Donnadieu C., Gayral G., Ballenghien M., Genestout L., Barbotte L., Mahla R., Haffray P., Klopp C., 2014. Development of SNP genotyping arrays in two non-model shellfish species. Mol. Ecol. Resources, 14, 820–830. doi: 10.1111/1755-0998.12230

Tableau 1 : Results of parentage assignment and effective population (Ne).

	Hatchery 1	Hatchery 2	Hatchery 3	Hatchery 4
Parentage assignment rate	89%	86%	94%	72%
Sires number represented (%)	19 (100%)	46 (84%)	13 (93%)	77 (64%)
Dams number represented (%)	22 (96%)	67(68%)	26 (96%)	41 (84%)
Full-sib families number represented (%)	146 (33%)	98 (52%)	157 (26%)	155 (26%)
Expected *Ne*	42	135	37	139
Realized *Ne* without offspring variance	41	107	36	105
Realized *Ne* with offspring variance	23	47	16	63

THE REGULATORY COST BURDEN ON U.S. AQUACULTURE FARMS

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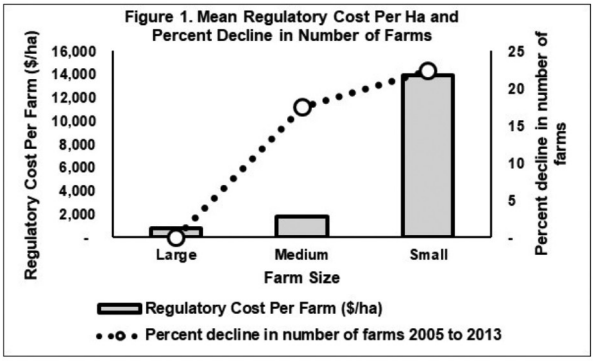
There is wide consensus on the need to reinvigorate and revitalize U.S. aquaculture. While there are multiple factors involved and variation among sectors of U.S. aquaculture, it also has become clear that the regulatory environment in the U.S. has played an important role in constraining growth of U.S. aquaculture. On-farm economic effects of the regulatory compliance burden were measured on U.S. baitfish and sportfish farms. Results showed that the average farm cost from regulations was $148,554 per farm; $7,383 per ha; and constituted 25% of the total costs of U.S. baitfish/sportfish production. Across the baitfish/sportfish industry, the total cost of regulatory compliance was $12 million per year. Only 1% of the total regulatory costs of U.S. baitfish/sportfish was associated with fees for permits and licenses, whereas 99% were indirect costs that resulted from time spent by farm personnel in monitoring, record-keeping and reporting; changes made on the farm, and lost/foregone sales. Regulatory costs were disproportionately (18 times) greater on small-scale than on large farms. Figure 1 shows that the number of farms that exited the industry between 2005 and 2013 increased with the increased regulatory cost burden. In addition to the substantial cost increases measured due to the regulatory environment, further analyses showed that the regulatory environment: 1) restricted access to markets; and 2) reduced farm efficiency as manpower was spent on regulatory compliance rather than innovations on the farm or increased marketing efforts.

Two additional studies have been initiated at the request of industry, one on West Coast shellfish and the other on trout. Observations to date from the West Coast shellfish survey include: 1) involvement of multiple regulatory levels at the county, state, and federal levels; 2) substantial overlap between regulatory agencies in the permitting process; 3) delays in permit approvals of up to 15 years; 4) no new farm permits in some states; 5) limitations to business expansion and growth in spite of strong market demand; 6) high start-up costs that prohibit new farms, due to regulations; 7) high legal costs to appeal permit decisions; and 8) inability to adopt more efficient practices due to restrictions on gear and technologies. From the trout survey, preliminary results show that: 1) more than half of respondents knew of trout farms that had gone out of business due to regulations; 2) nearly half of respondents had had unexpected and expensive changes due to regulations; 3) nearly half of respondents were forced out of markets due to regulations; and 4) small-scale trout farms were being regulated under a regulatory framework that was developed for large-scale heavy industry like coal, mining, and steel.



DIETARY SUPPLEMENTATION OF A MIXTURE OF SAPONIN-RICH PLANTS TO REDUCE AMMONIA-NITROGEN EXCRETION IN JUVENILE NILE TILAPIA (*Oreochromis niloticus*)

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The intensification in aquaculture production leads to increasing attention on the management of ammonia from its waste. Natural solutions such as *Yucca schidigera* extracts have shown beneficial effects. Their origin (harvested from the wild), however, call for alternative solution, equally efficient, in order to increase the sustainability of such solutions.

In this work, 240 juvenile Nile tilapias (*Oreochromis niloticus*, 24.7±0,7g) were randomly divided in 2 equal groups and supplemented for 3 weeks with different doses of a commercial mixture of saponin- rich plants (Norponin® OPTI, CTL: 0ppm, treated: 500ppm). The impact on fish growth and on water quality was monitored, with a particular attention to total ammonia-nitrogen excretion (TAN).

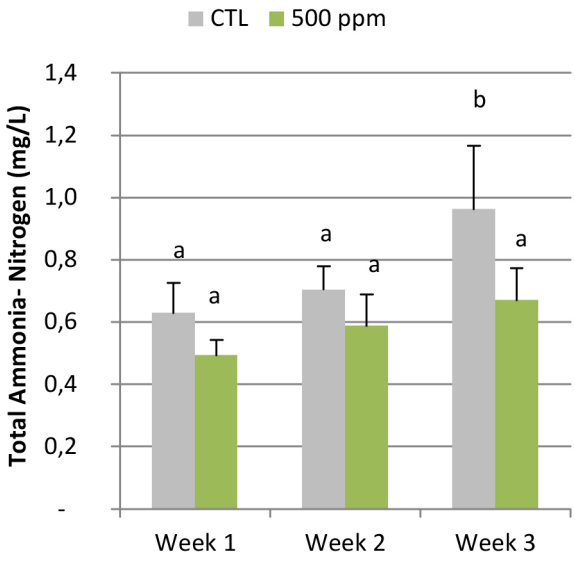
Supplemented fish had a numerically higher weight gain and a lower FCR than that of the control group (Table 1). Additionally, while TAN significantly increased in the control group over the trial period, it remained at the same level in the supplemented group during the length of the trial ($p<0,05$, Graph 1).

Overall, the present study demonstrates that a supplementation with 500ppm of the feed additive induced beneficial effects on growth performances of juvenile Nile tilapias at the same time as positively impacting TAN management.

TABLE 1. Zootechnical parameters of fish during the study

Group	CTL	500ppm
Survival rate (%)	100%	100%
Weight gain (g)	27.3	30.8
SGR	4.3	4.9
FCR	0.80	0.68

GRAPH 1. Evolution of the total ammonia-nitrogen over the trial period



BIOFLOC TECHNOLOGY AS A MEANS TO RECOVER SHRIMP FARM IN MOZAMBIQUE

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Aquapesca shrimp farm was established in Inhassunge near Quelimane, Mozambique on 1994. The farm, covering a pond area of 350 ha is located near the Bons Sinais River estuary, 20 km from the Indian Ocean. During the years 2008 to 2011 the average yearly production was 700 tons of organic *Penaeus monodon* shrimps, supplying work and livelihood to about 800 workers. Yet, on 2011 the farm was affected by white spot syndrome virus, eliminating production since.

On August 2017, the owners of Aquapesca and management decided to try the use of biofloc technology, chlorination of water used to fill the ponds and zero water exchange since, as means to overcome the diseases and recover production, in a pilot including two 1.2 ha each ponds.

The details of pond treatment, the effects on water quality and the successful results will be discussed in this presentation.

The technology used here may help in developing intensive, sustainable and profitable aquaculture in Africa.

Summary of Panaeus monodon production pilot

Pond #	Stocking date	PL/m^2	Harvest date	DOC	Average weight (g)	Survival %	FCR	Production Kg/ha
P04	13.1.18	33	16.4.18	93	22.8	95	1.3	7187
P05	17.2.18	37	10.5.18	82	15.0	97	1.3	5341

EFFECT OF WATER CLORINATION ON THE BIOFLOC, WATER QUALITY, PERFORMANCE OF SHRIMP *Litopenaeus vannamei* AND ITS RESISTANCE TO EARLY MORTALITY SYNDROME

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The effect of water chlorination on biofloc formation, water quality, performance of shrimp *Litopenaeus vannamei* and its resistance to early mortality syndrome (EMS) was studied. For which, four treatments were designed (T1 = Chlorination + molasses, T2 = Chlorination + without molasses, T3 = Without chlorination + with molasses and T4 = without chlorination + without molasses). The results of the study showed significant differences between some treatments, observing a lower final average weight (1.31 ± 0.23 g) and specific growth rate (SGR, 2.0 ± 0.6%/d) where it was grown without chlorination and without molasses (T4), with a lower survival (32.9 ± 2.8%) and biomass (3.4 ± 0.0 g/m2) in the treatment where it was cultivated with chlorination and without molasses (T2). The results of the stage where the organisms of each treatment (except those of T3, whose mortality was 100% before concluding this stage) were subjected to a challenge with a strain of the bacterium *Vibrio parahaemolyticus* that cause the syndrome of early mortality (EMS). In this challenge, the results showed only significant differences in cumulative mortality (%) in the treatment where it was grown without chlorination and without molasses (T4), where after 72 hours post-infection there was a lower accumulated mortality that remained until 84 hours post-infection. During the culture, the physico-chemical variables did not present significant differences between the treatments, although there was a trend of lower pH where it was cultivated with chlorination and without molasses (T2). While the water quality variables, only showed a concentration greater than 1 mg/l of ammonia in the treatments T1 (with chlorination and with molasses) and T2 at 21 and 14 days of culture, respectively. However, the concentration of nitrites in all the treatments exceeded 3 mg/l, reaching in some cases values higher than 25 mg /l. Also, in all cases, the alkalinity and total suspended solids (TSS) exceeded 250 mg/l and 100 mg/l, respectively, with TSS values greater than 500 mg/l at 35 days in the treatment where it was cultivated with chlorination and molasses (T1). In the water of culture, only values higher than 16 thousand CFU/ml of *Vibrio* spp were presented where it was grown without chlorination and without molasses (T4) and, from day 21 of culture said concentrations were very low in all treatments . On the contrary, in the same treatment (T4), the concentration of *Bacillus* spp showed high values at the beginning of the study, maintaining values higher than 12 thousand CFU/ml until the end of the study. During the study, a lower concentration of *Bacillus* spp was evident where it was cultivated with chlorination with and without molasses.

EFFECT OF NATURAL FEED ADDITIVE ON THE INFESTATION CAUSED BY *Sparicotyle chysophrii* (Monogenea: Polyopisthocotylea) IN GILDHEAD SEABREAM (*Sparus aurata*)

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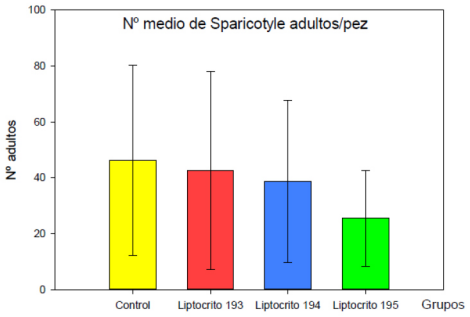
A study was undertaken to evaluate the efficacy of a natural feed additive to control de infestation caused by *Sparicotyle chysophri*i (Monogenea: Polyopisthocotylea) in Sea bream. The Sparicotyle is the most threatening ectoparasites for the Sea Bream culture in the Mediterranean (Sitja-bobadilla 2009), causing mortalities and it is frequently found in mixed infections with other parasites and bacterial infections (Padros and Crespo 1995).

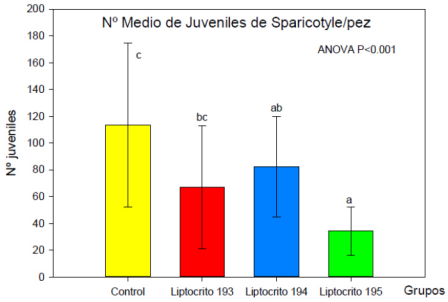
In the trial three different formulas of a feed additive were tried to control the infestation caused for this ectoparasite. For 60 days 4 groups of 60 fish each were feed with 4 different diets (CTR, AD193, AD194, AD195). Previously a stock of infected fish was collected from industrial farms and kept as a donors for the challenge. After those 60 days, a cohabitation infection was performed, following a ratio of 3:1 (3 non-infected fish by one infected fish). After one month of cohabitation growth, hematocrit, mucus production and recount of different stages of *Sparicotyle* were performed.

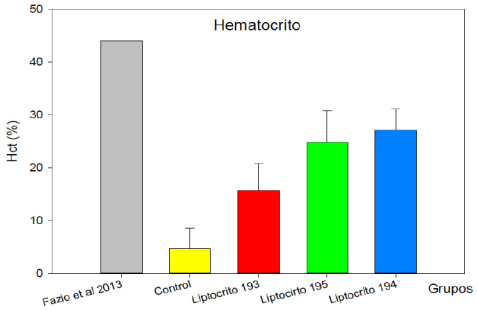
The group feed with ADD195 presented a significant reduction in the number of adults of *Sparicotyle chysophrii*, being basically immature parasites. Hematocrit levels and mucus production were higher in the groups feed with diets including natural feed additives.

The addition of LIPTOCITRO MMM (ADD195) in the feed has clear effect in the reduction of infection caused by *Sparicotyle chysophrii* in Sea bream.

The additive LIPTOCITRO MMM (195) has been tried in industrial conditions confirming the previous results, reducing the treatments needed to control Sparicotyle in more than 60%.







DENITRIFYING ACTIVITY AND SETTLING PROPERTIES OF AQUACULTURE BIOSOLOIDS TREATED IN SINGLE SLUDGE REACTORS

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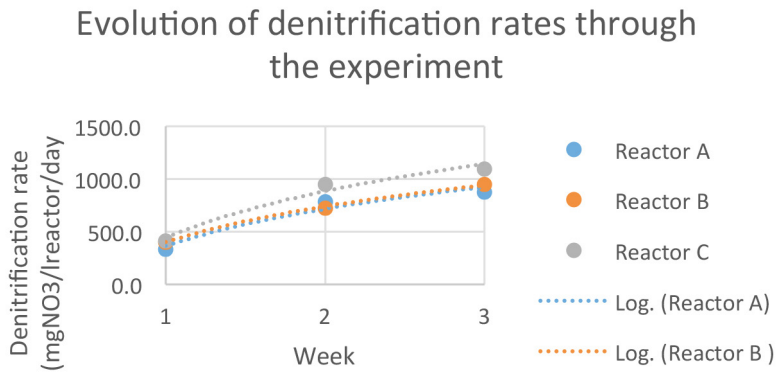
The control of nitrogen compounds in recirculating aquaculture systems (RAS) is often necessary to reduce water exchange requirements or to comply with environmental discharge regulations. NO_3 control in RAS is achieved using denitrification reactors which can be designed to operate with either extrinsic (i.e. ethanol), or intrinsic carbon sources originating from the biosolids generated within the system. The use of intrinsic carbon sources is of interest because these do not add to the operating costs. At the same time, the biosolids used for denitrification undergo a reduction in their initial volume, potentially reducing their associated handling and disposal costs.

This study aimed to understand the changes in denitrifying activity and the settling characteristics of biosolids as these were withheld in anaerobic reactors whose solids retention times (SRT) were controlled using a simple method based on Imhoff cone measurements. The reactors were operated at three SRT regimes, receiving a daily charge of drum filter backwash sludge and recirculating process water from a tilapia RAS. Denitrifying activity was studied by determining the reduction of NO_3 concentration at 1h intervals after daily charges. Settling characteristics were studied by unloading sludge samples into a conical settling basin and measuring the fraction of solids captured at the bottom of the basin at time intervals for one hour. Differences in denitrifying activity and sludge dry matter content where found for each SRT regime through the experiment. The settling velocities of sludge did not differ significantly between treatments.

The data gathered during this work is of use for aquaculture engineers to size single-sludge denitrification reactors, controlling for wastage of sludge biomass due to high water velocities inside the reactor.



IN VITRO STUDY OF a DOSE- AND TIME- EFFECT RESPONSE OF A COMBINATION OF ORGANIC ACIDS AND NATURE-IDENTICAL COMPOUNDS ON GILTHEAD SEABREAM HEAD-KIDNEY LEUCOCYTES

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Organic acids (OA) and botanicals have a long history of use as feed additives in animal production with the aim of improving intestinal health and performance. To the best of our knowledge, the available studies in aquaculture are very limited and little is known about their impact on the cells of the immune system. Aim of this study was to assess the biological properties of OA and nature-identical compounds (NIC) on head-kidney leucocytes (HKLs) viability, functionality and oxidative response in an *in vitro* model. HKLs were isolated from gilthead seabream (*Sparus aurata)* and incubated with a combination of citric and sorbic acid, thymol and vanillin (MIX) at 0, 50, 100, 250, 500, and 1000 mg/L for 30 min, 2 h and 4 h. At any assayed time, the MIX did not affect leucocyte viability (which was determined by flow cytometry using propidium iodide) nor phagocytic ability. Regarding respiratory burst, starting at 30 min the MIX at 50 and 100 mg/L induced a decrease in activity compared to control.

The relative gene expression of HKLs after being incubated for 30 min, 2 hour and 4 hours without (control) or with different concentrations of the MIX was also studied by real time PCR. The selected antioxidant genes were nuclear factor erythroid 2 (*nrf*-2), superoxide dismutase (*sod*), catalase (*cat*) and glutathione reductase (gr). The expression of interleukin 1-beta (*Il-1* beta) and interleukins 6, 7, 8, 15 and 18 (*Il-6, 7, 8, 15, 18)* as pro-inflammatory genes and interleukin 10 (*Il*-10) and transforming growth factor β (*tgf-β*) as anti-inflammatory genes was also determined. Different significant variations were detected in the expression of all the studied genes on HKLs after being incubated with the different concentrations of the MIX. The increases or decreases recorded were related to the MIX concentration and to the incubation time.

In conclusion, the present results showed that the MIX of OA and NIC did not have a negative impact on HKLs viability and the decrease in the respiratory burst activity in a dose-dependent manner underlines the potential antioxidant power of the MIX. The variation in the expression of antioxidant genes and interleukins suggests a possible modulation of the immune-response mediated by the MIX that needs to be further evaluated *in vivo*.

Acknowledgements: This research was funded by Vetagro S.p.A. and POR-FESR 2014-2020 Emilia Romagna grant.

CYTOTOXIC, BACTERICIDAL AND ANTIOXIDANT *IN VITRO* EFFECTS of OREGANO AND PURSLANE AQUEOUS AND ETHANOLIC EXTRACTS. POSSIBLE APPLICATIONS IN AQUACULTURE

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Oregano (*Origanum vulgare*) and purslane (*Portulaca oleracea*) are two well-known plants used since ancient times in traditional medicine and as food additives. Both plants present many beneficial properties that make them serious candidates to be included in fish feed because they can improve the immune system as well as the response to infections and stress. The aim of this work was to study the *in vitro* cytotoxic activity against a fish tumor cell line (PLHC-1), the bactericidal activity against three fish pathogen bacteria (*Vibrio harveyi*, *V. anguillarum* and *Photobacterium damselae*) and the antioxidant activity of aqueous and ethanolic extracts obtained from leaves of these plants. For the cytotoxic activity, PLHC-1 cells were incubated 24 hours with culture medium (control group) or with different concentrations of extracts (0.01, 0.05, 0.1, 0.25, 0.5, 0.75 and 1 mg/mL), while for bactericidal activity, the three selected bacteria were incubated 5 hours with PBS (control group) or with the different concentrations of extracts. Both activities were determined by the reduction of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide test (MTT). For the study of the antioxidant activity, three different concentrations of the extracts (0.1, 0.5 and 1 mg/mL) were tested by the 2, 2'-azino-bis-3-(ethylbenzothiazoline-6-sulphonic acid) test (ABTS).

Regarding the cytotoxic activity, aqueous and ethanolic extracts of oregano decreased PLHC-1 cells viability starting from 0.5 and 0.25 mg/mL respectively, while only 0.5 mg/mL or higher concentrations of ethanolic extracts of purslane decreased it. In the case of bactericidal activity, while only 1 mg/mL of aqueous extracts of oregano showed bactericidal activity against *P. damselae,* concentrations of ethanolic extracts starting from 0.5 mg/mL for *V. harveyi* and 0.25mg/mL for *V. anguillarum* and *P. damselae* already showed bactericidal activity. On the other hand, aqueous extracts of purslane starting from 0.25 mg/mL (for *V. harveyi*) and 0.75 mg/mL (for *V. anguillarum* and *P.* damselae) had also bactericidal activity. Furthermore, concentrations of ethanolic extracts starting from 0.1 mg/mL for *V. harveyi* and 0.25 mg/mL for *V. anguillarum* and *P. damselae* already showed bactericidal activity. Finally, both extracts of both plants showed notable antioxidant activity.

In conclusion, the present results showed that plants, oregano and purslane, present beneficial properties that make them interesting candidates to be included in the adequate concentrations as additive in fish fed, and their use could help fish treat or avoid infections, diseases and stress. These results need to be further evaluated *in vivo*.

Acknowledgements: This research was funded by MINECO co-funded with European Regional Development Funds (ERDF/FEDER) (grant number AGL2014-51839-C5-1-R) and by the *Fundación Séneca de la Región de Murcia* (grant number 19883/GERM/15, *Grupo de Excelencia*).

BREWERS BY-PRODUCTS AS ALTERNATIVE INGREDIENTS FOR PARTIAL SUBSTITUTION OF FISH MEAL IN AQUACULTURE FEED

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Aqua-feeds are formulated considering all the essential nutrients fishes need to keep healthy. They are **highly dependent on fish meal (FM) and oil (FO)**. Alternative ingredients which replace these marine components are required to result in sustainable and economical feeds. However, it depends on improving feed efficiencies and substituting away from FM and FO.

Brewers´ by-products stand **as a potential alternative for replacing FM in aquaculture feed due to: their availability in Europe** (6 million tons of brewer spent grain (BSG) and 0.8 million tons of brewer yeast (BY)); **their nutritional characteristics** (high content in protein; 50% and 25% in BY and BSG) **and the preliminary results** which demonstrate no differences in feed intake with 15% inclusion of BY in Senegalese sole feeding.

This project, funded by Life Program (LIFE16ENV/ES/000160), **aims to demonstrate the feasibility of the utilisation of BY and BSG in Sea bream and Senegalese sole (as model of sea fish) and trout (as model of fresh fish) feeding,** increasing aquaculture sustainability by providing 2 new sustainable raw materials for aquaculture feed.

Firstly, **the adequacy of by-products for aqua-feed production** will be studied and demonstrated at industrial scale. All legal, technical and administrative requirements for each stage of the value chain will be considered. Secondly, **fish growth trials and feeding efficiency** will be carried out. In a 1st trial, extreme levels of substitution of the FM with BSG and BY will be tested and, based on these results, these levels will be adjusted. Then, a 2nd trial will test previously adjusted diets to ensure the best growth and health of the fish. Finally, growth in weight and length, histological examination of the intestine and liver and health status of the fish will be evaluated.

The expected result is the **provision of 2 new raw materials for aqua-feed producing market, validated throughout 2 fish growth trials at semi-industrial scale.**

It will **improve the sustainability of the aquaculture in the future** by reducing 25 % of the environmental impact of aqua-feed production by replacing at least 15 % of the FM with a new raw material from brewer by-products.

USE OF PORCINE BYPRODUCTS AS FISH MEAL REPLACEMENT IN ONGROWING FEEDS FOR GILTHEAD SEABREAM: EFFECTS ON GROWTH, FILLET COMPOSITION AND IMMUNE STATUS

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Two porcine byproducts (hydrolyzed protein, PEPTEIVA and spray-dried plasma, APPETEIN, APC Europe) were used to substitute 5% fishmeal (FM) in a 48% protein-rich diet (7% FM) for gilthead seabream. Fish (initial weight = 60g) were on-grown for 3 months in 4x replicated 450L tanks connected to a recirculation system (IRTAmar©). At the end of the trial, samples of different organs (fillet, liver, plasma and skin) were taken to study the effect of these products on the composition and immune status of the fish. Growth in weight and length was also recorded.

The results obtained showed a significant positive effect on the growth (Table 1) of the fish although no differences could be found in terms of proximal and fatty acid composition of the fillet (Table 2) or in the plasma immune status (Fig 1)

No significant variations were detected in the immunoglobulin M levels (Fig. 1), natural haemolytic complement activity and bactericidal activity against *Vibrio anguillarum* in the serum from fish fed the different experimental diets.

Acknowledgments. This work has been financed through the H2020 MedAID project (No 727315).

	Final weight (g)	*Final size (LS, cm)*	*Fulton Index (K)*
Diet 1 (control)	173.8 ± 6.0 b	18.7 ± 0.2	2.65 ± 0.1
Diet 2 (Porcine plasma)	180.0 ± 4.4 a	19.0 ± 0.3	2.64 ± 0.2
Diet 3 (Porcine protein)	182.2 ± 8.4 a	19.0 ± 0.1	2.67 ± 0.3

Table 1.- Results in growth in weight and length and Fulton condition factor, letters indicate significant differences (ANOVA, P < 0.001).

	Diet 1 Control	Diet 2 Porcine plasma	Diet 3 Porcine protein
Water (%)	70.89±0.49	70.19±0.86	71.71±0.45
Protein (% DW)	62.00±3.01	61.44±2.54	69.28±2.81
Carbohydrates (% DW)	1.33±0.25	1.56±0.14	1.29±0.12
Lipids (%DW)	12.69±1.32	13.90±2.28	12.27±1.78
Ash (%)	2.12±0.23	2.49±0.34	2.53±0.55
Total Fatty acids (mg/g lipids)	735.05±72.65	665.76±12.96	693.32±84.14
Fatty Acid profile (% Total)			
Total Saturated	25.35±0.63	25.60±0.28	24.35±3.67
Tota Monounsaturated	30.46±1.47	31.55±1.21	29.04±4.92
20:4n-6 (ARA)	0.82±0.05	0.87±0.16	0.76±0.31
Total n-6 PUFA	10.52±0.20	11.26±0.24	10.78±1.73
20:5n-3 (EPA)	8.00±0.38	8.12±0.32	7.31±1.29
22:6n-3 (DHA)	13.64±0.75	14.14±0.90	12.78±2.56
Total n-3 PUFA	25.96±1.41	26.38±1.02	24.04±3.97
Total PUFA	36.48±1.57	37.64±1.25	34.82±5.65

Table 2.- Results in proximal (% dry weight) and fatty acid composition (% of total fatty acids) of the fillet at the end of the study

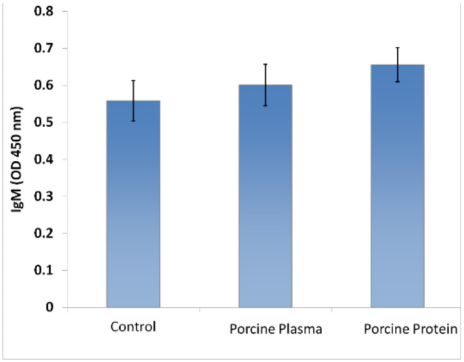

Fig 1.- Results of immunoglobulin in the serum of the fish at the end of the trial.

USE OF ESSENTIAL OILS IN THE FEEDS FOR GILTHEAD SEABREAM TO PREVENT INFECTIONS BY *Sparicotyle chrisophrii*

A. Estévez, E. Gisbert, J. Firmino, C. Trullás, E. Vilarrasa

Two essential oils (garlic oil –Arotec A- and a mixture of garlic oil with carvacrol and timol –Arotec G-, TecnoVIT, Spain) were used in the formulation of diets for gilthead seabream. The diets were used to feed 40 g gilthead seabream for 30 days. The experiment was carried out in triplicate tanks in IRTA using recirculation units. After 30 days of feeding part of the fish were used in a cohabitation trial with fish infected with *S. chrisophrii* in a ratio 2 non parasitized fish (receptors) : 1 infected fish (donors), with a total N=36 fish per tank. The cohabitation trial was carried out for 30 days. At the end, the fish were sampled from each tank separating receptors and donors. The fish were weighted, measured, individually stored in labeled bags and frozen at -20ºC. No differences were detected in the growth of the fish (Fig 1) along and at the end of the study. The number of parasites in each of the gills was determined in each individual fish, with 3 categories: adults, juveniles and larvae according to morphological characteristics. The fish from the control group not fed the essential oils enriched diets showed a significantly higher number of parasites whereas the fish fed Arotec G enriched feed showed a significantly lower number (Fig. 2). The presence of adults was significantly higher in the control and Arotec A fed groups showing a higher degree of reinfection with the parasite, compared with Arotec G group which showed few parasites mostly present as juveniles (Fig. 3). Thus, the preventive use of a diet enriched with Arotec G (garlic oil with carvacrol and timol) allows a lower presence of *S. chrysophrii* when the fish are exposed to highly infected fish.

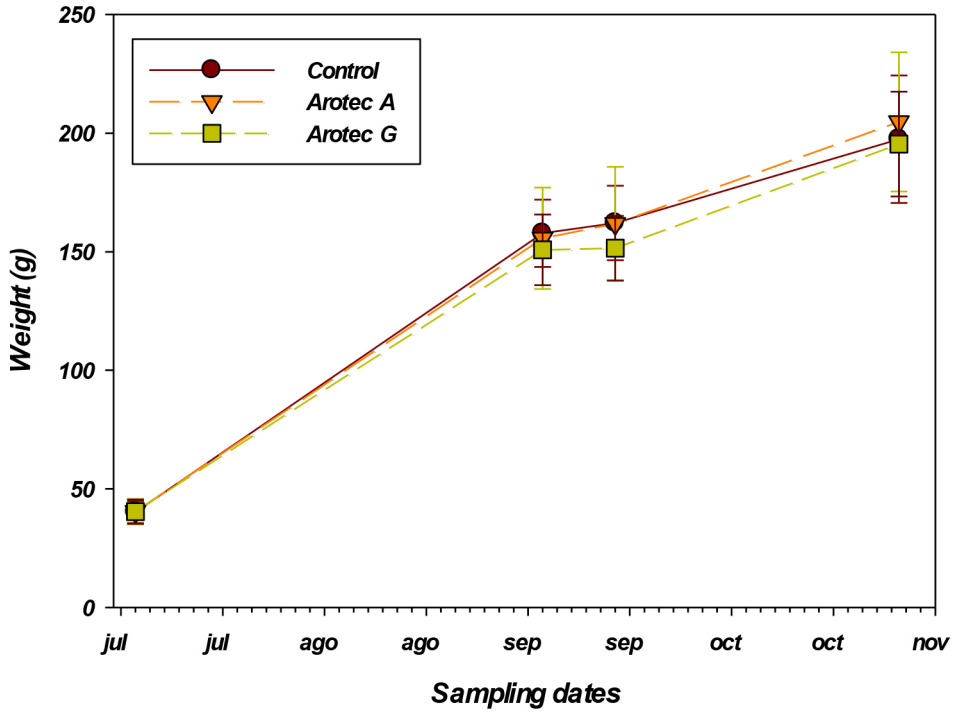

Fig. 1.- Growth of fish during feeding trial and cohabitation

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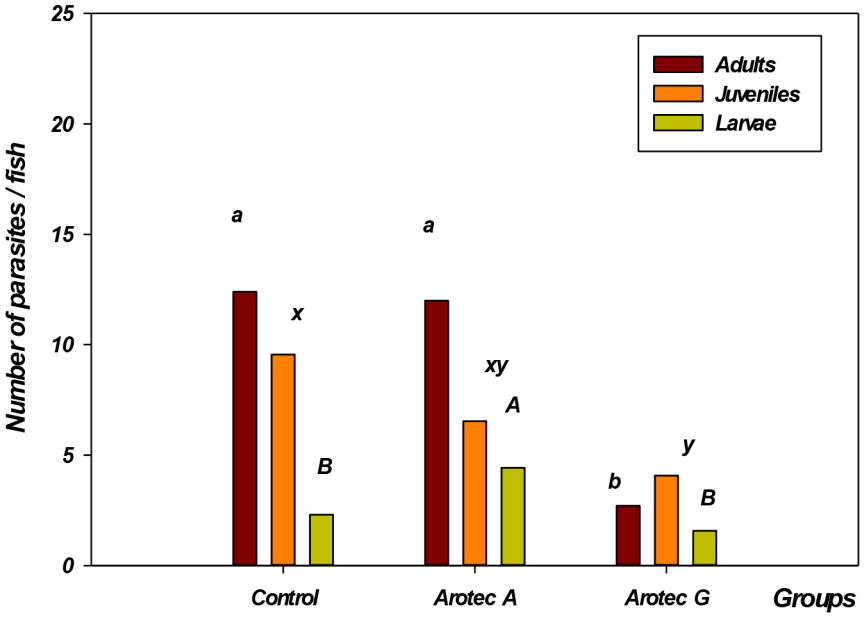

Fig 2.- Number of parasites per fish fed the experimental diets. Different letters show significant differences (ANOVA P<0.001) (a,b for adults; x, y for juveniles; A, B for larvae)

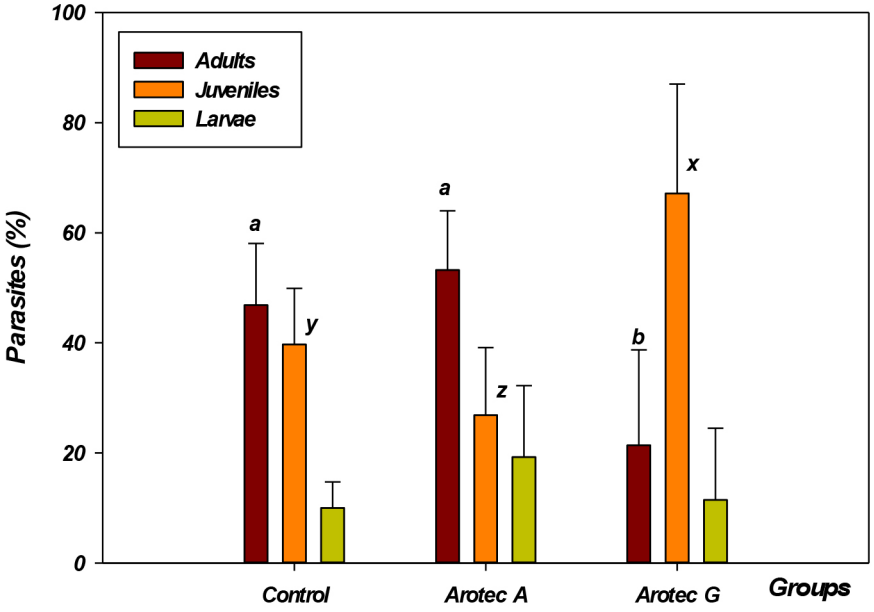

Fig 3.- Percentage of adults, juveniles and larvae of *S. chrisophrii* in the gills of the fish fed the experimental diets, different letters indicate significant differences (ANOVA, P<0.05, a, b for

POPULATION-SPECIFIC VARIATIONS OF THE GENETIC ARCHITECTURE OF SEX DETERMINATION IN WILD EUROPEAN SEA BASS *Dicentrarchus labrax* L.

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Polygenic sex determination (PSD) is generally considered an evolutionary unstable sex determination mechanism. In a species with PSD, the contribution of genetic and environmental components may vary between populations exposed or adapted to different environmental conditions. European sea bass (*Dicentrarchus labrax* L.) is an interesting model that combines both a PSD system and clearly distinct Atlantic and Mediterranean populations, with genetic substructures even within the Mediterranean Sea.

We studied 927 individuals from a full factorial mating scheme, crossing 60 sires sampled in four wild populations matching with the natural range of the species (15 sires per origin: North Atlantic, NAT; Western Mediterranean, WEM; North-Eastern Mediterranean, NEM; South-Eastern Mediterranean, SEM) with 9 dams from WEM. At 180 days post-hatching (dph), fish were measured for body weight (BW) and fork length (BL) and at 226 dph they were euthanized and sexed by visual observation of the gonads or using the squash technique. Sex was coded as a binary trait (1 for males, 2 for females). Genetic parameters (heritabilities and genetic correlations) for sex, BW and BL were assessed using a linear mixed sire model, accounting for the fixed effects of rearing tanks and sires origins, and the random effects of dams and sires nested into their origin (plus the fixed effect of sex for BW and BL). Using the same multivariate model, a weighted genome-wide association study (wGWAS) was carried out for sex using an iterative procedure, both on the global dataset and within each origin.

In the global dataset, 32.5% of fish were females, with significant variations amongst populations (38.0% in NAT^{a}, 39.0% in WEM^{a}, 25.1% in NEM^{b} and 32.3% in SEM^{ab}; χ^2=12.27, df = 3, *P*-value = $7 \cdot 10^{-3}$) and between dams (χ^2=34.72, df = 8, *P*-value = $3 \cdot 10^{-6}$) and sires (χ^2=124.56, df = 59, *P*-value = 10^{-6}). Sex showed a moderately high heritability (0.52 ± 0.17) and was relatively highly genetically correlated with growth-related traits ($r_{Asex/BW} = 0.69 \pm 0.12$, $r_{Asex/BL} = 0.66 \pm 0.13$).

The wGWAS (Table 1) revealed a phylogeographical pattern of the genetic architecture of sex determination in *D. labrax*, with a gradient of similarities from Atlantic to Eastern Mediterranean populations, which can be interpreted as an ongoing evolution of the mechanism from a common ancestral polygenic system. We did not find any linkage group common to all populations with groups of SNPs explaining more than 2% of the variance, which supports the hypothesis that the genetic architecture of the European sea bass polygenic sex determination model differs among population. This may be due to a different balancing of the genetic and environmental components, as well as with the appearance or functionalization of population-specific genes.

Table 1. Position on European sea bass chromosomes of QTLs explaining more than 2% of the variance for sex tendency in the global dataset and in each of the four offspring groups.

	1A	1B	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18-21	19	20	22-25	24	*x*	UN
Global			•				•	•					•			•								•	
NAT		•		•		•		•	•	•	•		•		•					•	•				•
WEM						•			•											•	•				
NEM		•	•			•						•												•	
SEM		•						•													•			•	

CLIMATE CHANGE, SEA SURFACE TEMPERATURE AND SALMON AQUACULTURE IMPLICATIONS

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Introduction

Salmon have biological and environmental requirements which must be met to enable effective culture. Only a number of countries throughout the world meet the necessary criteria, meaning production areas are limited. Consequently, for long term sustainability it is vitally important to evaluate the past, present and potential future characteristics of an area that may affect production. One of the most important parameters to consider is sea surface temperature which influences growth, health and welfare of farmed salmon. Changes in temperature may affect the site production, the spreading, distribution and severity of diseases and outbreaks, and the carrying capacity of the environment. Increased sea surface temperature due to global warming will therefore have implications for the salmon industry, potentially affecting how, and where salmon are farmed. Even gradual changes will have consequences for the sector and should be considered in national and regional aquaculture plans and strategies. Assessment of temperature changes across spatial and temporal scales can provide information to support the industry in evaluating opportunities and risks, and enable the development of adaptation or mitigation strategies where necessary.

Approach

This study involved an evaluation of past, present and potential future sea surface temperature to assess the implications of temperature change on salmon aquaculture. The analysis considered the main salmon producing countries, with a particular focus on Norway, and made use of global, regional and farm-scale temperature data and modelled outputs. The assessment also considered issues associated with using global and regional temperature data for local level assessment.

Conclusions

The results highlight how spatial and temporal assessment of temperature can be useful for the aquaculture industry when considering potential impacts and opportunities from climate change. Global, regional and local level assessment can be used for a holistic overview which is beneficial for strategic planning and management.

Acknowledgements

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 677039 (ClimeFish).

THE EFFECTS OF THREE *Bacillus*-FERMENTED VEGETABLE PROTEINS AS FISHMEAL REPLACERS IN JUVENILE WHITELEG SHRIMP *Litopaeneus vannamei*

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Two experiments (8 and 4 weeks) were conducted to evaluate the effects of three *bacillus*-fermented vegetable proteins as fish meal replacers in juvenile whiteleg shrimp. In both experiments 24 rectangular 50L tanks were stocked randomly assigned into triplicate groups of shrimp. The number of shrimp per tank and average weight for the first and second experiments were 40 at 0.5 ± 0.01g (mean±SD) and 10 at 4.08 ± 0.12 g (mean±SD) respectively. In the first experiment seven isonitrogenous and isocaloric diets were formulated and fed to shrimp in the semi-recirculation system to replace 30% fish meal in the control diet (CON) with fermented soybean meal + *Bacillus* spp (Soytide®), sterilized Soytide (SSO), fermented soybean meal + corn gluten meal + *Bacillus* spp (Aquatide®), sterilized Aquatide (SAQ), fermented soy protein concentrate + *Bacillus* spp (FSPC), fermented soybean meal + lactic acid (FSBL), and soy protein concentrate (SPC). Results from the first experiment showed weight gain, specific growth rate, and nonspecific immune responses of shrimp fed Soytide® and Aquatide® were significantly higher than those of shrimp fed CON and FSBL ($P<0.05$). The second experiment was carried out to assess the impact of CON, Soytide®, Aquatide® and FSPC on water quality parameters to include DO, pH, NO^-_2 and TAN. Six replicates of the four diets were divided into two treatment groups, with and without AquaStar® (probiotic bioremediation) treatment, to assess their impacts on water quality. The results of the second experiment will be summarized in the presentation.

INFLUENCE OF MATERNAL FACTORS ON EGG QUALITY IN ATLANTIC COD *Gadus morhua*

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Atlantic cod (*Gadus morhua*) is a key species in the Northern Hemisphere and an emerging species in aquaculture. Atlantic cod is a batch spawner in the early spring. Currently, egg quality is determined by fertilization rates and cleavage patterns. Although those measures provide reasonable indication of egg quality, knowledge of the molecular basis of embryonic development would provide better understanding and improvement of current practices of egg quality determinants. Maternal factors are essential for early embryogenesis, since proteins, substrates, nutrients and mRNA are deposited in the unfertilized egg during oogenesis. Those factors are activated through protein modifications and are in use until the zygote genome takes over. Very little is known about how those maternal factors influence embryonic and larval developmental. Cod aquaculture would greatly benefit from identifying genes and maternal factors involved in embryonic development since that opens up the possibility of finding links between regulatory factors of maternal origin and "egg quality".

In order to develop molecular criteria to assess egg quality in Atlantic cod, we stripped gametes from 12 parental pairs and obtained 4-7 egg batches from them during the 2018 spawning season. Eggs and sperm were stripped every 48-72, fertilized and incubated until full batch hatching. Samples were taken at six different embryonic developmental stages during the incubation period (unfertilized egg, zygote, blastula, gastrula, early somatogenesis and late somatogenesis) as well as newly hatched larvae for further molecular analyses. Conventional egg quality determinants such as fertilization rate, normal cleavage pattern and egg mortality were also recorded. Newly hatched larvae from all batches of 3-4 selected parental groups were transferred to larval rearing tanks using standard protocols from the Centre of Marine Aquaculture (CMA). Larval samples were taken at regular intervals for growth measurements and molecular analyses.

Differential expression of the stage-specific genomic markers will be analysed using Real Time PCR to understand the function of the temporal transcriptome activity during cod embryogenesis as well as its relation with the quality of the egg. Maternally gene expression will also be analysed to relate egg quality and maternal effect in the early development of cod.

Preliminary results have revealed significant egg quality variation throughout the spawning season within female, being significantly higher in the middle of the period (Fig. 1). However, egg quality seems to differ also among females what is an additional source of variation to be explained using gene expression developments on the way.

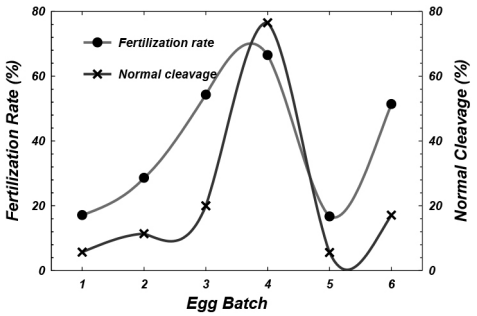

Fig. 1: Egg quality variation within a pair during spawning season.

GENETIC ANALYSIS OF BROODSTOCK, HATCHERY-PRODUCED OFFSPRING AND NATURAL POPULATIONS OF THE WEDGE CLAM *Donax trunculus*

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The wedge clam *Donax trunculus* constitutes an important fishing resource in Spain. Given that maintenance of stock quality is important, in this preliminary study, we investigated the effect of artificial reproduction in a hatchery facility. Genetic diversity and differentiation among a natural bed (Vilarrube; N=50), the broodstock (N=104) and their offspring (N=100) populations was analysed using 11 previously characterized microsatellites (Nantón et al., 2014). In order to reduce the number of amplifications, the previously optimized multiplex PCRs were redesigned. Multiplex 1 was used and the largest possible number of loci was optimized in a new multiplex PCR (Multiplex 2') (Table 1).

All microsatellite loci were polymorphic in the three samples and the mean of allele number ranged from 7.455 (offspring) to 9.545 (broodstock). The average expected (H_e) and observed (H_o) heterozygosities were 0.614 and 0.470 in the broodstock, 0.560 and 0.461 in the offspring, and 0.620 and 0.474 in the natural bed, respectively. Allelic richness ranged from 6.807 (offspring) to 7.418 (broodstock). Heterozygosity levels were very similar between samples but the difference between the mean of allele number was higher. Many studies have found that reductions in mean number of alleles can occur in the absence of marked changes in heterozygosity, when hatchery and wild populations were compared (An et al., 2011a, b). The allele lost is more important than a change in allelic frequency because the latter may change again by random drift but a loss of alleles cannot be recovered. Genetic differentiation analysis showed low but significant differentiation between the offspring and broodstock and also between offspring and natural bed populations (Table 2). Such differences could be related to a reduction in the effective number of contributing parents. Furthermore, genetic drift has probably played an important role in the observed loss of allelic richness, causing offspring to differentiate from broodstock and from natural population. Genetic drift may negatively affect the reproductive capacity of the stock because genetic factors are important in the production of high quality seed. Therefore, these data are useful to ensure the success of artificial seed production and to manage the production of wedge clam seed in hatcheries.

This work was supported by the Ministerio de Economía y Competitividad (Spain) through project AGL2016-75288-R AEI/FEDER,UE.

TABLE 1. Features of the Multiplex 2'.

Locus	Fluorochrome	Concentration (μM)
Dtr47	VIC	0.2
Dtr90	PET	0.2
Dtr108	NED	0.1
Dtr117	FAM	0.2

TABLE 2. Pairwise *F*st values (above diagonal) and corresponding *P*-values (below diagonal).

	Broodstock	Offspring	Natural bed
Broodstock		0.032*	0.000
Offspring	0.000		0.036*
Natural bed	0.339	0.000	

* significant after sequential Bonferroni correction.

OPTIMIZING OYSTERS DENSITY IN INTEGRATED PRODUCTION WITH FISH IN MARINE PONDS: HOW MANY ARE TOO MUCH?

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The growth, survival, water quality and total production of an integrated production using two different oyster densities and the same amount and proportion of fish were compared. The oysters, *Crassostrea gigas* triploids, were cultured together with meagre, *Argyrosomus regius*, white seabream, *Diplodus sargus* and mullet, *Mugil cephalus* in earthen ponds. The trials were performed at IPMA's, Aquaculture Research Station located at the Ria Formosa Natural Park in Olhão, Southern Portugal. Two replicate ponds of 500 m^2 surface and 1,5 m deep were used for two oyster density treatments (Low Oysters, LO, and High Oyster, HO) in an open system with daily water renewal ranging from 40 to 120%, daily fish feeding with automatic feeders (an average of 1,5% of daily feed ration) and bottom air injectors. The oysters were cultured in mesh bags attached to floating buoys distributed along longlines. Table 1 shows the different initial densities for both fish and oysters.

Results in Table 2 show lower growth rates for oysters in HO treatment, with no significant differences in survival. Phytoplankton was reduced by HO treatment at similar water renewal regimes (60-100%/day). In the same treatment meagre and seabream growth were higher with more oysters in the ponds but the levels of dissolved oxygen (DO) were significantly lower with less primary productivity (lower oxygen production during daytime).

The overall results indicate that HO treatment could affect negatively DO dynamics in ponds by limiting phytoplankton populations (top-down control) and this in turn may affect the amount of energy use in aeration and fish production. These results indicate that, for IMTA in ponds, the dynamic equilibrium between trophic groups interactions and growth with water quality and water renewal is critical since it needs continuous adjustments for optimum performance. Modelling may help to adapt IMTA production to each farm characteristics.

Acknowledgments: To project IMTA-EFFECT, for the financial support of this study (Atlantic Area Transnational Program nº 2009-1/123).

Table I – Initial number of individuals used for each treatment with corresponding mean weight and density.

Species	Treatment		Initial input	
	LOW OYSTER	HIGH OYSTER	Mean weight, g	Density, Kg/m^3
A. regius	2700	2700	175 ± 32.77	0.63
D. sargus	500	500	236 ± 19.12	0.16
M. cephalus	320	320	268.0 ± 94.59	0.11
C. gigas	**12000**	**24000**	1.5 ± 0.15	0.02/0.04

Table II – Main results and significant differences found between treatments

Parameter	Sig. between treatm.	Higher where?
Meagre growth	Yes	HO
seabream growth	Yes	HO
Mugil growth	No	-
Oyster growth	Yes	LO
Chlorophyls	Yes	LO
Average OD	Yes	LO
Average pH	Yes	LO
Average turbidity	Yes	HO
Aeration costs	Yes	HO

CHALLENGES TO AQUACULTURE EXPANSION IN EUROPE AND NORTH AMERICA: AN INDUSTRY PERSPECTIVE

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An analysis of aquaculture production in the EU, based on the FAO Fisheries Information system (FIGIS), shows that aquaculture production in the EU has fallen by approximately 8% since 2000, from 1.4 million tonnes in 2000, to 1.28 million tonnes in 2013. This results from significant drops in three of the top five major producing countries (France, Italy, Spain).

The United Kingdom and Greece are notable exceptions and have increased production of salmon (UK), and seabass and sea bream (Greece). Smaller producers in the EU have generally increased production, but not sufficiently to offset the fall in production in France, Italy, and Spain. Over the same period, production in Norway has increased by 154%, Canada by 27%, China by 100%, and Australia by 140%. The US situation is comparable to the EU. The annual growth rate between 2000 and 2013 in those countries (excluding the USA) ranged from 2.7% to 7.6%, compared to the average annual decrease of 0.8% in the EU28

The EU Horizon 2020-funded *Making Space for Aquaculture* (or AquaSpace) project produced a policy review (O'Hagan et al., 2017) to analyse potential hurdles to aquaculture development in Europe and North America. The development of the review included several steps: (i) questionnaire survey to industry, managers, academia, and other stakeholders such as the insurance sector; (ii) preparation of an analysis of survey results; (iii) discussion of the main outcomes at a high-level meeting in 2016 in Venice, Italy, which included representatives from key sectors from Europe, North America, and non-European GFCM[1] nations; (iv) preparation of the final review, including a series of invited industry case studies.

This paper reviews some of the key findings from an industry viewpoint and discusses how these can be integrated in policy discussion, with the objective of growing the aquaculture sector and providing food security for coming generations.

Table 1 shows some of the points raised by the industry case studies, as an example of the kinds of issues that are of major concern. A full list of issues will be presented, together with an analysis of similarities and differences across Europe and North America. Licensing delays, multiple requirements, and a lack of understanding by regulators and planners about the specificity of aquaculture are repeatedly highlighted both in these industry case studies and in questionnaire responses and are probably the single greatest challenge to growth.

Promotion of employment in rural areas was also frequently mentioned, which is linked to social license considerations. The latter revolve around the acceptance of the activity, rather than consumer acceptance of the product. In Europe and North America, public perception of aquaculture is considered to be negative, at least in segments of the popular press (Froelich et al 2017), but farmed fish now exceed wild capture for direct human consumption (FAO, 2016), and the diet of European consumers has reflected this for at least a decade (Cressey, 2009).

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The review indicates that European consumers place their trust in the major distribution chains, which has allowed the latter to grow the market with non-European products such as Nile tilapia (*Oreochromis niloticus*) and Vietnamese catfish (*Pangasius sp*.). European salmon is considered a high-quality product, with widespread acceptance in Europe and in export markets. The same is true for European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). European shellfish (51% of the EU farmed production) are largely cultivated, and it is recognised that products such as oysters, mussels, and clams are safe and nutritious .

It is noteworthy that although academic studies on consumer acceptance of aquaculture products continue to be published (e.g. Alexander et al., 2016), issues related to product placement and acceptance are not flagged by any of the industry case-studies developed in the AquaSpace policy and management review (O'Hagan et al., 2017).

The final part of this paper outlines a set of key measures proposed by industry in Europe and North America that are seen as fundamental to promote sustainable growth of aquaculture in the coming years.

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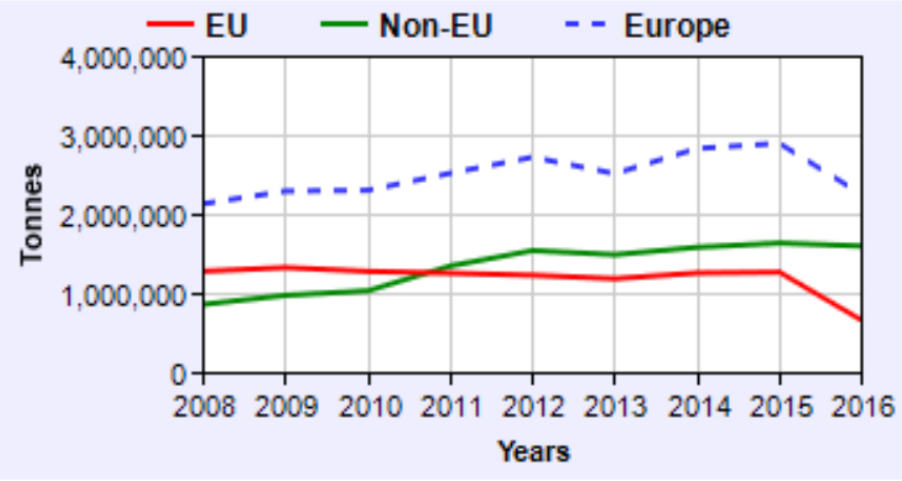

Fig. *1. Aquaculture production in Europe, sourced from EuroStat by means of the AquaPoint application. The drop in 2016 is an artefact due to unreported data.*

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Table 1. Summary of some industry comments on the challenges to aquaculture expansion in Europe and North America.

Keywords and country	Key points
Industry Salmonids Norway	• Salmon farming limits investment in other species • Aqua farmers are in the public focus, with camera teams regularly reporting from inside production and harvest facilities. How does that compare other meat production?
Academia Industry Salmonids Canada	• Many sections of both Atlantic and Pacific coasts are sparsely populated, with pristine waters, wharves, skilled mariners, and a need for employment • Regulation of Canadian aquaculture occurs via both provincial and federal governments, in often complex arrangements for each province
Management Regulatory Research Shellfish N. Ireland	• Conflicts with Nature Conservation designations • It can take many months/years from the date of application until new aquaculture licenses are granted
Industry Shellfish UK	• Distinguishing between the real and perceived risks from aquaculture is challenging for economic actors with no previous aquaculture related experience • Planners need to have a clear vision for aquaculture and its role in diversifying the rural economy and promoting long-term employment
Industry Shellfish USA	• 20-year process and nearly $2 million USD expense required to get the necessary permits for a new mussel farm • Many layers including public health permitting, local shoreline development permitting, disease and animal health permits, federal permits to ensure compliance with the Endangered Species Act, Clean Water Act, Marine Mammal Protection Act, Native American Treaty Rights

VALUATION OF REGULATORY ECOSYSTEM SERVICES FROM SHELLFISH FARMING

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The role of bivalve shellfish in top-down control of eutrophication is well established. The valuation of this regulatory ecosystem service normally uses nitrogen as a currency, and is often assessed on the basis of the nitrogen content in tissue and shell of harvested animals.

The nitrogen removed is then valued by comparison to costs of land-based treatment options, which can vary by 1-2 orders of magnitude, from cheaper alternatives such as point-source treatment plants to costly ones such as reconstructed wetlands (Ferreira & Bricker, 2016).

Other valuation methods include mathematical models such as the Farm Aquaculture Resource Management (FARM) model (Ferreira et al., 2007), which considers the overall net nitrogen removal by annualizing it over the whole culture cycle (Ferreira & Bricker, 2016). This approach gives higher estimates of N removal, and considers that the ecosystem service provided by the shellfish is in the removal of N in phytoplankton and organic detritus from the water column, even if the animals are in the water.

The paradigm underpinning the harvest approach is open to question, because filter-feeding bivalves do not remove dissolved nitrogen (as e.g. seaweed aquaculture does); in other words, oysters or mussels remove the *symptoms* of eutrophication, rather than the causative factors (sensu Bricker et al., 2003). Furthermore, by considering only animals removed from the water (i.e. harvested), this approach results in a valuation of zero with respect to nutrient regulation for a restored reef, if it is not harvested.
Although the modelling approach does consider the sequestration of organic material in the shellfish, regardless of whether the organisms are harvested, it still doesn't focus on the symptoms of eutrophication, because of the use of nitrogen as a currency for valuation of the service.

This paper presents a third approach, which is based on the valuation of the control of a primary symptom of eutrophication, chlorophyll. This is usually considered in terms of water transparency as a regulatory service, but it is as a rule almost impossible to assess—the drawdown of phytoplankton and other organic material can be estimated based on factors such as clearance rate, but the complex dynamics of any coastal system (Eq.1) make it very challenging to translate such estimates into changes in chlorophyll concentration.

$$\frac{dB}{dt} = P - G_s - G_z - M + advection\ \&\ dispersion \qquad \text{(Eq. 1[1])}$$

Although changes in underwater light climate due to shellfish aquaculture can of course be measured, fluctuations in turbidity e.g. driven by the tidal signal make it a challenge to link causality, and local-scale models such as FARM cannot address the connection between an aquaculture farm and broader-scale, system-wide chlorophyll draw-down.

The Shared Waters Enhancement and Loughs Legacy (SWELL) project uses a system-scale framework to examine shellfish carrying capacity. Because the SUCCESS framework makes an explicit link to the catchment nutrient loading through the application of an ecohydrological model, we were able to use the framework to develop regressions of (i) the reduction in land-based nitrogen loading (i.e. the influencing factor of eutrophication) and the reduction of chlorophyll percentile 90 (P_{90}) in different areas of Lough Foyle (UK/Ireland); (ii) the reduction of chlorophyll P_{90} in different areas of Lough Foyle due to shellfish (blue mussel, native oyster, Pacific oyster) aquaculture. We then used data on land-based nutrient removal to estimate the cost of reducing chlorophyll in the lough by 1 mg L^{-1}, and derived an equation for valuation of the ecosystem services of bivalves. When compared to the type of nitrogen removal calculation described above, we found that the present approach results in a substantially higher valuation of the regulatory service of eutrophication control.

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Furthermore, by using a system-scale framework such as SUCCESS, we are able to directly address key indicators used for assessment of ecosystem health, such as the EU Water Framework Directive (2000/60/EC) Biological Quality Elements (BQE) abundance and biomass of phytoplankton, and equivalent indicators used in the United States and Canada for eutrophication assessment.

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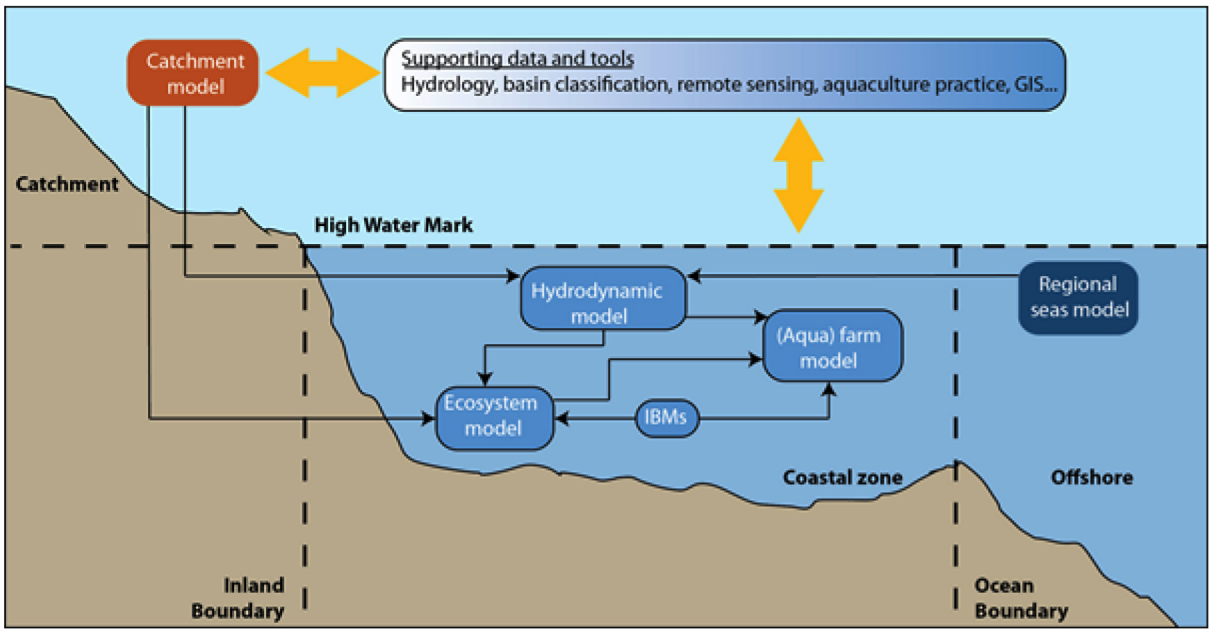

Fig. 1. The SUCCESS (System for Understanding Carrying Capacity, Ecological, and Social Sustainability) Framework for modelling system-scale carrying capacity.

FORECASTING CLIMATE CHANGE EFFECTS ON SHELLFISH AQUACULTURE: A MODEL ANALYSIS

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Global warming is a major impact of climate change. Changes in temperature trends and variability bring about associated shifts in hydrology and hydrography of water bodies, lead to primary production shifts, including patterns of occurrence of algal blooms, and may promote greater incidence of pathogens, all factors that have important impacts on aquaculture.

Practically all cultured aquatic animal species for human consumption are poikilothermic. Consequently, any change in temperature would have a significant influence on general metabolism, and hence on growth rates and production, reproduction, seasonality and even reproductive performance (e.g. relative fecundity, spawning frequency), susceptibility to diseases and toxicants, etc. The lower and upper lethal temperature and the optimal temperature range for shellfish species differ widely. Therefore, climate-change induced temperature variations are bound to have an impact on spatial distribution of aquaculture activities.

The objective of this work is to forecast direct and indirect effects of climate change on some of the most important cultivated shellfish species, to anticipate responses and assist in the adaptation of aquatic food production industries.

In order to achieve this objective, we have applied the following approach (Fig. 1):

i) Simulate different climate change scenarios based on regional, industry-relevant future projections of key environmental variables, using the regional models available within the CERES (https://ceresproject.eu/) project:
    a. Direct effect scenario (e.g. increase in seawater temperature)
    b. Indirect effect scenario (e.g. primary production shifts)
ii) Develop and calibrate individual-based models for key species (blue mussel, Mediterranean mussel, Pacific oyster and Manila clam) to determine the effects of climate change on individual growth.
iii) Scale-up the individual models to test consequences for shellfish populations, their environment, and economic performance, using the Farm Aquaculture Resource Management (FARM) model.
iv) Run these models under current and climate change scenarios for different species and locations.

This modelling approach enabled us to analyze changes in a) individual growth, b) farm productivity and profitability, and c) environmental services/effects, such as nitrogen removal, for each species-location pair. All these factors were sensitive to direct and indirect effects of climate change, such as water temperature changes and primary production shifts.

Some example results are shown in Table 1. While an increase in food (using chlorophyll *a* as a proxy) always leads to a higher end-point biomass, the effect of increased seawater temperature on growth depends on the optimal temperature range for each species.

Our results will help to understand of how climate change will influence shellfish aquaculture in the European Union, particularly bearing in mind that organically extractive aquaculture is 52% of the total EU production. The modelling tools developed here will help develop adaptive strategies allowing the aquaculture industry to anticipate, prepare, and adapt to adverse changes or future benefits.

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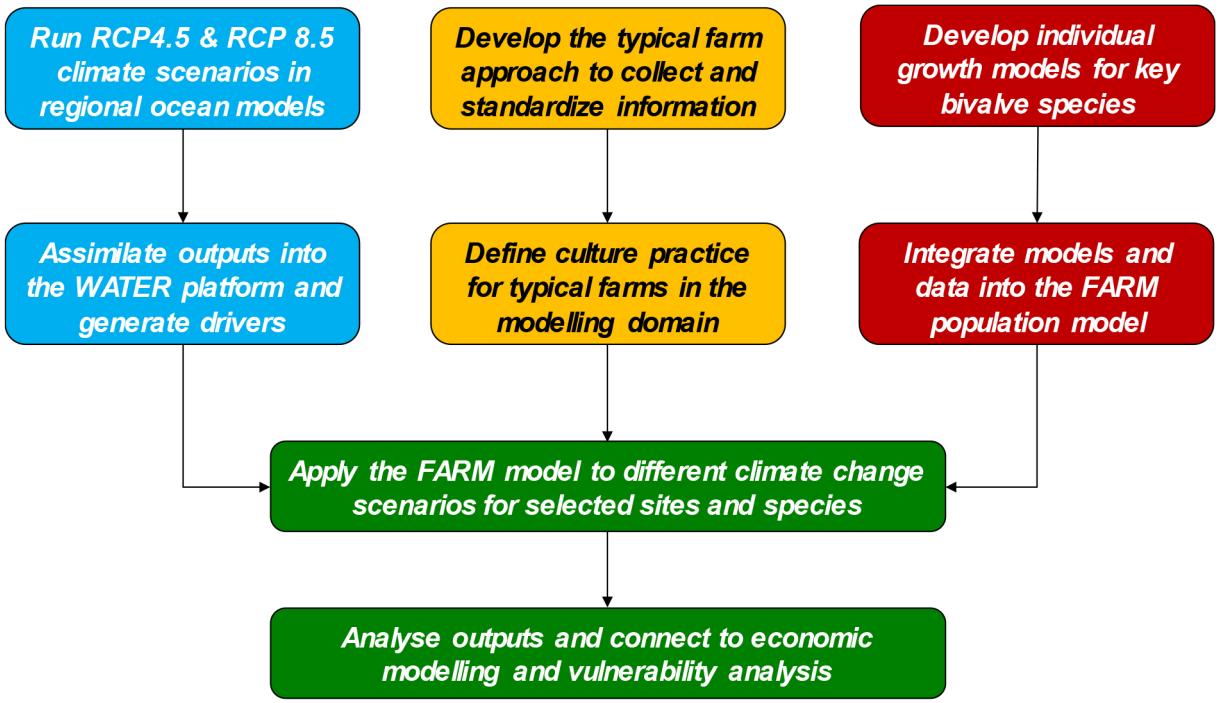

Fig. 1. General modelling framework for predicting effect of climate change on shellfish aquaculture.

Table 1. Comparison of some of the production and environmental effects of shellfish farming under different climate change scenarios obtained from the individual model.

Species	Integrated outputs over the culture cycle	Standard	+2°C (%)	+0.5 μg L^{-1} (%)
Pacific oyster	End-point biomass (g live weight)	89.3	+7.4	+7.3
	Chl-a removal (mg chl m^{-3})	121.3	+20.9	+15.5
	Net POM removal (g POM m^{-3})	75.0	+27.2	+3.2
	Net nitrogen removal (g N)	3.88	+27.6	+2.8
Blue mussel	End-point biomass (g live weight)	12.3	-3.3	+17.1
	Chl-a removal (mg chl m^{-3})	56.0	+5.5	+20.2
	Net POM removal (g POM m^{-3})	27.4	+9.5	+4.4
	Net nitrogen removal (g N)	1.20	+11.7	+2.5
Med mussel	End-point biomass (g live weight)	13.3	-35.3	+0.8
	Chl-a removal (mg chl m^{-3})	27.8	-23.0	+22.3
	Net POM removal (g POM m^{-3})	10.0	-23.0	+1.0
	Net nitrogen removal (g N)	0.44	-27.3	-9.1
Manila clam	End-point biomass (g live weight)	20.7	+33.8	+12.1
	Chl-a removal (mg chl)	39.6	+43.4	+18.4
	Net POM removal (g POM m^{-3})	11.3	+43.4	+4.4
	Net nitrogen removal (g N)	0.56	+39.3	+3.6

INTEGRATED CARRYING CAPACITY MODELLING FOR SHRIMP AND FINFISH CULTURE IN INDONESIA

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Introduction

In 2015, Indonesia harvested an estimated 15.6 million tonnes of aquaculture products, making it the second largest producer in the world, with about four times the combined production of Europe, Canada, and the United States. While a significant proportion of that harvest corresponds to seaweeds, i.e. the base of the food chain, development plans projected to 2030 focus on a range of carnivorous species, including grouper, barramundi, and various shrimp species.

The challenges presented by the annual growth targets for different species (6-18% y^{-1}) are very substantial, not only in terms of limitations with respect to feed, particularly availability of fish meal and fish oil, but also in the wider dimensions of environmental sustainability and ecosystem carrying capacity.

In order to illustrate the underlying issues, and in particular the challenges in the implementation of the Ecosystem Approach to Aquaculture, taking into account ecological sustainability, social balance, and harmonization of multiple uses, a well-tested framework for carrying capacity assessment is being tested in Pegamatan Bay, North Bali (Fig. 1). The rationale is that models of this kind can be used to support industry development, and help managers make informed decisions about aquaculture expansion.

Approach

The approach used for carrying capacity assessment builds on a combination of various models (Fig. 2), which together are used to understand the different components of the system, including (i) the catchment, which has diverse uses such as urban activity and agriculture, fish hatcheries, and shrimp cultivation; (ii) the inner bay, which is fringed by a coral reef, and produces about 1000 t y^{-1} of finfish in both plastic circle cages and traditional wooden cages; (iii) the extended bay area, which connects to the Java Sea, with the fast-flowing water in the Java Straits to the west.

Performance of land-based shrimp cultivation is one of the key indicators used in the *EcoShrimp* project.

Local-scale growth models for shrimp (Ferreira et al., 2015) are being used to determine land-based loading, and the system-scale ecosystem model will be used to determine the water quality at the inlet pumping stations for the shrimp farm.

At present, the loading of organic material and dissolved substances from land-based and inshore culture appear to be of the same order of magnitude, but it is unclear what proportion of the overall organic loading is aquaculture-related by comparison to other sources. Primary and secondary symptoms of eutrophication (*sensu* Bricker et al., 2003) are the key diagnostic features of ecosystem health, and will be calculated by means of a bay-scale ecosystem model.

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Fig. 1. General view of Pegamatan Bay. There is a large (1200 t y^{-1}) shrimp farm to the west, and the bay is also used for grouper and tiger grouper (artisanal) and barramundi (circle cage) culture.

Results and Discussion

An example of estimated loading from shrimp ponds is given in Fig. 3. The model will be calibrated for the Pegamatan Bay shrimp farm, and used to generate accurate daily loading of dissolved nutrients, organic matter, and chlorophyll. These results will be used by the catchment model, in combination with other loading data from agriculture and other uses, to drive the overall land inputs to the bay.

The approach applied by Cubillo et al (2016) to determine organic loading to the sediment from open-water finfish culture will be used to estimate internal loading to the bay, so that different management outcomes can be examined. The exchange regime with the shelf is also of importance, since that conditions the flushing capacity of the coastal area.

An important component of the *EcoShrimp* work is to ensure there is transferability of tools, to allow for a broad application. This will partly be achieved through a close integration of a stakeholder user group, and the delivery of simple screening models that (i) can synthesize the outcomes of complex modelling frameworks; (ii) are free and simple to use; and (iii) do not have extensive data requirements.

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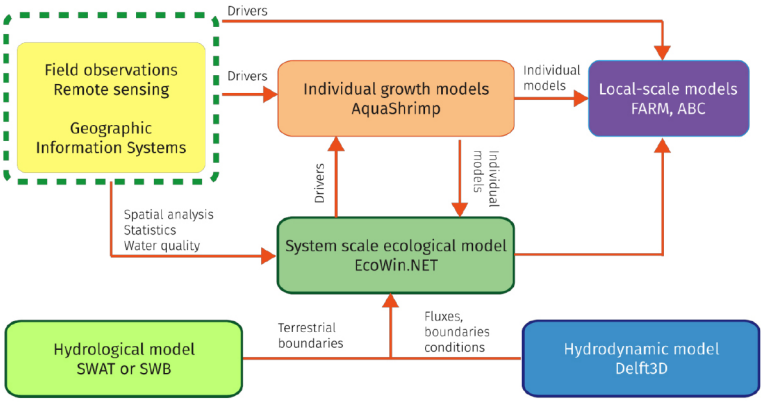

Fig. 2 –General modelling framework, showing the different types of models and respective interactions.

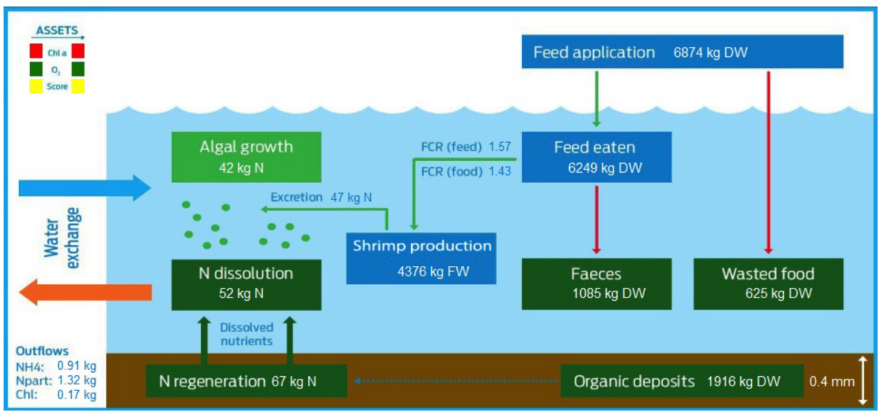

Fig. 3 – Example outputs from the FARM model (Ferreira et al, 2015).

INCREASING DHA LEVELS IN RAINBOW TROUT *(Oncorhynchus mykiss)*

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There are currently no plant oils available that can be considered as equivalent alternatives to fish oil. From our previous experiments we know that the isoflavone metabolite equol potentially increases tissue levels of docosahexaenoic acid (DHA). In addition, Ahiflower oil, a plant oil rich in SDA, is able to enhance the content of eicosapentaenoic acid (EPA) in rainbow trout. Therefore, it can be assumed that both ingredients modify the fatty acid biosynthesis and, subsequently the content of long chain polyunsaturated fatty acids (LCPUFA). The objective of this study was to evaluate the interactions of both mechanisms to improve the nutritional quality of rainbow trout.

Rainbow trout (initial weight 89.4±10.9g) were fed five diets in triplicates. A fish oil based diet served as reference diet (FV). The experimental diets contained a blend of Ahiflower oil and vegetable oils (AV). Three diets were supplemented with equol by 0.1, 0.2 and 0.3% DM of the diet (AV-EQ1, AV-EQ2, AV-EQ3). AV without supplementation served as negative control diet (C-AV). Fish were fed 1.6% of fish biomass for 56 days. Whole body and fillet samples were taken for chemical nutrient analysis. Fatty acid compositions were analyzed for whole body, fillet and liver samples.

At the end of the experiment, all groups had doubled their weight. No significant differences were found between treatments in terms of growth and performance parameters. Chemical nutrient composition of whole body samples was not significantly different between groups in terms of crude protein, crude lipid or crude ash content. In fillet, crude protein levels of AV-EQ1 and -EQ2 were significantly higher compared to FV. The content of EPA showed no consistent pattern between tissue samples but all AV-groups were characterized by higher liver EPA values than FV. DHA values of AV-EQ2 and -EQ3 were similar to FV in fillet, tended to be highest in whole body and were significantly higher in liver compared to FV.

The results of this experiment indicate that the combination of equol and Ahiflower oil can be fed to rainbow trout without impairing growth. In addition, high contents of dietary ALA and SDA due to Ahiflower oil inclusion seem to enhance EPA synthesis. Thus, fish of all dietary groups supplemented with Ahiflower oil showed higher liver EPA levels. Further, combining Ahiflower oil and equol seems to positively affect DHA levels in fish tissue. However, this effect seems to be dose-dependent as it was only found for 0.2 and 0.3% of equol in the diet.

TRACE MINERAL NUTRITION OF FISH AND SHRIMP: A SHORT REVIEW

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In the past two decades, increasing economic and sustainability constraints have pressured the aquaculture industry to significantly reduce inclusion rate of dietary marine feedstuffs. Despite great advances made by both academia and industry, gaps remain in a number of important areas of nutrition, which compromises the successful replacement of fish meal with alternative ingredients. Alternative ingredients of plant or animal origin are often limiting in concentration and availability of several essential nutrients. Supplementation of fish and shrimp diets with adequate levels of all limiting nutrients, including essential trace minerals such as Zn, Mn, Cu, Fe and Se, will be determinant in moving towards precision dietary formulation based on nutrients, rather than ingredients (e.g., fish meal). A better understanding of the role and essentiality of trace minerals for growth and health of aquatic species is recognized as key to helping meet the needs of challenging production conditions (i.e., high stocking density, disease outbreak, and resistance to antibiotics and other medicinal treatments). Dietary optimization, under commercial conditions, is imperative to continuous growth of nutritious and healthy farmed aquatic animals.

Although more limited than for terrestrial animals, evidence of the benefit of more bioavailable trace minerals, compared to inorganic trace minerals, for fish and shrimp is mounting. Supplementation with zinc amino acid complex (Availa®Zn; Zn-AA) improved growth and FCR of Asian sea bass at 50 ppm Zn (90 ppm dietary Zn), improved growth and immune response of Pangasius catfish at 50 ppm Zn (87 ppm dietary Zn), and improved growth and FCR while decreasing sea lice (*Caligus rogercresseyi*) infestation stage in Atlantic salmon at 60 ppm Zn (123 ppm dietary Zn). The performance mineral zinc methionine (ZINPRO®; Zn-Met) was shown to be 3 to 5 times more bioavailable than inorganic Zn ($ZnSO_4$), in meeting growth requirements of channel catfish fed purified and practical diets containing phytic acid, respectively. In addition, benefits of supplementing channel catfish diets with metal amino acid complexes vs. inorganic zinc were observed to go beyond growth performance, with Zn-Met being 3 to 6 times more effective than $ZnSO_4$ in protecting channel catfish against *Edwardsiella ictaluri*. Similar growth and immune responses were observed in whiteleg shrimp, when metal amino acid complexes were supplemented at half the level of a mixture of Zn, Mn, Cu, Fe and Se from inorganic sources. Alone or in combination with inorganic zinc (ZnSO4), zinc amino acid complex (Availa®Zn) resulted in significantly higher growth rate and immune response of shrimp than inorganic zinc alone. In addition, the supplementation of shrimp feed with astaxanthin and xanthophyll, in combination with iron amino acid complex (Availa®Fe) and Se-AA, has not only improved immunity, but also enhanced cooked shrimp color, according to consumer acceptance scores.

Trace mineral recommendations for optimal fish and shrimp growth and health performance will be discussed in this presentation.

DOES PUMPING RATE AFFECT PARTICLE RETENTION EFFICIENCY IN BIVALVES? THE CASE OF OYSTERS AND MUSSELS

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Bivalve particle retention efficiency has been traditionally attributed solely to particle size, with particles greater than a nominal threshold size assumed to be captured at similar high efficiency. However, recent findings, mostly driven by the use of new technologies, i.e. laser particle counters and DNA sequencing, have challenged this paradigm. A key component of the capture process could be driven by the water flow through the gills, which could affect the angle at which particles approach the filaments. In this study, the retention efficiency of the oyster *Crassostrea virginica* and the mussel *Mytilus edulis* has been measured and explored in relation to their pumping rates, which can be used as a proxy for water velocity for individuals of similar sizes. Preliminary results have revealed a species-specific effect of pumping rate on retention efficiency, with mussels showing similar retention across the observed range of pumping rates, but oysters showing a positive relation between retention and pumping for small particle sizes (Figure 1). These results are important for improving our understanding of bivalve energetics and growth but also bivalve-phytoplankton trophic interactions, which are critical in bivalve aquaculture sites where the cultured population could exert a significant effect on the structure of phytoplankton populations.

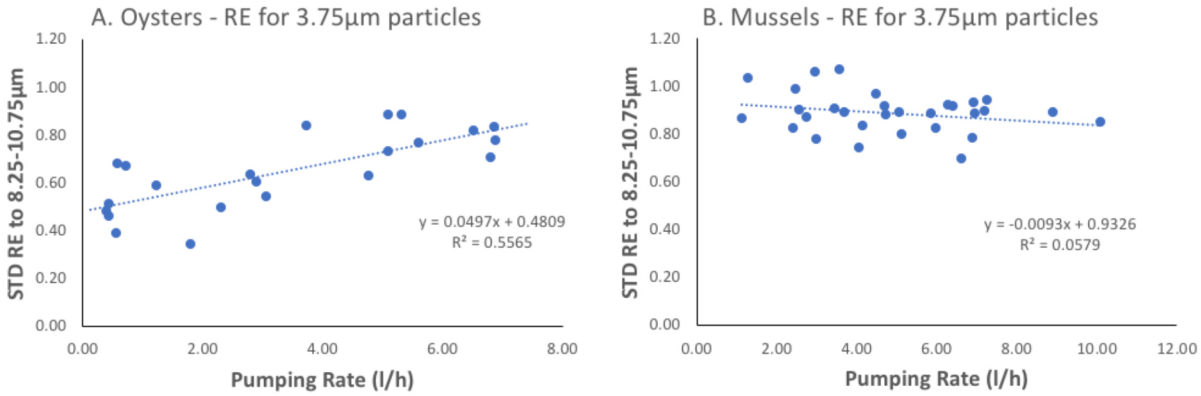

Figure 1. Standardized retention efficiency of oysters (A) and mussels (B) for 3.75µm particles for different pumping rates.

KNOWLEDGE FOR INCREASED PRECISION IN SEA LICE COUNTING ON FISH FARMS AND ESTIMATION OF OCCURRENCE

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Salmon lice, *Lepeophtheirus salmonis*, are parasitic and nourish on the skin and blood of salmonids. For the host, this infestation causes both reduced welfare and growth, and can cause increased mortality in aquaculture and wild populations. Good conditions for the reproduction and growth of salmon lice exist in net pens on fish farms as they contain a high number of potential hosts. Due to this fact, salmon lice constitute a serious problem and are a major challenge for the aquaculture industry. Treatment against salmon lice, surveillance and preventive measures incur significant costs for fish farmers. Further is it a legal obligation for fish farmers to monitor, control and report lice count in Norwegian fish farms every week. Repeated or high exceedance of given limits of salmon lice on the farms can trigger costly measures, such as requirements for reduced biomass or removal of fish from net pens. Production and a potential growth in the industry will be regulated by an action-based system, that is, based on a model recently developed to manage sustainable and predictable growth in the aquaculture industry.

The present project is assessing current methods for lice counting on fish farms and the related uncertainties associated with infestation estimation. The project is organized in four work packages, where the first work package documents current methods of lice counting, while the second work package focuses on practical testing in full scale. The third work package focuses on uncertainties with respect to lice count estimation and its relationship to sample size and heterogeneity in population distribution. The purpose of the fourth work package is to conclude on possible methodical advances and discuss how such activity could be standardized while maintaining the required flexibility to answer to local demands and constraints. A presentation of the project as well as results from the various work packages will be included in this presentation.

EFFECTS OF DIETARY IMMUNOSTIMULANTS ON THE IMMUNE CONDITION IN GILTHEAD SEA BREAM (*Sparus aurata*)

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The concept of maintaining the health of fish through the best possible nutrition is well accepted in modern aquaculture. Scientific evidence clearly indicates that dietary nutrients as well as additives have the ability to stimulate the immune system and to protect the fish from pathogenic diseases. A common strategy for maintaining fish health and to improve performance, immunostimulants have been used as dietary additives to improve weight gain, feed efficiency, and/or disease resistance in cultured fish.

\Under this context, an immunostimulant containing *Echinacea purpurea*, mannan oligosaccharides, β-glucans and vitamin C (INMUNOTEC®, TECNOLOGÍA & VITAMINAS, S.L., Spain) was evaluated in seabream during 75 days. The inclusion of the additive was tested at two doses (0.15 and 0.3%) and compared to a control diet devoid of the feed additive. Each diet was tested with four replicates (basal diet: 48% crude protein, 17% crude fat, energy: 21.7 MJ/kg feed). Preliminary results showed that the group supplemented with Immunotec at 0.15% were 5% heavier in body weight (82.0 ± 1.3 g) when compared to the control group (78.1 ± 1.0 g) ($P < 0.05$), while the BW in fish the diet containing the feed additive at 0.3% was similar to the control group (Figure 1).

In addition, the effect of the feed additive was evaluated in terms of oxidative stress markers (SOD, GST, TAC, LOP), several non-specific serological immune parameters (lysozyme, complement, bacteriolytic activity), as well as the transcriptomic profiling of the intestine. Results from different analytical approaches will be integrated and discussed in order to evaluate the performance and immune competence of fish fed this functional feed with the purpose of addressing new nutritional strategies to improve aquaculture productivity and solutions against different stressors that concern the sector.

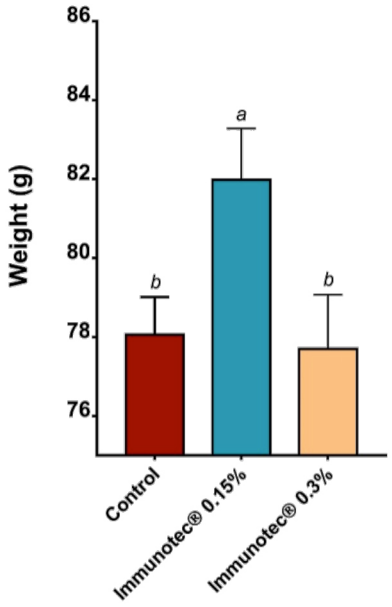

Figure 1 Final weight of juvenile gilthead seabream fed with three experimental diets with different additive inclusion levels. Different letters represent significant differences among groups ($p<0.05$).

PUBLIC ATTITUDES TOWARDS MARINE AQUACULTURE IN CANADA: INSIGHTS FROM THE PACIFIC AND ATLANTIC COASTS

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With 25% of the world's coastline, Canada is endowed with enormous potential for marine aquaculture. Its development on both the Pacific and Atlantic coasts, however, has come under increasingly intense public scrutiny and generated heated debate over many issues including First Nations territorial rights, impacts on wild fisheries, and environmental impacts. Provincial governments, industry alliances, scientists and ENGOs (environmental non-governmental organizations) have become increasingly embroiled in a communication tug-of-war to win the hearts and minds of the public. To date, however, there has been very limited community level research into the public's awareness of aquaculture, the information sources viewed as being most credible, and the issues that resonate the most.

This paper reports the results of a study undertaken in small coastal communities on Canada's Pacific (west) and Atlantic (east) coasts that investigated awareness of aquaculture, and the issues that people associate with its development. While many studies have focused exclusively on attitudes related to either finfish or shellfish farming, this study explores community perceptions related to salmon, shellfish and seaweed farming. Significant differences exist between the Atlantic and Pacific coast respondents in terms of their familiarity with and evaluation of different farming systems, the information sources that they rely upon, and their perceptions of the reliability of different information sources.

EXPLORING THE RELATIONSHIP BETWEEN NEW AQUACULTURE SYSTEMS AND FOOD SECURITY: INSIGHTS FROM THE BOLIVIAN AMAZON

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New aquaculture systems are emerging in regions outside the historical epicenter of aquaculture production in Asia. This increasing diversity of aquaculture systems has begun to attract research attention and is generating new questions about what social, environmental, and economic impact aquaculture can have for participants. Given aquaculture's potential to contribute to feeding the world, a key question that has emerged is how are these new aquaculture systems affecting food security. In Bolivia, a relatively new cluster of Tambaqui (*Piaractus Brachypomus*) culturing in earthen ponds has emerged and is experiencing rapid growth. The cluster is located in the central region of the country that forms the Southern fringes of the Amazon Rainforest. Despite Bolivia having one of the lowest per capita fish consumption rates in the world, and being the poorest and most food insecure country in South America, the new aquaculture system is showing signs of generating broad positive social, economic, and food security impacts. It is also revealing further insight into the nature of the relationship between small-scale entrepreneur driven aquaculture value chains and food security. This presentation, based on a value chain analysis using semi-structured interviews with 40 aquaculture farmers, 40 neighboring non-aquaculture producing agriculturalists, 26 aquaculture chain workers, and 16 key informants, outlines the pathways through which aquaculture is affecting food security for different chain actors in Bolivia. It then discusses some of the opportunities and barriers associated with scaling-up aquaculture system driven food security.

INSECTS AS A SUBSTITUTE FOR FISHMEAL

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To produce fish feed more sustainable, the industry substitutes fishmeal more and more by alternative sources of protein. The aim of the present project is to determine how the substitution of fishmeal by insect powder influences the extrusion process and the product quality. A Brabender twin-screw extruder KETSE 20/40 is used to produce the feed. Furthermore, the physical properties of the fish feed, such as bulk density, degree of expansion, hardness and floating behavior, are analyzed.

AMERICAN BULLFROG (*Lithobates catesbeianus*) AS AN EXPERIMENTAL MODEL FOR RANAVIUS INFECTION

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Amphibians are among one of the most diverse and ecologically important groups in the environment they inhabit, but the reduction of diversity on a global level and the threat of extinction of several species has brought them to another worrying position. In recent decades, several factors were listed as potential agents for the decline, especially the causes of anthropogenic origin, but emerging diseases such as chytridmicosis and ranavirose, have shown strong negative contributions to this community. Ranavirose is the term given to the disease caused by viruses of the genus ranavirus, especially the Frog virus 3 (FV3) species that is the most studied. His plurality of ectothermic hosts (fish, reptiles and amphibians), wide geographic distribution and high rates of infectivity and lethality made a notifiable disease required by national and international regulatory bodies. In the country there is still incipient studies which makes it difficult to estimate what the actual viral spread situation. Although case reports in the country since 2003 the only species currently affected is *Lithobates castebeianus*, popularly known as the American bullfrog. In our study we used it as an experimental model to evaluate the infection process that affects the animals and try to understand their clinical evolution.

Material and methods: For this we used a negative control, without viral presence, and three treatments with different viral concentrations (T1: $1{,}9 \times 10^{1}$ p.f.u./0,05mL; T2: $1{,}9 \times 10^{3}$ p.f.u/0,05mL e T3:$3{,}1 \times 105$ p.f.u/0,05mL). For 21 days the animals were observed seeking manifestation of clinical signs. Molecular analyzes of conventional and real-time PCR were used to confirm and quantify viral presence on infected animals, as well as correlate the histological tissue damage were observed.

Results and Discussion: PCR showed a low rate of infection in both larval and adult animals. To explain our results, we hypothesized the following:
1) The amount of inoculum applied in frogs was not enough to cause infection;
2) For the manifestation of clinical signs of ranavirose in this species there is a need for a cofactor;
3) The animals were infected with FV3, but recovered over the experimental period and,
4) The inoculum used has low virulence. We also discuss the susceptibility of *L. catesbeianus* when infected with FV3 and the presence of clinical signs associated with ranavirosis.

Taking into account all these hypotheses, additional work is needed for better understanding the epidemiology of *Ranavirus* infection.

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COMPARISON OF GENETIC DIVERSITY IN FOUR EUROPEAN HUCHEN *Hucho hucho* L. BROODSTOCKS FROM POLAND, GERMANY, SLOVAKIA AND UKRAINE

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European huchen (*Hucho hucho*) is a very valuable and one of the most endangered members of the Salmonidae family. Therefore, during conservation management, the study on genetic structure and diversity of European huchen stocks is very important. Four broodstocks of European huchen from: Poland, Germany, Slovakia, and Ukraine were investigated based on ten microsatellite DNA markers. In the present study the genetic variation and genetic distance between four selected broodstocks were evaluated. Additionally, the size of the genetic bottleneck and founder effect, that affected genetic variation, was examined.

The moderate genetic diversity (D=0.501-0.544 and I=0.924-1.047) in three European huchen broodstocks (Poland, Slovakia and Ukraine) was identified, while German broodstock was characterized by high genetic diversity (D=0.649 and I=1.254). Observed (Ho) and expected (He) heterozygoisty across the investigated loci in all broodstocks ranged from 0.483 to 0.686 and from 0.503 to 0.650, respectively. The reduction of genetic variation in all studied broodstocks, based on The Garza-Williamson index (M=0.146-0.279) and values of the heterozygoisty excess, was noted. Probably it was result of the founder or bottleneck effect. Based on genetic differentiation (Fst) and Nei's genetic distance the closest genetic relationship was identified between Polish and Ukrainian broodstocks. In contrast, the highest genetic divergence parameters (Fst and Nei's distance) were observed among German, Slovak and Ukrainian broodstocks.

IMPROVING LUMPFISH (*Cyclopterus lumpus*) GROWTH AND ROBUSTNESS: EFFECTS OF REDUCED NUTRIENT DENSITY AND FUNCTIONAL ADDITIVES

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Sea lice remains one of the largest challenges of the salmon aquaculture industry, and there is an increasing demand for de-licing strategies that do not negatively affect the health of the fish. The use of cleaner fish as a method of controlling infection has become well established in the European salmon aquaculture industry in recent years, and farmers indicate good efficiency, reducing the need for medical or mechanical treatments.

Lumpfish (*Cyclopterus lumpus*) are well adapted to colder temperatures and are less complicated to farm than other lice eaters such as Ballan wrasse (*Labrus bergylta*). The Norwegian production of lumpfish juveniles reached 30 million individuals in 2017, and is expected to increase further in 2018. Nutrient-dense feed is a factor believed to influence rapid growth, development and health of lumpfish. However there is a request from the market to reduce the growth rate to comply with vaccination guidelines from manufacturers (i.e. 500 degree days from vaccination to sea transfer) and still have efficient lice eaters in the cages.

In this RFF Nord-funded project in collaboration with Nofima (#267738) a feeding trial was carried out at Tromsø Aquaculture Research Station, with fish delivered from Center of Marine Aquaculture. Triplicate groups of lumpfish juveniles (average initial weight 2 g) were fed 3 diets with 2 different gross energy and protein levels for 12 weeks. A functional additive pack including krill, immunostimulatory components, prebiotics, and increased levels of vitamins was present in all diets, however the third diet contained a slightly modified additive pack.

Survival was high throughout the growth period, with average survival above 93% for all diets and an average final weight of 57g. No difference in feed intake or growth performance was observed between the 3 tested diets. Cataracts presence was evaluated after 12 weeks of feeding with clear differences between diets. Liver pigmentation was also enhanced by the diets. At the end of the feeding trial, randomly selected fish from the two best performing diets were labelled and transferred to duplicate challenge tanks for atypical *Aeromonas salmonicida* bath challenge. After 41 days, survival was improved with 30% between diets. These results indicate that a well-balanced reduced nutrient diet has a positive impact on various health parameters, however an impact on growth was not identified.

A NORWEGIAN CULTIVATION PROGRAM FOR THE SUGAR KELP *Saccharina latissima*

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To investigate possible geographical and seasonal differences in growth, biofouling and chemical composition of cultivated *Saccharina latissima* along the Norwegian coast, nine different locations from south (58,1 °N) to north (69,8 °N) were compared. Fertile *S. latissima* sporophytes from the nine origins were shipped to the seaweed hatchery at SINTEF where the seedlings were produced simultaneously and under similar environmental conditions. In February 2017, after 6 weeks in the hatchery, the juvenile seedlings were transported to the cultivation sites and deployed at the sea farms on vertical droppers at 1-2 and 8-9 m depth. In the period from April to September 2017, registrations were made every second to fourth week at all locations. Growth measurements (length, width, density, weight), morphological and biofouling studies and collection of biomass for chemical analyses of amino acids, internal nitrate, carbon/nitrogen (CN), carbohydrates and heavy metals.

The results showed good growth on most stations from the start. The biomass production was particularly high in Central Norway in the period until June, before large portions of the biomass was lost due to heavy biofouling during summer. In Northern-Norway, growth was highest in late summer and autumn.

The content of amino acids, internal nitrate and CN had a clear seasonal variation at all locations, and variations were also found between the cultivation depths as well as between the locations. The results suggest good possibilities for seaweed cultivation along the Norwegian coast, with a prolonged production season north of the Arctic Circle.

Fig. 1: The Norwegian coastline showing the location (with coordinates) of the nine different stations used in the cultivation program

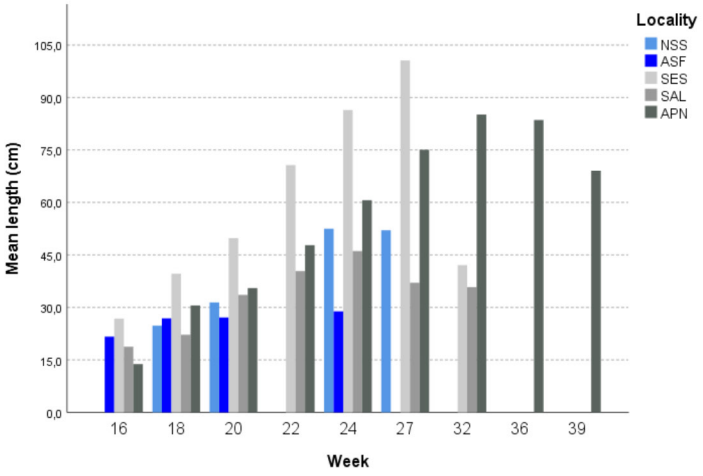

Fig. 2: The growth in length (cm) from five of the cultivation stations from week 16 to 39

APPLICATION OF THE LIFE CYCLE APPROACH TO THE SET-UP OF A PILOT AQUAPONIC FACILITY IN BELGIUM

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Introduction

Aquaponics is a technique which combines recirculating aquaculture systems (RAS) with hydroponic cultivation. In the last decades aquaponics gained increasing attention as a sustainable way of producing fish and vegetable for human consumption (Karimanzira et al., 2017). However, previous studies, suggest that the environmental and economic sustainability of aquaponics is still a controversial subject matter. Flexible assessment tools such as Life Cycle Assessment (LCA) and Life Cycle Costing (LCC), allow one to investigate the whole process, from the design up to the operating phases (Boxman et al., 2017; Forchino et al., 2018). The aim of the present study is the combined application of LCA and LCC to highlight critical aspects related to the construction of an indoor pilot aquaponic facility in Belgium.

Materials and Methods

The aquaponic system (total volume = 19 m^3) is hosted in a 104 m^2 aerated concrete building. The RAS is composed of 6 GRP rearing tanks (total volume = 6.4m^3), 2 GRP sump tanks (1m^3), a GRP swirl separator (3m^3), a drum filter (100W; backwash pump 1.1kW), a moving bed biofilter (1.5m^3), 2 circulation pumps (500W), an air blower (1.3kW), a UV sterilizer (90W) and a heat pump (1.1kW). The system was designed for a yearly production of 700kg of tilapia (*Oreochromis niloticus*) and 3 tons of vegetables. Hydroponic cultures are arranged on 3 levels and made of 33m^2 of deep water cultures and 17 m^2 of NFT equipped with LED lighting (7.2kW). The building is equipped with a double flow ventilation system (1kW) and an air heater (2kW). For both LCA and LCC, system boundaries were set using a cradle-to-gate approach and all the primary data used for the calculation were collected during the construction phase of the system. Input data were grouped in 4 categories: (1) "BUILDING" (i.e. all the materials used to construct the building hosting the aquaponic system), (2) "RAFT" (i.e. the hydroponic cultivation equipment), (3) "Pumps" (i.e. air and water pumps), (4) "RAS" (i.e. the recirculating aquaculture unit). The functional unit is defined as "the building of an aquaponic indoor system for the production of 700kg of tilapia and 3 tons of vegetables." Calculations were performed using the SimaPro® software.

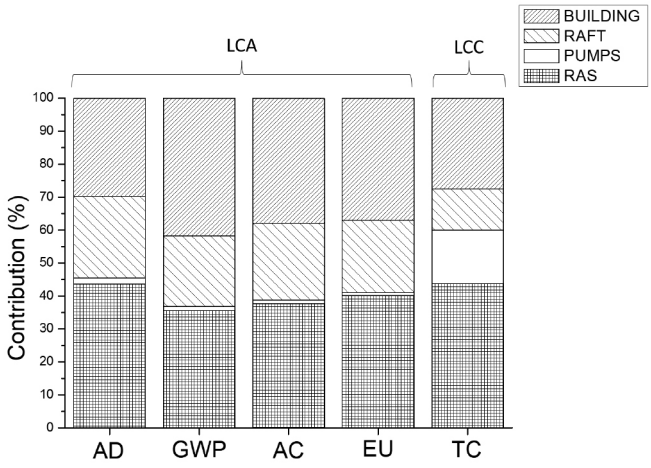

Figure 1. LCA and LCC contribution analysis. AD: Abiotic Depletion; GWP: Global Warming Potential; AC: Acidification; EU: Eutrophication; TC: Total Cost.

(Continued on next page)

Results

The results of LCA and LCC contribution analysis are reported in Figure 1. For all the LCA impact categories the main contributions were found for "BUILDING" (AD = 29.77%; GWP = 41.80%; AC = 37.97%; EU = 37.10%), RAS (AD = 43.67%; GWP = 35.53%; AC = 37.70%; EU = 40.12%) and RAFT (AD = 24.71%; GWP = 21.46%; AC = 23.24%; EU = 21.87%), while impacts linked to "PUMPS" appeared definitely limited (AD = 1.85%; GWP = 1.22%; AC = 1.09%; EU = 0.92%). On the contrary, this latter category showed an important contribution for LCC representing about the 16% of the total expenditure. However, concerning this analysis, the higher economic burdens are linked to the "RAS" equipment (43.76%) and "BUILDING" (27.54%) while the contributions of "RAFT" appeared to be lower (12.48%).

Discussion and Conclusion

Even if previous studies suggested that Life Cycle Thinking represents a useful approach to evaluate the burdens of an aquaponic system, only a limited number of work is available in the literature, nowadays (Boxman et al., 2017; Forchino et al., 2017; Forchino et al., 2018; Maucieri et al., 2018). In this respect, the present work represents an interesting case study, focusing the attention on the impacts linked to the construction of an aquaponic system. A specific element of strength of the study presented here is the use of only primary data. The combined application of LCA and LCC underlined that "RAS" equipment represented the most relevant critical issue in the system set up, in terms of both environmental and economic impact. The extended lifespan considered for the building hosting the aquaponic system (25 years) limited the contribution of this category to the overall impacts. The comparison of LCA and LCC results showed that environmental and economic impacts are, in some instances, not correlated. This appeared quite clearly in relation to "PUMPS" category, which showed the lower percentages of contribution for all the impact categories of the LCA but represented a quite important item of expenditure for LCC. Thus, only the combined application of LCA and LCC will allow to get a more complete picture of the sustainability of aquaponics.

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TENCH (*Tinca tinca*) RESTOCKING THROUGH AQUAPONICS: A CASE STUDY

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Introduction

Aquaponics, resulting from the combination of recirculating aquaculture and hydroponics, is considered a feasible option to develop sustainable and resilient agricultural production systems (Goddek et al. 2015). This technique can also represent an option for producing fish and/or vegetables species for restocking purposes. The tench, (*Tinca tinca*), is one of the most important commercial fresh water species in eastern countries of Europe being an important one for the diversification of fresh water aquaculture (Kujawa et al, 2011) and, at the same time, it is currently categorized as "near threatened" in the Red List, as its natural propagation had a severe reduction due to some environmental and biological limiting factors, as maintenance of river beds, construction of barriers, water pollution and the introduction of invasive alien species, such as the catfish (*Ameirius melas*) and the goldfish (*Carassius auratus*) (Zerunian, 2007). Given this commercial and conservation interest of the tench, this study was focused on its suitability to the aquaponic practice and on its resilience to sub-optimal water conditions, induced by possible disequilibrium of the system.

Materials and methods

The aquaponic facility is located in the Valle Averto national nature reserve (Venice lagoon, Italy) and managed by the World-Wide Fund for Nature Italy (WWF Italy) and it consists of two separately working sub-units: i) Deep Water Culture (DWC) and ii) Media Filled Bed (MFB). The sub-units are completely similar in structures, except for the presence of an additional biofilter in the DWC subunit. Both the sub-units are made up of one fish tank (volume = 1 m^3), a grow bed (total volume = 0.9 m^3; cultivated surface = 3 m^2) and a sump (volume = 0.2 m^3). In both the sub-units, lettuces were cultivated at a density of 30 plant m^{-2}. In each sub-unit, 350 individuals of tench, (total biomass about 10 kg) with an average weight of about 30 g, were reared in the PVC tank and fed with commercial extruded pellets (Veronesi - W.W. FISH 2©) (Feed daily ration = 1 % of total biomass). Tench resilience was tested with a 30 days trial, from May – 16th, 2017 to June – 14th, 2017. During this period, biofilters in the two sub-units worked differently. MFB sub-unit had a functional biofiltration, while DWC biofilter had a lower yield in transforming ammonia into nitrates, causing an accumulation of NH_4 and NO_2 in the water. Every seven days, fish growth was assessed by randomly selecting and measuring a sample of 30 fish from each of the two sub-unit populations. Fish biometric parameters considered were their weight, standard length and width (distance from the ventral fin insertion to the insertion of the dorsal one). Moreover, pH levels, concentration of dissolved oxygen (DO), ammonia (NH_3), nitrites (NO_2) and nitrates (NO_3) were daily measured using a portable photometer. Statistical analyses were accomplished using SPSS (version 25.0). At the end of the experimental trial, to pursue the goal of reintroduction, fish were released in inland water of Veneto Region.

Results

During the experiment, DWC and MFB biofilters worked differently, thus fish in the DWC and the MFB tanks were reared in different water quality: in the DWC sub-unit, ammonia ranged between 0.8 and 1.6 $mg \cdot l^{-1}$, while nitrites between 0.8 and 3. $mg \cdot l^{-1}$. In the MFB sub-unit concentration of ammonia and nitrites were lower, ranging between 0.05 and 0.4 $mg \cdot l^{-1}$. Tench reared in different water conditions in the sub-units showed similar mortality rate, below the 2 per cent. In the same way, estimated FCRs where similar. Indeed, fish reached similar size: in one month, fish in both the sub-units gained about 7 g in weight (about the 25% of their initial weight), 1 cm in length and 0.40 cm in body width (Table 1). T-Tests considering all the biometrical parameters chosen to describe fish growth did not show any statistical difference between fish reared under different water conditions in the two subunits.

Discussion and Conclusion

During the experimental trial, fish mortality was almost zero (under 2%) resulting similar between fish exposed to high concentration of harmful metabolites, ammonia and nitrites, and fish reared in water with optimal values of the same molecules. Moreover, fish did not show any sign of distress related to the methaemoglobinemia, caused by the accumulation in high concentration of nitrites (Svobodova et al., 2005). Indeed, in daily controlled fish, neither tissue convulsions and cramps nor lethargic behavior nor dirty dark brown colored gills were observed. Tench reared in different water conditions

(Continued on next page)

reached a similar size during the same time interval. According to our results, the tench, known to be a resilient species to pH variations and lack of oxygen in water, resulted to be a suitable species to be introduced in an aquaponic system. Specimens tolerated high concentration of harmful metabolites such as NH3 and NO2, even for a fairy long time, which means that tench may overcome the difficulties related to possible malfunction of the system such as biofilter breakdown, without great loss for the farmer.

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Table 1 Resume of sub-unit yield in terms of fish growth and lettuce production

	DWC		**MFB**	
	t_0	t_f	t_0	t_f
Fish mortality rate	6 deaths (1.71%)		5 deaths (1.42%)	
Mean fish weight (g)	27.82 ± 8.88	35.18 ± 8.84	28.01 ± 9.04	35.36 ± 9.62
Mean fish length (cm)	11.10 ± 1.28	12.28 ± 1.53	11.35 ± 1.81	12.63 ± 1.33
Mean fish width (cm)	3.07 ± 0.44	3.45 ± 0.40	3.12 ± 0.62	3.53 ± 0.38
Total fish biomass (kg)	9.74	12.10	9.80	12.20
Total feed given (g)	3121.63		3247.39	
FCR	1.21		1.26	
Lettuce mortality (%)	0		0	
Mean lettuce weight (g)	4.81 ± 0.56	180.25 ± 39.29	4.82 ± 0.64	158.80 ± 46.93
Total lettuce biomass (kg)	0.43	16.22	0.43	14.29
Mean lettuce yield ($kg \cdot m^{-2}$)	5.4		4.76	

DEVELOPING PRECISION FISH FARMING: UNVEILING LINKS BETWEEN OBSERVABLE BEHAVIOUR AND PHYSIOLOGICAL STATES IN FARMED FISH

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Precision Fish Farming: improving fish farming through technology and automation

Although intensive fish farming has become increasingly industrialised over the last decades, most aspects of present day fish farm management practices are still predominantly manual and experience based. To illustrate this, it is useful to consider any operation at a fish farm as a cyclical process consisting of four distinct phases; Observe, Interpret, Decide and Act (OIDA-cycle, Figure 1). In the *Observe* phase, data on the fish in the cage is collected (e.g. by observing the fish from the surface or through submerged cameras). The farmer then interprets these observations based on experience (*Interpret*), obtaining a perception of the welfare, health and other states of the fish in the cage. These perceptions are the basis for experience based decisions (*Decide*) on if the fish population should be subjected to some action (*Act*). Following observations of the fish (*Observe*) reveal if the action had the intended effect.

Analogous to experiences from Precision Livestock Farming, Precision Fish Farming (PFF) aims to increase the human control in fish production. This is necessary to improve our ability in ensuring good fish health and welfare, while maintaining efficient production and environmental sustainability. The PFF approach seeks to increase control by applying technological solutions and automation principles to the phases in the OIDA-cycle. *Observe* may be improved by e.g. intelligent sensors quantifying features in the data rather than providing raw data such as video, while *Interpret* may be improved through e.g. predictive models or estimation techniques. *Decide* may be made more objective by e.g. applying artificial intelligence methods, while *Act* may be automated using automated actuators.

SalmonInsight: unveiling links between behaviour and physiology

The implementation of PFF-methods in modern fish farming is presently biased toward *Observe*. The number of technologies available and commonly used for observing fish in fish farms is substantially higher than the number of technological solutions available for the other phases. As behavioural expressions may arise due to underlying physiological responses, identifying the links between observed behaviour and the physiological states of the fish has great potential for optimisation of operations, and represents a natural next step towards achieving PFF. The recently funded research project SalmonInsight aims to unveil the links between behaviour and physiology in farmed salmon to thus address this challenge. The project will run for 4 years, and will feature laboratory studies where fish are observed using measurement principles possible to use in commercial cages in parallel with being subjected to physiological sampling. As such, SalmonInsight will pave the way for attaining the next steps towards full PFF application in the salmon farming industry.

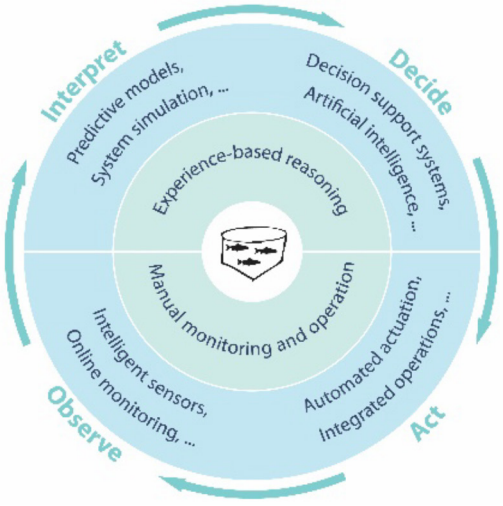

Figure 1: The OIDA-cycle illustrating the application of PFF to fish farming.

NUTRITIONAL AND HEALTH PERFORMANCES OF DIETARY SHRIMP HYDROLYSATE AND KRILL MEAL IN RED SEABREAM (*Pagrus major*) FED LOW FISH MEAL DIET

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Fish meal replacement in diets formulated for carnivorous species is still limited by the negative consequences on feed palatability, fish growth performances and as well by the adverse effects on fish physiology and immunity. In this context, the use of new functional raw materials and additives is being more and more popular to counteract mentioned side effects of fish meal alternatives, especially those of vegetable based raw materials.

A trial was implemented to assess the nutritional and health performances of two crustacean based raw materials, krill meal (KM) and shrimp head hydrolysate (SH), in red seabream juveniles when fed a low fish meal diet. Four dietary treatments were formulated: a positive control diet containing 40% fish meal (FM), a negative control diet (25% FM) where FM was replaced by soy protein concentrate and balanced with free amino acid supplementation, and this latter basal diet supplemented with either 5% KM or 5% SH. Fish (initial body weight: 8.5g; n=30 per tank; triplicate) were fed the diets for 15 weeks and after the final weighting, whole fish, blood and tissues were sampled for analysis of fish proximal composition, response of some parameters of innate immunity and morphometric parameters of anterior intestine. Remaining fish were then challenged with *Edwarsellia tarda* and mortality kinetics were followed until their stabilization (11days). Beside the feeding trial, feed dry matter and protein digestibility were controlled in red seabream, with inclusion of an inert marker and feces collection during 3 periods of 10 days.

At the end of the feeding trial, FM replacement resulted in a significant decrease of fish and feed performances and negatively impacted the response of innate immunity ($P<0.05$). Resistance to the bacterial challenge was significantly impacted too. KM and SH both resulted in fully restored, or superior, zootechnical and health performances when supplemented to low fish meal basal diet and when compared to high fish meal diet. However, SH supplementation significantly over-passed KM performances for most of the investigated parameters: fish growth and feed performances, feed DM and protein digestibility, gut health, survival to bacterial challenge and response of innate immunity.

Results will be discussed with a focus on the differences of protein quality (solubility and peptide profiles) between KM, SH and FM and how they could have influenced diet performances (palatability, nutrition and health), with positive outcomes for fish and, more broadly, for aquaculture development.

IMPLEMENTATION AND QUALIFICATION OF AN OZONATION SYSTEM FOR THE TREATMENT OF EFFLUENTS FROM EXPERIMENTAL FISH FACILITIES

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The French Agency for Food, Environmental and Occupational Health & Safety owns certified experimental fish facilities in Brittany to work on infectious agents and anthropogenic pollutants susceptible to alter fish health and welfare. The issue of decontamination of effluents from these experimental facilities is crucial.

In order to increase the level of safety for the environment and reduce operating costs, we recently installed a decontamination system coupling activated carbon and ozonation. Ozone (O_3) is a powerful oxidant with high disinfectant capacity and activated carbon absorbs a very wide range of chemical molecules.

Effluents of our experimental installations (25 cubed meters per hour maximum; fresh and seawater), which operate in open circuit, are initially treated with activated carbon and then with O_3. Ozone is produced from dried and purified air compressed by two generators arranged in redundancy. The system allows to apply until 7 ppm of O_3, for a maximum residual CT (concentration multiplied by time) of 21 for three minutes in a first step, continuously controlled by two redox probes at the input and end of the circuit. Qualification of the O_3 system was done by measuring the reduction of the microbial flora but also the inactivation of RNA bacteriophages inoculated in the effluents before and after treatment, thanks to the integration of sampling points in the circuit. Reduction levels of 99.997 and 100% were obtained for total flora and bacteriophages, respectively. Staff safety is ensured by installing destructors and ozone detectors at different locations. Ozone detection and any system defects induce the closing of solenoid valves placed at the outlet of the water tanks supplying the installations, making impossible the discharge of contaminated water to the environment. An emergency chlorination system completes the process.

This chemical and biological decontamination system has multiple advantages: large volume of treated effluents, reduce exploitation and maintenance costs, high safety level of the installations and possibility to realize simultaneously chemical and biological expositions.

UNDERSTANDING THE ROOTS OF SOCIAL PROTESTS AGAINST MARINE FARMING DEVELOPMENT IN BRITTANY FRANCE

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Aquaculture is promoted as a key sector of blue growth strategies all over Europe. Among the different groups of species, seaweed aquaculture is valued for its potential uses in biotech another important component of the blue growth. Over the last decade strong contestation against shore occupation by shellfish and seaweed farming was observed in Brittany, France. New installations or extension of shellfish or seaweed farms on the near shore or in open sea was almost impossible due to the protest of local citizens quickly relayed by regional environmental Non Governmental Organisations. Protests against Public Maritime Domain occupation slowed the development of shellfish farming and halted seaweed farming which was at its beginning.

To understand the reasons and the nature of this protest an inventory of articles published in local and regional newspapers mentioning conflicts between citizens and marine aquaculture was realised. The aim was the identification of projects of shellfish and seaweed farming accepted by the administration and facing conflicts at local level. Then three projects were chosen for further investigation, one being a mussel farm near the Mont Saint Michel Bay, the second an abalone farm and the third seaweed farming. The analysis of press articles provides an overview of the roots for contestation, actors and their arguments as well as its impacts. Based on these results, ways to avoid conflicts are discussed.

AQUACULTURE FOR GLOBAL HEALTH: SUPPORTING THE UNITED NATIONS SUSTAINABLE DEVELOPMENT GOALS WITH REMOTELY OPERATED VEHICLES

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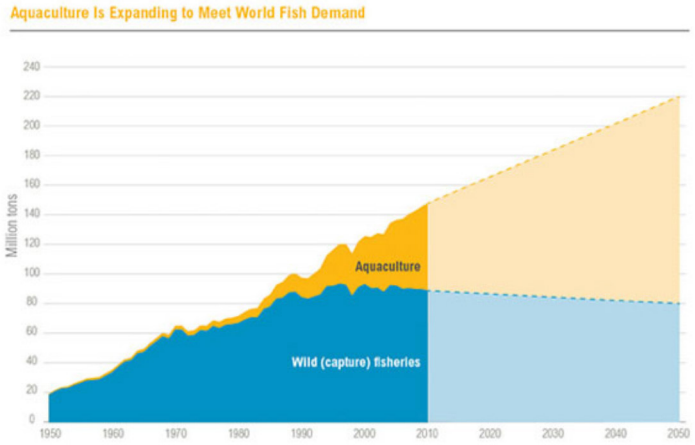
Aquaculture is a key source of food, nutrition, income and livelihoods for millions of people around the globe. As of 2016, the world capita fish supply reached more than 20 kg, with aquaculture providing half of all fish for human consumption. Moreover, fish continues to be one of the most-traded food commodities worldwide. There is tremendous potential for the oceans and inland waters of the world to contribute significantly to food security and adequate nutrition for the global population - expected to reach almost 10 billion by 2050.

As the global demand for viable protein rapidly increases, the aquaculture industry must recognize the vast responsibilities involved in providing said nutrients. Unmanaged aquaculture expansion can cause pollution and rising levels of carbon dioxide in the atmosphere contributing to ocean acidification. Unmonitored, subsurface assets such as nets, moorings and weights can cause catastrophic and irreversible damage to indigenous ecosystems and fish populations.

Deep Trekker's presentation will speak to the use of Remotely Operated Vehicles (ROVs) to promote good governance, participatory decision making and best practices in aquaculture. The aim of this technology is to harmonize the environmental, social and economic aspects of living aquatic resources to ensure equitable benefits for the world-wide community. The use of underwater monitoring systems, such as an ROV, has proven to be a cost effective, efficient way for fish farmers to comply with global regulations, ensuring healthy fish crop, efficient harvest and intelligent environmental protection.

Support for ROV methods will come in the form of case studies - from Canada, Norway and Chile, involving site managers, enforcement officers and researchers - evaluating the effects of aquaculture sites on the surrounding environment down to the microscopic level. Highlighting both large offshore pen usage and smaller inland farms, Deep Trekker will advocate for maintaining environmental awareness at all time and harvesting a viable, healthy protein to assist in the feeding of the growing global population.



RAINBOW TROUT RESISTANCE TO *Flavobacterium psychrophilum*: A GENOME WIDE ASSOCIATION STUDY IN A FRENCH POPULATION AFTER A NATURAL DISEASE OUTBREAK

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Health management is a major issue for sustainable aquaculture. *Flavobacterium psychrophilum* (Fp), the causative agent of bacterial cold water disease (BCWD) is responsible of important economic losses in rainbow trout farming. Resistance to the disease is heritable and several Quantitative Trait Loci (QTL) with moderate effects have been detected, indicating that selective breeding may be efficient. However, in most studies, the resistance to Fp was assessed after experimental infectious challenges using injection as route of infection, which is not representative of the natural infection as it bypasses external barriers (e.g. skin, mucus) likely to play a protective role. In this study, we aimed at describing the genetic architecture of the resistance after a field outbreak in a French trout population, using a medium-throughput genotyping array (Affymetrix OD Axiom 57K SNP array).

A natural outbreak of BCWD occurred in a farm from *Les Aquaculteurs Bretons*, a French breeding company in a cohort of 2,000 fish derived from 10 factorial mating design (69 dams, 97 sires in total). Dead fish were removed daily. At day 95 surviving fish were euthanized. All fish were stored for further genotyping. The presence of Fp in dead fish was checked at different time points until day 60, when mortality reached a plateau (30%) and Fp was no longer detected. Fish that died after day 60 were thus considered as resistant. Resistance was assessed as STATUS, a binary trait (dead/alive-resistant), and as time to death (TTD), the number of days between the onset of the disease and the day fish succumbed to the disease (value of 61 assigned for resistant fish). Using microsatellite genotyping, 1,733 fish were correctly assigned to a single mating pair. Those fish were used to estimate pedigree-based heritability for the two traits. For the genome wide association study, 720 individuals were sampled (290 resistant and 430 dead fish) and genotyped with the 57K SNP array. After genotype quality control, 706 fish genotyped for 30,030 validated SNP could be used. The association analysis was carried out using BLUPF90 software on TTD and STATUS separately.

Heritability was 0.33 for TTD and 0.27 for STATUS. We detected seven low-effect and two moderate-effect QTL and several relevant candidate genes involved in the inflammatory immune response were located inside those QTL. Our study confirms the main features of the genetic architecture of resistance to Fp (no major QTL, several QTL with moderate effects) and that implementing genomic selection should improve resistance. Moreover, some QTL were the same as the ones detected previously in other trout populations infected experimentally with different bacterial isolates, confirming that they may drive a core set of resistance mechanisms.

This work was funded by the European Maritime and Fisheries Fund (EMFF) and French Gouvernment via the national public body in charge of agriculture products and seafood FranceAgrimer (n°2015-0638)

CULTIVATED COPEPODS *Acartia tonsa* IMPROVE SURVIVAL FIVEFOLD AND GROWTH THREEFOLD IN JAPANESE YELLOWTAIL *Seriola quinqueradiata*

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For the last decades the cultivation of Japanese yellowtail (*Seriola quinqueradiata*) has stagnated at an average production close to 150 000 tons, with one of the bottlenecks for increased cultivation being a reliable production of high quality larvae and juveniles. Currently, the larval production is suffering from low survival rates, in addition to having a low retention rate of the swim bladder. Common practice is to use rotifers as feed for Japanese yellowtail larvae. Although being convenient to use, they have major drawbacks related to size and maintaining a good and stable nutritional quality. Copepods, on the other hand, are a natural part of the diet of most marine fish larvae and are generally considered to better meet the nutritional requirements of the growing fish larvae. This trial aimed at comparing the effect of live feed quality on growth, survival and the rate of opened swim bladder in Japanese yellowtail by feeding either enriched rotifers or the cultivated copepod *Acartia tonsa*.

Newly hatched larvae were stocked in rearing tanks of 1,5 m3, each housing 8000 larvae. The water temperature was 21-25 °C, and fluorescent lighting was used in combination with natural light between 06:00 and 17:00. The larvae were either fed enriched rotifers or newly hatched nauplii of *Acartia tonsa* at a density of 10-40 individs/ml, and each treatment had three replicates. Larvae were sampled at 6 dph and 11 dph (n = 20) for measurements of total length (TL) and retention of swim bladder. At the end of trial (16 dph) all remaining larvae was counted to determine survival and used for measuring the TL and body weight.

A significant (P = 0,012) difference in TL was observed by 6 dph, where feeding the Japanese yellowtail larvae copepods had resulted in longer larvae (5,21 ± 0,11 mm, compared to 4,62 ± 0,18 mm for larvae fed rotifers). At the end of the experiment the larvae fed copepods measured 8,52 ± 0,28 mm TL and weighed 7,04 ± 0,40 mg, which compared to the average rotifer-fed larvae at 5,5 ± 0,16 mm TL and 2,15 mg body weight, was significantly more. In addition, the survival rate at the end of trial was more than 5 times higher for the larval group fed copepods, ending at 40,3 ± 6,6 % compared a survival of 7,6 ± 4,2 % for the larval group fed rotifers. All results are presented in table 1. No difference was observed with regards to retention of swim bladder.

Based on these results, copepods have shown to be an excellent initial food for Japanese yellowtail larvae and the potentials for improving the larval quality and survival are profound.

Table 1. Total length (mm), survival rate (%) and body weight (mg) of Japanese yellowtail (*Seriola quinqueradiata*) fed enriched rotifers or cultivated copepods (*Acartia tonsa*). All values are significantly different (Welch´s T-test; P = 0,012).

	6 dph	16 dph		
Live feed	**Total length (mm)**	**Total length (mm)**	**Survival rate (%)**	**Body weight (mg)**
Copepods	5,21 ± 0,11	8,52 ± 0,28	40,3 ± 6,6	7,04 ± 0,40
Rotifers	4,62 ± 0,18	5,50 ± 0,16	7,6 ± 4,2	2,15 ± 0,09

DEVELOPMENT OF A LAND-BASED RECIRCULATING INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) SYSTEM

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Two experiments were conducted to develop a land-based recirculating integrated multi-trophic aquaculture (IMTA) system using a combination of abalone (*Haliotis mariae*) and Asian seabass (*Lates calcarifer*) as fed species, and brown mussel (*Perna* sp.) and seaweed (*Ulva fasciata*) as extractive species. The first experiment was conducted to determine the effect of sea bass densities of 20, 40 and 60 individuals per 500-liter tank on water quality and the growth and survival of the cultured species in the system. Sampling of all species was done every two weeks to check their growth. Water samples were taken every two weeks for measurement of ammonia, nitrite, nitrate, phosphate, and silicate. Measurements of temperature, dissolved oxygen and salinity were done daily. Growth of abalone and mussels were higher in fish densities of 20/tank and 40/tank, respectively, while growth and survival of seabass were not significantly different between densities. Biomass of seaweeds decreased during the experiment period. Temperature, dissolved oxygen and salinity were within optimum levels. Ammonia levels decreased as nitrite increased but in some cases it remained high while nitrates did not increase, indicating that nitrites were not converted to nitrates most likely due to the lack of efficient bio-filtration in the mussel tanks. The second experiment compared 24 versus 12 hours water recirculation versus flow-through to determine the effect on water quality and growth of cultured organisms. Growth of seaweeds was better at 24 h recirculation system but there was no significant difference in growth of abalone, seabass and mussel at different water supply regime, thus, for economic reasons, partial recirculation (12 h) may be adopted in land-based recirculating IMTA system.

MICROEUKARYOTE COMMUNITY AND THE BIOCHEMICAL COMPOSITION OF THE BIOFLOC IN A NILE TILAPIA CULTURE IN BFT WITH REUSE WATER

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The biofloc technology (BFT) functions by promoting microbial proliferation in culture units, such that they exploit the nitrogenous waste and recycle nutrients, improving the quality of the water throughout the culture process. However, although the microorganisms sustain the system, information that refers to its composition and abundance is scarce. Studies that refer to the microbial community of the biofloc, principally focus on the composition and abundance of the communities of bacteria. There is a lack of interest to evaluate the microeukaryote community (MEC) present in BFT systems despite their contribution to the efficient operation of the system through benefiting the species in cultivation. The objective of the study was to evaluate the biochemical composition and abundance of the microeukaryote community during the cultivation of Nile tilapia in biofloc with reuse water.

The study was evaluated over a period of 25 weeks. A completely randomized design was used with two treatment groups: tilapia culture in biofloc (TB) and tilapia culture in biofloc with reuse water (RWTB), with three replicates. The experiment was conducted using two macrocosm-microcosm systems. The systems consisted of one main culture tank or macrocosm (3 m^3) and three experimental units or microcosm (0.2 m^3). The quality of the water in the culture system was evaluated during the study. Every week, the biofloc samples (10 mL) were revised with an inverted microscope and the groups of microorganisms and genera of the MEC were registered. Total abundance, richness, indices of diversity, evenness and dominance were estimated. The proximate composition of the biofloc samples (humidity, crude protein, total lipids, ash and nitrogen-free extract was determined. The percentage of protein, lipids and ash from the biofloc was evaluated at weeks 14, 18 and 25. For the data analysis, the Pearson correlation coefficient and one-way ANOVA were used ($p < 0.05$).

The MEC of both treatments was comprised of microalgae, ciliates, flagellates, amoebas, rotifers, nematodes and annelids; the richness and total abundance in the RWTB group (74 genera, 161.52 ± 80.60 org mL^{-1}) was significantly greater than in the TB group (63 genera, 139.54 ± 81.39 org mL^{-1}) (Table 1). The abundance in TB and RWTB varied in relation to the temperature, sedimentable solids, NH_4-N, and NH_3-N. The dominance index in RWTB (0.59) was statistically greater than in TB (0.57). The genus of microalgae *Nitzschia*, *Monoraphidium* and *Scenedesmus*, the ciliate *Paramecium*, and the genera of rotifers *Lecane* and *Lepadella* were observed for a period ≥ 13 week. The proximate composition of the biofloc varied with time in both treatment groups, with an increase in protein (47%) and a reduction in lipids (2%) by the end of the study. The results suggest that the reused water serves as an inoculum and favours the richness and abundance of the MEC in less time, which contributes to part of the nutritional requirement of the tilapia culture in biofloc systems.

Table 1. Microeukaryote community and biochemical composition of the biofloc.

VARIABLE (mean ± SD)	TB	RWTB
Microeukaryote community		
Abundance (NMP org mL^{-1})	139.5 ± 80.8[a]	151.6 ± 79.8[b]
Richness (Total number of genera)	63[a]	74[b]
Shannon's diversity (H', nats ind^{-1})	0.83 ± 0.6	0.79 ± 0.6
Pielou's evenness (J')	0.51 ± 0.3	0.49 ± 0.3
Simpson's dominance (D')	0.57 ± 0.3[a]	0.59 ± 0.3[b]
Biochemical composition (%)		
Protein	36.83 ± 7.7	35.90 ± 8.1
Lipids	4.14 ± 2.9	4.45 ± 4.5
Ash	11.39 ± 1.3[a]	13.61 ± 0.9[b]

TB= tilapia culture in biofloc, RWTB= tilapia culture in biofloc with reuse water, S.D.= standard deviation, NMP = most probable number, rows with different subscripts show significant differences between treatments ($p < 0.05$).

MODELLING THE ECOSYSTEM OF A MULTITROPHIC INTEGRATED PRODUCTION OF FISH, OYSTERS AND MACROALGAE IN EARTHEN PONDS

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Ecopath models were built on data provided by three different integrated multitrophic aquaculture treatments carried out in replicate earthen ponds of about 500 m^{-2} and 1.5 m depth each, located in Olhão, Southern Portugal. The experiment was supported by the ERA-Net COFASP IMTA-Effects project. The aim of the models was to describe and explain the results of the treatments, which included the growth of three fish species (meagre, white seabream and grey mullets) raised with or without oysters or macroalgae accordingly: Fish, Oysters and Macroalgae (FOM); Fish and Oysters (FO); Fish and Macroalgae (FM).

The three models comprised 9 to 10 functional groups: 1. Meagre; 2. White Seabream; 3. Grey Mullets; 4. Macroinvertebrates; 5. Oysters; 6. Zooplankton; 7. Macroalgae+Periphyton; 8. Phytoplankton; 9. Feed. 10. Detritus. Feed was considered as a second detritus group. The oysters were not included in the FM model. In the FO model, a fishery was introduced to represent the weekly partial removal of macroalgae+periphyton from the pond. The diet matrix assumed that meagre and seabream fed only on fish feed (based on the results of fatty acid and isotope analyses, reported in another abstract). To better understand the results of the three multitrophic models these were compared with a previous model based in a nearby ecosystem: a Ria Formosa re-naturalized pond (RF).

In the IMTA systems the trophic web was very simplified and of linear type (Fig. 1). Meagre and bream had a trophic level of 2 (in nature it is around 4 for meagre) because they fed exclusively on feed. The total amount of energy throughout the IMTA systems, fueled by the feed, is at least 12 to 14 times higher than in RF (Table 1). In FM model, the total energy and biomass were lower, when compared with FOM and FO models, since no oysters were produced. The net production in the IMTA systems was negative since a large quantity of energy is imported (artificial feed) and converted into biomass (mainly for meagre). The FO net production was closer to RF model due to the amount of phytoplankton biomass that was higher in this treatment, compensating the total respiration of the system.

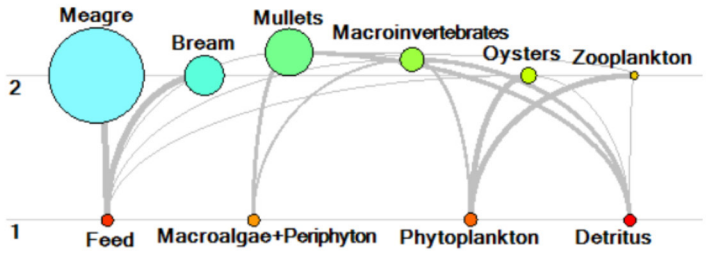

Figure 1. FOM model flow diagram. The size of the circles is proportional to the biomass of the functional groups.

DYNAMIC MODELLING OF A MULTITROPHIC INTEGRATED PRODUCTION OF FISH, OYSTERS AND MACROALGAE IN EARTHEN PONDS

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Ecosim models were built based on Ecopath models previously developed from data provided by three integrated multitrophic aquaculture treatments carried out in earthen ponds located in Olhão, Southern Portugal (see previous abstracts), in the framework of ERA-Net COFASP project: IMTA-Effect. The main aim was to adjust the results of the Ecosim model to the observed productions of fish (meagre, white seabream and mullet) and oysters in the fish+oysters+macroalgae treatment (FOM), including selected environmental data as response functions. The second aim was to use the data observed in the other treatments as forcing /response functions, and also use possible intra or interspecific relationships between the functional groups to simulate the observed productions in fish+oysters (FO) and fish+macroalgae (FM) treatments.

The experimental results pointed out to:

- Higher meagre final weights in the ponds with oysters (FOM and FO);
- Higher bream and grey mullet final weights in the ponds with macroalgae (FOM and FM);
- Higher oyster weight in the ponds without macroalgae (more phytoplankton!) (FO);
- Lower turbidity and higher dissolved oxygen (DO) in ponds with oysters.

These results indicate an improvement of meagre growth due to the co-production with oysters. Oysters clean up the water, which enhances meagre feeding, and indirectly improve phytoplankton growth and increases DO. Mullets grew better in ponds with more macroalgae and periphyton, since periphyton and associated fauna is one of its main feeding resources. White seabreams possibly suffered from competition with meagre in FO treatment. With cleaner waters and less macroalgae, meagre could feed more efficiently than breams.

Ecosim FOM model simulates the growth of the four species produced and the variation of the other functional groups (Figure 1). In the model, phytoplankton decreases sharply by the end of the experimental period, causing the decrease of oysters' growth rate. The environmental forcing functions were not sufficient to reproduce what happened in the other treatments, being necessary to include in the model the possible competition between macroalgae and phytoplankton and between meagre and bream.

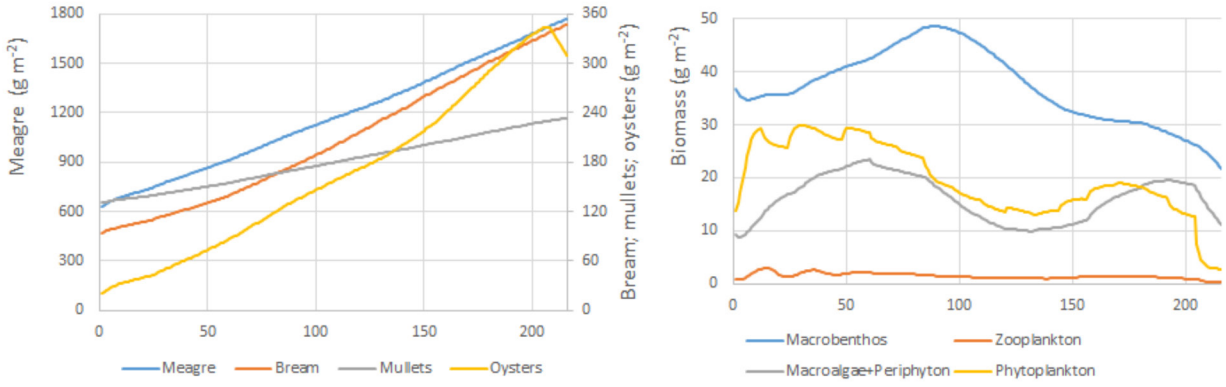

Figure 1 - Simulation of the groups' biomasses over the experimental period (days).

SUPPLING SOY OIL WITH POTASSIUM IODATE TO THE DIETS FOR CHINESE SOFT-SHELLED TURTLE (*Pelodiscus sinensis*)

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Mash feed is commonly used for Chinese soft-shelled turtle (*Pelodiscus sinensis*) in China due to easy handling. In order to upgrade palatability and adjust the nutrient composition, the soy oil normally is added to the mash feed before feeding the turtle. Iodine is the source of thyroid hormones that are proved to be involved in lipid metabolism. The aim of present study therefore was to determine the effect of mesh diet with gradient level of soy oil and potassium iodate on turtle growth, body composition and lipid metabolism. Nine diets were formulated in basis of a commercial feed that serviced as the control. The diets are designed in basis of 3×3 factorial design. The soy oil (5, 10 and 15%) and potassium iodate (KIO_3; 50.6, 101.2 and 151.8 mg kg^{-1}) inclusion levels were set to be two factors. The turtle experiment was designed according to a completely randomized design. Each diet was fed to one group of fish (n=25, average initial weight was 12.9 g). The feeding period lasted for 87 days. The results showed that adding KIO_3 to the mash feed significantly reduced weight gain, specific growth rate (SGR), feed intake and protein efficiency ratio (PER). 101.2 mg kg^{-1} KIO_3 in the diet negatively affected FCR. The group fed the diet with 5% soy oil exhibited significantly lower final weight and higher FCR than the control. The group fed the diet with 10% soy oil showed reduced feed intake and increased FCR. The significantly lower final weight, weight gain, SGR, PER was detected in the group fed the diet with 15% soy oil. Some hepatic gene expression involved in lipid metabolism was affect by soy oil. The diet with 15% soy oil significantly increased ACSL1, CPT1a and FABP gene expression levels, and reduced PPARγ gene expression. KIO_3 (151.8 mg/kg) increased ACSL1 gene expression. In conclusion, adding more than 5% soy oil and more than 50.6 mg kg^{-1} KIO_3 to the mesh feed impaired turtle growth, and affected the genes related with lipid metabolism.

Table 1 Effect of soy oil on turtle growth and feed utilization

	Soy oil, %			
	0	5	10	15
Initial weight, g	13.02±0.32	12.83±0.22	13.05±0.52	12.85±0.16
Final Weight, g	26.17±1.22a	22.78±0.23b	21.42±1.02bc	19.43±0.43c
Weight gain, g	13.14±1.44a	9.95±0.46ab	8.37±0.60bc	6.58±0.39c
SGR	0.80±0.074a	0.66±0.032ab	0.57±0.025bc	0.48±0.024c
Feed intake, g $day^{-1}fish^{-1}$	0.29±0.016a	0.23±0.013ab	0.21±0.014b	0.23±0.029ab
FCR	1.96±0.22b	2.04±0.097b	2.17±0.081b	2.99±0.26a
Nitrogen retention, %	15.37±1.81	15.89±0.18	14.27±1.43	15.96±2.29
PER	3.45±0.17a	2.99±0.15ab	2.60±0.23bc	2.31±0.041c
Survival rate, %	90.67±2.67	96.00±4.00	92.00±4.00	97.33±1.33

A NEW TYPE OF SURFACE FILAMENTOUS APPENDAGE OF *Edwardsiella tarda*: CHARACTER, COMPOSITION AND FUNCTION

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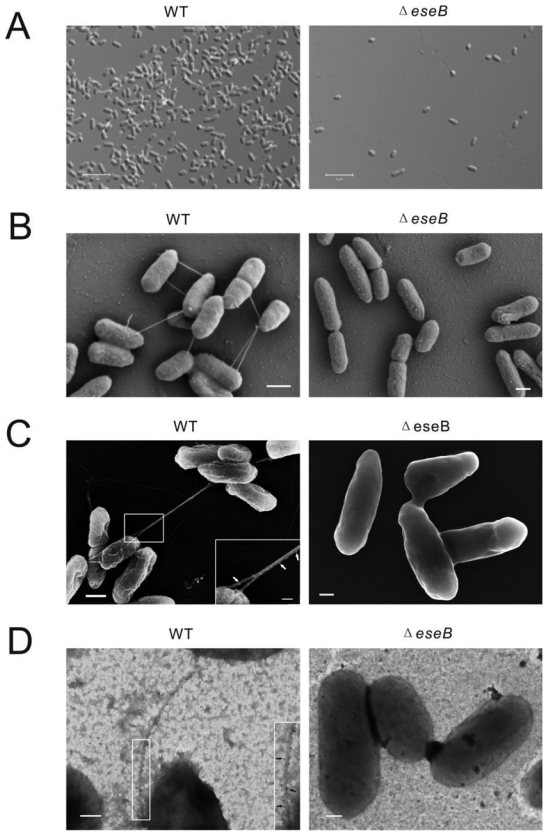
In this study, we demonstrate that EseB (a type III secretion system (T3SS) component of *Edwardsiella tarda*) forms a new type of filamentous appendage on the surface of *E. tarda* and is required for biofilm formation. EseB filaments play important roles during biofilm development.



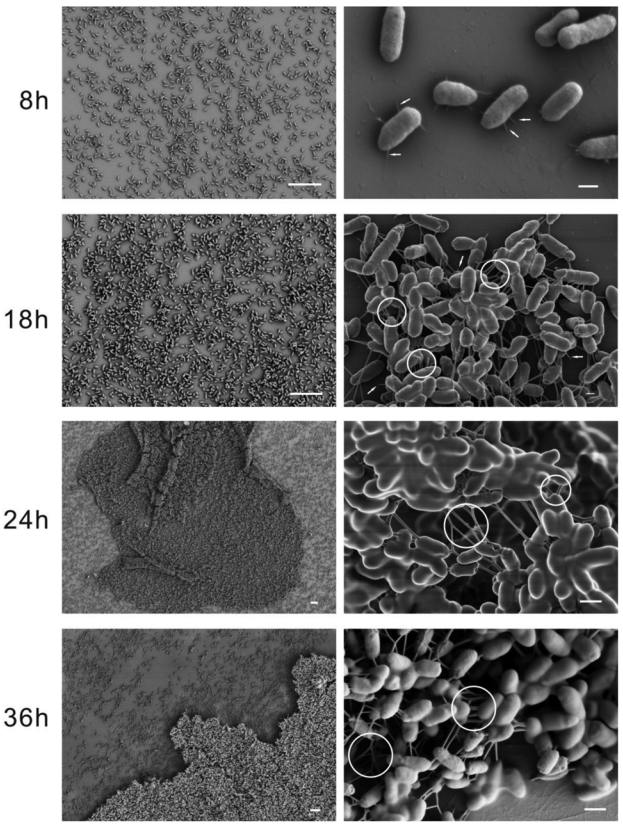


Figure 1 **Figure 2**

Figure.1 (A) SEM micrographs showing Wild Type (WT) and Δ*eseB* of *E. tarda*. Filamentous surface structures with a diameter of ~10nm were observed between adjacent cells of WT, but no such structures were found on Δ*eseB*.; (B and C) Immuno-FESEM and Immuno-TEM micrographs showing WT and Δ*eseB* immunogold labelled with the EseB antiserum and protein A-coated gold-particles with a diameter of 10 nm. The gold-particles (arrows) stained filamentous surface structures on WT (arrows), but not Δ*eseB*. The lower-right insets showed enlarged views of the respective boxed areas.; (D) Immunofluorescence staining showing WT and Δ*eseB* stained with EseB antiserum. The antiserum stained filamentous surface structures on WT (arrows) but not on Δ*eseB*.

Figure.2 Dynamic analysis of biofilm formation and EseB filaments. At 8 h, 18 h, 24 h, and 36 h postsubculture, *E. tarda* wild-type cells that settled on the coverslips were assayed by SEM. At 8 hps, EseB helps *E. tarda* to attach to the coverslips, as indicated by the white arrows, and at 24 and 36 hps, EseB helps to connect and support *E. tarda* cells.

IMPROVING THE SUSTAINABILITY OF TILAPIA CAGE FARMING IN BRAZIL: AN EMERGY APPROACH

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The accelerated and disorderly expansion of aquaculture can lead to economic, social, and environmental problems. In this sense, it is necessary to prioritize the adoption of practices that aim for sustainable production. The aims of the present study were to identify the contributions from nature and economy in the system of tilapia cage farming. In addition, emergy synthesis was utilized to evaluate whether the use of periphyton as a complementary food and the reduction of storage density improve the sustainability of this production system.

Three different production managements were evaluated and compared: using traditional stocking density adopted by farmers (80 kg m^{-3}) with 100% of the daily recommended feed and without substrates for periphyton (TRAD); traditional stocking density (80 kg m^{-3}) with 50% of the daily recommended feed and with substrates for periphyton (TDS); lower density (40 kg m^{-3}) with 50% of the daily recommended feed and with substrates for periphyton (LDS). We calculated using emergy synthesis the transformity (Tr), renewability (%R), emergy yield ratio (EYR), emergy investment ratio (EIR), emergy loading ratio (ELR), emergy exchange ratio (EER), and emergy sustainability index (ESI) of the distinct production managements.

The results showed that tilapia cage farming is highly dependent on resources from economy, and feed is mainly responsible for this. The proportion of resources from economy in each treatment was 87% in the TRAD, 78% in the TDS, and 64% in the LDS. On the other hand, the participation of renewable resources from nature was higher in LDS treatment with 36%, compared to 22% in TDS and 13% in TRAD.

TDS management presented the best result due to the lower value of transformity, which indicates a better process performance to generate a service or product, since this management can produce more tilapia by less emergy invested compared to TRAD and LDS. The renewability (%R) shows that the systems using periphyton (TDS and LDS) are more renewable and less dependent on the resources from economy than the traditional cultivation system (TRAD). The EIR, ELR, and EER indicators had higher values in TRAD, while EYR and ESI were higher in the management that adopted periphyton as a complementary food (Table 1). This occurred because there was a decrease in the use of resources from economy and an improvement in the use of renewable natural resources.

The results of this study indicate that the adoption of practices and management that reduce the dependence on resources from economy and increase the use of renewable resources help to make aquaculture a resilient and more economically fair activity with lower negative environmental impacts.

Table 1 – Emergy indexes for different situations evaluated.

Indicator	TRAD	TDS	LDS
Tr (seJ/J)	2.80E+05	1.52E+05	3.74E+05
%R	12.87%	21.79%	35.85%
EYR	1.15	1.28	1.56
EIR	6.77	3.59	1.79
ELR	6.77	3.59	1.79
EER	1.18	0.90	1.09
ESI	0.17	0.36	0.87

A 3D BIOPHYSICAL MODEL TO ASSESS THE IMPACT OF FINFISH FARMING IN KUWAIT BAY

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This work presents the development of a coupled biophysical model to assess the potential impact of finfish farming in the coastal waters of Kuwait. Kuwait Bay is an example of a highly impacted ecosystem. The input of nutrients and other pollutants through rivers and anthropogenic sources of different nature, together with the particular hydrodynamic conditions of the Bay, has impacted the marine environment, threatening fish, human health and ecosystem function. The expansion of aquaculture in coastal areas is known to be as one of the extra-nutrient sources into the ecosystem.

Finfish farms are likely to have a harmful impact on the surrounding environment depending on their size, nature or location. Fish waste and uneaten food can accumulate at the sea bed under the fish cages, causing an impact on the benthos, or might be transported by the currents. Frequently, the associated excess of nutrients and organic matter have led to eutrophication and oxygen depletion, with the consequent stress or kill of aquatic organisms. The spread of diseases, parasites or antibiotics that might affect wild species have also been associated with these farms.

Coupled hydrodynamic-ecosystem models are useful tools for analysing the impact of finfish farms by accounting for the most relevant physical and biological processes involved. Models can also help to find the optimal location of finfish farms in terms of minimizing the environmental impact or can be used to investigate Integrated Multitrophic Aquaculture (IMTA) strategies. In this study, Delft3D-FLOW and Delft3D-ECO were used for modelling the dynamic flow fields and the water quality, respectively. Estimates of the farm waste into the environment were used as model inputs. These were calculated as a function of the size of the cage, the number of fish per unit volume, the excretion per fish and the food waste. Simulations were performed for different scenarios of food waste, including good and poor feeding practice. Remediation strategies were also tested, including the effectiveness of hypothetical macroalgae farms to remove the excess or nutrients, and selecting alternative locations for the finfish farms to reduce their impact.

OPTIMIZATION OF COMMERCIAL COPPER ANTIFOULING COMMONLY USED IN AQUACULTURE

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Introduction

Biofouling is a complex and existing problem in every sector of European aquaculture. Problematic areas include biofouling in infrastructures (submerged structures such as cages, nets and containers, or equipment such as pipes, pumps, filters and collector tanks) and in cultivation species (mussels, seabass, seabream, oysters, etc). Within 10 years, the choice and availability of anti-fouling paints will be considerably more restrictive in Europe. Antifouling is used by the aquaculture industry for several reasons: 1. To maximize the deployment time of marine cages nets. 2. To reduce nets wear by reducing the number of cleanings and nets management required per production cycle. 3. To kept clean nets for long periods of time (from 8 to 12 months), thus to provide optimal breeding conditions for growth and health. The aim of this preliminary work is to study the accumulation of heavy metals from antifouling paints in biological community.

Materials and methods

Antifouling paints were applied to two types of nets (Nylon and Dynema) commonly used in fish farms. Prior to any treatment, the nets were disinfected with a HALACID dilution for one hour. Once disinfected, they were dried in the oven at 50ºC in 24 hours, and impregnated with the different commercial antifouling products, Flexgard VI and Flexgard XI, specific to fish farms, at five different concentrations (0, 25, 50, 75 and 100%). Already painted nets were fixed in pvc frames cages at 4 meters depth during 6 month. Once collected, the samples were frozen at -20ºC until processing. Heavy metals concentration was measured in three taxonomic groups: algae, bryozoans and sponges.

Results

There was a lineal relationship between fouling biomass and paint Cu concentration of biota products. Fouling community structure, exposed to several amounts of Flexgard VI and Flexgard XI paints with different Cu concentration, showed important differences. Also changes were observed depending on type of nets used, Nylon or Dynema. The great differences in heavy metals accumulations between functional group studied indicate that paints concentration is a key factor to take into account. Dynema net accumulated lower biomass and metals than Nylon net. Our results suggest that lower concentration of commercial paints shows the same results than a higher one, which could be used to reduce environmental impact.

EVIDENCE-BASED CRITERIA FOR CHOOSING TESTING OF POOLED OVER INDIVIDUAL SPECIMENS IN AQUATIC ANIMAL HEALTH SURVEILLANCE

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Testing of pooled specimens as part of surveillance for aquatic animal diseases is preferable to testing individuals when the prevalence of infected samples is low and the concentration of the target analyte is high in infected animals. However, use of pooling raises important questions: *for which combinations of prevalence and load can pooling be justified; should pooling recommendations differ for systemic vs. localized infections; are there published data to support recommendations for numbers of specimens (e.g. 2, 5 or 10) to pool?*

A systematic review of published studies in aquatic animals was undertaken using standard search terms in PubMed and CAB Abstracts. OIE-listed and non-listed diseases were included. Abstracts were screened and relevant information was extracted and summarized from the full papers. An expert group was convened to review the collated evidence and develop guidelines for designing pooling studies. Fourteen relevant papers were identified in the search, most evaluated the use of qPCR. Most authors concluded that pooling reduced sensitivity compared with testing of individuals but supporting data for pool sizes was minimal. Only two papers specifically mentioned the concept of pooled sensitivity and pooled specificity. These two outbreak studies provided useful comparative data (virus isolation and qPCR for salmon alphavirus and infectious salmon anaemia), but no papers reported cycle threshold (Ct) values.

Pooling is logistically necessary for testing early life stages but is optional in other situations. For decisions about risk associated with movements of animals and animal products, scientific evidence is needed to justify pooling over individual animal testing. The evidence should be sufficient to withstand legal scrutiny. The preferred design for generating evidence to support choice of pools sizes is field studies with parallel testing of both individual specimens and these same specimens in randomly-created pools that mimic real-life surveillance scenarios (e.g. post outbreak testing when no clinical disease is evident) supported with sensitivity and specificity data, and costs of both options. Individual and pooled Ct values should be reported even if the final decision is presence/absence. Use of experimentally-infected animals and spiking experiments were considered inferior sources of data to evaluate effects of pooling. Studies are also needed to describe the distribution of Ct values in naturally-infected but healthy aquatic animals, as this information can provide a sound basis for simulation studies of potential effects of pooling and also inform the design of laboratory experiments.

THE DEPENDANCE OF THE PRODUCTION CYCLE OF EUROPEAN FLAT OYSTER, *Ostrea edulis* (LINNAEUS, 1758) ON THE TIME OF HATCHING

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Cultivation of European flat oyster, *Ostrea edulis* (Linnaeus, 1758), in the Mali Ston Bay, Croatia, has a long history, i.e. it exists since the Roman era. It is still only oyster species present in this region and its farming is of great socio-economic importance. Traditional farming technology is entirely based on the spat collection from the natural environment. The production cycle lasts 2 to 2,5 years. In recent years, the initial phase of production (spat collection) suffered a dramatic decrease in the number of larvae available from the nature. Artificial spawning in hatcheries presents the only alternative that could ensure sufficient quantities of spat to secure the existing production and create the base for its expansion. The aim of this project was to test out-of-season spawning in hatchery, and to compare differences in growth period of oysters in dependence of the season in which they were deployed on long-lines.

Oyster hatchery consisted of broodstock facility, algae production, larval rearing and nursery area. *O. edulis* broodstock was established by collecting adult specimens from their natural environment during 2009. Specimens were kept in controlled conditions within a close recirculation system.

First conditioning started in November with spawning in January, while second started in February with spawning occurred in April. During both condition periods, feeding regime included combination of the flagellates *Tetraselmis suecica, Isochrysis galbana* (60%) and diatoms *Chaetoceros muelleri and* Cylindrotheca closterium (40%). The daily amount of feed was calculated at 3% of dry tissue weight of the shellfish.

Larvae were raised at the stocking density of 7 individuals per milliliter under optimal water quality conditions and a constant temperature of 21±1ºC. They were kept in stationary tank systems until developed pediveliger stage. In this stage they were transferred to recirculation system which was constructed in order to produce clutchless spat. During this phase feeding regime consisted of 60% of the flagellates mixture (*Tetraselmis suecica, Isochrysis galbana* and Pavlova lutheri) and 40% of diatoms mixture (*Chaetoceros muelleri and Skeletonema costatum*). The density of microalgae in the culture tanks was maintained at 100 cells/μl of *I. galbana* equivalents. Spat was kept in this system until day 15th after metamorphosis.

For the next phase, spat was transferred to the nursery facility and kept on constant temperature of 21±1ºC in close recirculation system with upwelling installations. Feeding regime consisted of the same ratio of the flagellates (*Tetraselmis suecica, Isochrysis galbana, Thalassiosira pseudonana*) and diatoms (*Chaetoceros muelleri and Skeletonema costatum*), while the density was maintained at 150 cells/μl of *I. galbana* equivalents. After three months in the nursery system spat was placed into lantern-nets and employed on the long-lines in the ambient sea water.

Spat from January reached consume size of 60 mm in December of the same year, while spat from April reached the consume size in July next year.

NUTRITION OF ROBALO (*Centropomus undecimalis*) JUVENILES

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There exist a number of articles on the protein requirement through a compounded diet, some data on lipid, and even the muscle composition of snook according to salinity. Feed consumption and gastric evacuation were described, and recently intense nutritional studies were undertaken at University under lab conditions, on line with previous ones on barramundi (AADCP, S'pore) and its main requirements. Such studies summed up on ingredients basically fishmeal and soybean meal, *in vivo* digestibility and formulations to find optimum protein and a ratio P/E on a basis of 3 trials (P constant, E variable; E constant and P variable and P/E constant. It allowed getting a basal diet with two major ingredients and mg protein kJ^{-1} DE for an optimum weight gain in clear seawater. Such study led to a pilot phase in floating cages and later a commercial production at large scale in SEA. Such data helped initiate trials on robalo for optimum protein with fish caught from the wild; in mind a development of fish culture in Yucatan area. Moreover, *in vivo* digestibility on marine and land meals led to start *in vitro* digestibility with digestive enzymes of robalo. Present results led to a grower feed produced in a local feed mill (MC) to sustain pilot projects initiated in Merida area. "New" formulations are oriented towards (i) a replacement of fishmeal by poultry by-products and single cell protein (ii) mineral mixture composition to smooth effects of diluted seawater regarding osmorregulation, for a species qualified as carnivore. First grower feeds derived from previous trials sustained already several operations of fish culture in the vicinity. Therefore the nutritional physiology approach was successful and final product in terms of organoleptic properties and fish texture proved the adequacy to culture conditions. Further concerns will be on reproduction and genetic diversity, fish fatty liver (steatosis) plus a reference to wild specimens in particular for taurine content. All those operations proceed from R&D and sustain fish culture projects in Yucatan.

SOME ASPECTS OF NUTRITIONAL PHYSIOLOGY OF WILD JUVENILES *Epinephelus morio*

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There was a prerequisite to identify the impact of seasonality on enzymes of *E. morio*, a colosal carnivorous, as a candidate species for aquaculture in Yucatan area. Juveniles (88 to 260g) were captured from the wild to measure their physiological conditions during three marked seasons: rainy, dry and North wind. After a short period of acclimatation in the laboratory the following parameters were measured: blood indicators (glucose increased during North and rainy periods, $p>0.05$) and liver digestive enzymes (amylase, glucosidase, $p>0.05$) The seasonality effected on amylase from intestine (43U mg protein^{-1}) and glucosidase from ceca ($p<0.08$) and metabolic enzymes (GK, PK, G6P-DH, 6PG-DH, FBPase) without difference ($p>0.05$). Even of fluctuation in natural resources in its environment, there is a relative stability in fish physiology, as well at digestive level or for metabolic response. Grouper can regulate both main pathways with or without carbohydrates. But preferentially without carbohydrates; there is a route of glycogenesis that could be a source for glucose especially in case of high demand when chasing at preys. But in case of maintenance, and after a stress of capture, fish go back to a stable physiological status compatible with captivity. As other carnivorous, *E.morio* presented a certain metabolic plasticity that helped to cope with variations in its environment (whether in rainy or dry season primarily). *E.morio* juveniles caught from the wild and acclimated to lab conditions accepted readily the compounded feed, a mixture of fishmeal (FM) and soybean meal (SBM). Due to its high need for protein, and the constant increase cost of FM, the candidate species could not be cultured sustainably with a current feed. Adequate dietary energy could reduce protein degradation and then FM content. The paper deals with carbohydrate (cbh) from two botanical origins and two processed ways; taking into account a putative limited cbh tolerance. The trial was conducted during 60 d. in 500 L tank with practical diets including wheat or cornstarch plus a control diet without starch. Survival at end was above 80% and weight gain (2.8g d^{-1}). Energy partition in kJ kgMBW^{-1} d^{-1} did not indicate difference in maintenance while native corn starch produced best results in weight gain, survival and metabolic activity. The level of activity, quite low, helped maintain retained energy (RE). 15% starch in feed was well tolerated by this species that could be raised with wheat or corn as an energy source. 20% of starch induced de glucokinase activity in liver.

THE ROLE OF DENSITY IN SEX DETERMINATION OF THE EUROPEAN EEL AND THE IDENTIFICATION OF EARLY SEX MARKERS

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Contrarily to most mammals, the majority of fish doesn't have heteromorphic sexual chromosomes (type XY/XX). In most fish, the gonad development is extremely labile and for some of them, the sex can be influenced/determined by environmental factors. This is observed in eel, where the proportion of males increases with the number of individuals at a given location or in aquaculture conditions. Two main questioning have emerged in the eel sex determination domain: **when? and how ?** does the environment affect the sex of animals.

To investigate both questions we recently conducted a 3.5-year study aiming at understanding the effect of both density and individual growth rate on sex determination (the how?). We used the von Bertalanffy growth model with mixed effect to fit our data and take into account both the reiteration of measurements on the same fish and the fact that each individual grew in different density conditions. To identify the time-window (the when?) during which the environment influence the sex of eels, we targeted genes susceptible to be differentially expressed between ovaries and testis at different stages of development in aquaculture conditions. In parallel, we conducted field studies to identify early sex markers in areas known to produce more males or more females.

Using qPCR, we detected testis-specific expressions of *pre-mir202*, *dmrt1, amh,* and *gsdf* and ovary-specific expressions were obtained for *aromatase, zar1, zp3* and *foxn5*. We showed that gene expressions in the gonad of intersexual eels were quite similar to those of males, supporting the idea that intersexual eels represent a transitional stage towards testicular differentiation. The combined expression of six of these genes allowed the discrimination of groups according to their potential future sex and thus, this appears to be a useful tool to estimate sex ratios of undifferentiated juvenile eel. Concerning density and growth rate, our data clearly indicate that future males reach their asymptotic size faster than future females for both length and weight, even when we set up a similar asymptotic size for both sex. Differences in growing pattern occur between 6 and 12 months of rearing in our aquaculture conditions. These differences progressively lessen as males enter in silvering phase and both slopes cross at 1225 days for length and 1515 days for weight, when males and females reach a size of 400 mm or 123 g. Finally, future females tended to present higher Coefficient Factor than future males, suggesting a central role for available energy in triggering the development of one sex.

Our findings gathered throughout both experiment (aquaculture and field context) support the hypothesis of a metagametic (environmental) sex determination and also suggests that the estimation of the quality of the environment, made by juveniles' eels, is one of the key factors influencing sex determination.

SEX DETERMINATION OF THE EUROPEAN SEA BASS: DECIPHERING THE ROLE OF TEMPERATURE OVER THE WHOLE LARVAL PERIOD TO ENHANCE FEMALE PRODUCTION

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The European sea bass *Dicentrarchus labrax* is a major aquaculture species with a polyfactorial sex determination system (genetic + temperature). In this species, more males are produced when larvae are exposed to high temperatures during the early stages. The temperature profile used in farming conditions to promote rapid larval and juvenile growth also induces high masculinization rate (60-100%). However, future females readily present higher growth rates compared to males. In addition, a proportion of up to 30% of males will precociously mature in the first year, further slowing down the growth of these individuals. Therefore protocols that limit masculinization, maximize female bias, and reduce male precocious sexual maturation will have a strong positive impact on farming productivity. Obtaining both experimental husbandry conditions that favour females and reliable early sex markers of fish would be optimal tools to improve production.

In order to identify the timeframe during which temperature affects phenotypic sex, we transferred fish batches from 16°C to 21°C every 2 weeks, during 8 months (leading to 16 transfers). The first transfer occurred at 11dph. Then, periodically (12, 33, 54, 75 and 96 day post hatching, dph), some larvae (total 740) were sampled in the different tanks for methylome and gene expression analysis.

Following the first transfer, high mortality occurred so that sex-ratio was reliable only after the second transfer. In February 2018, fish from the first 9 transfers were macroscopically sexed (Figure), as the smallest ones reached a critical minimal size of 45 g. As predicted, the proportion of males was generally higher than the proportion of females. Nevertheless, it appeared that fish transferred from 16°C to 21°C at 54 and 75 dph produced a higher proportion of females (respectively 45% and 47%). The fish sampled for molecular analysis at all time-points still have to be analysed. Expression of key genes (*sox17, sox19, gr1, gr2, mr, hsd11b, aromatase, dicer1, amh, dmrt1* and *star*) will be evaluated and we hope this will provide accurate predictors of the phenotypic sex for future applications in aquaculture.

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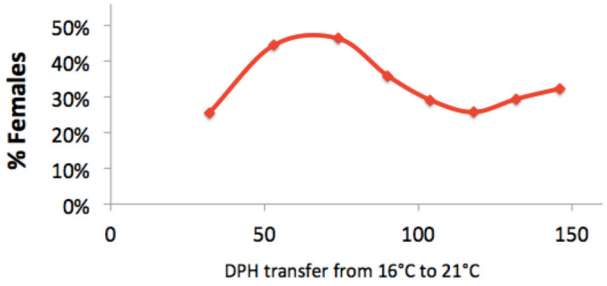

EFFECTS OF DIFFERENT DIETARY OLIGOSACCHARIDES ON GROWTH OF *Clarias gariepinus* IN RECIRCULATED AQUACULTURE SYSTEM: TWO YEARS RESULTS

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In this study, African catfish, *Clarias gariepinus* as a candidate aquaculture species was cultured in recirculating aquaculture system (RAS) with minimum water changes (10% weekly). We aimed to determine the effects of different dietary oligosaccharides (FOS, GOS and MOS) on growth of *C. gariepinus* larvae in RAS environment for two years' trial. The study was conducted in two trials under the same conditions (2016 and 2017) in 21 (40 L) fiberglass tanks (stocking rate 40 fish/tank) for 92 days larval rearing period at Aquaculture Research and Application Unit of Agricultural Faculty of Ankara University.

For each year, the inflow of freshwater was maintained at 3 L/min over first 30 days, 3,5 L/min over the second 30 days and 4,5 L/min during last phase (last 30 days') of the study. The ranges of physicochemical parameters for two years, such as water temperature, saturation, dissolved oxygen, nitrite and ammonium were recorded 25±1°C, %80±5, 7.8±0.5 mg/L, 0.03±0.02 mg/L NO_2^- and 0.06±0.03 mg/L NH_4^+ respectively in the RAS environment. 20-25 days old fish after hatching were used (for first year IW: 0.21-0.23 g, TL 2.91-2.93 cm; second year IW: 0.20-0.23 g, TL: 2,96-3.00 cm). The commercial diet (50% crude protein) with supplemented 1‰ and 2‰ levels of furucto oligosaccharide (FOS1-FOS2), galacto oligosaccharide (GOS1-GOS2) and mannan oligosaccharide (MOS1-MOS2) in 3 replicates with a control groups (C) were used throughout the experiments. Every 10 days' live weight and total lengths of fish were measured and growth parameters were calculated. The first and second years' trials is summarised in Table 1 and Table 2. The 92 days' feeding experiments findings showed that fish larvae reached for first year TL: 13.03±1.99 cm (C) to 16.71±2.05 cm (MOS2) and W: 12.11±2.67 g (C) to 15.93±1.88 g (MOS2); for second year TL: 17.55±0.70 cm (C) to 19.58±1.35 cm (MOS2) and W: 18.44±2.28 g (C) to 22.28±1.78 g (MOS2). At the end of the study, generally enhanced growth performances and survival rates were observed in African catfish fed on diet containing 2‰ mannan oligosaccharide (MOS2) ($p<0.05$). When compared with the data of two years, the growth parameters of the second year gave better results than the first year. It may be thought that this difference may be caused by the differences in broodstock used in both trial. Another reason may be the increase in the amount of feed (ad libitum) to prevent cannibalism in the second year. In conclusion MOS2 and GOS2 supplementation could be used as healthy growth promoter in *C. gariepinus* diets.

Table 1. Effects of different oligosaccharides on growth of *Clarias gariepinus* larvae in RAS system: First year results 2016

	Treatments						
	Control	***FOS1***	***FOS2***	***GOS1***	***GOS2***	***MOS1***	***MOS2***
Initial weight (IW) g	0.23±0.07^a	0.21±0.06^a	0.22±0.06^a	0.21±0.07^a	0.22±0.06^a	0.23±0.07^a	0.22±0.06^a
Final weight (FW) g	12.11±2.67^a	12.50±2.69^a	13.00±2.09^a	13.93±2.53^{ab}	15.45±1.51^{bc}	14.94±2.47^{bc}	15.93±1.88^c
Initial total length (ITL) cm	2.92±0.25^a	2.91±10.27^a	2.90±0.32^a	2.91±0.36^a	2.91±0.27^a	2.93±0.31^a	2.91±0.22^a
Final total length (FTL) cm	13.03±1.99^a	13.22±1.64^a	13.79±2.11^a	14.02±1.71^a	16.26±1.73^b	15.59±2.42^b	16.71±2.05^b
Live weight gain (LWG) g	11.88±2.66	12.29±2.69	12.78±2.08	13.72±2.53	15.23±1.50	14.71±2.47	15.71±1.89
Daily weight gain (DWG) g	0.13±0.04	0.13±0.04	0.14±0.03	0.15±0.04	0.17±0.04	0.16±0.04	0.17±0.04
Specific growth rate (SGR)	4.31±0.35	4.44±0.30	4.43±0.32	4.56±0.35	4.62±0.28	4.54±0.36	4.65±0.33
Feed conversion rate (FCR)	1.50	1.35	1.48	1.62	1.19	1.52	1.43
Survival rate (SR %)	77.5	80	80	77.5	85	82.5	85

Table 2. Effects of different oligosaccharides on growth of *Clarias gariepinus* larvae in RAS system: Second year results 2017

	Treatments						
	Control	***FOS1***	***FOS2***	***GOS1***	***GOS2***	***MOS1***	***MOS2***
Initial weight (IW) g	0.24±0.07^a	0.23±0.06^a	0.24±0.08^a	0.24±0.07^a	0.23±0.06^a	0.23±0.03^a	0.23±0.05^a
Final weight (FW) g	18.44±2.28^a	20.25±1.68^b	20.55±2.42^{bc}	20.62±2.15^{bc}	20.75±2.85^{bc}	20.79±2.48^{bc}	22.28±1.78^c
Initial total length (ITL) cm	2.96±0.24^a	2.96±10.24^a	2.99±0.30^a	3.00±0.30^a	2.97±0.25^a	2.99±0.33^a	2.99±0.29^a
Final total length (FTL) cm	17.55±0.71^a	18.27±1.52^{ab}	18.93±1.16^{bc}	19.07±1.50^{bc}	18.63±1.35^{bc}	18.29±1.07^{ab}	19.58±1.35^c
Live weight gain (LWG) g	17.25±2.28	18.86±1.68	19.19±2.43	19.24±2.15	19.41±2.87	19.43±2.47	20.78±1.76
Daily weight gain (DWG) g	0.19±0.05	0.21±0.05	0.21±0.05	0.21±0.05	0.21±0.06	0.21±0.05	0.23±0.06
Specific growth rate (SGR)	4.47±0.31	4.88±0.30	4.90±0.39	4.88±0.34	4.93±0.34	4.92±0.17	4.99±0.23
Feed conversion rate (FCR)	1.70	1.71	1.63	1.68	1.66	1.61	1.51
Survival rate (SR %)	82.5	80	82.5	85	80	77.5	82.5

Mean values with different superscripts are significantly different ($p<0.05$)
LWG = FW (g) – IW (g)
DWG = LWG / Number of days at the end of the period
Specific growth rate (SGR. %/day) was determined as: SGR= 100 x (Ln final weight (g) – Ln initial weight (g))/ (period in days)
Feed conversion ratio (FCR) = feed fed (g)/ live weight gain (g)
Survival (%) = 100 x (final number of fish)/ (initial number of fish)

ELIMINATION OF OFF-FLAVOUR COMPOUNDS AND DISINFECTANTS IN RECIRCULATING AQUACULTURE SYSTEMS (RAS) APPLYING HYBRID CERAMIC MEMBRANE MODULES – RESULTS OF LABORATORY SCALE TESTS

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Recirculating aquaculture systems (RAS) have gained increasing importance in industrial fish production during the last decades. There are several advantages of the defined environmental conditions in closed loop systems. However RAS operators are facing two main challenges: Preventing the outbreak of serious diseases and controlling the organoleptic quality of the product, i.e. the smell and taste.

In order to reduce the risk of infections caused by several microorganisms (bacteria, viruses, fungi) or parasites tanks, filters and other equipment have to be cleaned carefully when changing the stocking etc. Several disinfectants (mainly based on the activity of chlorine species) are used in aquaculture (e.g. chloramine-T, also known as Halamid® Aqua) and treated water is usually discharged separately. The earthy or musty taste, well known as "off-flavour", is mainly caused by the accumulation of metabolites of different cyanobacteria or actinomycetes in the muscle and fat tissue of the fish. The most prevalent compounds among them are geosmin and 2-methylisoborneol (2-MIB). Since these substances are not eliminated by the typical water cleaning process in RAS several days of depuration in fresh water without addition of feed is the only strategy to reduce the concentration of the off-flavour components. This process is not only time- and resource-consuming, but is also causing a decrease of individual weight and lipid content.

In order to infiltrate a ceramic membrane element with (activated) carbon an organic polymer was used as precursor which was pyrolyzed in a subsequent thermal treatment step. Applying the hybrid membrane module enables to combine membrane micro- or ultrafiltration and adsorption in one process.

First experiments in laboratory scale were carried out using single channel ceramic membrane modules (outer diameter: 10 mm, length: 250 mm; 40…45 % open porosity). Different elements were characterized e.g. for nitrogen and water flux. Adsorption tests were carried out using model solutions (deionized water with either 4-chlorophenol (4-CP) – representative for the organochlorine disinfectants or the off-flavour compounds geosmin/2-MIB).

Water flux varied between less than 10 and up to approx. 170 l/(m^2 h bar) – depending on the structure of the ceramic body (pore size, support characteristics) for the favoured precursor. The adsorption capacities were comparable or even significantly higher than data for adsorption on granular or powdered activated carbon described in literature. Adsorption capacities determined were up to approx. 330 mg/g carbon for 4-CP (initial concentration of model solution 210 mg/l) or between approx. 44 and 50 μg/g carbon for geosmin and 2-MIB (initial concentration 10 μg/l for each compound), respectively.

Further experiments focused on the competitive adsorption, i.e. unspecific binding of other organic compounds in aqueous media as well as the regeneration of the loaded membrane adsorbers (desorption using hot air or steam). Scale-up of the membrane modules applied (19-channel elements) and optimization of carbon infiltration process are subject of current work.

EFFECTS OF ARABINOXYLAN-OLIGOSACCHARIDES AND XYLANASE ON GROWTH PERFORMANCE, NON-SPECIFIC IMMUNITY AND INTESTINAL MICROBIOTA OF AFRICAN CATFISH *Clarias gariepinus*

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The application of endo-xylanases is found almost ubiquitously in commercial poultry feeds and, to a slightly lesser extent, in commercial pig feeds. Xylanase has found its inclusion in aquafeeds circa the 1980's due to the positive results found in the above-mentioned industries. The working mechanism of xylanase in animal feeds are twofold: Firstly, by hydrolysing the non-starch polysaccharide fraction in plant materials through random cleaving of the β-1,4-linkages of the xylan backbone. This breakdown of the cell wall leads to improved nutrient assimilation. Secondly, the use of xylanases produces fermentable oligosaccharides, such as arabinoxylan-oligosaccharides (AXOS), which has recently been showed to have potential prebiotic properties. AXOS has shown to stimulate the growth of specific microbiota in the digestive tract of animals. Studies have shown that both AXOS and xylanase supplementation can shift microbial communities in the gastro-intestinal tract of animals, while concurrently, stimulating the growth of specific health beneficial bacteria. These fermentative bacteria also utilize undigestible ingredients to produce short-chain fatty acids (SCFA), which have shown to enhance gut health and can also be used as an additional energy source by the host.

A 91-day feeding trial was done on African catfish in a recirculating aquaculture system (RAS) at the University of Stellenbosch, South Africa. Treatment diets consisted out of a basal diet with three levels of AXOS and xylanase each. Plant ingredients (maize, soybean oilcake and wheat bran) constituted the majority of the basal diet, completely substituting the inclusion of fishmeal. Each treatment was replicated six times, while each replicate contained six catfish (± 500g). Growth parameters tested include: significant growth rate; feed conversion ratio and growth percentage. Haematological assays include: Haematocrit values and innate immunity parameters, such as serum lysozyme activity, serum total protein and serum immunoglobulin. Intestinal microbiota was quantified using the automated method of ribosomal intergenic spacer analysis (ARISA). The production of SCFA in the posterior part of the large intestine was also measured by means of gas capillary. The aim of this study was to evaluate and compare the extent of the effect of xylanase and AXOS, as they both can be seen as alternatives for the other, as a valuable feed additive to improve aquafeeds.

SEEKING THE OPTIMAL DOSE OF MICRO NUTRIENTS IN ARTIFICIAL DIETS FOR MARINE FISH LARVAE

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Artificial micro diets (MDs) are considered to become a replacement for common live feed *Artemia salina* in the production of marine fish larvae. However, malformations are still a serious issue during early weaning of marine larvae. Some key vitamins, such as A, D2, D3 and K, are discussed in that context. Therefore, these vitamins were subject of the presented study in which malformations were evaluated in relation to the vitamin content of the artificial diet.

Two micro diets were produced each with a high and a low content in vitamins as described above. All four treatments were compared to larvae reared on Artemia as control group.

Sea Bass larvae *(Dicentrarchus labrax*) were reared, following the standard protocol from commercial hatcheries, and the following feeding regime was applied: Artemia nauplii were fed at 8 days post hatching (dph) for the first time. At 20 dph MDs were administered and were co-fed with Artemia for 5 days. Until 49 dph larvae were only given MDs. The control group was fed Artemia nauplii from 8 dph. After 20 dph enriched Artemia were fed. All experiments were conducted in triplicates of 60 liter tanks, with an initial stocking density of approximately 135 larvae/l.

Larval growth was determined by measuring length and dry weight every five days. Survival was evaluated by counting the remaining larvae in each tank at the end of the experiment. To evaluate the occurrence of malformation, 50 Larvae per tank were sampled at 30, 40 and 49 dph and double stained in Alcian blue and Alizarin red.

Preliminary results show that there was no significant difference in growth parameters between all four MD groups. However, growth performance of MD fed groups was reduced in comparison to larvae reared on Artemia only. Similar results could be shown for larvae survival.

The amount of malformation was significantly reduced in fish of both treatments with high vitamin concentrations in comparison to the low vitamin groups. Nevertheless, Sea Bass larvae fed with Artemia only had the lowest percentage of malformations in this experiment.

SALINITY ADAPTATION OF *Totoaba macdonaldi* LARVAL STAGES

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Totoaba macdonaldi is an endemic fish from the Sea of Cortez, its bladder represents a billon black market in China, it has been declared as endangered species by Mexican Federal Government. It inhabits the Colorado Delta River Marine Reserve, which supports high productivity, biomass and endemism of several species and is a marine nursery area that used to receive huge volumes of fresh water from the Colorado River, although currently only receives occasional pulses. The cause of populations reductions of this fish has been controversial (mainly attributed to broodstock overfishing, but also to loss of freshwater habitat). We exposed some Totoaba larva in different developmental stages (2, 7, 9, 19, 25 and 45 days after hatch) to salinity challenges in the range from 3 to 40 psu in short-(48 hours) acclimation trails. The Totoaba were directly transferred. Blood osmotic pressure (OP) and survival were evaluated. Young larva (<D19) are osmoconformers with external salinity, their OC increases progressively during development and their IP fluctuates until D19. Thereafter early juvenile stages (>D25) develops a fully euryhaline behavior, able to osmoregulate and at this stage maintains its internal blood OP regardless that from external media in 332±4 to 327±2 (11 psu) after 48 hpa. Survival patterns associated to salinity indicates that young Totoaba larva (D2-D3) is dependent of marine, but mostly hyper–saline water but soon in development (D6-D8) extreme salinities (5 or 40 psu) are lethal, they have higher survival in 11 and 26 psu. There is a tendency to reduce survival trough D14-D16 in which at 11 psu continues to have higher survival. Larva are more sensitive to extreme intervals with the worst survival around D23-D27 when post-flexion larva (10-15 mm) transition occurs, but after D28-D30 extreme salinities were the best to survive for this fish under our experimental conditions. The global IP average of *Totoaba macdonaldi* is 362±35mOsm.Kg-1(12±1psu).

EFFECT OF DIFFERENT FEEDING PROTOCOLS ON DAILY PROTEOLYTIC ENZYME ACTIVITY IN GILTHEAD SEABREAM JUVENILES

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Many studies have assessed digestive enzymes activity in response to diverse feed ingredients and water environmental conditions. However, the circadian rhythm of the digestion process and the factors influencing these rhythms have scarcely been examined. The aim of the present work was to compare the daily activity pattern of two key proteolytic enzymes (acid proteases and trypsin) in the gilthead seabream (*Sparus aurata*) fed with different protocols.

Juvenile fish (17.66 ± 0.38 g) were distributed into 4 groups (250-L tanks, 19.5 ± 1.0 °C, photoperiod 11L/13D). All groups were fed a daily ration of 2 % body mass during the daylight with different feeding protocols: (a) 1 meal (08:30 h); (b) 3 meals (08:30, 13:30, and 18:30 h); (c) 5 meals (08:30, 10:30, 13:30, 16:30 and 18:30 h); and (d) continuous feeding. Fish were acclimatized during 2 weeks and then were sampled every 4 hours during a 24 h cycle. Total acid protease activity in the stomach was analyzed using hemoglobin, at pH 2.0. Trypsin activity in the intestine was analyzed using BAPNA at pH 8.5.

Not significant variations were observed in the pepsin daily activity in none of the feeding protocols, excepting the fish fed 3 times that exhibited a peak at the beginning of the dark cycle (Fig. 1A). However, juveniles fed 5 daily meals showed higher trypsin activity during daylight with a considerable decrease during the dark period. Similarly, in fish with continuous feeding, trypsin activity increased gradually during the daylight, reached maximum levels around 21:00 h, and decreased drastically afterwards. On the other hand, fish fed 1 and 3 times a day did not show significant variations in the trypsin activity (Fig. 1B).

According to our findings, feeding frequency and timing have a major influence on the daily pattern of enzymatic activity, especially during the intestinal phase of digestion. It seems that splitting the daily ration in several meals leads to higher trypsin activity during the daylight and the beginning of the dark period.

Acknowledgments: Grants AGL2014-52888-R from MINECO (Spain) with FEDER/ERDF contribution, and WISEFEED funded by the European Union's H2020 programme (Marie Skłodowska-Curie grant No 691150). NG supported by BES-2015-071662 from MINECO.

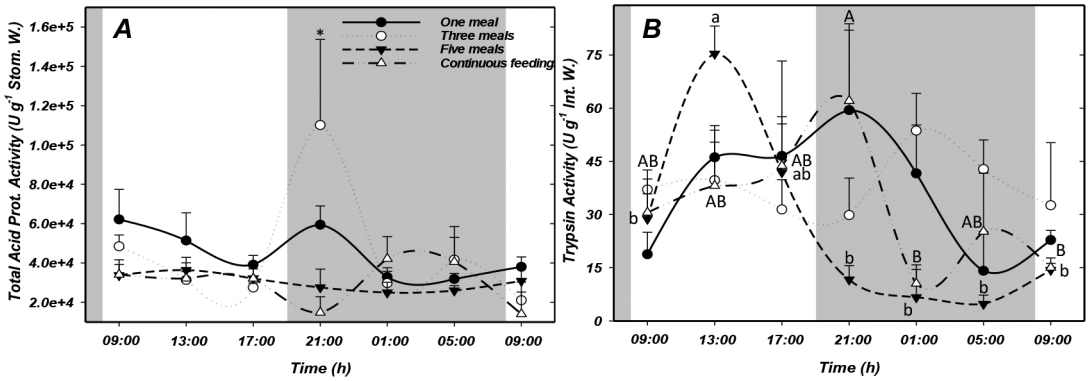

Fig. 1. Daily changes in the activity of (A) total acid proteases and (B) trypsin, in *S. aurata* fed with different daily feeding protocols. The grey areas indicate the dark period. In each protocol, sampling points not sharing the same symbol or letter are significantly different (Tukey test, $p < 0.05$).

FEEDING PROTOCOL MODULATES THE DAILY GASTROINTESTINAL TRANSIT IN GILTHEAD SEABREAM JUVENILES

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Improving the growth performance in farmed fish is a permanent challenge. There is a vast knowledge in feed formulation and digestibility of different ingredients. However, few studies have addressed the gastrointestinal tract (GIT) parameters *per se* as fundamental factors that should be considered in the digestive process. Therefore, this work aimed to evaluate the changes of the ingesta transit rate throughout the GIT in response to different feeding protocols in gilthead seabream (*Sparus aurata*).

Juvenile fish (17.66 ± 0.38 g) were distributed into 4 groups (250-L tanks, 19.5 ± 1.0 °C, photoperiod 11L/13D). All groups were fed a daily ration of 2 % body mass during the daylight with different feeding protocols: (a) 1 meal (08:30 h); (b) 3 meals (08:30, 13:30, and 18:30 h); (c) 5 meals (08:30, 10:30, 13:30, 16:30 and 18:30 h); and (d) continuous feeding. Fish were acclimatized before sampling during two weeks. To determine the GIT transit (filling and evacuation time), fish were alternatively fed with diets with and without Yttrium, as an inert marker. Yttrium was measured separately in the stomach and intestine by ICP-MS.

Stomach and intestine became full 4 to 12 h after feeding, depending on the protocol (Fig. 1A, B) and were emptied between 8 to 12 h and 12-16 h, respectively (Fig. 1C, D). Fish fed one single meal showed the fastest filling rate, both for the stomach and the intestine, although their evacuation time were comparable to the rest of the groups. Fish fed five daily meals showed the fastest stomach evacuation rate; however, their intestine evacuation was similar to the rest of the groups. Juveniles with the continuous daily feeding had the longest intestine evacuation time (around 16 h).

Our results indicated that feeding the gilthead seabream with a single meal, allocating the whole daily ration, leaded to a very fast stomach transit. However, splitting the daily ration in several meals resulted in longer transit times, especially during the intestinal digestion period. These findings highlight the importance of the feeding protocol on the GIT transit time, and in the duration that the ingesta is exposed to different luminal chemical environments and digestive enzymes, and, therefore, in the efficiency of the feeding practice.

Acknowledgments: Grants AGL2014-52888-R from MINECO (Spain) with FEDER/ERDF contribution, and WISEFEED funded by the European Union's H2020 programme (Marie Skłodowska-Curie grant No 691150). NG supported by BES-2015-071662 from MINECO.

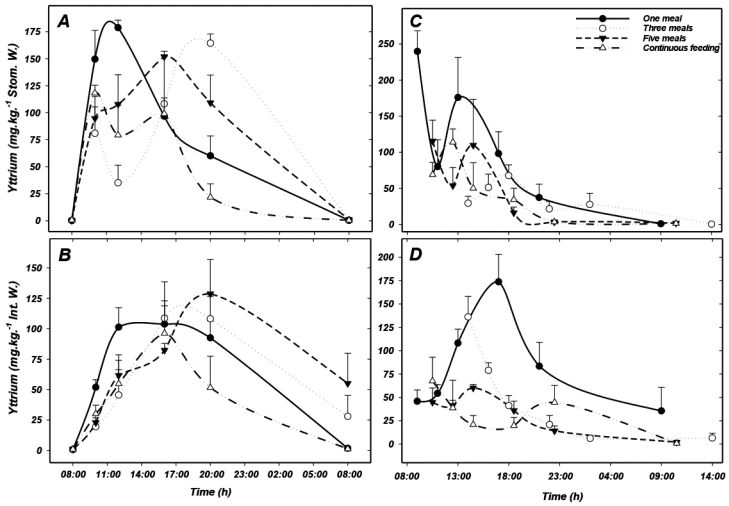

Fig. 1. Daily changes in the Yttrium content (mean ± SEM) in *S. aurata* GIT, fed with different daily feeding protocols, indicating (A) stomach filling, (B) intestine filling, (C) stomach evacuation, and (D) intestine evacuation. The gray areas indicate the dark period.

ECONOMICAL SUSTAINABILITY OF TAMBATINGA (*Colossoma macropomum X Piaractus brachypomus*) AND FARM SIZE INFLUENCE

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Production of Tambaqui and its hybrids like tambatinga represent 20% of total fish production in Brazil. Production farms show a large range of sizes and no studies have assessed the scale effect. Thus, in the present study, indicators of sustainability were determined for farms of different sizes.

Sixteen farms comprised of earthen ponds operated in semi-intensive systems, with total pond area between 0.1 and 220 ha, were studied in the Brazilian Northeast region. A survey to obtain investments, operating costs and incomes was performed in 2016-2017. Data were used to determine indicators of economical sustainability, which were compared through the different farm sizes.

The principal economic indicators (figure 1) show a large sustainability of small farms. There is a diminution of sustainability in all farms above 12 ha. This inverse relationship is principally explained by larger productivity and high capacity to sale fish in best markets at smaller farms, increasing profit margin. Moreover, the larger farms show expense proportionally larger regarding vehicles, infrastructure and labor.

In conclusion, in this regional economic context, it is more interesting to promote smaller farms (≤ 12 ha) allowing a more sustainable economic development. Larger farms need suitable local infrastructures like feed factory and freezers to ensure a more regular sale of fish to increase profitability.

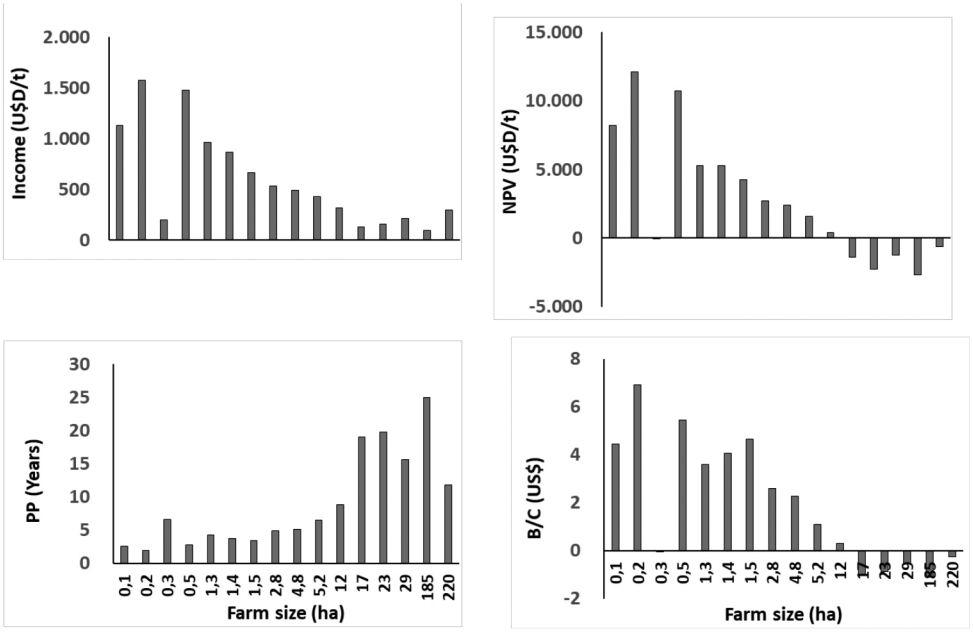

Figure 1 Scores of the principal economic indicators of the studied farms. X-axis are the farm size (ha) sorted in ascending number. NPV= Net Present Value. B/C = Benefit-Cost Ratio. PP= Payback period.

WHAT CONTRIBUTION AND ROLE CAN THE STURGEON AQUACULTURE AND THE CAVIAR PRODUCTION CHAIN HAVE TODAY IN FAVOUR OF THE CONSERVATION AND PROTECTION OF STURGEON SPECIES?

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During the past three decades a private fish farmer and his family played an important role in the conservation and restocking of *Acipenser naccarii*, one of the highly endangered Italian sturgeon species. Without this private initiative supported by several sturgeon experts and enthusiasts this species would otherwise be most likely extinct today. The presentation reflects the highlights on this 30 years long journey of attempts to culture and maintain this species while also discussing the role a fish farmer can play in sturgeon conservation, especially in a country where public facilities are not yet established neither for ex situ conservation nor for the reproduction and rearing fish for release. In fact since the seventies about fifty wild sturgeon caught in the River Po and other Italian rivers have previously been gathered and maintained in a trout farm close to Brescia and thereafter in a sturgeon farm not far from Milan. This initial group of fish became the broodstock and was maintained in raceways with spring water coming from a small lake of about one hectare. Since 1988 some of the adult fish were used to produce on a yearly basis fingerlinds through controlled reproduction. They have been raised up to adult sub-sizes and have since been used for repopulation actions. More than 600.000 specimens were released in natural waters of the native rivers of this species, and good results started to appear in these last years. Now the family company is involved also in the business of caviar and sturgeon productions, but always with a particular attention to the quality of the fish and the maintenance of the species purity of those reared.

MODELLING THE APPLICATION OF THE IDEAL PROTEIN CONCEPT IN SHRIMP

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The ideal protein concept (IPC) works on the premise that an animal requires the ten proteinogenic essential amino acids (EAA) in a ratio relative to lysine (or some other quantitatively determined amino acid) as defined by their own natural body amino acid composition. Using a quantitative determination of a key amino acid this ratio is then applied to define the quantitative requirements of all the other EAA. Because of this relationship and the capacity to model protein and energy demand, using standard factorial bioenergetic modelling (FBM) methods, by extension it also becomes possible to model demand for each of the ten essential amino acids.

There are several steps to facilitating this modelling of the quantitative requirements for the ten EAA. The first step is to have a factorial bioenergetic model that predicts energy and protein demand of a healthy, growing animal. This requires a predictive growth model, a series of body composition equations, and determination of key utilisation coefficients of energy and protein and having estimates of maintenance requirements for energy and protein. In addition to these standard parameters for development of a FBM, we also need a robust analysis of the amino acid composition of the whole animal with varying body size. This information then needs to be paired with a similarly robust assessment of the quantitative requirements for any of the EAA, usually as defined using a simple dose-response study. Each of these different parameters can then be integrated together to create an adaptation of the FMB that includes predicative outputs for the EAA. In this presentation, we will explore the application of the IPC in combination with the FBM to define the quantitative requirements for each of the ten EAA for shrimp. From these derived estimates, we will then compare across different shrimp species and different animal sizes to assess various applications of this modelling strategy.

Table 1. Estimations of EAA requirements in shrimp based on Empirical and IPC estimations

EAA	**LYS**	**MET**	**HIS**	**ISO**	**LEU**	**PHE**	**TRP**
Empirical Estimations							
As % Diet*	2.0	0.9	0.8	1.0	1.7	1.4	0.2
As % Protein	5.1	2.4	2.0	2.5	4.3	3.5	0.6
As ratio to LYS	100%	47%	39%	49%	84%	69%	12%
Ideal Protein Concept Estimations							
Ratio to LYS	100%	34%	30%	60%	119%	62%	14%
As % Protein	5.1[#]	1.8	1.5	3.0	6.0	3.0	0.8
As % Diet*	2.0[#]	0.7	0.6	1.2	2.4	1.2	0.3
Modelling Estimations							
As % Protein	6.5	2.2	1.9	3.9	7.8	4.0	0.9
As % Diet*	2.6	0.9	0.8	1.6	3.1	1.6	0.4

* assumes a 40% protein diet. # assumes requirement for LYS as 5.1% of protein.

Origanum vulgare AND *Cinnamomum zeylanicum* EXTRACTS AS WELFARE PROMOTERS FOR GILTHEAD SEABREAM (*Sparus aurata* L.)

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Farmed fish are exposed to a variety of stressors during aquaculture practices which can affect fish performance, growth and survival. However, as aquaculture fish is intended for human consumption, it is important to search for compounds with potential anti-stress properties, increased safety and low cost. Interest in natural products has increased while *Origanum vulgare* and *Cinnamomum zeylanicum* are of the mostly used medicinal herbs with antibacterial, anaesthetic and antioxidant properties in fish. Essential oils may be safe at low concentrations but display dose-related toxicity. DNA strand breakage acts as a biomarker of genotoxicity, strongly related to induced stress.

Presently, the potential genotoxic effect of dietary cinnamon and oregano essential oils supplementation was assessed in gilthead seabream (mean weight: 3.75±0.21g). Commercial fish pellets were over-coated with four essential oils. Four experimental fish group were fed with diets supplemented with essential oils, CIN1: 1% *C. zeylanicum*, CIN2: 2% *C. zeylanicum*, OR1: 1% *O. vulgare* and OR2: 2% *O. vulgare*, while a control fish group (C) was fed with commercial fish diet. Genotoxicity was assessed by comet assay, 90 days post-treatment. DNA migration (tail moment) was measured in hepatocytes and gut cells. All data were statistically analyzed using SPSS 17 while differences were considered significant at $p<0.05$ using one-way ANOVA followed by Tukey post hoc test.

According to comet assay results (Fig.1), DNA migration in hepatocytes was lower when fish were fed with diets supplemented with essential oils compared to control group. In case of cinnamon oil, DNA migration was significantly lower ($p<0.05$) indicating a potential geno-protective effect. Dose-related geno-protective effect and antioxidant activity of cinnamon has been assessed before while DNA repair system after induction of genotoxic lesions may be significantly related. No differences, compared to the control group, were mentioned in gut cells apart from the group OR2 where significant ($p<0.05$) DNA damage was induced. The genotoxicity in this case is probably related to the reduced feeding activity, which was observed only in this experimental group due to extra strong flavor of oregano oil. Increased DNA migration is expected, as starvation is a well-known stress factor for farmed fish. Presently, *O. vulgare* and *C. zeylanicum* essential oils were assessed as potential anti-stress agents. Their use as fish feed additives promoting fish welfare under intensively reared conditions should be considered, while the long term effects of phytomedicines exposure on fish has to be elucidated.

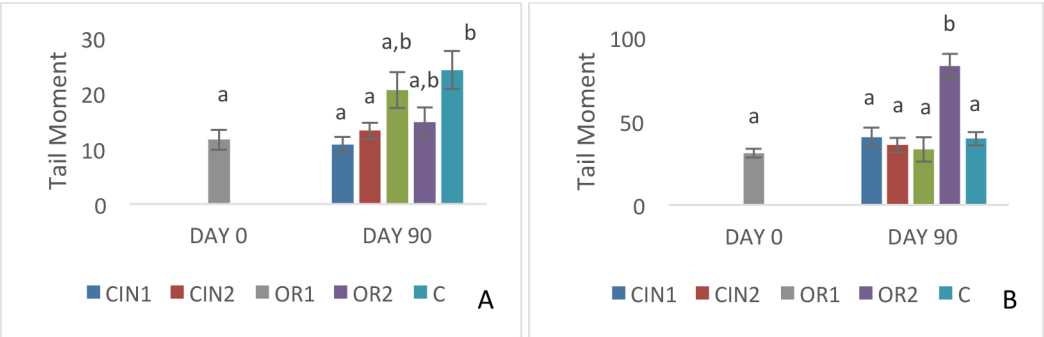

Figure 1. DNA damage induced in A. liver cells and B. gut cells

SEX CONTROL IN COMMON CARP: THEORETICAL AND PRACTICAL ASPECTS

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Sex control in common carp *Cyprinus carpio* is especially important for production increase in conditions of tropical or subtropical climate where females are significantly larger than males. Also, raising of all-female progenies makes it possible to prevent reproduction in cases when fish reach maturity before attaining market size.

The same as in other aquaculture species, sex control in common carp is connected with elaboration and application of the hormonal sex reversal technique. In the case of the XX/XY system of chromosome sex determination, which was revealed in common carp, this technique may be used in two different ways for production of all-female progenies: directly, by feminization of all commercially raised fish by estrogen treatment, or indirectly, by crossing previously obtained by androgen treatment sex-reversed XX males with normal XX females. The second way (genetic sex regulation) is regarded as optimal taking into account food safety and environmental concerns.

Successful genetic sex regulation in common carp was demonstrated repeatedly from the beginning of the 1980s. There are two factors, which help this technique to be effective for practical application. First, sex-reversed common carp males (with XX genotype) do not have abnormalities in development of sperm ducts (as is observed in rainbow trout); this permits the stripping of sperm from sex-reversed males. Second, the chromosome system of sex determination in common carp works, as a rule, without any autosomal influence (as is observed in Nile tilapia) and crosses of sex-reversed XX males with normal XX females results in female offspring only.

Usually, for achievement of sex reversal, fish were fed with food containing 100 mg/kg of androgen methyltestosterone (MT) for 35-40 days. The important issue is to determine which parameter, fish age or size (weight), should be used as a practical indicator for the onset of MT-treatment. Based on results of many experiments on sex reversal, it seems that fish weight (rather than their age) should be used for this purpose. It can be recommended that the weight of fish should be monitored during nursing; when it reaches 3-5 g, the MT-treatment should be started. The age of fish at this period usually varies from 1 to 2 months.

Some conditions of fish rearing before and during androgen treatment can influence results. Prior to using in experiments on sex reversal, progenies can be stocked for nursing in earthen ponds; nursing in ponds is technically simple and fish can consume some natural food. During MT-treatment water temperature should be kept at 25ºC or higher. Apparently, the process of sex differentiation becomes shorter at higher temperature and it is covered more completely by the period of androgen influence. Also, androgen treatment can be more effective when fish are kept in recirculating water systems. This is possibly caused by accumulation in the water of some active soluble androgen metabolites released by fish consuming MT-containing food.

GROWTH RATE AND SEX SEGREGATION IN NILE TILAPIA CROSSES OBTAINED USING YY MALES AND NORMAL XX FEMALES OF DIFFERENT ORIGIN

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Nile tilapia *Oreochromis niloticus* has become a popular food fish for human consumption in the Unites States. Most of the tilapia sold in the country are imported; however domestic production of tilapia increases. It is a popular species for raising in recirculating and aquaponic systems.

Sex control in tilapia is important because males grow significantly faster than females. The genetic sex regulation in tilapia by using YY males is considered more acceptable compared with direct masculinization of fish taking into account food safety and environmental concerns. The genetic sex regulation in tilapia presumes crossing YY males with XX females. Females used in crosses can be originated from different lines. In this case, production of fish for commercial rearing is based on crossbreeding, or crossing of animals belonging to different lines of the same species. It is recommended to test many cross combinations in order to identify the best crosses.

The purpose of this study was to investigate comparative growth rate and sex segregation in four crosses obtained using YY males and normal XX females of different lines from commercially available sources. Characteristics and origin of fish parents are presented in Table 1. All four crosses were obtained at the same time and were raised in recirculation water systems under the same conditions for 78 days. Then fish were stocked in a recirculating system with 12 946-liter (250-US gallon) tanks for grow-out comparative raising. Each cross was raised in three replicative tanks; 50 fish were stocked per tank. During 166-day raising, fish were fed 2-3 times a day to satiation; water temperature was kept at 28 ºC. Mean initial and final weights as well as other parameters are presented in Table 1. The percentage of males in crosses varied from 79 to 100%. Cross 2 appeared to be superior and surpassed other tested crosses with regard to growth rate by 58% or more. This cross was obtained using females from Genetically Improved Farmed Tilapia (GIFT) line. Based on results of this trail, it can be suggested that crossing YY males with females from lines previously selected for high productivity (as compared with non-selected lines) results in appearance of highly productive crosses. This suggestion will be checked in further studies.

Table 1. Parameters of comparative raising and sex composition in four Nile tilapia crosses.

Cross No.	Characteristics and Origin of Parents		Survival, %	% of Males	Mean Initial Weight, g (±SD)	Mean Final Weight, g (±SD)	Daily Growth Rate, g/d
	YY Males	Females					
1	Red[a]	Red[c]	99.3	79.2	21.1±3.7	402.4±93.0	2.3
2	Red[a]	Dark[d]	99.3	79.2	21.4±5.8	702.4±150.7	4.1
3	Red[a]	Blotched[e]	100	84.7	18.6±3.5	433.6±127.2	2.5
4	Red[b]	Blotched[e]	100	100	17.5±4.7	447.0±93.9	2.6

[a] Obtained from Miami Aqua-culture, Inc. (Boynton Beach, FL); [b] Obtained from Fishgen Ltd. (Swansea, Wales); [c] Obtained from Miami Aqua-culture, Inc. [d] GIFT line; obtained from Miami Aqua-culture, Inc., [e] Red fish with large dark patches; obtained from Louisiana Specialty Aquafarm LCC (Robert, LA).

ADVANCED TECHNOLOGY FOR INTENSIVE MARINE RAS: ANALYSIS OF THE IMPLICATIONS OF THE USE OF ELECTROCHEMICAL OXIDATION

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This work is focused on the application of the electrochemical oxidation technology as an alternative to conventional treatments, i.e. biological filters, in intensive marine recirculating aquaculture systems (RAS), with the aim to overcome two main drawbacks: the accumulation of harmful pollutants and the consequent need for make-up water to dilute their concentration. The validity of the proposed technology has been evidenced by the evaluation of the performance of a system prototype (ELOXIRAS®[1]) over a culture cycle of gilthead seabream (*Sparus aurata* L.) and European seabass (*Dicentrarchus labrax*) □involving a total culture volume of 20 m^3 and a biomass density up to 29 kg/m^3□, which is introduced by the present document as related to:

- The development of the fish. Instantaneous growth rates of 0.88 and 1.07 %/day have been obtained for gilthead seabream and European seabass, respectively, as indication of an increase in the fish size from 42-76 g to 140-206 g. These values are equivalent to those obtained in a comparison with a biofiltration-based RAS, conducted under identical conditions: 0.92 %/day, for gilthead seabream; and 0.91 %/day, for European seabass —from 56-82 g to 159-232 g—.
- The quality of the culture water. It has been assessed based on: (i) ammonia and their oxidation derivatives, (ii) organic matter, and (iii) populations of microorganisms. Table 1 shows that the level of pollutants has been kept within a suitable range throughout the entire cycle —despite reduced water renewal rates as expected for intensive RAS, requiring less than 240 L of new water per kg of fish produced—, with no significant accumulation of nitrate and faster removal kinetics —up to 93 % for ammonia— compared to typical values obtained when using biofilters. Table 2 shows that the disinfection capacity of the treatment —of at least 3 logarithms— contributes to the stabilization of the microbiota of the culture tanks.

In conclusion, based on the above results, two potential beneficial impacts are derived:

- The adequate control of pollutants and microorganisms guarantees the optimal development of the fish. In addition, faster removal kinetics of these pollutants enables the possibility of working with higher biomass densities.
- The reduced nitrate formation —compared to that of biofilters— leads to a minimization of the amount of make-up water required, with the consequent savings and reduced environmental impact.

[1] Detailed description and 3D video of the ELOXIRAS® system at: https://www.youtube.com/watch?v=d6HcH7TPyvE.

Acknowledgements: This project has received funding from the EU H2020 R&I programme under GA No. 698494. This abstract reflects only the author's view and the Agency is not responsible for any use that may be made of the information it contains. The authors also acknowledge the valuable contributions and research assistance of Institut de Recerca i Tecnologia Agroalimentàries.

TABLE 1. Averaged and maximum values of the most concerning pollutants in the culture tanks and maximum removal efficacies per pass through the electrochemical cell.

Pollutant	Mean value (± Std. deviation)	Max. removal efficacy (%)
TAN[a] (mg $N\text{-}NH_3/L$)	0.55 ± 0.42	93
Nitrite (mg $N\text{-}NO_2^-/L$)	0.08 ± 0.11	-
Nitrate (mg $N\text{-}NO_3^-/L$)	2.5 ± 3.1	-
COD[a] (mg O_2/L)	< 30	40

[a]TAN: total ammonia nitrogen; COD: chemical oxygen demand.

TABLE 2. Bacteriological survey in culture tanks to determine total bacteria and Vibrionaceae species in TSA and TCBS growth media, respectively.

Bacterial species	Bacteria count (CFU/mL)
Total bacteria	$1 \cdot 10^2$-$2 \cdot 10^3$
Vibrio spp.[a]	$3 \cdot 10^1$-$7 \cdot 10^2$

[a]Vibrionaceae bacteria are responsible for pathologies that may frequently affect farmed gilthead seabream and European seabass.

FIRST RECORD OF LEVELS AND DISTRIBUTION OF MICROPLASTICS IN COASTAL SEDIMENTS COLLECTED NEAR AN AQUACULTUE SITE IN THE SOUTH WEST OF NORWAY

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Plastic waste is of increasing concern in the aquatic environment. A large portion of the plastic waste is produced onshore and reaches the marine environment, which is considered the main sink of plastic debris. Floating plastic particles accumulate in pelagic habitats. However, due to the biofilm formation they eventually sink and accumulate on the seafloor together with non-buoyant by design plastic particles posing risk to the benthic communities.

Aquaculture makes wide use of durable plastics, which are used for tanks, fish cage collars, pond liners, netting, rope, and floats, among other items. In the context of global plastic pollution of the oceans, aquaculture is a tiny contributor, although estimation of the size of the contribution remains a knowledge gap and lost or derelict gear from aquaculture can be a locally important contributor. In the present study, a preliminary assessment of the occurrence, levels and chemical characterization of 9 type of polymers in sediments near an aquaculture located in the Boknafjorden (Norway) is presented. Plastic microparticles were extracted by flotation from 5 kg sediment samples, purified by a multi-step combined enzymatic and mild oxidant reaction and finally analysed by sequentially visual microscopic inspection and thermal desorption pyrolysis gas chromatography/mass spectrometry (GCMS-pyr). Most of the detected polymers were identified as polypropylene, polyethylene, polyvinylchloride followed by polyethylene terephthalate, polystyrene and polyamide. An attempt to identify and quantify plastic based paints and antifouling agents was performed. Preliminary results are presented.

MAPPING MICROBIAL COMMUNITY SHIFTS DURING BIOREMEDIATION OF SHRIMP FARMING WATER

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Bioremediation is a reliable and economic tool for improving water quality and developing more environmentally sustainable aquaculture operations. The processes underpinning the efficacy of the bioremediation technology involve complex and dynamic shifts in the microbial composition throughout the system. Understanding the underlying microbiological processes that support the bioremediation technology is important to enable its broad implementation, which could help safeguard the aquaculture industry in the face of biosecurity and environmental challenges. The current study investigated the changes in microbial communities across different stages of a bioremediation system that has been implemented in a shrimp farm. Microbial characterisation was performed using a combination of single genes (16S and 18S rRNA) and shotgun metagenomic sequencing on samples collected before and after water had been subjected to sand filters and high rate algae ponds (HRAPs). This analysis revealed different groups of bacteria and eukaryotic microorganisms dominating the different stages of the bioremediation system. We identified a suite of microorganisms that were either enriched or depleted from the water by the bioremediation technology. The microorganisms differentially abundant across the system include several species of cyanobacteria, non-sulfur bacteria, rod-shaped bacteria, dinoflagellates, green algae and brown algae. The presence of common shrimp pathogens was investigated in the sequencing data and also by quantitative PCR (qPCR). This assessment showed either the absence or very low relative abundance of shrimp pathogens, including the pathogenic strain of *Vibrio parahaemolyticus* (Vp_{AHPND}) and White Spot Syndrome Virus (WSSV). Overall, our findings indicate that the bioremediation technology has the potential to contribute to the improvement of both physico-chemical and microbiological water parameters. The reorganisation of the microbial communities observed throughout the bioremediation system enables the production of improved-quality water that could be re-used in the production process (water recycling) or be safely discharged into the environment.

EXPLORING THE MOLECULAR MECHANISMS DRIVING THE PROTECTIVE EFFECTS OF RNA INTERFERENCE THERAPY AGAINST WSSV INFECTION

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RNA interference (RNAi) therapy represents one of the most promising strategies to minimize the devastating effects of viral infections in shrimp aquaculture. Previous studies have shown improved survival and growth rates in animals that had their RNAi machinery activated before being infected with pathogenic viruses. However, the molecular mechanisms underlying the antiviral protective effects of RNAi against shrimp viruses have not been fully elucidated. The current study aimed to characterize the hemocyte transcriptional profile mediated by the RNAi therapy in animals that exhibited successful antiviral response and survival to white spot syndrome virus (WSSV). To do so, juvenile shrimp (*Litopenaeus vannamei*) were injected with a cognate double-stranded RNA (dsRNA) targeting the WSV421 gene (encoding the WSSV major envelope protein VP28) and then challenged with a lethal dose of WSSV. The expression profile of key immune-related genes was assessed by high-throughput quantitative PCR (qPCR). The antiviral state promoted by the RNAi therapy (injection of dsRNA-VP28) resulted in the survival of 72% of the animals infected with WSSV as well as induced the modulation of genes involved in antiviral defense, oxidative response, cellular homeostasis and regulation of the cell cycle. We have also showed that the dsRNA-VP28 is effective in promoting an antiviral protection against WSSV not only by rearranging the host transcriptional responses but also by limiting viral replication. The set of genes identified in this study could aid the development of management strategies (*e.g.* marker-assisted breeding programs) designed to control the impacts of viral disease outbreaks on shrimp production.

EFFECT OF FEED ADDITIVES ON THE UTILIZATION OF MICROALGAE *Nannochloropsis* sp. FED TO ATLANTIC SALMON *Salmo salar*

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Supplying quality protein and lipids for Atlantic salmon feeds is a challenge. Fishmeal and fish oil in present-day salmon feeds are to a large extent replaced with plant-derived ingredients. Nutritional requirements of salmonids cannot be met solely from plant-derived ingredients. There is thus a need for more reliable, scalable and sustainable protein and oil sources with good nutrient profiles. Microalgae are promising alternative sources, and their production is expected to grow to supply the feed ingredient market. We have observed that nutrient digestibility values of microalgae-included feeds were lower compared to the reference feeds in Atlantic salmon (Gong et al. 2018). In the present study we investigated the potential of feed additives in stimulating the digestive system, to improve the utilization of the nutrients in the microalga *Nannochloropsis* sp.

Four diets were formulated; control diet without the microalga *Nannochloropsis* sp. (CT), a diet containing 10% of the microalga (NC), and two diets (NZ and ND) containing 10% of the microalga and two different commercial feed additives. Fish (initial av. weight 227 g) were fed the experimental diets for 10 weeks. Feed consumption was monitored daily. At the end of the feeding period, fish weights were recorded before collecting the samples of interest; distal intestine, fillet, whole body and faeces of fish. Growth, survival, gut morphology, digestibility, and quality of fish and fillets were assessed to understand the effect of the experimental diets.

The specific growth rate and feed conversion ratio of the fish did not suggest any positive influence of the additives. Although the viscerosomatic indices of the study groups were not different, hepatosomatic index of the ND-fed fish was significantly higher compared to the NC-fed fish. Samples are currently being analyzed and results from the work will be presented at the conference.

This study was part of the Research Council of Norway project Alger4laks (No. 260190), funded under the COFASP ERA-NET project MARINALGAE4aqua). Yangyang Gong is supported by a fellowship from the China Scholarship Council.

Gong, Y., Guterres, H.A.D.S., Huntley, M., Sørensen, M., Kiron, V. 2018. Digestibility of the defatted microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. when fed to Atlantic salmon, *Salmo salar*. Aquaculture Nutrition, 24(1): 56-64.

GROWTH PERFORMANCE AND GENE EXPRESSION ANALYSIS OF THE WHITE SHRIMP (*Litopenaeus vannamei*) FED WITH ADDITIVE MICROENCAPSULATED PHENOLIC EXTRACTS

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The phenolic composition and antioxidant capacity of extracts of leaf, bark and fruit of the *Malpighia umbellata* Rose were assessed as nutraceutical antioxidant additive in shrimp culture feeds. Results revealed leaves as an excellent source of both total phenolics (46.91 mg/g dry extract) and carotenoids (424.63 μg/g dry extract). The HPLC-DAD analysis indicated that leaves were a relevant source of flavones, bark of phenolic acids, whereas fruits of flavonols. A kaempferol-3-O-glycoside accumulated in the red mature fruits (15.84 mg/g dry extract) was the major phenolic compound of *M. umbellata*. The mature fruits showed the highest contents of flavonoids (17.462 mg/g), anthocyanins (2.15 mg/g), and carotenoids (46.23 μg/g), whereas the unripe ones the highest contents of total phenolics (29.21 mg/g), condensed tannins (4.57 mg/g), and vitamin C (51.17 μg/g). The 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) scavenging activity and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) inhibition assays revealed important antioxidant properties for the leaves, bark, and fruits of this species. The treatments were performed using one control (microencapsules alone) and three concentrations of microencapsulated leaves phenolic extracts:, T1: 1 mg 10 g^{-1}; T2: 5 mg 10 g^{-1} and. T3: 10 mg 10 g^{-1}. As antioxidant nutraceutical additives, the microencapsulated phenolic extracts showed significant differences. The gene expression of CAT and proPO were significant higher ($P< 0.05$) with the addition of *5 mg 10 g^{-1}* (**T1**) and *10 mg 10 g^{-1}* (**T3**) of microcapsule, respectively (Fig. 1).

In the present study, CAT as part of the antioxidant system is involved in the destruction of H_2O_2 generated during cellular metabolism. GPx enzyme is characterized by its high reaction capacity but relatively little affinity for the substrate. It has two functions: the catalytic and the peroxidative. The reaction catalyzed by the enzyme glutathione peroxidase allows, through oxidation delglutathione, the detoxification of hydrogen peroxide and various hydroperoxides. The microencapsulated phenolic extracts of *M. umbellata* played the role as supporting antioxidant enzimes and that reduced the H2O2 and hydroperoxides produced by the *L. vannamei*.

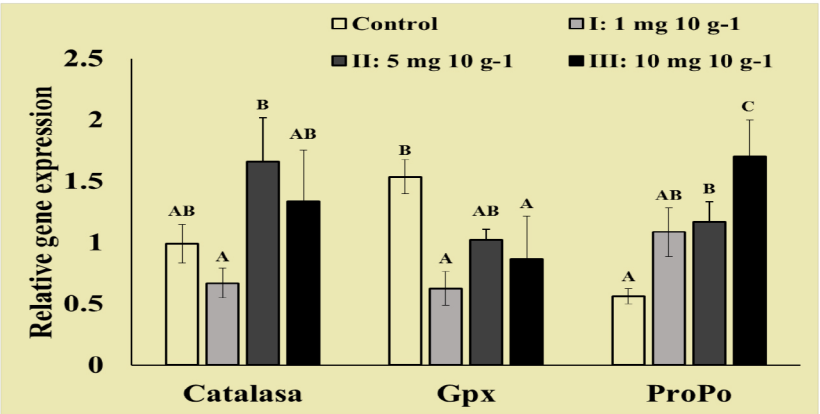

Figure 1. Relative mRNA expression of SOD and lysozyme in hemocytes and, HSP70, HSP90, chymotrypsin, and trypsin in hepatopancreas of *L. vannamei*. Values are mean ± SE (n = 9). Different superscript indicates significant differences (P<0.05).

GROWTH PERFORMANCE AND GENE EXPRESSION ANALYSIS OF THE WHITE SHRIMP (*Litopenaeus vannamei*) CULTURED IN ZERO-WATER EXCHANGE AND SUPPLEMENTED WITH *Bacillus licheniformis* AND MOLASSES

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Superintensive shrimp farming is characterized by high stocking densities. In these systems, there is zero- water exchange, feed intake and nutrients are controlled; and the accumulation of flocculated particles (bioflocs) formed by aggregates of algae, bacteria, protozoa, faeces, and uneaten feed is favored. Several studies have pointed out that microorganisms present in biofloc systems contribute to stimulate shrimp immune response and growth. Therefore, the aim of this study was to determine the effect of *Bacillus licheniformis* BCR 4-3 and molasses on growth performance and gene expression of white shrimp (*Litopenaeus vannamei*) cultured with zero-water exchange. The effects *B. licheniformis* BCR 4-3 and molasses on growth performance and gene expression in *L. vannamei*, cultured at high stocking density and zero-water exchange, were evaluated in bioassays with treatments by triplicate. Bioassay 1 (growth):

I) Control, biofloc without additives; II) Bioflocs + molasses; III) Bioflocs + bacilli; IV) Bioflocs + bacilli + molasses. Bioassay 2 (gene expression): I) Control, bioflocs without additives; II) Bioflocs + bacilli + molasses. Survival, growth in weight, nitrogenous wastes, total suspended solids (TSS), inorganic (IM) and organic matter (OM), and the expression of crucial immune, digestive, and stress-related genes were determined. Survival was higher and the growth was increased at lower stocking density. Reduction of ammonium concentration was found in treatments with bacilli and bioflocs. TSS and organic matter were either within or above the optimal range for shrimp culture. The expression of superoxide dismutase, lysozyme, HSP90, chymotrypsin, and trypsin genes were down-regulated, whereas mRNA HSP70 transcription was up-regulated in treatments with molasses, bacilli, and bioflocs. Zero-water exchange, *B. licheniformis*, and molasses promotes biofloc formation that decreases ammonium but stressing culture conditions such as high stocking density, high TSS, and suboptimal temperature and oxygen concentration affect shrimp growth and stress control, digestive, and immune capacity.

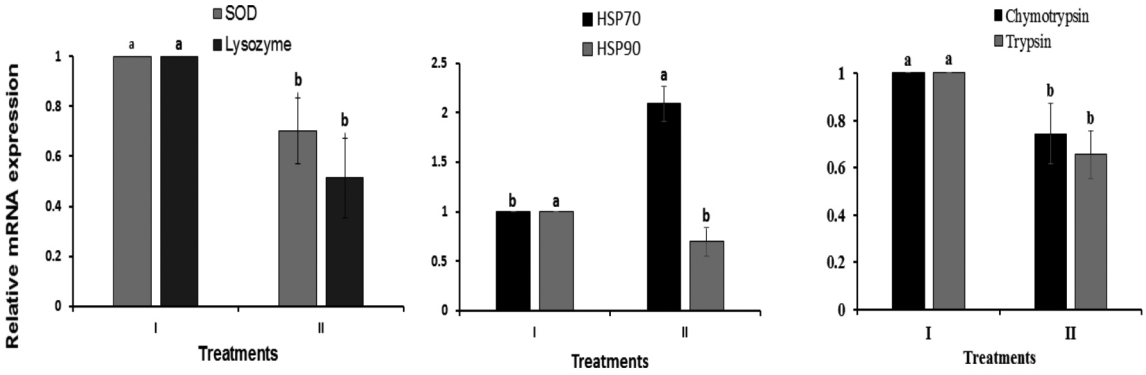

Figure 1. Relative mRNA expression of SOD and lysozyme in hemocytes and, HSP70, HSP90, chymotrypsin, and trypsin in hepatopáncreas of *L. vannamei.* Values are mean ± SE (n = 9). Different superscript indicates significant differences (P<0.05).

HUMORAL IMMUNE PARAMETERS IN SERUM AND MUCUS OF ATLANTIC SALMON (*Salmo salar*) SMOTLS

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Aquaculture is the human fed sector with higher growing rate and great impact in both economic and social levels. Chile is one of most productive countries in the world which have increased its fish production exponentially during last decade focused in the production of salmonids species, mainly Atlantic salmon (*Salmo salar*). In fish, it is widely known that the innate immunity is the main defence resource to fight against foreign agents, which response in captivity conditions is of special relevance. Fish mucus produced by physical barriers as skin, gill and gut are rich in humoral compounds and provides fish of one of most potent first lines of defence against pathogens. Skin secretes mucus continuously and its involvement in the innate immunity appears to be of great importance although its knowledge is still limited. With this study we aimed to compare the constitutive status of humoral immune parameters of serum and skin mucus of Atlantic salmon smolts.

When antimicrobial activities of Atlantic salmon smolts were studied (Fig. 1) we could observed that alternative haemolytic activity of complement was higher in serum as in skin mucus was non-detected (Fig. 1A). Moreover, higher bacteriostatic activity in mucus was showed when compared with serum against two fish pathogenic bacteria analysed (Fig. 1B,C). Interestingly, in a similar manner of complement activity, peroxidase activity was greater in serum whilst in mucus was near to non-detectable values contrarily to what happens with other fish species as gilthead seabream. Otherwise, anti-protease activity was similar in both serum and mucus samples whether protease activity was non-detected in any sample type studied (data not shown).

Work funded by postdoctoral VRIEA-PUCV-2017 project and *Fundación Séneca de la Región de Murcia* (Saavedra Fajardo program, Grant no. 20407/SF/17). Fish were kindly donated by Dr. Fernando A. Gómez (*Laboratorio de Genética e Inmunología Molecular*, PUCV).

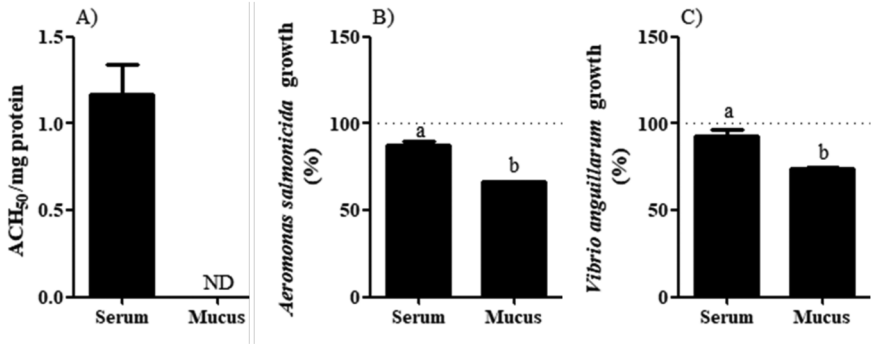

Fig. 1: Antimicrobial function in the serum and skin mucus of Atlantic salmon. A) Alternative complement activity (ACH_{50}/mg protein); bacteriostatic activity (measured as bacterial growth; %) against B) *Aeromonas salmonicida* and C) *Vibrio anguillarum*. Data represented as the mean ± standard error of the mean. Letters denote statistical differences between sample types. ND non-detected. Dotted line means total bacteria growth in culture medium.

COMPARATIVE STUDY OF ANTIMICROBIAL FUNCTION IN SERUM, SKIN MUCUS AND GUT ASSOCIATED LYMPHOID TISSUE OF YELLOWTAIL KINGFISH (*Seriola lalandi*)

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Aquaculture in Chile is one of the most important economic sectors of the country. However it is focused mainly in the production of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), so its great dependence to this species makes it vulnerable and strictly conditioned. Yellowtail kingfish (*Seriola lalandi*) has been proposed as the fish species with highest potential in Chile because of its special growing characteristics, the available technology and its market price. However, its antimicrobial function is almost completely unknown whether it is a powerful immune response in fish. Taking into account its relevance, we studied the antimicrobial function of humoral factors in the serum and in the two largest physical barriers in fish, the skin-secreted mucus and in the intestine as gut associated lymphoid tissue (GALT) of yellowtail kingfish juveniles. Additionally, the pattern of expression of antimicrobial peptides and other immune related coding genes were analysed in different tissues.

Our results showed that complement activity was higher in serum than intestine (Fig. 1A), whether bacteriostatic activity against *Aeromonas salmonicida* was in serum and intestine (Fig. 1B). Interestingly, highest bacteriostatic activity against *Vibrio anguillarum* (*Va*) was observed in intestine (Fig. 1C) suggesting a more specialized specific response against this bacteria as the gut is a site of adhesion.. Otherwise, anti-protease activity was higher in mucus whilst protease was in intestine, as happens with peroxidase activity (data not shown). Strikingly, several antimicrobial peptide coding genes showed greater expression in muscle, skin or intestine as most of the rest of immune-related genes studied (data not shown) which might be correlated with antimicrobial function in mucus or intestine, respectively. Our results contribute to a better knowledge of immune response of yellowtail kingfish although further studies are needed to characterize it.

Work funded by postdoctoral VRIEA-PUCV-2017 project.

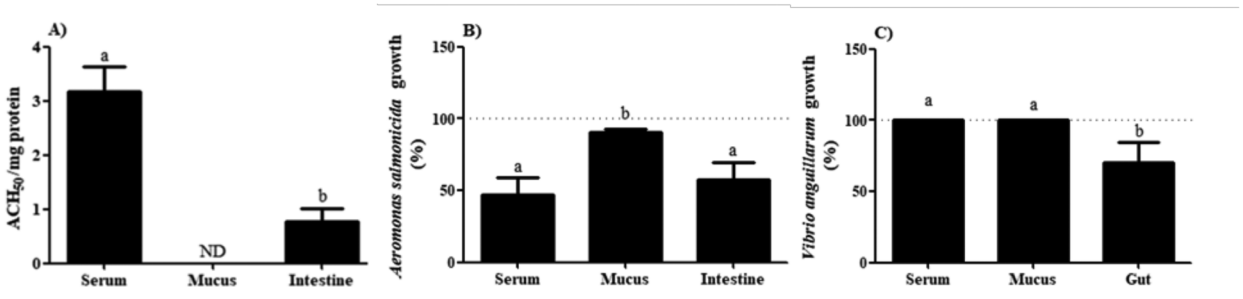

Figure 1: Antimicrobial function in the serum, mucus and intestine of yellowtail kingfish. A) Alternative complement activity (ACH_{50}/mg protein); Bacteriostatic activity (measured as bacterial growth; %) against B) *Aeromonas salmonicida* and C) *Vibrio anguillarum*. Data represented as the mean ± standard error of the mean. Letters denote statistical differences between sample types. ND non-detected. Dotted line means total bacteria growth in culture medium.

In vivo IMPACT OF YEASTS β-GLUCANS ON IMMUNE RESPONSE IN HEAD-KIDNEY PACIFIC RED SNAPPER (*Lutjanus peru*) LEUCOCYTES CHALLENGED WITH *Aeromonas hydrophila*

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β-glucans are considered an immunostimulant that acts on fish non-specific defense mechanism. However, few studies have reported the effect of β-glucans diets on the fish innate immune response after bacterial challenge. Therefore, the present *in vivo* study attempts to describe the immunostimulatory impact after bacterial challenge with *Aeromonas hydrophila* in Pacific red snapper fed diets supplements with glucans derived from *Yarrowia lipolytica* (N6), *Debaromyces hansenii* (Dh004) and *Saccharomyces cerevisiae* (Zymosan A as positive control) during 4 weeks. *In vivo* assay was carrying out using head-kidney (HK) leucocytes from Pacific red snapper after bacterial challenge with *A. hydrophila* at 24 and 48 hours. Our results (Fig. 1) revealed that the respiratory burst activity was only decreased in challenged fish fed Zymosan A diet after 48 h post-challenge whilst the nitric production in HK leucocytes was inhibited in challenged fish fed all experimental diets. Similar results were observed in the peroxidase activity which was reduced in HK leucocytes from control and Dh004 groups at 24 hours and from N6 and Zymosan A groups at 48 hours post-challenge. Regarding SOD and CAT activities (data not shown) in leucocytes from HK, the values of CAT activity increased in challenged fish fed Zymosan A diet respect to unchallenged ones at 24 and 48 hours post-challenge whilst SOD activity not showed alterations between experimental groups.

The project was funded by CONACYT/Mexico grants: INFR-2014-495 01/225924 and PDCPN2014-01/248033. F.A. Guardiola thanks the *Fundação para a Ciência e Tecnologia* (FCT) (Portugal) for his grant (SFRH/BPD/104497/2014).

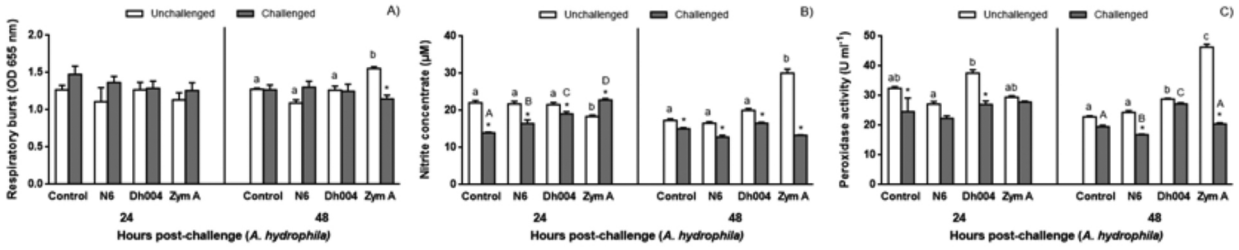

Fig. 1: Respiratory burst activity (A), nitric oxide production (B) and peroxidase activity (C) in HK leucocytes of Pacific red snapper specimens unchallenged (control) and challenged with *A. hydrophila* at 24 and 48 hours post-intraperitoneally injection, previously fed experimental diets [non supplemented (control); supplemented with β-glucan from *Yarrowia lipolytica* (N6); supplemented with β-glucan from *Debaromyces hansenii* (Dh004); and supplemented with glucans from *Saccharomyces cerevisiae* (Zymosan A) during 4 weeks. Bars represent the mean ± SEM (n=6). Asterisks denote significant differences unchallenged and challenged groups (T-test; $p<0.05$) whilst small (unchallenged) and capital letters (challenged) denote significant variations between experimental groups at each sampling time (ANOVA; $p<0.05$).

Sterigmatomyces halophilus β -GLUCAN STIMULATES SEVERAL IMMUNE-RELATED GENES IN PACIFIC RED SNAPPER (*Lutjanus peru*) PERIPHERAL BLOOD LEUCOCYTES CHALLENGED WITH *Aeromonas hydrophila*

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β-Glucans are naturally occurring polysaccharides that are produced by bacteria, fungi and yeast. In fish, β-glucans are considered an immunostimulant that acts on non-specific defense mechanism. Glucans derived from the yeast cell wall (*Sterigmatomyces halophilus*, β-Gluc/Sh) has been used for this purpose in the present study. Therefore, *in vitro* assay using peripheral blood leucocytes (PBLs) from Pacific red snapper was carry out in order to evaluate the effects of β-Gluc/Sh and zymosan A (positive control) on the expression of immune-related genes (*ecCu/ZnSOD*, *icCu/ZnSOD*, *MnSOD*, *CAT*, *IL-1β*, *IL-10, IL-12, IL-17)* during 12 and 24 h and after bacterial challenge with *Aeromonas hydrophila* at 24 h. For this, relative gene expression was analyzed in PBLs using Real-Time PCR (qPCR) and the 2^{-DDCT} method. Our results clearly showed the ability of leucocytes to strongly react to β-Gluc/Sh with an increase in cytokine gene expression, particularly the *IL-1β*, *IL-10* and *IL-17* genes (Fig. 1). Interestingly, superoxide dismutase genes (*ecCu/ZnSOD, icCu/ZnSOD, MnSOD*) were up-regulated in leucocytes stimulated with β-glu/Sh compared with control and zymosan treatments (data not shown). Catalase mRNA gene was higher up-regulated in those leucocytes stimulated with zymosan A and β-glu/Sh after challenge test compared with control group (data not shown). These results confirm that the β-glucan derived from *S. halophilus* yeast can lead to transcription up-regulation of the genes involved in anti- and pro-inflammatory responses, which might explain positive effects of β-glucans on disease resistance.

The project was funded by CONACYT/Mexico grants: INFR-2014-495 01/225924 and PDCPN2014-01/248033. F.A. Guardiola thanks the *Fundação para a Ciência e Tecnologia* (FCT) (Portugal) for his grant (SFRH/BPD/104497/2014).

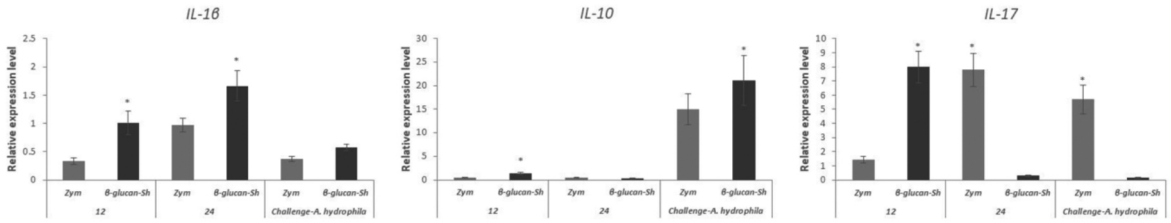

Fig. 1. Gene expression in peripheral blood leucocytes (PBLs) stimulated with β-gluc/Sh and zymosan A (as positive control) at 12 and 24 h and post-challenge with live *A. hydrophila* (1 x 10^8 cfu ml^{-1}) at 24 h. Bars represent the mean ± SE (n=9). Asterisks denote significant differences between treatments and control group at 95% of confidence level when the statistic F value did not exceed the theoretical value.

IMMUNE RESPONSE OF GILTHEAD SEABREAM (*Sparus aurata* L.) AFTER AN ORAL CHALLENGE WITH V*ibrio harveyi*: A TIME COURSE STUDY

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Aquaculture as intensive fish farming method is subjected to important challenges as dealing with infectious diseases provoked by pathogens. One of most threating pathogens are bacteria since the abusive use of antibiotics has elicited resistance problems causing high mortality rates and so, non-viable economic losses in the aquaculture sector. For that reason, understanding the fish immune responses upon bacterial infections is crucial. In the current study was performed to shed some light about the immune response of gilthead seabream (*Sparus aurata*) after oral challenge with the fish pathogen *Vibrio harveyi*. Interestingly, our results showed that *V. harveyi* orally administered activates B lymphocytes and inhibits head-kidney (HK) leucocyte phagocytosis in gilthead seabream (Fig. 1).

F.A. Guardiola thanks the *Fundación Séneca de la Región de Murcia* (Spain) for his grant (Saavedra Fajardo program, Grant no. 20407/SF/17). Y. Valero thanks the *Vicerrectoría de Investigación y Estudios Avanzados* of the PUCV for her postdoctoral funding. This work was partly supported by the Spanish Ministry of Economy and Competitiveness, co-funded by European Regional Development Funds (Grant no. AGL2014-51839-C5-1-R) and *Fundación Séneca de la Región de Murcia (Grupo de Excelencia*. Grant no. 19883/GERM/15).

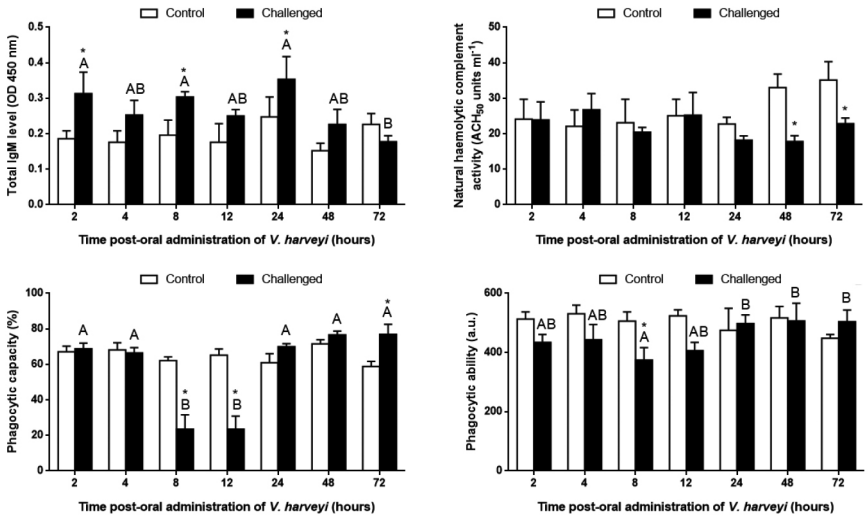

Fig. 1. Total IgM levels (OD 450 nm); natural haemolytic complement activity (ACH_{50} mL^{-1}); phagocytic capacity (%); and phagocytic ability (a.u.) in the serum and head-kidney leucocytes from gilthead seabream unchallenged (control) and oral-challenged with *Vibrio harveyi* after 2, 4, 8, 12, 24, 48 and 72 hours. Bars represent the mean ± standard error of the mean (SEM; n=6). Different capital letters denote significant differences of challenged group regarding time (one-way ANOVA; $p< 0.05$) whereas asterisks denote differences between unchallenged and challenged fish (Student's *t* test; $p< 0.05$).

GENE EXPRESSION OF LIPID METABOLISM IN GILTHEAD SEABREAM (*Sparus aurata* L.) LIVER FED DIETS ENRICHED WITH FENUGREEK SEEDS

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The present study was carry out to determine the potential effect of the dietary intake of fenugreek (*Trigonella foenum graecum*) seeds on the expression of several lipid metabolism-related genes in liver of gilthead seabream (*Sparus aurata* L.). Fish were fed four experimental diets with increased fenugreek incorporation levels [0% (control), 1 %, 5 % and 10%] during 8 weeks. The results showed that the highest fenugreek supplementation in aquafeed increased the gene expression implicated in the lipogenesis (*fas, srebp1, ppar* α, β and γ), lipid mobilization (*apo1a*) and lipid storage program (*lpl* and *hsl*) in the liver of gilthead seabream (Fig 1). The gene expression of the pro-inflammatory markers studied (*cox 2* and *il-1*β) was not affected by fenugreek supplementation, except to fish fed the lowest inclusion level (1%). Therefore, the dietary inclusion of fenugreek not compromise negatively gilthead seabream nutritional status, not induce an inflammatory response and could enhance the efficiency of nutrient conversion, assimilation and growth performance, as well as could

F.A. Guardiola thanks the *Fundación Séneca de la Región de Murcia* (Spain) for his grant (Saavedra Fajardo program, Grant no. 20407/SF/17). This work was partly supported by the Spanish Ministry of Economy and Competitiveness, co-funded by European Regional Development Funds (Grant no. AGL2014-51839-C5-1-R) and *Fundación Séneca de la Región de Murcia (Grupo de Excelencia*. Grant no. 19883/GERM/15).

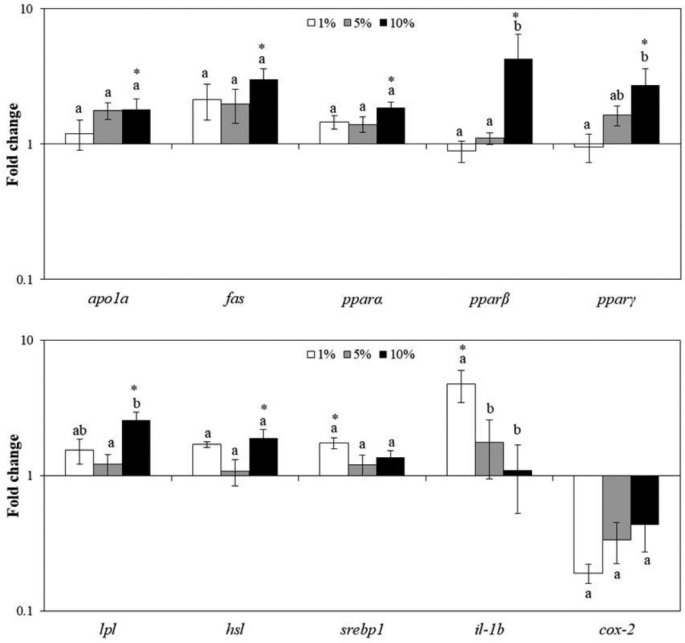

Fig. 1. Gene expression in liver of gilthead seabream fed diets containing 0% (control), 1%, 5% and 10% of fenugreek for 8 weeks. Data are expressed as fold change (mean ± SEM, n=20), obtained by dividing each sample value by the mean control value at the same sampling time. Asterisks denote significant differences between non-supplemented (control) and supplemented groups ($P \leq 0.05$). Different letters denote significant differences among supplemented groups ($P \leq 0.05$).

A HOLISTIC APPROACH TO INVESTIGATE THE ESTABLISHMENT AND DYNAMICS OF THE BLOOD-MICROBIOTA (BACTERIA, PROTISTS AND VIRUSES) IN A MARINE INVERTEBRATE

Yannick Gueguen, Samuel Dupont, Anna Lokmer, Bruno Petton, Eve Toulza, Erwan Corre, Jean-Christophe Auguet, Caroline Montagnani, David Pecqueur, Laure Guillou, Christelle Desnues, Julien de Lorgeril, Guillaume Mitta, Jean-Michel Escoubas

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Numerous studies have highlighted the importance of microbial communities in the environmental adaptability and evolution of macroorganisms that host them. The microbiota of most metazoans is located on external surfaces such as skin, teguments or in cavities in contact with the outside. Moreover, it is generally accepted that the circulatory system of healthy animals is sterile. Nevertheless, some marine invertebrates such as oysters escape this rule and possess an internal microbiota associated with the hemolymph called hemo-microbiota. The existence of this hemo-microbiota constitutes a paradox since the hemolymph contains circulating hemocytes that play a key role in the anti-infectious response of the oyster. The hemolymph can thus be considered as an ecological niche in which immune cells and micro-organisms coexist. An attractive hypothesis is that the hemo-microbiota could be the result of a coevolution process leading to the "domestication" of a microbial community participating in the holobiont fitness.

In order to explore this hypothesis, we have undertaken the characterization of the entire microbial community (viruses, bacteria and protists) living in oyster's hemolymph using global analytical approaches (metabarcoding and metagenomics). In order to determine the impact of the oyster's genetic background on the hemo-microbiota establishment and dynamics we used genetically differentiated oyster families, produced in hatchery and bearing contrasted phenotypes, especially in respect to summer mortality syndrome. These animals were then transplanted in natural environments (during infectious and non-infectious periods) in order to study the impact of the environment on the composition and dynamics of the hemo-microbiota. Our results showed that the hemo-microbiota is a complex microbial community composed of viruses, bacteria and protists. Moreover, the hemo-microbiota (bacteria and protists at least) is different from the whole oyster microbiota, suggesting that the hemolymph compartment could be considered as an ecological niche. All together our results suggest that the structure of hemolymph microbial consortia is mainly shaped by environmental factors. For bacterial and protist community, oyster genetic background had a fine-tune effect only on these communities structure. Whereas, oyster's genetic background played a key role in the dynamics of the viral community especially in the natural environment during the infectious period.

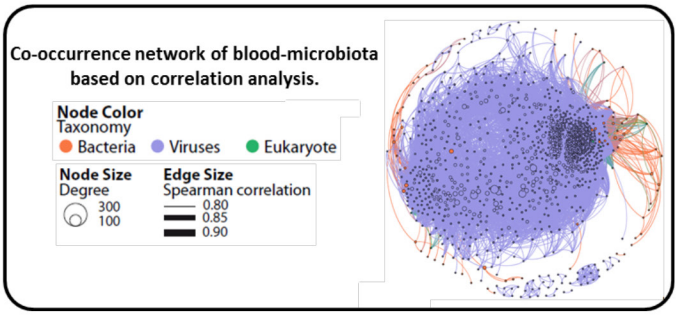

Figure 1: Relationship between the different compartments of oyster blood microbiota

ASSESSMENT OF THE EFFECTS OF D-LIMONENE ON THE HEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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In recent years, research on the use of essential oils from plants in fish has increased. Essential oils derived from plants with water or water steam distillation methods. Essential oils are complex mixtures of different organic molecules-terpenes, alcohols, esters, aldehydes, ketones and phenols. It has effects antimicrobial, antifungal and regulation of intestinal microbiota in organisms with their complex mixtures. Essential oil of the orange peel included 94.74 % D-limonene. D-limonene is listed in the Code of Federal Regulation as generally recognized as safe (GRAS). It is used in perfumes, soaps, foods, gum, and beverages. Also, it is clinically used for dissolve of cholesterol-containing gallstones, support of normal peristalsis, relief of heartburn and stomach sickness. D-limonene has chemopreventive activity against many types of cancers. Therefore, the aim of this study is to assess the effects of D-limonene, which is derived from orange peel essential oil, on the hematological and biochemical blood parameters of rainbow trout.

Four D-limonene supplemented (0%, 0.5‰, 1‰ and 3‰) diets (CP/CF: 45/15) prepared. Four groups fish were stocked 100 L aquarium (55 fish per aquarium) with free flow water system for triplicate study in 90 days feeding trial. The fish were fed twice daily mid-morning and mid-afternoon to apparent satiation. Dissolved oxygen, pH, temperature and conductivity of the water were 7.32±0.28 mg L^{-1}, 7.7±0.3, 18.9±0.6 °C and 559.3±44.5 µS sec^{-1}, respectively.

At the end of the experimental feeding period; feed conversion ratio was significantly decreased, relative growth rate and specific growth rate were importantly increased by feeding 1‰ rate D-limonene additives ($p<0.05$). Erythrocyte, hematocrit and hemoglobin were significantly increased at the feeding with 0.5‰ and 1‰ supplemented diet groups ($p<0.05$). Mean corpuscular hemoglobin concentration, albumin was significantly changed between groups ($p<0.05$). Triglycerides was significantly decreased at the feeding with 0.5‰ and 3‰ supplemented diet groups ($p<0.05$). Cholesterol was significantly decreased at the feeding with 1‰ supplemented diet group ($p<0.05$). Results of the study showed that, usage of D-limonene in rainbow trout diets could positive effect both growth performance and health status on the fish.

Table 1. Growth performance of groups

	Control	0.5‰	1‰	3‰
RGR	276.98±11.05^{a}	338.91±12.15^{b}	369.66±3.15^{c}	321.30±6.15^{b}
SGR	1.47±0.03^{a}	1.64±0.03^{b}	1.72±0.01^{c}	1.60±0.02^{b}
FCR	1.46±0.06^{c}	1.16±0.03^{b}	1.06±0.02^{a}	1.25±0.03^{b}

Abbreviations: RGR: Relative growth rate (%), SGR: Specific growth rate, FCR: Feed conversion ratio. Means with different superscripts in rows are significantly different ($P < 0.05$).

Table 2. Hematological and biochemical blood parameters

	Control	0.5‰	1‰	3‰
RBC	3.63±0.145^{b}	4.46±0.12^{c}	4.51±0.10^{c}	3.38±0.12^{a}
HCT	31.00±0.57^{a}	33.66±0.33^{b}	36.00±0.63^{b}	30.66±0.55^{a}
HB	8.48±0.15^{a}	10.34±0.19^{b}	10.60±0.34^{b}	7.87±0.24^{a}
MCV	85.90±4.02	75.69±2.45	79.99±2.50	91.08±3.23
MCH	23.50±1.09	23.20±0.50	23.54±0.91	23.48±1.37
MCHC	27.38±0.57^{ab}	30.74±0.74^{c}	29.50±1.17^{bc}	25.69±0.65^{a}
GLC	98.30±4.27	94.61±6.48	81.77±3.89	80.83±11.49
ALB	0.56±0.05^{a}	0.58±0.03^{ab}	0.58±0.04^{ab}	0.64±0.01^{c}
GLB	3.17±0.38	3.23±0.40	2.71±0.18	2.67±0.14
TPRT	3.73±0.43	3.81±0.41	3.30±0.22	3.31±0.15
TRIG	108.06±3.20^{b}	76.47±2.16^{a}	103.26±1.60^{b}	83.24±4.30^{a}
CHOL	1.46±0.06^{c}	1.16±0.03^{b}	1.06±0.02^{a}	1.25±0.03^{b}

Abbreviations: RBC: Erythrocyte, HCT: Hematocrit, HB: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, GLC: Glucose, ALB: Albumin, GLB: Globulin, TPRT: Total protein, TRIG: Triglycerides, CHOL: Cholesterol. Means with different superscripts in rows are significantly different ($P < 0.05$).

Ps: This study supported by TUBITAK, which number is 115O907.

GENOMIC PREDICTION ACCURACY FOR GROWTH TRAITS IN PACIFIC OYSTER USING A MEDIUM DENSITY SNP ARRAY

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Selective breeding has major potential for improvement of production traits in bivalve shellfish, including the widely farmed Pacific oyster. There are a limited number of existing breeding programmes for oysters, and they focus on mass or family selection to improve growth rates and disease resistance. Genomic selection can improve selection accuracy, and therefore genetic gain, in breeding programmes, but has not yet been widely tested for bivalve species. In the current study, a population of ~850 oysters were measured for traits related to juvenile growth (wet weight, shell length and shell height). These oyster were all genotyped using a recently developed medium density oyster SNP array (40K SNPs). The pedigree was reconstructed using the genetic marker data, and linear mixed model approaches were used to estimate genetic parameters and to estimate breeding values. All three traits were heritable, with estimates ranging from 0.21 to 0.36 using the pedigree, and from 0.186 to 0.272 using the genomic kinship matrix. Additionally all three traits show high genetic (>0.86) and phenotypic (>0.73) correlation, indicating that any of the traits may be a suitable measure for early growth rate. Using a cross-validation approach, genomic prediction accuracy (using GBLUP) for shell length was high (0.67), approximately 7 % higher than the value obtained using pedigree information (PBLUP). While juvenile growth is unlikely to be a major target trait for genomic selection, it can be considered as an exemplar polygenic trait. Therefore, these results are promising for the future application of genomic prediction in oyster breeding programs, and suggest that genotype data could be used in lieu of pedigree recording to make genetic progress for key economic traits in bivalve shellfish.

BIVALVE AQUACULTURE-ENVIRONMENT INTERACTIONS IN THE CONTEXT OF CLIMATE CHANGE

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Coastal embayments are at risk of impacts by climate change drivers such as ocean warming, sea level rise and alteration in precipitation regimes. The response of the ecosystem to these drivers is highly dependent on their magnitude of change, but also on physical characteristics such as bay morphology and river discharge, which play key roles in water residence time and hence estuarine functioning. These considerations are especially relevant for bivalve aquaculture sites, where the cultured biomass can alter ecosystem dynamics. The combination of climate change, physical and aquaculture drivers can result in synergistic/antagonistic and nonlinear processes. A spatially explicit model was constructed to explore effects of the physical environment (bay geomorphic type, freshwater inputs), climate change drivers (sea level, temperature, precipitation) and aquaculture (bivalve species, stock) on ecosystem functioning. A factorial design led to 336 scenarios (48 hydrodynamic x 7 management). Model outcomes suggest that the physical environment controls estuarine functioning given its influence on primary productivity (bottom-up control dominated by riverine nutrients) and horizontal advection with the open ocean (dominated by bay geomorphic type). The intensity of bivalve aquaculture ultimately determines the bivalve–phytoplankton trophic interaction, which can range from a bottom-up control triggered by ammonia excretion to a top-down control via feeding. Results also suggest that temperature is the strongest climate change driver due to its influence on the metabolism of poikilothermic organisms (e.g. zooplankton and bivalves), which ultimately causes a concomitant increase of top-down pressure on phytoplankton. Given the different thermal tolerance of cultured species, temperature is also critical to sort winners from losers, benefiting *Crassostrea virginica* over *Mytilus edulis* under the specific conditions tested in this numerical exercise. In general, it is predicted that bays with large rivers and high exchange with the open ocean will be more resilient under climate change when bivalve aquaculture is present.

IMPACT OF CLIMATE CHANGE ON THE PRODUCTION COSTS IN HUNGARIAN CARP AQUACULTURE

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Pond culture is the dominating sub-sector in Hungarian aquaculture with an output of 13,014 t from 26,480 ha of fish ponds in 2016. It is characterized by low-input farming technologies, with reliance on natural pond food sources and high dependence on environmental conditions, which make the farming technology sensitive to climate change.

A food web integrated dynamic simulation model was developed and implemented in Programmable Structures of Direct Computer Mapping based methodology, in order to forecast the effects of climate change on pond hydrology, ecosystem and production of cultured fish species. NORESM 4.5 and 8.5 climate scenarios were used for the prediction, considering a single geographical location on the Southern Great Plain. The model was validated by industrial production data. Economic calculations were made in order to analyse the impact of climate change and of different pond management strategies on farm-level economic performance. Yields and used amount of feed, stocking material, manure and water were determined by model outputs, while assumptions were made on further inputs (labour, capital costs and overhead costs). Unit prices of inputs prevailing in 2016 were used all over the time frame investigated.

Simulated results show that capital costs and labour costs per unit production will decrease owing to improved yields. Feed costs per kg production will also decrease, as a result of increased zooplankton formation. In contrast, increased evaporation rates will increase water costs per kg production. However, calculated benefits of climate change override the increased costs in case of all management scenarios (10 different feeding rates and 8 stocking densities) considered in the model. Regarding difference between the pond management scenarios, extensive technologies benefit more from climate change than the ones with higher production intensity. This may have a managerial implication, because average cost minimizing strategies occur at more extensive technologies in the future, than now. Nevertheless, farmers choosing the maximum profit per hectare as an ultimate goal, might not change the technology according to our simulation results.

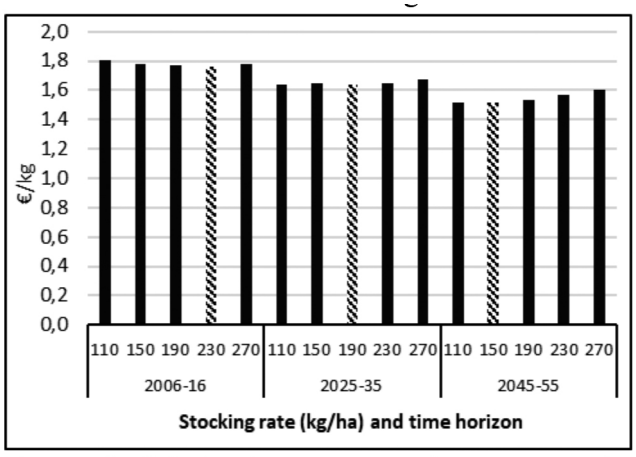

Figure 1 Calculated average production costs for different production intensities and under different time horizons (using RCP4.5 climate scenario). Feed and manure management is the same across different stocking rates. The cost minimizing strategy is indicated by diagonally striped columns.

EFFECT OF DIFFERENT SOLVENT RATIO USAGE ON LIPID YIELD OF *Schizochytrium sp.*

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In order to get well and long living conditions, mankind need of high quality and healthy foods. To this end, the sustainability of omega-3 and omega-6 fatty acids required for vital functions and the development of vegetative-derived products are inevitable.

Studies have shown that microalgae *Schizochytrium sp*., due to its high lipid content, is an important lipid source for both food and its additives and better lipid yield is possible with methods like osmotic shock, thermal shock, vortexing sonication, etc.

In this study, the effects of solvent usage with different ratios on the amount of lipid were investigated for spray dried algae powder *Schizochytrium sp*. (MarinBio, Turkey). Samples were subjected to ultrasonic water bath (40kHz) for 10 minutes at 60°C. Methanol:chloroform (1:2) was used as solvent. Ratios of (1:2), (1:10), (1:20), (1:50) (sample:solvent) were used, the highest lipid yield was obtained as 13.36% with (1:50) solvent ratio. The lowest lipid yield was 1.40% with (1:2) solvent ratio. With usage of (1:10) and (1:20) ratio of solvent, lipid contents were detected as 8.15% and 11.60%, respectively.

Our results suggest that usage of high ratio of solvent plays an active role in the destruction of the cell wall and increases the amount of lipid of *Schizochytrium sp*.

GENETIC PARAMETERS OF RESISTANCE TO GASTROENTERITIS (RTGE) AND RAINBOW TROUT FRY SYNDROME (RTFS) IN RAINBOW TROUT *Oncorhynchus mykiss* AND THEIR GENETIC CORRELATIONS WITH PRODUCTION TRAITS

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Two bacterial diseases without vaccine hamper rainbow trout production : Rainbow Trout Fry Syndrom (RTFS) due to *Flavobacterium psychrophilum* (10% – 30% mortality at fry stage), and gastroenteritis (RTGE) due to *Candidatus arthromitus* (> 30 % mortality at 300g-3 kg), the latter essentially when plant-based diets are used at temperatures above 16°C. The usual solution to RTGE is to stop feeding during summer, which limits the expression of genetic gain for growth and decreases benefits from breeding. Heritability (h^2) estimates of resistance to both diseases and their genetic correlations (r_g) with production traits are needed to design efficient breeding programs, to improve production sustainability, decrease antibiotherapy and antibioresistance and to anticipate the impact of global warming on breeding.

Three sib groups (n = 2000 sibs per group) of 600 rainbow trout families created by partial factorial mating design were derived from selected line of "Les Aquaculteurs Bretons" and DNA pedigreed with microsatellites. The 1st group was submitted to a natural challenge in fishfarm (*in campo*) to RTFS. The 2nd group was challenged to RTGE also *in campo* since the first summer. The 3rd group was slaughtered at a mean body weight of 1932g (18 months of age) and phenotyped for body weight (BW), gutted and fillet yields (Carc% and Fil%) and muscle fat content (Fat%). Genetic parameters were estimated using VCE software with Carc%, Fil% and Fat% represented by the residual of the numerators of these ratios to individual BW.

All traits presented medium to high h^2 indicating for the first time the possibility to select against mortality to RTGE. r_g with BW were negative for resistance to RTGE (-0.35) and positive for resistance to RTFS (+0.25). r_g between resistances and other production traits, as well as between both diseases, were not different from zero.

It is proposed to include RTGE in breeding index combining production traits and resistance to RTFS in order limit antibiotherapy, facilitate feed substitution and anticipate climate warming.

Acknowledgements
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	BW	Carc%	Fil%	Fat%	RTGE	RTFS
BW	**0.32 ± 0.04**	-0.14 ± 0.08	-0.04 ± 0.10	0.07 ± 0.08	**-0.35 ± 0.10**	**0.25 ± 0.09**
Carc%		**0.54 ± 0.08**	0.51 ± 0.07	0.09 ± 0.08	-0.08	-0.13
Fil%			**0.28 ± 0.04**	0.26 ± 0.08	-0.02	-0.17
Fat%				**0.54 ± 0.04**	0.18	0.05
RTGE					**0.31 ± 0.04**	0.01
RTFS						**0.42 ± 0.04**

Tableau 1. Heritabilities (h^2 ± SE) of body weight (BW), carcass and fillet yields (Carc% and Fil%), muscle fat content (Fat%), Rainbow Trout Fry Syndrom (RTFS) and the gastroenteritis (RTGE) and genetic correlations ± SE (above the diagonal)

ENHANCING GROWTH AND QUALITY OF SEA BREAM (*Sparus aurata*) LARVAE BY ADDING COPEPODS (*Acartia tonsa*) TO THEIR DIET

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Successfully rearing larval stages of marine fishes is still a major bottleneck in aquaculture operations. This holds not only true for the establishment of new aquaculture candidate species, but also poses a major source of uncertainty for established species. A major challenge and frequent reason for rearing failures is the provision of appropriate food. The two most widely used prey items used for start feeding of marine species are brine shrimps and rotifers, which despite their convenience in use have major drawbacks related to size and nutritional quality. Almost all marine fish larvae feed at least partly on copepod life stages, hence mimicking natural diets to avoid malnutrition is a labour intense, yet promising approach in circumventing negative effects associated with the use of inappropriately sized prey items and insufficient nutritional quality feeds.

In two industrial scale Sea bream (*Sparus aurata*) larval rearing experiments we added copepods to traditional feeding regimes based on brine shrimps and rotifers. During the first experiment larvae received either daily rations of copepods or were fed alternately fed on brine shrimps and rotifers and only received copepods every other day. During the second experiment larvae were co-fed on rotifers, brine shrimps and copepods.

During the first experiment, larvae reared on copepods grew faster during the initial rearing period and showed significantly less gill deformities (**Figure 1**), a widespread problem in sea bream culture. During the second experiment, differences were less clear between the copepod fed groups and the control groups, pointing at a too little copepod dose. Future work should be directed towards defining a minimum threshold of copepods co-fed with traditional live feeds to maximize positive effects of copepod on growth and health of larval fish.

The experiment clearly demonstrates that copepods can successfully be utilized as a high-quality replacement for low quality rotifers and brine shrimps in commercial scale start feeding, resulting in faster growing and healthier juvenile fish with lower losses due to mortality and bone malformations discarded during sorting procedures.

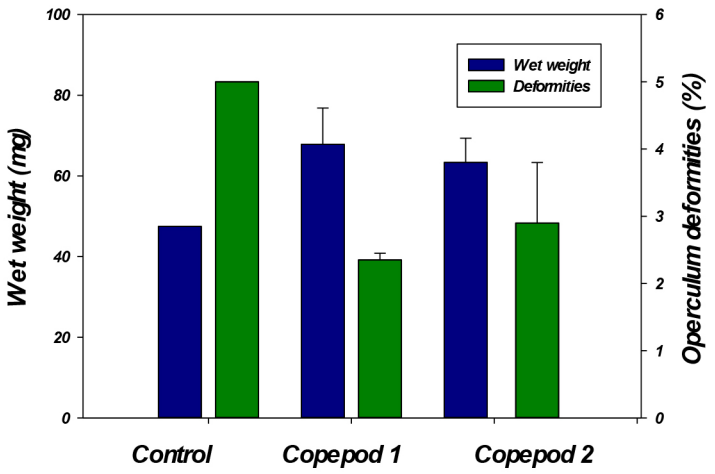

Figure 1 *Wet weight and operculum deformities of larval* Sparus aurata *at 62dph.* 'Copepod 1' *received one copepod meal per day from 4-11 dph (day post-hatch) and one copepod meal every other day thereafter.* 'Copepod 2' *received one copepod meal from 4-8dph.* 'Control' *received rotifers and brine shrimps.*

STUDY OF SOLVING THE UNEMPLOYMENT PROBLEM BY ORNAMENTAL FISHERY TRADE: AN OVERVIEW OF WEST BENGAL

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This title framing on "Solving the unemployment problem by ornamental fishery trade brings peace in our family in the new prospective" has been taken, because different problems can be solved by this subject.

Earning livelihood, taking responsibility of the family and properly maintaining the family is very difficult in this time to the poor people.

The way of earning a normal income has been found out and even there is a possibility of being a remarkable job in future. And the ingredients to give pleasure to the family have been hidden in it.

It is a suitable job for the developing and under-developing countries like us. It has a far-spreading result which is able to strengthen the Economical, Political, and Social bound in our society.

As the concept is very ideal for this country, because most people are unemployed, below poverty, some of them are addicted, some family members are involved in quarreling every day, lead an unhappy life and they have little social respect comparatively.

As it has been got honored as social business, so the business can be started in the level of basic, advanced and corporate. In an international field it has spread out and it is able to bring foreign money. The economical development, socialization and understanding have been increased among persons, families and society. To involvement in different constructive works in society has been increased. In some fields they have taken a role of leadership. Overall they have turned into an icon.

Introduction

To pet the colored fishes was my hobby from my childhood. Continuously I am engaging myself to hobby, observing by natural breeding and cross breeding make my eagerness grow to cultivate new fishes. In this way collection of fishes and success of breeding has helped me to come from hobby to ornamental fish farming and from cultivation to this research field. I begin to write down my experiences with my simple interest. But I cannot be pleased to my limited knowledge of this subject. So I have gone to different colored fish breeders and dealers from whom I have come to know about fish breeding process in scientific way. Besides, aquatic plant experts have also helped me. Moreover many books on this subject have helped me to fulfill my objective work. My friend circle and many well wishers have encouraged me to present this subject as researchable work.

In the year 1984 when I was student of class III, then the situation of my family was below poverty level. I would get 10 paisa for Tiffin. Collecting those I would buy colored fishes like Molly, platy, Swordtail etc. with 50 paisa per piece. From that day gradually development is started.

In the year 1990 after passing School Final Examination, my direct fight was started with want of family. From 1996 Earning, taking responsibility of family and social demand of family members have gradually been fulfilled from culture of colored fishes. My works have been published in different papers. It has also encouraged the other youth by showing the way of earning by my training and publishing some books on it
I have got honor at home and abroad. Even different Print Media, Wave and Tele Media have published my works.

Now I am family man. Their education, health, socialization have been taking care for this field which will help the next generation to go forward.

(Continued on next page)

I have shown that this work will encourage and influence others. So I have presented my research paper. I also hope that this subject will be important not only in social science, but to the unemployed people, interested persons, cultivators of Aquarium plants, students, Teachers, Traders and to Researchers also.

Indian Scenario

On which situation this subject is standing on:

The Indian civilization is not today, it began from Vedic period. If we look back, we can see that according to Matsya Puranas the 'Fish' appeared and was mythological introduced in our religion as Abtar of God Sri Bishnu. There we follow the Fish has been known to the people as enemy, friend, holistic, favor, and savior. So from the pre-historic age all the people have accepted the Fish in society from different views. So for this reason a mixed concept has been seen in Bharatbhumi (Indus civilization).

Particularly in this time there has been seen a systematic co-relating matter in spite of different languages, opinions, dresses, foods etc. As a result in Indian thought it is seen to give a special respect to all the matters, even a reflection of an equal distribution is seen. In this sphere not only concentrate on the value of land life, but the same priority has been given to underwater life.

Taking the fish as food has been started from the pre-historical time. The many decades have passed away, at the time of Sumerian, Babylonian, Assyrian and Missourian period, the fish was used not only as food, but it was taken as great pleasure, blessing , savior module. In the middle age the same concept is flowing. After passing long time this concept has been reached to the modern civilization and this fish has been remarked as a social, economical and environmental balance to be maintained.

Among the mile stones during this long term historical journey of time line different long gaps have been seen.

At present study this 'Fish' has been introduced by another name also, as 'Ornamental Fish' which will take a role of helping hand filling up the long gaps.

Now the unemployment problem has been grown up as a great social problem in India and as a result more other problems have been raised which is bringing an unexpected awful massage to the Sovereignty, democratic, socialist, secular country like India, though India has reached to the developing country from underdeveloped country, yet in the sphere of the population, birth and death rate, increasing of life span, education, health, socio-economy, socio-status etc. an unparallel parametric situation has been seen. Though before it applying different types of methods a way of getting way of solving process has been found out and applying those more or less implement has been got in some spheres.

Here a way of normal income can be got by the Ornamental Fish farming and in this social system this is very helpful new perspective.

Though there is differentiation in our environment of India the seasonal change is wonderful gift of our Indian nature of wild life. Here there is widely desert, likewise there is also oasis. Again there is table land too on the high mountain. Beside there are many rivers, canals, watery lands, estuaries, streams, lakes etc. spreading over the plains.

"Where there is Water, there is Fish". Though this mission is going forward towards fulfillment in India, yet there have been seen different gaps in it, such as there are fishes of different types, shapes, colors, sizes, fins in seas, rivers, canals, drains, ponds, lakes, water reservoirs, etc. among whose a large amount of fishes are at the mouth of destruction being neglected and careless, because besides traditional food fish lovers in Bengal, a little of other communities have taken fish as food. As a result a little number of people has selected some number of fishes in their plate and planning to cultivate them they have commercialized the field.

Generally it is said that it is a destructive game to demolish wonderful differentiation of nature surrounding the traditional food fishes of whole environment. Not only the environment is destroyed, but the total social management is going to ruin.

In the sphere of Ornamental Fishery Trade it has been seen a unique endeavor in present India to make them live smoothly, reproduction, development, to save the endanger aquatic species and to return back in natural world, which is neglected, unknown, unrespectable to the eyes of common people or fishermen and other communities. Even now they have been playing in our drawing room with special respect, besides good looking scenario has been seen by income with these ornamental fishes in the country like India, to set up a peaceful family relation and by earning of foreign currency and with it a powerful economical back ground has been developed.

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Particularly, an Ornamental Fish Marketing field has been built up surrounding the places of Howrah, Kolkata, Hooghly, 24 Parganas of West Bengal, where a popular market (Hat) has been going on during previous 100 years, yet no any research work is seen here till now regarding this field, which could be future implementation scope for the future generation.

There has been seen a huge gaps in the sphere of ornamental fishery knowledge, training, technology, skill development and also in research in India.

Here some people of below poverty level, refugees, and landless persons started this business in Bengal surrounding Howrah, Dasnagar etc. places. They have gradually prospered in business. Now the newcomers of different communities have started to come in this business. Even the people from primary to higher educated young are showing their little interest in this business.

According to Indian environment and socio situation, it has been seen that this business can be started in three types of levels, e.g. basic level, advance level and corporate level which depend on endeavors, economic conditions, and infrastructure of men.

It has been seen in many sphere that the fisherman families of West Bengal, who earn their livelihood by catching fishes do not get expected evaluation economically, socially, politically. Most families suffer from economical drawback. There is a lack of infrastructure of food, wear, shed among them. As a result they are always facing with socio inequity, quarrelling, many difficulties and social respect. It is not matched with rapidly changeable social system.

In India, it is lightly indicated that the different problems are shoot down most easily by aquarium fish farming and trading.

Here one side priority has been given on particular fish catching, eating and marketing. For this a gap has been seen here. It is seemed by observing the aquarium fish units of different places that it solves the different kind of problems and increases the flow of development.

It is seen here that the ornamental fish trade was an off-bit job, but recent year it has been seen by study that it can move to a remarkable job, because many people have involved with this trade and many of them are spending their lives smoothly by this livelihood. Many organizations e.g. Government, NGO and self organizations are motivating them, even they have taken responsibility of plans, projects, subsidies, training, up-gradation etc. Besides, Government has taken some rules and regulations about it which will develop this field more, but different fish organizations and individual people of India are not conscious about it. Even a cloud of fear has covered them. Here the gap which is seen that there is a lack of understanding among Government, Ngo and common traders.

A monopoly economy policy is seen to hold this ornamental fish business in India, because there is no printed price tag on the most products. There is also no evidential record from where these are coming, how it is being sold and how much the end users are satisfied. So at present the business belong to an unorganized position, but it is necessary to move it quickly in organize sector for current situation of Indian Socio economy. But it is not only time factor matter, there are other factors also behind to move it from unorganized sector to organized sector. A lengthy gap has also been seen here.

Though the Ornamental fish trade shows the light of hope here and there haphazardly, there are lack of publicity, training, and program, project about it among the Government and non-government and self interest in India. Days have come to work with endeavor to the social media to work consciously with special priority with this sound able matter.

A large number of Indians have taken fishes easily not only as a food, but for other reason also. So it is a great opportunity for our society. It is necessary to plan a better country by human resource, which will create a field of freedom in the society.

Literature Review

Before our Epics Mahabharat and Ramayana another unwritten literature Purankatha is noted. Besides it four Vedas have been known which the core of knowledge is. Here social environment, philosophy, political, economy, health, activities, birth-death, freedom etc. all the matters are mentioned and discussed. How it is applicable – all have been discussed. Even ethics, rules, civics, etc are consciously flourished. 5000 years of this tradition have been passed away, yet its importance is not faded, moreover gradually it is being got important day after day.

It is seen that this Matsya Purana which is the core and ancient among the 18 Puranas where Lord Vishnu appeared in this aquatic world being the symbol of Fish. ('Brihat O Adi Matsya Puran', written by Sri Monmaharshi Krishna Dwipayan Vedbyas, translated by Poet Bhaktadas, 2014).

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Now-a-days this fish is so widely spread out that it is not understood without giving deeply importance - as purity, good luck, food, economic development, beautification, pleasure, wonderful eye-catching, saving environment and many others.

Fishes are very important and fruitful.

Following the time-chart we see that the life in all the countless forms that it in today begins in the sea four thousand million years ago. The earlier fossil evidence we have seen that it is very simple organism consisting of thread-like chains of cells, dating back about 3 thousand million years. Ichthyologist has given us fossil evidence covering much of the early geological history of the development of the fishes is insufficient. Some comes from the Cambrian period of about five hundred million years ago- with fossil remains of invertebrate mollusks, the immensely prolific trilobites, and the clam like brachiopods, but more importantly it comes from the Devonian period. During this period, between 360 and 410 million years ago, the placoderms (plate-skinned fishes) were by far the most abundant.

First Jawed fishes. Jawless Fishes spread into lakes and rivers. Rapid growth in Coral Reefs is seen Silurian Period of 410-440 millions ago. After that age of fishes Jawless and Jawed Fishes exist simultaneously, ancestor of amphibians and ammonites appeared. This time is called Devonian period 360-410 million years ago. (Peter W. Scott. Dorling Kindersley, 1994.

At present the fish culture is very popular particularly in Bengal, Behar, Orrisa. The infrastructure is ready. In other States also the work on aqua pisciculture has been going on in India. (Fish Culture in India, Indian Council of Agricultural Research, New Delhi, Editor- M.G. Kamath by K.H. Alikunhi, Research Officer, Pond Culture, Central Inland Fisheries Research substation Cuttack Pages-3)

The total area of India is 32,67,500 square Kilometer. Its land area is 15,200 Km in length. As the coastal area is less broken, so it's lengthy is only 5,700km. The position of India is 7th for this area in the world.
Its position is 2nd for its population than China. (From Wikipedia, the free encyclopedia, geography of India. September, 2017.)

70% of the total rain fall in India is for the appearance of south-west monsoon wind blowing in summer, but it is not routine wise, it also changes for different causes. So giving priority to the irrigation System of India it has been trying to develop the fishery and agriculture. At present 21% of agricultural land is being taken under irrigation process. The volume of water in rivers in India is almost, 1,67,251crore square meter, among which 66,600 crore square meter water is useful for farming. Still now it is not possible to use the total water. Till 2000 year 23 thousand crore square meter water has been used.

This is known from an editorial column of a Monthly Magazine 'Aqua culture, Asia' volume-viii, no.3, July to September, 2003 that -Talk aimed at reforming global trade in agricultural products collapse on 14th September in Cancun, Mexico as expected one of the main striking points was the issue of agricultural subsidies and tariffs. As discussed in this column previously, these are mainly imposed by developed nation to protect their domestic industries from foreign competition which are mainly developing nations.

A key difference in Cancun over previous talks was that developing nations banded together much more strongly than they have in the past to lobby for agricultural reform and to oppose four new proposals about foreign investment and competition added to the agenda primarily by the US and European interest.

The collapse of talks in Cancun is a blow to the prospect of developing a global trade treaty by the end of 2004, set at a meeting 2 years ago in Doha. Many advocacy groups celebrate the collapse as a victory for developing countries. Greater solidarity among proponents of agricultural reform is a welcome development but the collapse of the Cancun WTO talk is in no way beneficial to developing countries.

Global trade is not bad. The economic and social benefits are well documented. The real problem is that the global training system as it exists today is not fair. It is heavily biased towards rich countries which continue to prop up inefficient agricultural sector with heavy subsidies (Forcing there consumer to pay more tax for the privilege of buying more expensive products) Why finding of imported products with quotas and tariffs.

The outcome of the failed negotiation at Cancun is that this status quo has been preserved, and so the global trading system will continue to favor reach nation. The collapse of the Cancun talks is a lost opportunity for both developed and developing countries; it is not a will for any one. We need agricultural reform, let hope that the navigations are more successfully next time.

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Aqua culture, Asia is an autonomous publication that gives people in developing countries a voice. The views and opinion expressed herein are those of the contributors and do not represent the policies or position of NACA. NACA is an intergovernmental organization that promotes rural development through sustainable aqua cultural seeks to improve rural income, increase food production and foreign exchange earnings and to diversify farm production. The ultimate beneficiaries of NACA activities are farmers and rural communities.

In India the central Government has brought some rules, regulation and Acts on Ornamental Fish Trading. The Gazette of India extraordinary part-11, Section-3, Sub-section-1 published by Authority No. 395, New Delhi, Thursday, May-23, 2017/ JYAISTHA 2, 1939.

At present Government has taken some Rules, Regulations and Acts regarding Ornamental Fish Farming, Trading, Hobby and Research. For this most Ornamental Fish Community are not satisfied in this step. They are making a group and tried to move against the rules. Most members of the Community have wanted that the Government be quite flexible about this rules, regulations and acts. They think that many common people of the State like West Bengal are involved in this business. Most of them are poor and below poverty level, some of them are economically quite healthy, but they are unconscious about society environmental situation. The very little percentage of fish traders is generally traders. To find out those persons is very difficult in such new small scale industry.

However, it has been seen that the policy of Government and the movement of the Organization are justified for the society's development and implements. But both of voice has to give priority to each other with soft acceptance of new perspective.

PeTA India. Animals are not ours. To Eat, Were, Experiment on, Used for Entertainment, or Abusing any other Way.

India Passes New Rules to protect animals.

The Indian Fisheries Act-1897 [Act No.-4 of 1897]

To follow the rules and regulations we see a fear and frustration among the ornamental fish farmers, traders and hobbyists. As a result this business cannot run properly in this time, even a small business which has come in a light after long time journey and it has a good prosperous and sound health in future. In such situation it has been seen the possibility of dropping out of this industry to these traders. They are thoughtful about this matter. To organize these unorganized sectors a tendency has been seen among them.

So they have called all and invited to gather under an umbrella.

A venue of the meeting of such organization is quoted here.

Colored fish traders have been invited to take membership of WBOFA and to protest against these rules. WEST BENGAL ORNAMENTAL FISH ASSOCIATION(WBOFA)

Meeting Venue: Swamiji Sporting Club, 44/1/1, Santragachhi, PS. Shibpur (Natun Rasta) Howrah- 711104 on Monday 05.06.2017. at3p.m.

Aquarium Fish in troubled waters.

The Telegraph. Monday, June 19, 2017. Soumitra Das. RULE CLOUD ON LIVELIHOOD OF 200,OOO
A pond is in Howrah where ornamental fishes are bred. They have been doing so for years without any harm to the fish.

Cement fish tank in Howrah under the shed of netting. Meeting of the West Bengal Fish Association was held in Howrah to protest the new rules. A school of Cichlids Inside and Aquarium and Picture by Sanjoy Chattopadhyay, June 18, it is not the protector of Gaumata alone; it extends its safety net to cover aquarium fish as well.

In a fit of kindness towards our finned friends, the center is about to impose such stringent rules on the trade of breeding selling ornamental fish that grass roots "Fish Farmers" and traders all over the country will soon go hungry to bed.

Bengal is responsible for about 80% India's production of ornamental fish and many people entirely depend on it for their daily meals have neither the means, nor the technical expertise to follow these "lopsided" rules.

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The livelihood of about 200,000 people- mostly self employed and with little capital to speak of –is threatened following the May 27 notification of the Ministry of environment, forest and climate change in the Gazette of India: extraordinary, which will enable the center to impose these "impossible" rules.

The rules have been tentatively titled the prevention of cruelty to animals (aquarium and fish tank animal shop) Rules 2017, and they were published by the center in exercise of the powers conferred by sub-section (1) and (2) of section 38 of the prevention of cruelty 2 animal's act, 1960 (59 of 1960).

Ironically, most Government Institute and the State Fisheries department and other Stakeholders and in the dark about the online notification. Not even Benfish, a Government Organization that runs a project for ornamental fish breeding, has no knowledge on the order.

The Marine Product Development Authority of India, which functions under the commerce ministry, has been paying subsidies to ornamental fish farmer for many years. It is not aware of the notification, too.

Deepak Nopany, a leading Calcutta-based exporter of ornamental fish, says: "The ramifications are huge. We collect fish from far-flung places. These rules are not feasible. The bulk of people involved are young. It will be a catastrophe. I am entirely dependent on these suppliers."

The notification came like a bolt from the blue. The ornamental fish trade is part of the unorganized sector, but soon after they discovered it on line, the breeders and sellers hurriedly formed the West Bengal Ornamental Fish Association (WBOFA), which is yet to be registered.

Spearheading the movement against this notification is Indranil Banerjee, who is acting as a spokesperson for WBOFA. "The rule same to have been formulated by somebody who is totally out of touch," Banerjee says.

He explained why the rules will be impossible for them to follow and not for financial reasons alone.

Rule: Veterinary and Infrastructure facilities – every aquarium shall (a) have a fisheries veterinarian or a fisheries expert employed full time for the purpose of overseeing the health care of all the fish tank animals in its collection; (b) have appropriate facilities for treatment room.

WBOFA speak: How is it possible for someone who earns a meager living breeding ornamental fish to hire a veterinarian or a fisheries expert employed "full time".

Rule: No aquarium shall be allowed in temporary stalls in fair or markets or exhibitions in which permanent facilities are lacking.

WBOFA speak: what about the Gallif Street market where 500-700 stalls sell such fish every Sunday? These small-time traders belong to a registered body, the Baghbazar Sakher Haat Byabasayee Samity.

Rule: Every Aquarium shall take adequate care to locate and design all Pucca buildings, including the visitor facility, in such a manner that the fish tank animal display area is not masked and the cleanliness and the hygiene of the aquarium is not affected.

Fish tanks or aquarium are not placed in the following location namely: (i) in direct sunlight or near radiators; (ii) in a place where are there is always light, or where it is always dark; (iii) in a place where rain water can enter the tank,

The master plan referred to in sub-rules (1) And (2) shall inter alia include all-round development in aquarium for a period of ten years and which shall be revised every 5 years along with detail lay out plan prepared on the basis of them adopted by the aquarium indicating fish tank animal, display animal, visitor facilities, support infrastructure for fish tank animal upkeep and health care building for administration and maintenance unit.

WBOFA speak: won't these rules shut down thousand of makeshift shops and hatcheries? Some fishes cannot breed without sunlight. Without rainwater, some others cannot get 'eggbound'. Without sunlight the pigmentation of Goldfish and other species like Barb and Tetra will not become richer.

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Rule: Every aquarium, as a save- guard against access to unregulated visitor and fish tank animals being subject to provocation and pilferage, shall design appropriate barrier along the fish tank and shall have on display appropriate signage discouraging visitor approaching the fish tank at close proximity or from touching the fish tank.

WBOFA speak: how can this rule be implemented when most shops hardly have any space to speak of? The question of a barrier does not arise when there is hardly any shop space.

Rule: Maintenance of records and inventory space-(1) every aquarium shall maintain a record of the acquisition, deaths and disposal of each species of fish in its collection in the manner and in the format determined by the State Animal Welfare Board.

WBOFA speak: Is it possible for traders without any infrastructure whatsoever to maintain such elaborate record? And even if they do so, how can this be presented to the board, about whose existence no one in award of.

Rule: No aquarium of fish shop shall display or shell-(a) bowls for keeping fish tank animal; or (b) fish tank with a capacity less than 13 gallons or 60 liters of water for keeping fish tank animals.

WBOFA speak: The minimum size of an aquarium has been fixed at 60 liters. But the most popular ones hold only 50 liter of water. Fish bowls that hold 10-12 liter have been banned. But small fish like Fighter thrive and even breed in bawls. And most children love this.

Rule; Every aquarium and fish shop shall ensure that the quality of the water in the fish tank is maintained at all times and is within the permitted range of salinity, Ph, hardness and temperature specified show as to meet the health and welfare needs of the fish tank animals.

WBOFA speak: This is absolutely not necessary in the case of fresh water fish. Siphoning is good enough for maintaining balance.

Rules: It is prohibited to keep or sell the following species namely……..(a least of the species and group banned, along with their common and scientific name, follow).

WBOFA speak: 95% of popular marine fishes are banded. Some examples: 46 varieties of butterfly fish; 16 types of angel fish; 3 types of File and Trigger fish; Four types of Damsel fish; Four types of Clown Fish; Two types of Bat fish; 5 types of Eels (Eels are being exported to foreign countries as edible fish); 8 types of Wrasses, Blennies, Dragonets, Gobies and parafish; 4 types of Anthias, Groupers and Grunts; 6 types of Tang; 9 types of Lionfish, Stonefish and Pipefish; 4 types of Boxfish, Razorfish and Mantis Shrimp; All Octopus species (Octopus is also an edible item) and Jellyfish.

WBOFA speak: We have nothing again the ban on Cetaceans (A whale, Dolphin or Porpoise), Penguins, Otters and Manatees. These are banned items according to the wild life protection Act, 1972. But why ban the sale of most marine fish?

Repeated attempts last week by Metro to contact Ravi S. Prasad, joint Secretary, Ministry of environment, forest and climate change, who has signed the notification and others in the Ministry in Delhi, elicited no response.

WBOFA members meet at Santragachi on June 5th to start a movement against the notification. Jayanta Mandal, 46, who is among the more prosperous of breeders, says what started as a hobby became a profession.

A group of young men work under Mandal. Their own tiny hatcheries in their house as well. “We live around 3a.m to collect daphnia and plankton to feed fish. It is a strenuous job and we have to keep an eye on the tanks 24x7, “says Mandal, who has won several awards. “We never got any training. We learn on the job.”

Sourav Ghosal, a young importer says the rules are not ‘commercially viable.’

Debasis Ghosh, a small traders, sums it up; “There is no questions of cruelty. We treat the fish like babies. Unless we keep them healthy and alive they won’t sell.’

The clinching argument against the notification comes from Sunirmal Das, secretary, WBOFA: “If the Government is so concerned about cruelty to fish why does it not ban the manner in which life table fish is often battered to death before cleaning?

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Conclusion of Literature Review:
It is clearly understood from literature review that there are different types of problems, possibilities, present situation and future scopes in ornamental fishery field .It has been seen that many people are involved with this business. Many people have no monetary sound. Even this business stands on still now on an unorganized sector. The time is very necessary here to bring this long time business in an organized sector, even education, expertise and awareness are most necessary.

Now time has come to the organizations of ornamental fishermen communities to be conscious more and it is also necessary to apply the governmental policy in systematic way and equally. Even it is necessary to take it with softening respect among Government, government-undertaken Org, non-government organizations and self organizations. To keep special respect on the rebuilding development policy systems an applied is very necessary for forth coming decades. .

More people will be benefitted by earning livelihood by this business and helpless, poor men too can make the family happily and smiling. These people will take the responsibility of leadership and will be dignified personality in the society.

This business will take the position from the local to international market. Importance will be increased economically; a large amount of the foreign currency will be gotten, the economical structure will be strengthening in our country, and the social systems of the country will run normally with pleasure.

Research Questions
How will be the unemployment problem solved by Ornamental fishery trade and how will the families get the happiness? Such pattern of research question has been followed here. e.g. The name, age, address, contact number, relation among family members, possibilities and difficulties of business, even their individual views about past, present and future of this business has been presented here as a question. Some questions have also been kept here free and others are bounded.

How is the implement percentage of two years business?
Is research and training facilities upgraded?
From where do the resources come?
From where have you got inspiration?
How types of problems do you face in your business and which method do you apply for solving the problems?

Objectives
To solve unemployment problem by peaceful co-existence is main object and for this the subject **Ornamental Fishery Trade** has been chosen here. Especially importance has been given on different types of beautiful multi-colored fin fishes and on good-looking Aquatic plants which are set up into an Aquarium. It has been informed that the cultivation of Aquatic plants has an international market. How such unknown common matter can widely spread in India and how it is possible to strengthen the economical structure of our country within a short period. I want to show this here. It has also been known that economically many backward families have got their socio-economical respect and many families have been established in society and many are hopeful they can also be economically established. They believe in this theory **"Small Capital, Short Space, Little Time, Normal Income."**

It has been seen that aquatic plants and ornamental fishes are co-related and they maintain an environmental balance.
- Solving the unemployment problem
- Ornamental fishery farming is necessary for socio-economic development.
- How can happiness bring to our families?

Approach:
Case Study. Positivistic approach.

Methodology:
Empirical research based on surveys and interviews with testable hypothesis. Mixed methods and action research.

Conclusion:
Ornamental fish farming trade is in West Bengal i.e in India. It is very important to know whether it is technically innovative or not.

In India there are many people whose conditions are very weak for different difficulties, especially for their economic, knowledge and for social respect. As a result lack of peace and soft understanding is shown in such kind of people and their families. In society among such situation a bad side and sign is shown in the socio scenario.

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How this problem and problem solving process is presented and how far it is spread out – it is also an important matter. Besides, how is the nature of innovation and what is their achievement?

It is very significant question and till now India does not get any more entrance, expand acceptance in international field of ornamental fishery trade. So this trade will have to travel alone far away. When the thinking and knowledge of many men will get more importance, then this trade will get entrance in open field.

This trade belongs to an unorganized sector. This can be organized. It is an off-bit job which can be bringing to the main stream of social business; It has huge possibilities to make it in an international market leaving the regional field.

The helpless and poor people can take part in economical development shortly, they can also set up an environment of peace and happiness among the own families, can get self respect in society, they have entrance and empowerment, can give leadership and will be distinguished personality by this trade in the developed country like India.

A clear visibility and transparency about this trade is necessary in this time to the people of ornamental fishery community and interested people, though the consciousness of the people, their influence and contribution is necessary too more. By using the social media its publicity will be increased too much and will prepare a plan, project and implementation with taking the all people in all stages of the society.

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THE STATE OF THE WORLD'S AQUATIC GENETIC RESOURCES

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The sustainable use, management and conservation of aquatic genetic resources (AqGR) for food and agriculture are crucial for the continued development of aquaculture, and there is an urgent need to preserve and better manage our aquatic genetic diversity to enhance its contribution to food security, nutrition and livelihoods. Expanding genetic improvement of key AqGR represents a significant opportunity to enhance the efficiency of aquaculture production globally. FAO is currently engaged in a number of activities, in support of its member countries, to meet these needs. A key initial activity is the preparation of a report on *The State of the World's Aquatic Genetic Resources for Food and Agriculture* (the Report). This represents the first ever, country-driven, global assessment of AqGR, and is now available on line (*www.fao.org/aquatic-genetic-resources*). The Report is supported by FAO's Commission on Genetic Resources for Food and Agriculture and was guided by FAO's Committee on Fisheries. Ninety two countries provide extensive information on the status of their AqGR through a structured reporting process, covering farmed aquatic species and their wild relatives in areas within national jurisdiction. This can be considered to provide a truly global perspective as these reporting countries account for more than 96 percent of total global aquaculture production. This process, in itself, has enhanced engagement, built capacity and strengthened information systems related to AqGR in many member countries.

This presentation provides an overview of the key findings in the Report. It highlights that current data on aquaculture production does not fully reflect the broad diversity of AqGR in use and identifies a need to record information at a genetic level (e.g. as "farmed types") as well as at the species level. The Report further recognises capacity building needs for future assessment of AqGR. The importance of selective breeding and other approaches to genetic improvement is highlighted, as is the significance of both *in situ* and *ex situ* conservation. The Report identifies that some degree of genetic management is applied in 60% of species reported under culture but recognises aquaculture's continuing dependency on wild stocks, emphasizing the interconnectivity between ecosystem conservation, sustainable fisheries and aquaculture development. The Report includes five detailed Thematic Background Studies which address identified gaps in knowledge including on: i) genome-based biotechnologies in aquaculture, ii) incorporating genetic diversity and indicators into statistics and monitoring, iii) genetic resources for microorganisms of relevance to aquaculture, iv) genetic resources of farmed seaweeds, and v) genetic resources of freshwater aquatic macrophytes.

Finally, the presentation identifies possible next steps, including recommendations to assess, explore and develop mechanisms to monitor the status and trends of AqGR, including through the establishment of a global information system and a registry of farmed types and stocks of wild relatives. FAO has been called upon to prepare a Global Plan of Action on AqGR, underlining the importance of sustained funding for the continued development, use and conservation of AqGR.

CHEMICAL AND MICROBIAL ASSESSMENT OF SOME FRESH WATER FISH SPECIES IN SELECTED AREAS OF LAGOS STATE, NIGERIA

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Investigation was conducted to assess the nutritional qualities of some fresh water fish species (*Tilapia zilli, Clarias gariepinus, Chrysichthys nigrodigitatus and Sarotherodon melanotheron*) on sale for consumption in the Badagry, Epe, Ikorodu and Ojo areas of Lagos state, Nigeria. A total of 100 Fish samples (25 specimens each) with a weight ranges between 250-680g were collected from these locations.

Samples were iced and taken to the Biochemistry and Pharmaceutical laboratory sections of the College of Medicine University of Lagos, Idi-Araba, Lagos, Nigeria. The iced samples were maintained in alternate layers of ice until spoilage was noticed. Chemical analysis was conducted using Spectrophotometer (Jenway, GN 6400) and Atomic Absorption Spectrophotometer (Shimadzu AA 650, Sohxlet Extraction Apparatus, Osk, Japan 158). Thiobarbituric acid (TBA), The acid value (AV), peroxide value (PV), Iodine value, free fatty acids (FFA) and Saponification value were determined, while microbial analyses was carried out to determine the overall quality of the samples. Result indicates higher TBA, acid values was within the standard limits, higher iodine value and variations in the microbial count among the fish species within the same location. The obtained results are indications of notable quality improvement thus, lead to a high quality fish in terms of the taste, colour, odour, shelf life and market value. The high iodine value recorded for in this study from different locations suggests that the fish species contain high level of unsaturated oil and as such could reduce the risk of heart diseases, high cholesterol, depression, anxiety, low immunity, cancer, eye disorders and ulcers in humans when consume. None of the fish samples exceed the limit for bacterial count, thus, demonstrating no risk on the public health.

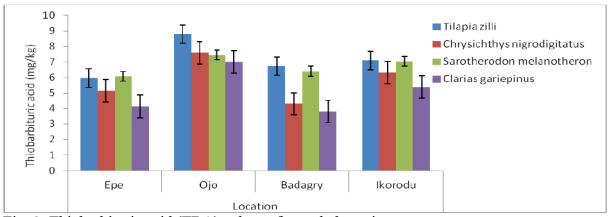

Fig. 1: Thiobarbituric acid (TBA) values of sampled species

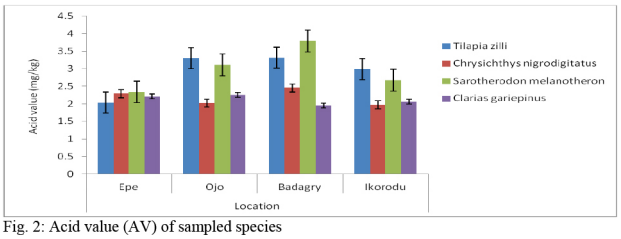

Fig. 2: Acid value (AV) of sampled species

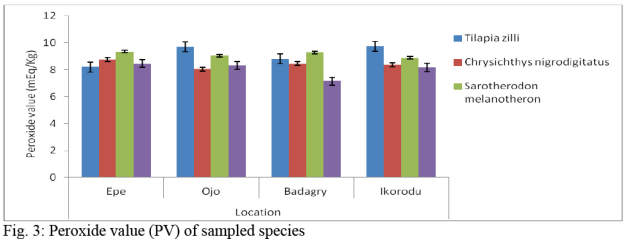

Fig. 3: Peroxide value (PV) of sampled species

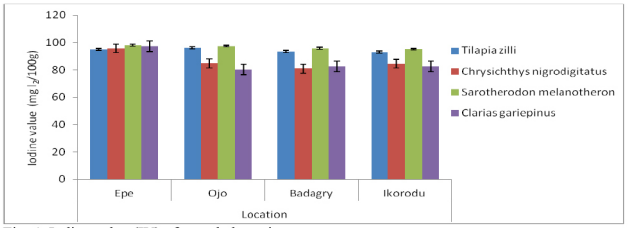

Fig. 4: Iodine value (IV) of sampled species

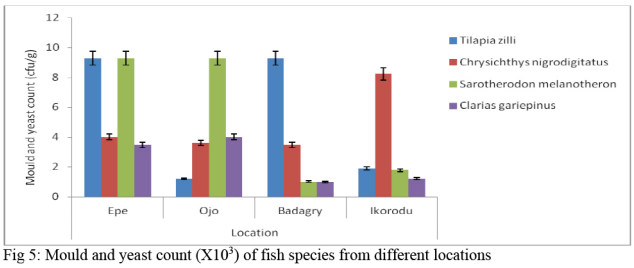

Fig 5: Mould and yeast count ($X10^3$) of fish species from different locations

POTENTIAL IMPACTS OF INDUSTRIAL-SCALE KELP FARMING ON COASTAL ECOSYSTEMS

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Farming of marine seaweeds is currently receiving attention in the Western world. The reason for this is the resources that cultivated seaweed and kelp can provide for the growing demand for raw materials within the food and feed industry, for medicine and cosmetics, and biofuels. The industry is fast expanding and by 2050, Norway alone is stipulated to produce 40 mill. tons of cultivated kelp, representing a value of 4 billion Euro per year. The global kelp farming presently amounts to 30 mill. tons, with China being the main producer.

Cultivation must be done in a sustainable manner if we are to preserve the environment while ensuring a long-term, profitable industry for kelp cultivation. Currently, we know little about the effects of cultivation on the natural coastal ecosystems inhabited by plants, algae, and animals, and if large-scale kelp cultivation will alter the natural ecosystem balance and food webs.

Here, we will present an overview of the physical, biological, and biogeochemical processes that influence the impact of kelp cultivation on natural ecosystems, and the following environmental trade-offs. Large-scale kelp farms will physically alter water currents, absorb sunlight, and provide physical hideaways and colonization structure for fauna. Performing photosynthesis, kelp take up nutrients and CO_2, and export oxygen and organic matter (detaching leaves) to its surroundings during growth. Thus, positive environmental impacts of kelp farming include reducing excessive nutrients and ocean acidification through nutrients and CO_2 uptake, which may provide mitigating efforts towards eutrophication and climate change. Negative impacts include depletion of limited water column nutrients and disposal of large amounts of kelp on the seafloor, which can lead to seafloor oxygen deficiency, change in natural biodiversity, and poor ecological condition. We will provide the theoretical basis for assessing the environmental impacts of kelp farming and status quo on the research efforts undertaken to understand the trade-offs of large-scaled kelp farming for the marine environment, with relevance for future sustainable management.

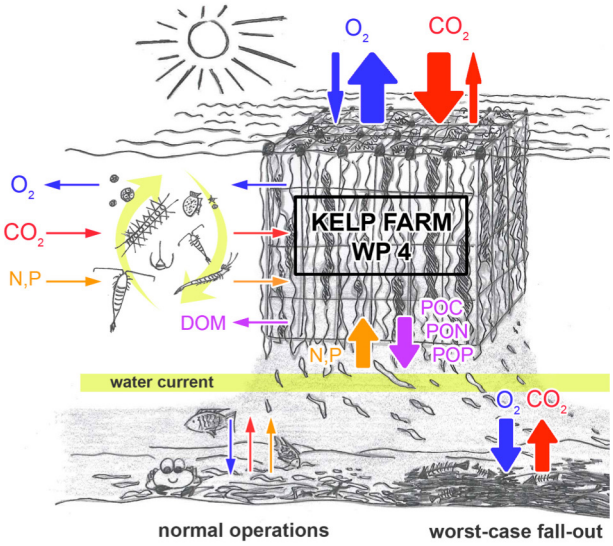

Figure 1. Illustration of the biotic and abiotic interactions of kelp farming on the marine environment, the foundation for assessing the environmental trade-offs of large-scale kelp farming.

EARLY INTRODUCTION OF COPEPODS AND DRY DIET DURING START FEEDING PERMANENTLY ENHANCES GROWTH FOR FARMED ATLANTIC COD (*Gadus morhua*)

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Atlantic cod larvae were fed using three different start-feeding protocols (see table 1); **control 1**- early introduction of artemia nauplia and dry feed, **control 2**- late weaning and **copepod 1**- early introduction of copepods and dry feed were compared in a design using triplicate tanks. Growth was measured from 2 days' post hatch(dph) hatch until 150 dph (see Fig. A and D). Survial was measured at day 85 post hatch (Fig. C). At day 190 deformities and individual weights were registered. 75 fish from each treatment were pit-tagged and transferred to one tank for on growing (common garden design). Pit tagged fish were transferred to a sea cage at 230 dph. Weight of pit tagged fish were measured at 320 dph and weight gain was measured at an individual level (Fig. B).

Fish from Cop 1 was significantly heavier than Con 1 and Con 2 except at 66 dph (Fig. A) and at 190 and 320 dph (Fig. B). There were no significant differences between treatments in survival at 85 dph (Fig. C) and in growth from day 2- 29 dph. Our results showed a long-term effect of copepod (fed from 23-45 dph) on larval cod.

Table 1: Description of feeding protocols

Age	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	16-23	23 24 25 26 27 28 29 30 31-38 39-45	Weaning complete 45 46 ----------56
Control 1				
Rotifer	100%		50% - 0%*	
Artemia			0.5 mill day^{-1}	
Dryfeed		1	10 25 35 44	
Control 2				
Rotifer	100%		50% - 0%*	
Artemia			0.29 mil/feed 7 feeding	0.8 mill/feed; 5 feeding; Weaning 100%-0%
Dryfeed				10-15 15-50
Copepod 1				
Rotifer	100%		50% - 0%*	
Copepod			N6 0.5 mill day^{-1}	
Dryfeed		1	10 25 35 44	

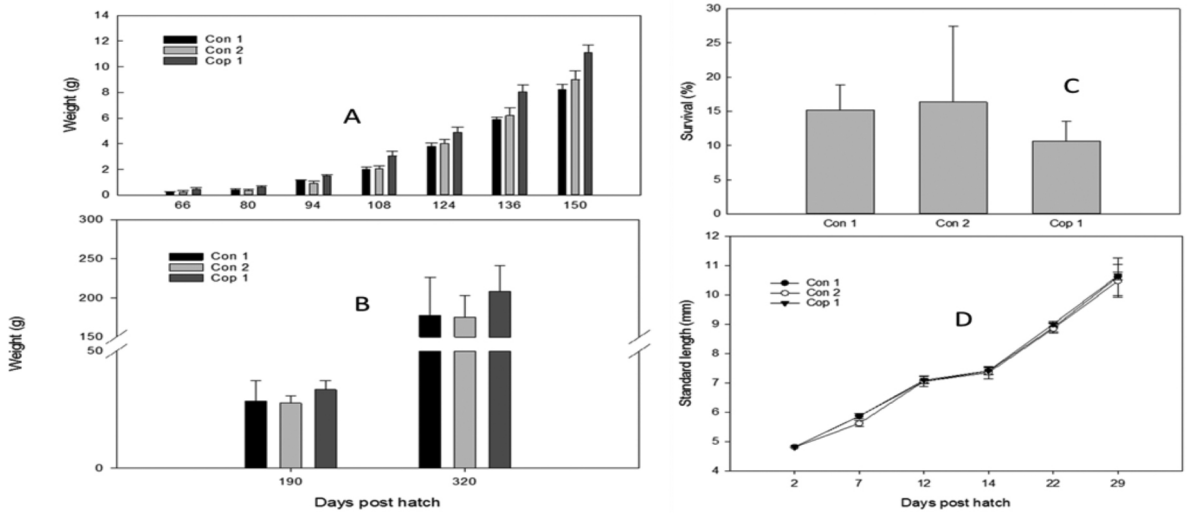

Figure A-D: Growth and survival of Atlantic cod at different treatments.

QUALITY VALIDATION OF THE BALTIC SEA MUSSEL *Mytilus edulis*-like BY FLESH WATER CONTENT

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Mytilus edulis (L. 1758), a commercial important blue mussel species in Europe, is also produced in Aquaculture. In the Baltic Sea, a low salinity environment, a mixture of *M. edulis* and *M. trossulus* (Gould, 1850) occurs, named as *M. edulis*-like. To guarantee steady production the stock is regularly monitored for condition index (CI), shell growth rate or soft body weight. A high CI coincides with a large size, high flesh contents and high biomass. Aquaculture producer often choose growth rate for monitoring but this factor excludes further important parameters like the shell proportion, the flesh weight and the flesh water content. It is disputed if the shell growth provides sufficient information about the total flesh production or is insufficient as a result of other shell variables.

This is the first study which tested over 218 days, about 8 months, under controlled experimental conditions the consequences of starvation upon the general condition of blue mussels under low salinity conditions (Baltic Sea). We found a decrease of blue mussels CI (loss of 74% (20.0 to 5.2)) and soft body weight (loss of 72% (1.95g to 0.54g)) and an increase of flesh water content (95% to 98.4% (+3.4%)). See also Figure 1. Higher flesh water contents reduces the product quality, which is of highest interest for Aquaculture producers. Prolonged starvation periods diminish the nutritional value of the final product for the consumer. The incorporation of water in body tissue seems a good quality indicator to provide information about the current nutritional status of the tested mussels. We highly recommend to consider this factor to allow more detailed conclusions to be drawn about the quality of Aquaculture produced mussels.

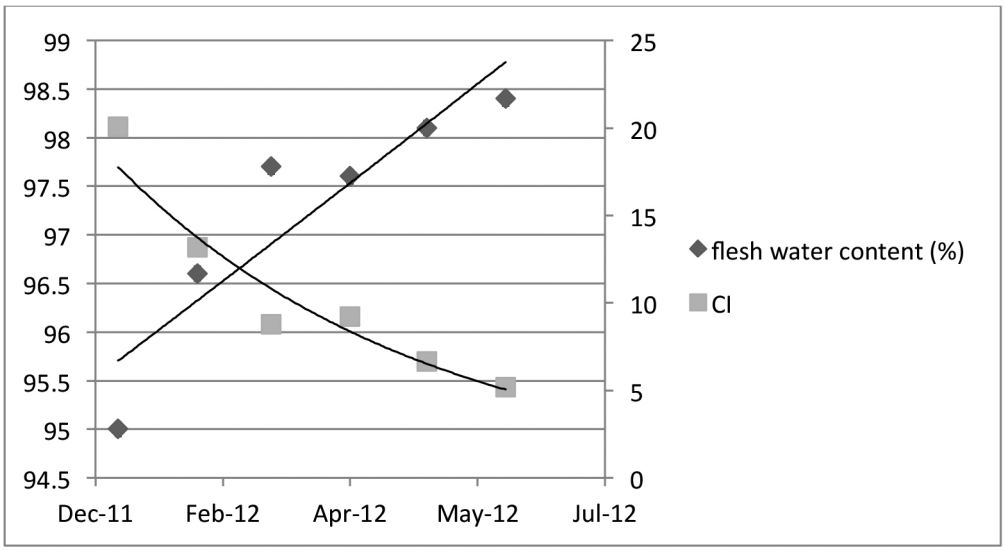

FIGURE 1. Condition Index (CI) and the flesh water content (%) of starving blue mussels (n=100) over the experimental time of 218 days.

CO-CULTIVATION OF BIVALVES AND MACROALGAE: MULTI-TROPHIC AQUACULTURE WITHOUT A FED SPECIES IN SWEDEN

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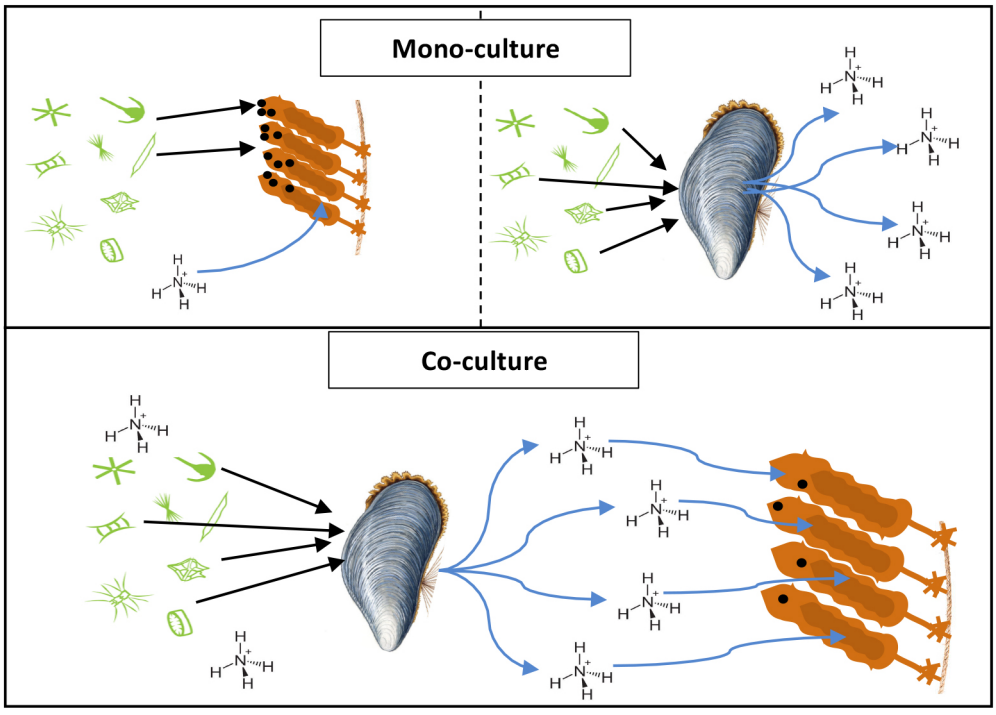
Population rise is putting increasing pressure on food production systems. Aquaculture is globally the fastest growing food-producing sector, but is still underrepresented in the West. In Sweden there has been an emphasis on cultivation of extractive species, which are widely regarded to hold promise as sustainable species for mass cultivation. However, commonly cultivated extractive species such as the blue mussel still produce potentially harmful waste products, primarily in the form of ammonium of which high levels can contribute to local eutrophication and environmental degradation. A semi-enclosed system was designed to cultivate the seaweeds *Saccharina latissima* and *Ulva lactuca* with two bivalve species, the blue mussel (*Mytilus edulis*) and the Pacific oyster (*Magallana gigas*). Bivalves as much as doubled ammonium concentration in comparison to ambient environmental levels, which resulted in enhanced seaweed growth. Bivalves were also seen as a potential novel biofilter, causing a reduction in epiphyte coverage on seaweeds. Nutrient analysis showed that seaweeds reduced ammonium concentration to ambient, pre-bivalve levels. Further, increased levels of ammonium from bivalves resulted in significantly higher tissue content of nitrogen in seaweeds. These findings present compelling evidence for the potential of integrating macroalgae and bivalve cultivation to increase seaweed yield and quality, and reducing local environmental impacts associated with bivalve cultivation.



EFFECT OF INUNDATION TIME ON GROWTH, QUALITY AND SURVIVAL OF PACIFIC OYSTER (*Magallana gigas*) FOR SITE-SPECIFIC (OFF-BOTTOM) AQUACULTURE IN THE OOSTERSCHELDE, THE NETHERLANDS

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In this study, a field experiment on the effect of inundation time on growth, quality and survival of Pacific Oysters (*M. gigas*), was carried out at an intertidal off-bottom cultivation site. The cultivation site was located in the central part of the Oosterschelde, the Netherlands. By using off-bottom cultivation techniques, farmers are able to control the inundation time of the oysters by adapting the height of baskets attached to the lines. Longer inundation has an effect on food condition and therefore on the growth of oysters but shorter inundation can increase the survival of the oysters.

Oysters (8-12 mm hatchery reared spat) were cultivated in hanging baskets in an off-bottom farm. For the experiment two lines, to which the baskets were attached, were adjusted to 0.61m and 1.27m below mean sea level, resulting in a inundation time for the oysters of 65% for the upper and 88% for the lower line. At the start of the experiment 6 baskets (three per inundation time, 6 mm mesh) were each stocked with 5000 live oysters (visually checked). Every six weeks the mortality in every basket was recorded and a subset of 50 oysters was measured for shell length (mm) and the wet weight (g) of the oyster spat. At the end of the experiment the condition index (CI) of the oysters was calculated as: [dry tissue weight (g) × 100] / dry shell weight (g).

The experiment was carried out from May 27th 2014 until April 19th 2016.

Results show that oyster growth rates, especially in spring, were higher at 88% inundation time (SGR - 8.5 mg/d), than at 65% inundation time (SGR - 7.5 mg/d (figure 1). This same pattern was also measured in the oyster wet weight. Overall there was an oyster survival of 17% for the 65% inundation treatment and 11% for the 88% inundation treatment (P=0.000).

The condition index differed between the treatments (P=0.000) and was 4.33 ±0.98 and 4.85 ±1.05 for an inundation treatment of 65% and 88% respectively for April 2016.

This study concludes that a longer inundation time results in higher GR and CI, probably caused by better food conditions. In contrast, shorter inundation times promotes oyster survival. This can be a result of a shorter exposure time to the oyster herpes virus.

It is recommended to increase the inundation time of the complete oyster culture cycle but decrease inundation time in periods with high mortalities (e.g. in spring due to herpes virus).

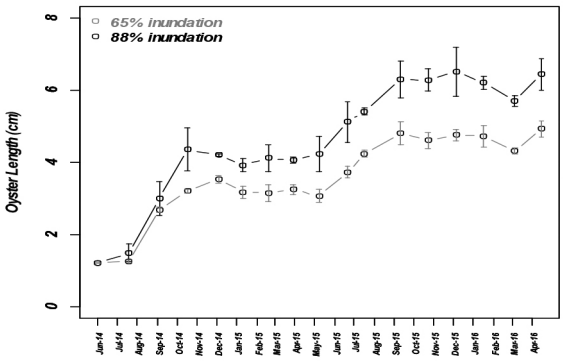

Figure 1: Oyster spat size at different inundation times. Grey line = 65% and black line =88% inundation of the experimental period

NFLUENCE OF *Origanum vulgare* IN THE DEVELOPMENT OF *Lophiosilurus alexandri*, A CARNIVOROUS SPECIES

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Oregano is a phytogenic additive that improves fish performance and survival. *Lophiosilurus alexandri* is a carnivorous species that presents cannibalism in the early stages of life. The objective of this study was to evaluate the influence of the inclusion of oregano during feed conditioning, as well as its possible effects after this phase when using extruded diets for *L. alexandri* juveniles.

For food conditioning (Phase 1), 2112 juveniles were distributed in 16 tanks connected to a water recirculation system, where they received moist diets containing 0; 1.5; 2.0 and 2.5% oregano for 12 days. In Phase 2, 66 animals from each tank were selected and kept in the original tanks for 30 days with supplementation with oregano (phase 1). In both phases, feeding was performed three times a day and the photoperiod was set at 12:12 LD. The pH, temperature and total ammonia remained controlled at 7.4 ±0.4, 26.79±0.8°C and 0.24±0.26 mg/L, respectively. At the end of each experimental phase, three animals from each replicate were euthanized (285 mg/L eugenol) and fixed in bouin for analysis of intestinal morphometry. Weight (g) and length (mm) were measured for performance evaluation. Data were submitted to the homoscedasticity and normality test (Shapiro-Wilk) followed by ANOVA and TUKEY test ($p < 0.05$).

At the end of phase 1 no significant differences were recorded for average final weight (0.89±0.06g), average weight gain (0.68±0.28g), specific growth rate (4.53±0.80%) and length (74.00 ±1.12mm). For phase 2, differences were not found for average final weight (3.91±0.19g), average weight gain (1.22±0.13g), specific growth rate (8.17±0.57%) and length (68±0.9 mm). Survival in phase 1 was lower in the 2.5% treatment and higher in the inclusion of 1.5% (Table 1). In phase 2, survival was lower in control and higher for treatments with supplementation. The height of intestinal villi was the greatest when 2.0% and 2.5% of oregano were used in phase 1. For phase 2, higher intestinal villi heights were recorded in treatments with 1.5% and 2.0% of oregano inclusion.

In conclusion, addition of oregano to diets for juveniles of *L. alexandri* increases the survival and height of the intestinal villi of the animals.

TABLE 1- Survival and intestinal morphometry of juveniles of L. alexandri, fed with oregano in the two experimental phases.

Variable	Oregano levels				
	0.00%	1.50%	2.00%	2.50%	p-value
		PHASE 1			
Survival (%)	78.40±6.79^{ab}	83.14±7.91^{a}	72.72±4.42^{ab}	64.01±11.57^{b}	0.03
Intestinal morphometry (µm)	168.93 ±42.35^{b}	293.00 ±85.62^{ab}	316.58 ±44.58^{a}	330.7 ±76.18^{a}	0.01
		PHASE 2			
Survival (%)	81.98±8.61^{b}	95.45±1.24^{a}	93.56±2.87^{a}	92.42±5.10^{a}	0.008
Intestinal morphometry (µm)	394.05±15.94^{ab}	447.39±35.56^{a}	442.97±46.76^{a}	356.35±32.56^{b}	0.01

Means followed by the same letter do not differ statistically by the Tukey test (P> 0.05).

EXTENDING SHELLFISH CULTURE INTO OPEN OCEAN EXPOSED SITES IN NEW ZEALAND

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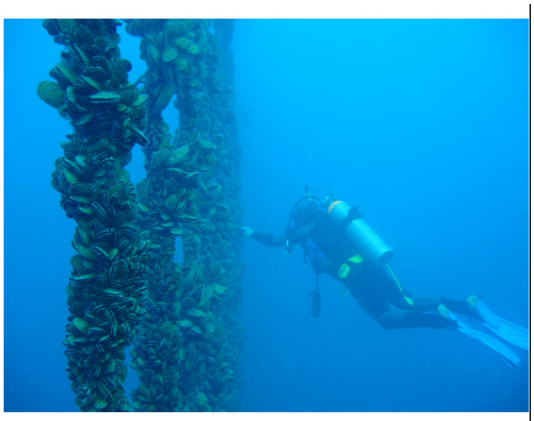
New Zealand's $264M shellfish aquaculture sector is eager to grow in response to globally increasing demand but is constrained by limited access to sheltered inshore farm space. The new frontier is open ocean aquaculture (OOA), where large tracts of consented space are available but farming is challenging. If this space were fully utilised, it would more than double NZ's total aquaculture estate.

Inshore farming methods lack durability and are expensive to maintain when extended into the high-energy open ocean. While futuristic open ocean finfish systems are rapidly evolving, systems for shellfish OOA are in their infancy. New engineering concepts and new farming approaches are needed to maximise the sustainable production potential of the challenging OOA environment and give industry confidence to invest.

A major part of the project is to develop new tools and methods needed to cost-effectively farm shellfish in this new frontier, potentially realising ~$300M p.a. in new export revenue. Solving the technical challenges and giving industry confidence to invest will unlock the potential of this new aquaculture frontier.

This presentation will discuss the OOA development to date, the potential species and the path going forward.



Diver with Greenshell mussels *Perna canaliculus* at a New Zealand open ocean site.

THE EFFICACY OF SUSPENDED BROODSTOCK CAGES AS A RESTORATION STRATEGY FOR THE EUROPEAN FLAT OYSTER *Ostrea edulis* LINNAEUS, 1758: A CASE STUDY IN THE SOLENT, UK

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The European flat oyster *Ostrea edulis* (Linnaeus, 1758) has been subject to anthropogenic and natural pressures for centuries, however, the industrialisation of fishing vessels has increased their exploitation to an unsustainable level, driving many populations to the brink of functional extinction. The Solent, is no exception; a boom-and-bust fishery, invasive species, pollution and disease led to a severe population crash during 2007 from which it has failed to recover despite fishery closures. Within one Solent harbour, oyster densities have decreased from 8.0 ±2.7/m^2 to 0.1 ±0.1/m^2 (mean ±SE) over 19 years. All locations sampled within the Solent during 2017, in this study and by the Southern Inshore Fisheries and Conservation Authority stock assessment, record oyster densities <0.84/m^2; causing concern over the reproductive viability of this population and a lack of suitable settlement substrate. Size class data shows a narrowing of maximum shell length and wet weight over a two year period, indicating a continued removal of larger individuals, leading to further reductions in potential larval output, and a lack of recruitment. This steady decline of the native *O. edulis* populations, combined with the culinary demand for oysters that rapidly reach marketable size and are consistently producible, has resulted in the introduced Pacific oyster *Magallana gigas* being the focus of many aquaculture facilities within Europe, and further afield.

Due to the extremely low densities of *O. edulis* throughout its biogeographic range across Europe, restoration of this native species to a self-sustaining population will require a multifaceted long-term intervention strategy. The first approach has been to develop a novel aquaculture system (Fig. 1) designed to suspend from marinas, and other floating structures, to house protected broodstocks of *O. edulis* within coastal systems. High and low density stocks were tested in micro-reef structures that allow for vertical stacking and suspension. Brooding of veliger larvae was observed from May - September 2017 with peak activity occurring during June, 10.7% of the sample population. Survival remained >94.2% for May and June, however, survival reduced to 78.8% in July. Survival increased during August, September and October to 81.6, 82.7 and 85.8% respectively. No difference in the mortality between high and low density stocks was observed at each location, however, variation between locations, even in close proximity, was observed and suggests microgeographic variations in environmental conditions are influencing survival. These results support the hypothesis that placing broodstock from fishery areas into protective cages can provide a viable source of larvae, thus should be considered for future restoration activity alongside other methodologies.

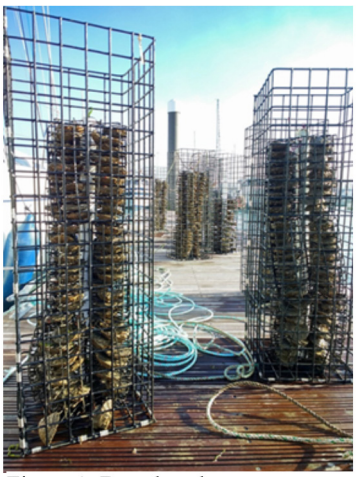

Figure 1. Broodstock cage system.

PROTEOME CHANGES OF EURASIAN PERCH *Perca fluviatilis* FILLET FROM TWO DIFFERENT REARING SYSTEMS INFLUENCED BY FROZEN STORAGE

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Unpleasant off-flavors and formation of volatiles can develop due to oxidation of PUFA in fish fillet. In addition, protein oxidation can lead to loss of protein functionality. On the other hand rearing systems cab be considered as an important parameter which may affect on the flesh quality.

Forty eight market size Eurasian perch from two different rearing systems (traditional pond culture and recirculating aquaculture system (RAS)) were used in the present study. Fillets from fish were randomized packed in plastic bags, labelled and stored at -20 °C. Firmness, chemical and proteomics analyses were executed on the first day and after 4, 8 and 10 months of storage.

Table 1. Shows the TBARS, protein carbonyls concentration, firmness and liquid loss changes. Lipid and protein oxidation results showed a significant in the fillet from both systems during the storage time. RAS fillets showed a higher firmness and liquid loss compare to the pond fillets. Proteomics in combination with western blotting were used to investigate the changes in protein and protein carbonyls (Figure 1A, B). SDS-PAGE analysis revealed that the bonds intensity were decreased or disappeared in the fillets from both systems. Western blot showed an increasing trend in the amount of protein carbonyls in both groups but RAS fillets had higher oxidized protein compare to the pond system.

ne onth)	TBARS		Carbonyls		Texture		Liquid loss	
	RAS	Pond	RAS	Pond	RAS	Pond	RAS	Pond
	0.28 ± 0.01^{Aa}	0.28 ± 00^{Aa}	1.9 ± 0.01^{Aa}	1.38 ± 0.02^{Aa}	1533.67 ± 216.9338^{Aa}	1239.84 ± 246.2972^{Aa}	0.2 ± 0.05^{Aa}	0.15 ± 0.6^{Aa}
	0.33 ± 0.01^{Aa}	0.34 ± 0.02^{Aa}	$3.1+0.01^{Ba}$	$2.78+0.02^{Ba}$	913 ± 142.1937^{Ba}	688 ± 142.391^{Bb}	0.5 ± 0.09^{Ba}	0.41 ± 0.14^{Ba}
	0.56 ± 0.05^{Ba}	0.52 ± 0.02^{Ba}	$3.82+0.05^{Ba}$	$3.1+0.02^{Ba}$	813 ± 244.8469^{Ba}	$613^{\pm}162.0893^{Ba}$	1.1 ± 0.18^{Ca}	0.7 ± 0.17^{Cb}
	0.73 ± 0.04^{Ca}	0.69 ± 0.02^{Ca}	4.38 ± 0.04^{Ca}	3.94 ± 0.02^{Ca}	644 ± 210.5875^{Ca}	518 ± 185.2541^{Cb}	2.31±0.18Da	1.54 ± 0.17^{Db}

TABLE1. Oxidation parameters in fish fillets during frozen storage at -20°C

Different capital superscript letters in a column indicate significant differences ($p<0.05$) within each rearing system. Small superscript letters in a row indicate significant difference ($p<0.05$) between rearing systems at the same time point

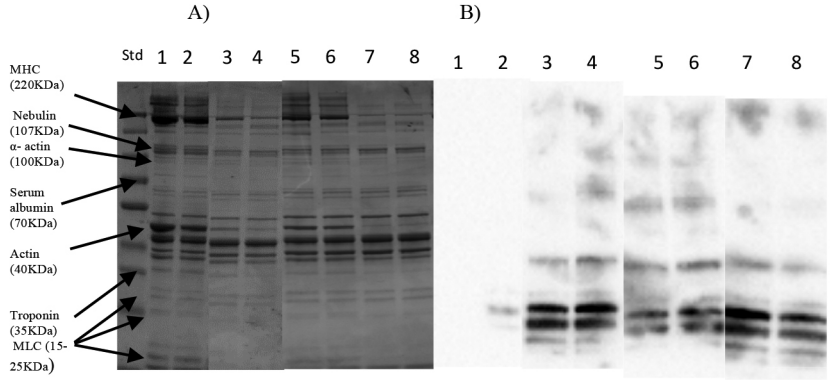

Figure 1. Perch fillet from RAS and pond systems during frozen storage at -20 °C (pond fillet 1) at t = 0, (pond fillet 2) 4 months, (pond fillet 3) 8 months, and (pond fillet 4) 10 months, (RAS fillet 5) at t = 0, (RAS fillet 6) 4 months, (RAS fillet 7) 8 months, and (RAS fillet 8) 10 months, with (A) SDS-polyacrylamide gel electrophoresis (B) immunoblotting against protein carbonyl groups.

AQUACULTURE'S POTENTIAL ROLE AS A SUSTAINABLE PROTEIN SOURCE

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The potential for expanding aquaculture in order to feed future generations has been emphasized in several recent articles. The environmental sustainability of the sector has, however, been contested, depending upon the production system and environmental impacts evaluated. Of the environmental impacts evaluated, several include important dimensions of planetary boundaries, such as global warming, eutrophication, land occupation, water consumption, and ecotoxicity. Many of these sustainability conclusions have lately been based upon Life Cycle Assessment studies, an ISO-certified (ISO 14044) environmental framework that is able to consider emissions throughout the whole value-chain. In the present research we evaluate these results and the potential of using different interventions, in the type of species farmed, feeds used, production methods, genetic strains, spatial planning, and fish processing, to lower the environmental footprint of farmed fish. This enables better understanding about aquacultures role for more sustainable dietary portfolios that keep within the planetary boundaries.

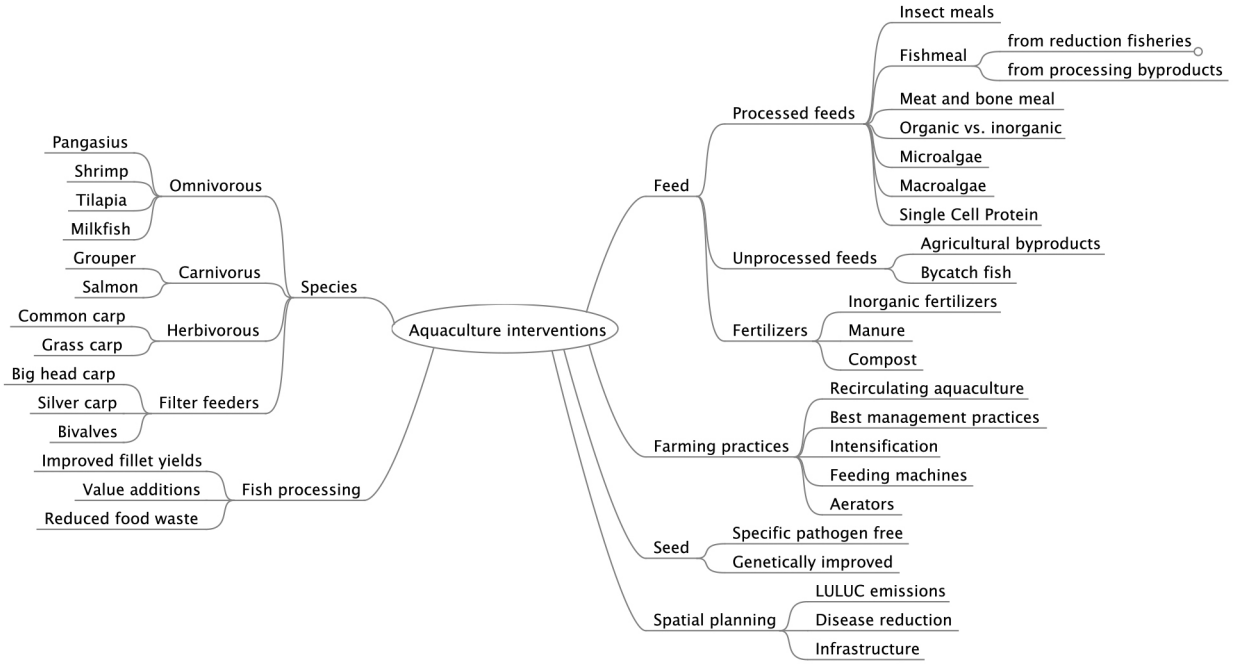

Figure 1: Key interventions that may allow aquaculture to become a more sustainable addition to the future global food portfolio.

REUSE OF RAGWORM PROCESS WATER FOR SHELL FISH CULTURE. A LANDBASED IMTA APPROACH

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In the region Zeeland, the Netherlands, ragworms (*Alitta virens*) are farmed on a large scale (37 ha). The ragworms are farmed for several applications, mainly as bait for angling and as maturation feed in shrimp farming. The production of *Alitta virens* takes place in large rectangular ponds (up to 450 by 10 meter). Saline water is supplied from the adjacent tidal water system Eastern Scheldt, via a flow through system. Ragworms are fed daily with high protein pellets. The feed load and water retention time are based on the actual standing stock and water quality. The effluent of the ragworm farm contains high nitrogen and phosphorus concentrations, which could be utilized for microalgae and shellfish production. Lab experiments and field measurements on nitrogen and phosphorus content in the water, ragworms and sediment were carried out to reveal the feasibility of combining ragworm farming with microalgae (*Skeletonema costatum*) and oyster culture (*Magallana gigas*). Nutrient balances for N and P for the three components (Building Block 1, 2 and 3, figure 1) were calculated, analyzed and tested under different temperatures, feed loads and retention times. Additionally, a model was developed to evaluate the potential of microalgae and oyster production on ragworm effluent for different scenarios/setups.

Results show that 10-25% of the nitrogen in the feed becomes available in the water column for microalgae, representing a 50-100% increase of the concentration of dissolved inorganic nitrogen (DIN) in the water. Although N:P:Si are unbalanced, these extra nutrients could result in a significant increase in the productivity of microalgae and shellfish. Optimization of the production process of micro algae and shellfish can be achieved by pond retention times adaptation and pre- and post-treatment of water streams in this Integrated Multi Trophic Aquaculture approach.

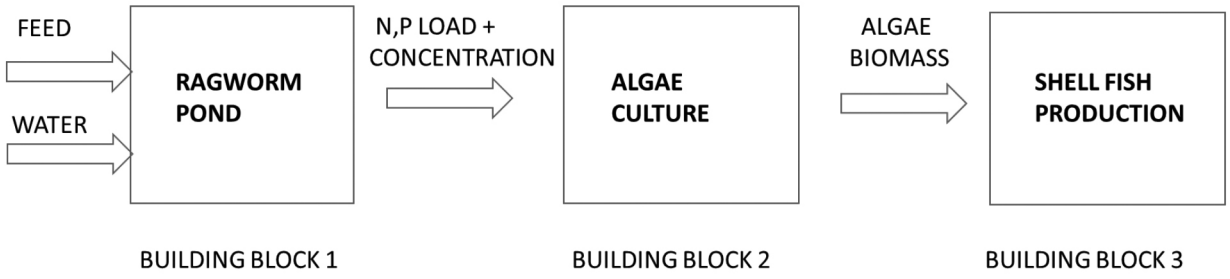

Fig. 1 Conceptual scheme coupling ragworm famring to algae culture to shellfish

THE EFFECT OF THE SOURCE OF DIETARY CAROTENE AND ENVIRONMENTAL COLOR ON THE BODY PIGMENTATION OF THE CLOWN FISH *Amphiprion ocellaris*

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In the marine aquarial market, there is a particularly high demand for the clown fish *Amphiprion ocellaris* due to its body color; however, its pigmentation often decreases whilst it is maintained under controlled conditions. Therefore, in order to maintain the intensity of the body color of this species, the present study evaluates the effect of the source and concentration of dietary carotenoids, in addition to the background color and light spectrum of the clown fish culture units on body pigmentation, with the purpose of establishing the most efficient conditions for sustainable production.

Through two experiments the effect of the source and concentration of dietary carotenoids in the feed of the clown fish *Amphiprion ocellaris,* in addition to the background color and light spectrum, on the survival, growth and body color was evaluated. In the first study (E1), lutein and astaxanthin were used as sources of carotenoids and were incorporated into the diet in three different percentages: 0.5, 1 and 1.5 %, in addition to a control diet without the addition of the pigment sources. In the second study (E2), two background colors of the fish tanks were evaluated: black and white, and two ambient light spectrums (white and red). In both experiments, the survival (90-100%) final weight (0.45 g and 0.72 g average in E1 and E2, respectively) were similar between treatments. With respect to color, the fish fed with 0.5 and 1 % of astaxanthin had a reddish pigmentation and a skin carotene content that was significantly greater ($P<0.05$) in comparison with fish fed the lutein supplemented diet and the control diet (Table 1). In addition, the fish of the fish tanks with a black background exhibited a more intense red coloration when compared to fish kept in tanks with a white background, without the light spectrum having an apparent effect on the fish (Table 1). The results of the experiments demonstrate that the source and level of inclusion of carotenoids in the diet of the clown fish affect the accumulation of pigments in the tissue and in the coloration of the skin, in addition the results indicate that the expression of the color can also vary due to the background color of the culture units. Therefore, it is necessary that these variables are considered during the controlled production of the species, such that they can compete in the market with wild specimens and make their production sustainable.

Table 1. Results of body pigmentation and total tissue carotene content in clownfish.

T	*L**	*a**	*b**	mg g^{-1}
		E1		
A(0.5)	58 ± 2^{ab}	41 ± 6^{a}	64 ± 2^{ab}	15 ± 0.7^{a}
A(1)	56 ± 5^{ab}	35 ± 9^{a}	61 ± 4^{ab}	13 ± 1^{a}
A(1.5)	51 ± 5^{a}	34 ± 5^{ab}	54 ± 6^{a}	9 ± 0.8^{b}
L(0.5)	64 ± 6^{b}	21 ± 5^{c}	61 ± 2^{ab}	5 ± 0.7^{c}
L(1)	64 ± 5^{b}	22 ± 3^{c}	65 ± 6^{b}	6 ± 0.9^{c}
L(1.5)	63 ± 5^{b}	21 ± 6^{c}	58 ± 11^{ab}	9 ± 0.5^{b}
TC	55 ± 8^{ab}	23 ± 6^{bc}	58 ± 5^{ab}	5 ± 1^{c}
		E2		
BBl	57±6	29 ± 7^{ab}	64±5	
BR	56±8	17 ± 8^{a}	53±6	
NBl	53±10	33 ± 10^{b}	60±8	
NR	56±9	29 ± 8^{ab}	62±8	
SL	55±6	39 ± 10^{b}	62±5	

T= Treatment. L*, *a** y *b**= average ±D.E of color space values Lab. mg g^{-1}= Total carotene content (mg g^{-1}) in the skin of the fish BBl= White background/white light, BR= White background/red light, NBl= Black background/ white light NR= Black background/ red light SL= Black background/ without light. TC= control of E1.

INFLUENCE OF TRYPTOPHAN CONCENTRATION AND FEEDING TIME FOR MEAGRES (*Argyrosomus regius*) FED STRESS-ATTENUATING DIETS

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In previous experiments, our group has reported that dietary tryptophan (Trp) supplements can modulate the stress response in the meagre (*Argyrosomus regius*), a promising aquaculture species in the Mediterranean area. However it is necessary to determine the right concentration of this amino acid and the feeding period suitable for minimising the stress response. For that purpose, experimental cultures were designed with 2 factors: Trp concentration and feeding time. Several meagre batches (40.8±3.3 g body weight) were cultured under the same zootechnnical conditions though fed different diets (control, 0.25%, 0.5% and 1% Trp added on dry feed) during different time periods (1, 3 and 5 days). At the end of each period, fish were firstly sampled for basal values (no stress), following they were exposed to air for 3 min, and sampled 1h and 6h post-stress. Stress markers in plasma were analysed: cortisol, glucose, lactate, and proteins.

The best results (see Figure 1) in terms of minimising differences between basal and post-stress status were obtained for 3 days-05Trp and 3 days-1Trp. It seems that longer or higher –Trp treatments would reduce the secondary stress responses (metabolites), though cortisol (primary response) would keep stable only for short-term 05Trp treatments.

This work has been financed through the DIETAPlus project (National Aquaculture Plan, FEMP funds). M.Herrera's contract is supported by INIA-FSE.

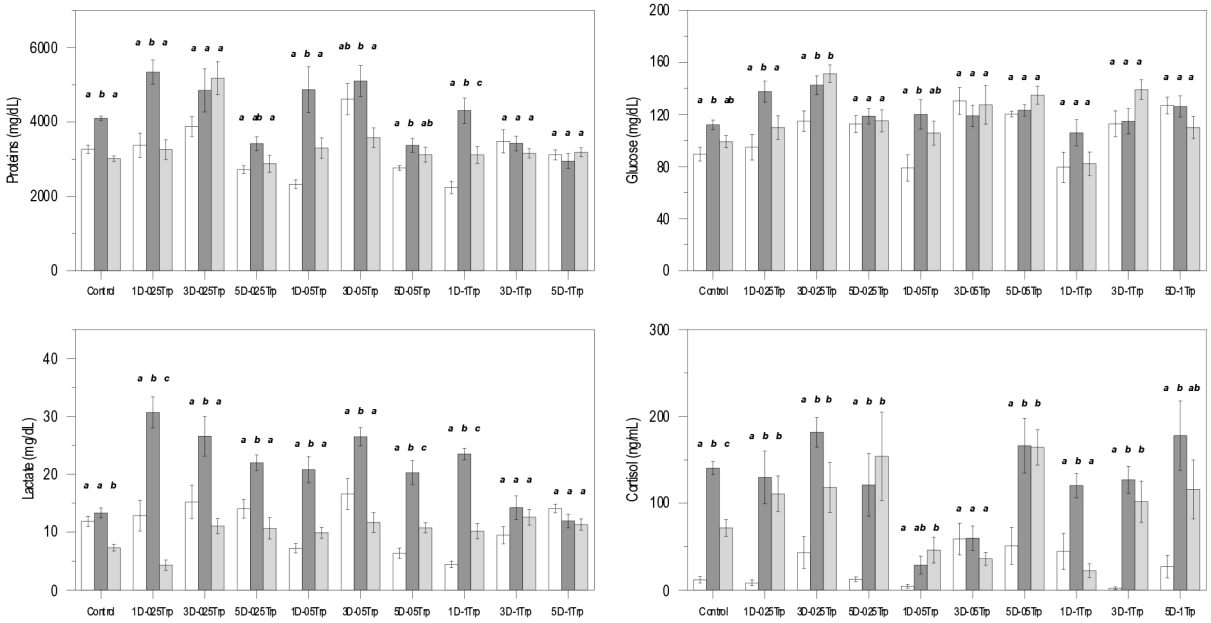

Figure 1. Plasma proteins, glucose, lactate and cortisol (mean±SE) in meagre submitted to the experimental treatments. Different letters indicate significant differences among basal (□), 1h (■) and 6h(■) post-stress.

UPDATE ON FISH DISEASE SITUATION IN NORWAY 2017

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In 2017, Norway produced 1.207800 tons of Atlantic salmon (*Salmo salar*), 60000 tons of rainbow trout (*Oncorhynchus mykiss*) 5500-6000 tons captive wild caught Atlantic cod (*Gadus morhua*), 1600 tons of Atlantic halibut (*Hippoglossus hippoglossus*), 500 tons Arctic char (Salvelinus alpinus) and 2-300 tons turbot (*Scophthalmus maxima*).

Salmon lice (*Lepeophtheirus salmonis*) infestation represents one of the most significant challenges to Norwegian aquaculture, and increased resistance to anti sea lice chemicals is a problem of great concern. The fish farming industry, the Norwegian government and research institutions are investing in the development of non-chemical methods to control sea lice. Cleaner fish eating sea lice are used by a large number of fish farmers. In 2016, fish health personnel reported that mechanical de-liceing resulted in an increased level of mechanical injury and mortality in treated fish. This is still the situation in 2017.

Infection with salmonid alphavirus (SAV) remains the most serious virus disease in sea-farmed salmonids. In total, 176 new sea-farms were registered affected in 2017. This is a significant increase compared to 2016. Change in regulations with more mandatory screening for SAV is probably contributing to the increase.

Infectious salmon anaemia (ISA) was diagnosed in 14 farms in 2017 compared to 12 farms in 2016.

Infectious pancreatic necrosis (IPN) was diagnosed in 23 - 27 salmonid farms in 2017. This is much lower than the peak year of 2009 when IPN was diagnosed in 223 farms. Use of QTL strains of salmon combined with increased focus on eradication of 'house strains' of virus is probably the most important reasons behind the reduction in number of cases in recent years.

Heart and skeletal muscle inflammation (HSMI) was in 2014 removed from the Norwegian national list of notifiable diseases. Reported cases from the Norwegian Veterinary Institute, NVI (93) and private laboratories (90) indicate a similar situation in 2017 as in 2016.

Cardiomyopathy syndrome (CMS), also known as 'heart rupture,' wasdiagnosed by NVI on 96 sites in 2017. Considering reported cases from private laboratories (100 cases), this indicates an increase over recent years.

While AGD (*Paramoeba perurans*) remains an important parasitic infection, the disease was not as severe in 2017 as it was in 2014. Gill disease occurs during all phases of salmonid culture. Chronic gill inflammation is a particularly significant and recurring problem. Bacterial ulcers continue to be a problem in farmed fish particularly in Northern Norway. Yersiniosis (*Yersinia ruckeri*) continues to affect an increasing number of farms and in recent years, there appears to be an increasing trend towards clinical outbreaks in large sea-farmed salmon especially in Mid-Norway.

Production losses remain a significant problem in Norwegian aquaculture.

RISKS OF TRANSMITTING INFECTIOUS DISEASES TO FARMED SALMON THROUGH THE USE OF CLEANER FISH

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Cleaner fish, both wild-caught and farmed, are widely used for delousing farmed salmonids. At present, mandatory fallowing requires that all fish, including cleaner fish, are removed from the farm after each production cycle of salmon. Due to sustainability concerns, a change in Norwegian legislation has been proposed, which would allow reuse of cleaner fish over several production cycles. The Norwegian Scientific Committee for Food and Environment (VKM) assessed the risks of infectious disease spread to the next production cycle of salmonids in such a scenario. We focused on those pathogenic agents that potentially can be transmitted from cleaner fish to farmed salmonids, using a qualitative approach.

The disease status of wild-caught cleaner fish is, in general, poorly known. Therefore, wild-caught cleaner fish represent a higher risk of transmitting infection than farmed cleaner fish. Stocking and screening of wild-caught cleaner fish prior to their introduction to the net pens could mitigate the risk.

Amoebic gill disease is the only known example of a disease in Norway for which there have been evidence-based suspicions of transmission from cleaner fish to farmed Atlantic salmon. The risk of bacterial diseases being transmitted to salmonids is considered low, due to vaccination programmes, although this should not be disregarded. *Viral haemorrhagic septicaemia virus*, represents a high risk of pathogen transmission, depending on the prevalence in wild-caught cleaner fish. Wrasses are susceptible to *Piscine myocarditis virus* infection and can therefore be considered potential reservoirs for this agent. The risk of cleaner fish transmitting this virus to salmonids is considered moderate. Salmonid alphavirus (SAV), the agent that causes Pancreas Disease, is not considered to infect cleaner fish. The risk of transmitting SAV to salmonids varies, depending on environmental, management, and regulatory factors.

Lumpfish are never or very seldom, reused. The probability of transmitting infection and disease to the next production cycle due to retaining cleaner fish for future reuse, therefore applies almost exclusively to wrasses. The probability will generally increase if cleaner fish are replenished from different sources. Potential risk-reduction measures include avoiding reuse of cleaner fish that have been in contact with salmonids experiencing disease outbreaks; periodic complete fallowing, including cleaner fish; quarantine stocking and fish health inspection; and reuse of only a small proportion of cleaner fish.

Very little is known about the pathogenic potential of *Vibrio* spp., lumpfish flavivirus, lumpfish ranavirus, or *Piscirickettsia salmonis* in cleaner fish. The lack of basic knowledge of disease development and the absence of specific diagnostic tools for cleaner fish infections are factors limiting a complete and evidence-based evaluation of the risks.

The overall conclusion by VKM is that sustainable use of cleaner fish to fight salmon louse requires that it is not able to transmit diseases to farmed salmon.

INTERGRATED AQUACULTURED BASED ON SUSTAINABLE WATER RECIRCULATING SYSTEM FOR THE VICTORIA LAKE BASIN (VICINAQUA)

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Lake Victoria is the second largest freshwater lake in the world and of key socio-economic importance for the region, supporting a population of around 30 million through large scale fishing, agriculture, tourism and other local industries. Despite its crucial importance, Lake Victoria has suffered the consequences of overexploitation of its resources (mainly fish stocks) and alarming pollution. One of the main challenges around the lake is poor sanitation and water provision infrastructure and measures and solutions on water supply and sanitation are urgently required.

VicInAqua, a project under the Horizon 2020 research and innovation programme, is developing innovative multipurpose self-cleaning water filtration solutions adapted for sanitation of different wastewater systems to be reused in Recirculation Aquaculture Systems (RAS) around Lake Victoria. The main goal of this project is to enable the supply of clean water to RAS and agriculture through a single solution for water treatment of different waste water streams. The system has an autonomous power supply by renewable energy (PV, biogas, TEG) and is remotely monitored with sensor technologies. The technology development and demonstration at pilot scale is combined with capacity building of local and regional actors. Solutions offered by VicInAqua are focussed on robustness, energy efficiency and economic viability in order to be adapted to the local challenges and to achieve high acceptance in peri-urban areas, where the sanitation infrastructure are poor and the demand for water is high.

The VicInAqua system is primarily designed to use for fish cultivation as here high quality water use is essential. For the pilot system, a Tilapia hatchery using RAS technology was designed and constructed. RAS conserve water and reduce waste discharges. In the pilot facility different types of membranes for water treatment will be tested. This hatchery will produce high quality fingerlings to supply pond aquaculture in the area. The RAS is tailored to the local conditions and the output of the self-cleaning membrane bioreactor. The pilot will ultimately be up-scaled for adoption by aquaculture operators around the lake.

DOWNSCALING CLIMATE IMPACTS AND DECARBONISATION PATHWAYS IN EU ISLANDS AND ENHANCING SOCIOECONOMIC AND NON-MARKET EVALUATION OF CLIMATE CHANGE FOR EUROPE, FOR 2050 AND BEYOND

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According to the fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), 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 are needed. In the field of Climate Change (CC) 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, in order to implement and promote efficient measures and investments. EU are particularly vulnerable to Climate Change consequences. However, the broad resolution of available climate models does not allow to stem valid statements for most of these islands.

The SOCLIMPACT project aims at modelling and assessing downscaled Climate Change impacts and low carbon transition pathways in European islands and archipelagos 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 (Cyprus and Malta; Baltic Islands, Balearic Islands, Sicilia, Sardinia, Corsica, Crete, Azores, Madeira, Canary Islands and French West Indies) by:

- Contributing to the improvement of the economic valuation of climate impacts and related policies for the EU´s Blue Economy sectors, by adopting revealed and stated preference methods.
- Increasing the effectiveness of the economic modelling of climate impact chains, through the implementation of an integrated methodological framework (GINFORS, GEM-E3 and non-market indicators), in the analysis of climate-induced socioeconomic impacts in 11 EU islands case studies, under different climate scenarios.
- Facilitating climate-related policy decision making for Blue Growth, by ranking and mapping the more appropriate mitigation and adaptation strategies.
- Delivering downscaled and accurate information to policy makers, practitioners and other relevant stakeholders, about the environmental and socio-economic consequences of global Climate Change in the EU Blue Economy.

By executing a work programme corresponding to the above objectives, SOCLIMPACT aims at allowing decision makers from all Small Island Member States and other EU islands and Outermost Regions to get a sound knowledge about the possible climate induced impact chains and a comprehensive evidence-based framework for research and policy. By using EU islands as living labs with a high replicability potential, SOCLIMPACT will provide stronger methodologies to better measure and asses the associated costs, benefits and risks of global Climate Change in other EU coastal regions.
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ELEVATED BEDS: A SOLUTION FOR EUROPEAN NATIVE OYSTER RESTORATION?

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In contrast to *Magallana* species that build three-dimensional reef structures, the European flat oyster *Ostrea edulis* tend to lie flat on the seabed as singletons; in part a consequence of current fishery practice. However, it has been argued that this renders native oysters prone to siltation in depositional environments and subject to competition for habitat with other benthic epifauna, including invasive species such as the slipper limpet, *Crepidula fornicata*. Data have shown that an elevated habitat provides for an environment with lower bacterial counts and reduced sediment loads, and potentially places oysters in faster flow regime with greater oxygen concentrations. Indeed, elevation of native oysters has been shown previously to result in more efficient particle clearance compared to those on the seabed. However, raising oysters above the seabed requires more effort and intervention, and would require a change in fishery practice. Further work is required to constrain the optimum height from the seabed, sediment load and water flow at which physiological performance is most productive for the native oyster. Herein we introduce a project to quantify the physiological performance of *O. edulis* at different elevations from the seabed. It will be argued that the prosecution of small scale experiments to constrain optimal environmental conditions represents a cost-effective approach to support native oyster restoration prior to the large-scale deployment of oyster reefs in the environment.

Figure 1. One 1 m^3 gabion to be placed on the seabed. This gabion holds a density of 240 oysters, elevated to a height of 0.8 m from the seabed.

MANAGING BIOFLOCS OPERATIONAL PARAMETERS MINIMIZES THE RISK FOR HEPATOPANCREATIC NECROSIS DISEASE (AHPND) OUTBREAKS

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Biofloc technology is a mature ecosystem composed by a microbial community. It has been reported to improve water quality, feed utilization and shrimp health. Therefore, Biofloc systems are claimed to provide protection against bacterial diseases, and the establishment of such systems can be managed by applying probiotic bacteria which also seems to be an alternative for the control of diseases. In this study, it was tested if bioflocs operated in different ways show a different capacity to control AHPND. Additionally, it was verified if *Bacillus*-based probiotics would allow to control the disease independently from the operational parameters.

L. vannamei postlarvae (PL22 - 0.025±0.01g) were cultured (30 shrimp/10L tank) for 21 days in five different experimental bioflocs: No bioflocs (clear water); Autotrophic bioflocs (cultured at C/N < 10) with and without probiotic supplementation; and Hetetotrophic bioflocs (cultured at C/N >15) with and without probiotic supplementation. The commercial probiotic mixture (**Sano**life®PRO-W) was added to the water every 48 hours. At the end of the period, an AHPND challenge test was performed in 3 different ways: 1- Shrimp cultured in the bioflocs were challenged in new seawater without bioflocs; 2-Shrimp cultured in the bioflocs were challenged in that same biofloc suspension; 3-Non-experimental shrimp were challenged in the different biofloc suspensions.

Overall, survival was lowest when shrimp were challenged in new seawater (Fig1A), independently of the biofloc suspension in which the shrimp were cultured before. When challenged in bioflocs, survival was highest in Heterotrophic bioflocs. Survival in Autotrophic bioflocs was lower, but could be compensated by the use of **Sano**life®PRO-W (Fig 1B;1C). These effects were independent of whether the shrimp has been cultured before in the bioflocs or not.

These results illustrate the protective effects of bioflocs and that operational parameters of biofloc systems the degree of disease risk. In addition, this study showed the potential of using **Sano**life PRO-W® in combination with bioflocs for the case these would not be able to provide protection of the shrimp against AHPND.

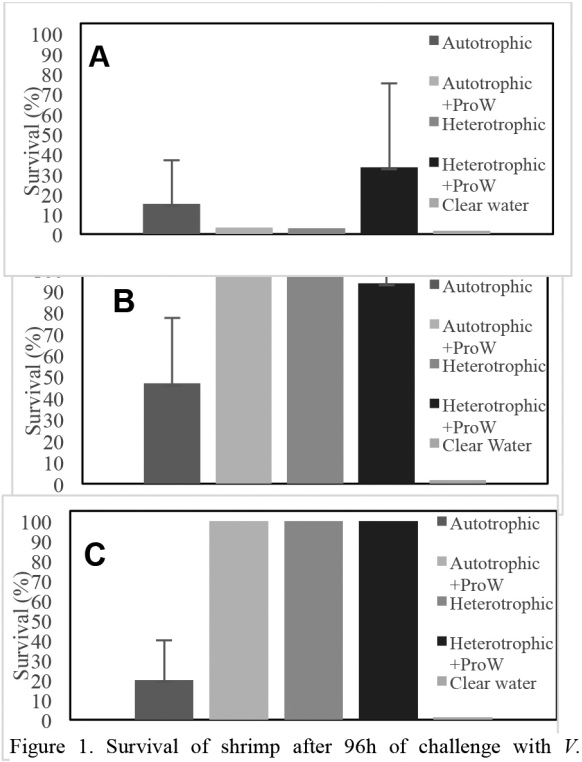

Figure 1. Survival of shrimp after 96h of challenge with *V. parahaemolyticus* according to challenge method A: from biofloc to seawater (A), challenge method B; from biofloc to biofloc (B), and challenge method C: from RAS to biofloc (C)

MITOCHONDRIAL METABOLISM UNDER PROLONGED OCEAN ACIDIFICATION AND WARMING IN EUROPEAN SEA BASS HEARTS

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Ongoing climate change is leading to warmer and more acidic oceans, with possible impacts on marine species, including fish. In our study, we raised larvae of European sea bass (*Dicentrarchus labrax*) from 2dph until juvenile stage (6-8 months) under six scenarios of ocean acidification and warming (OAW): 2 temperatures (ambient in the Bay of Brest and ambient plus 5°C) and three acidification conditions (400, 800 and 1200 μatm CO_2).

We measured mitochondrial respiration in permeabilised heart fibres following a SUIT protocol. Juveniles from all conditions were measured at both acclimation temperatures to compare acute and long-term effects of temperature on mitochondrial respiration. We found increased OXPHOS respiration rates with acutely increased assay temperature, while they were compensated at the respective acclimation temperature. Although mean values of respiratory control ratios indicate fully coupled mitochondria in all treatments (RCR^+ >4), some measurements in each treatment indicate a small degree of uncoupled mitochondria. The proportion of uncoupled mitochondria increased in cold acclimated fish under acute warming.

While effects of temperature on heart mitochondria are clearly visible in European sea bass, we only saw effects of acidification in the cold acclimated fish, were low acidification levels led to mitochondria with lower capacities, while warm acclimated fish were not affected.

We therefore conclude that European sea bass from their northernmost population (northern France) will be able to cope with and acclimate to high acidification levels in warmer temperatures. They appear to even favour temperatures above 15°C, which may enable them energetically to cope with ocean acidification in the English Channel and the Southern North Sea.

EMERGING DISEASES AND BIOSECURITY STRATEGIES FOR SHRIMP FARMING

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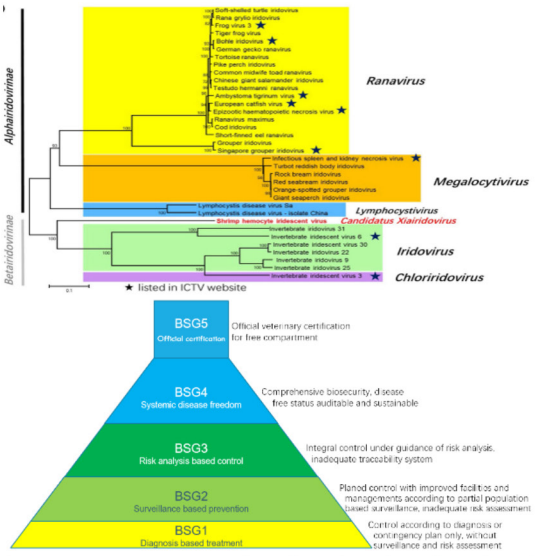
Emerging diseases became new threats for the shrimp farming industry of China. Pathogens including shrimp hemocyte iridescent virus (SHIV), *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) (Vp_{AHPND}), *V. campbellii* causing AHPND (Vc_{AHPND}), *Enterocytozoon hepatopenaei* (EHP), covert mortality nodavirus (CMNV), and yellow head virus genotype 8 (YHV-8), etc. were found in farmed shrimp. Coinfections with two or more pathogens were frequently detected and increase difficulty for confirmative diagnosis. Multiple pathogens, emerging diseases, and complexity of occurrences in shrimp farming industry require urgently a comprehensive resolution for disease control and health management.

Biosecurity concept was introduced both for national or provincial aquatic animal health services and aquaculture establishments. Five biosecurity grades based on implementation of biosecurity measures were suggested, which include diagnosis based treatment (BSG1), surveillance based prevention (BSG2), risk analysis based control (BSG3), systemic disease freedom (BSG4), and official certificated compartment (BSG5). Each biosecurity grade is standalone system and can be upgraded to next one. Farms can consider to achieve which biosecurity grade based on their background, conditions, management, capability, and investment. Biosecurity plans for breeding centers, hatcheries or nursery farms is in developing based on practices in the collaboration of some hatcheries. Twelve principle measures for biosecurity in shrimp farms were recommended and will be implemented in shrimp farms. Demonstrations of biosecurity system implemented in a shrimp farm and a shrimp hatchery have been started by evaluation of risks in these establishments and followed by development of standard operation procedures (SOP) with consideration of risks.



	BS0	BSG1	BSG2	BSG3	BSG4	BSG5
Cleaning of disinfection	-/+	++	++	+++	+++	+++
Ecological control and barriers	-/+	++	++	+++	+++	+++
Disease diagnosis and reporting	-	+++	+++	++	++	++
Contingency plan	-	+++	+++	++	++	++
Water barrier	-	-	+++	+++	+++	+++
Targeted prevention	-	-	+++	++	++	++
Surveillance	-	-	+	++	+++	+++
Disease-free larva	-	-	+	++	+++	+++
Critical control points	-	-	+	+++	+++	+++
Measures based on risk analysis	-	-	+	++	+++	+++
Disease-free screening for all families	-	-	-	-	+++	+++
Comprehensive audit system	-	+	++	++	+++	+++

THE USE OF SWIM TUNNEL STUDIES IN DEFINING ENVIRONMENTAL THRESHOLDS FOR ATLANTIC SALMON AND CLEANERFISH IN EXPOSED AQUACULTURE

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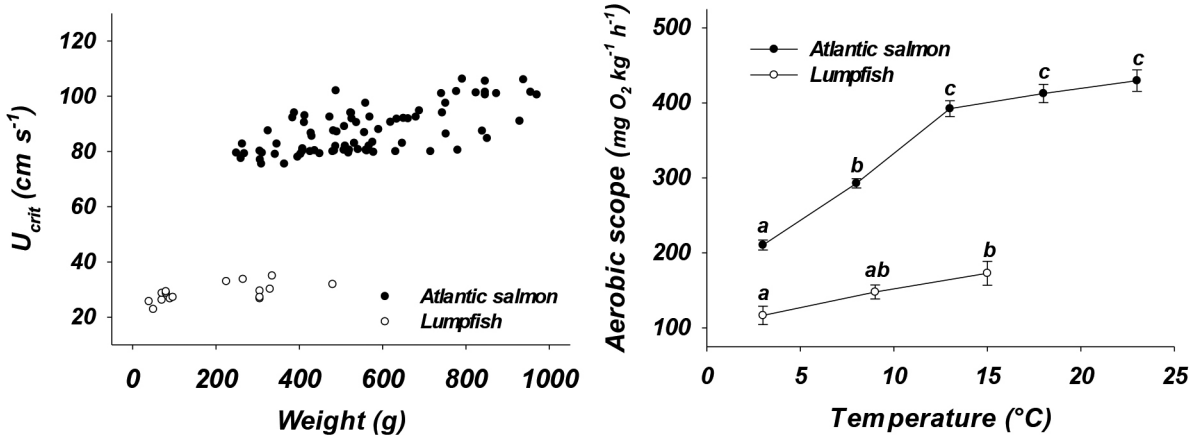
To ensure sustainable growth in Norwegian salmon aquaculture, an important strategy is to move production to more exposed locations that are characterized by more waves and stronger water currents. This poses many new challenges in infrastructure, technology and management. More importantly, these new sea cage environments may exceed the physiological limits of the fish in terms of swimming capabilities and aerobic capacities. Brief periods of very strong currents may cause fatigue and even death, while prolonged periods of intermediate currents may compromise growth and behaviours. To explore what sort of current conditions Atlantic salmon can endure, we have performed a range of swim tunnel studies to measure swimming performance and metabolic rates over a range of relevant environmental and biological conditions such as temperature, salinity, hypoxia, size, ploidy, group size, amoebic gill disease and duration of current exposure. All these factors affect the environmental limits of Atlantic salmon, where especially size, temperature and disease are important to consider when evaluating the suitability of new exposed farm locations. In addition, we have also investigated the environmental limits of the lumpfish, which is the most commonly used cleaner fish in the ever-persistent battle against sea lice in salmon aquaculture. Lumpfish are physiologically fundamentally different from salmon and overall much smaller. They are sluggish fish with poor swimming performances, a much narrower thermal niche, and have a limited response to exercise stress. Hence, in terms of acceptable animal welfare, they do not belong in exposed sea cages. Moreover, many currently used locations likely exceed the environmental limits of lumpfish which pose significant risks of poor welfare, and could explain some of the high mortalities for this species in salmon sea cages. Atlantic salmon may, however, thrive when future production moves to more exposed locations.



IMPACT OF FINFISH AQUACULTURE ON SULFATE REDUCTION, SEDIMENT OXYGEN DEMAND AND BENTHIC NUTRIENT RELEASE AND ITS IMPACT TO BENTHIC-PELAGIC COUPLING IN GANGIN BAY IN THE SOUTH COAST OF KOREA

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We investigated sediment geochemistry, microbial metabolic rates such as sulfate reduction rates (SRR) and sediment oxygen demand (SOD), and benthic nutrient fluxes (BNF) to assess the environmental impacts of finfish aquaculture on biogeochemical and ecological processes in the Gangjin Bay, Korea. Due to the enhanced sedimentation rate (126.2-189.5 g SPM m^{-2} d^{-1}) and organic carbon contents (2.16–2.37%) resulting from massive finfish aquaculture, SRR and SOD at the farm sites were 2-5 and 4-16 fold higher than those measured at the control site, respectively. The SR at the farm sites accounted for 94.9–145% of total organic carbon (C_{org}) oxidation, which was responsible for the accumulation of sulfide and ammonium in pore-water. Benthic nutrient (NH_4^+ and PO_4^{3-}) release resulting from the C_{org} oxidation at the farm site accounted for 325–530% and 257–936%, respectively, of the potential demand of N and P for primary production. Overall, accumulation of sulfide and ammonium and the enhanced SRR, SOD and BNF at the fish farm sediments suggest that continued loading of organic materials associated with the finfish aquaculture ultimately induces a deleterious impact on the coastal ecosystems via benthic-pelagic coupling.

Table 1. Environmental parameters, depth integrated (0–10 cm) inventories of the pore water, solid phase constituents and rate measurements in sediments

Parameter		July, 2013		September, 2013	
		Farm	Control	Farm	Control
DO (% saturated)		51.5	100	92.0	94.2
Sedimentation rate (g SPM m^{-2} d^{-1})		126.2	77.7	189.5	99.8
Food input (kg m^{-2} d^{-1})		1.04	0	1.91	0
POC (%, dry wt.)		2.16 (±0.14)*	1.47 (±0.01)	2.37 (±0.43)*	1.58 (±0.05)
Pore water (mmol m^{-2})	NH_4^+	14.0 (± 2.44)*	6.57(±0.97)	8.74 (± 1.10)*	6.69 (± 0.60)
	PO_4^{3-}	2.96 (± 0.24)*	0.91(± 0.20)	1.30 (± 0.14)	1.21 (± 0.27)
	HS^-	6.68 (± 3.85)*	1.08(± 0.10)	1.91 (± 0.30)	1.50 (± 0.13)
	Fe^{2+}	0.31 (± 0.01)*	0.95(± 0.17)	0.33 (± 0.06)*	0.81 (± 0.17)
Solid phase (mmol m^{-2})	AVS[(a)]	1023 (± 58.0)*	197 (± 87.7)	1959 (± 403)*	107 (± 27.3)
	CRS[(b)]	6054 (± 896)*	2614 (± 268)	5774 (± 949)	4524 (± 683)
	Fe(II)	3187 (± 298)*	1725 (± 160	2228 (± 171)*	1510 (± 267
	Fe(III)	3086 (± 307)	3064 (± 150)	5512 (± 438)*	3570 (± 300)
	Tot. Fe	6272 (± 171)*	4963 (± 152)	7781 (± 486)*	5080 (± 224)
Rate (mmol m^{-2} d^{-1})	SOD	98.8	43.1	205	42.5
	SRR	35.9 (± 4.25)*	10.1(± 0.58)	114(± 20.6)*	7.75 (± 0.68)

Values represent average ±1 SD on the duplicate or triplicate samples; [(a)]acid volatile sulfur; [(b)]Cr reducible sulfur *The farm site is significantly different ($p < 0.05$) from the control site

EVALUATION OF PARASITICIDAL POTENTIALS OF *Moringa oleifera* ON THE CONTROL OF *Ichthyophthirius multifilis* AND ITS KEY LIFE STAGE "THERONTS" ON *Clarias gariepinus* JUVENILES

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Ichthyophthirius multifilis, a ciliate protozoan known to be a pathogenic parasite in freshwater systems, found on a variety of freshwater fish, *Clarias gariepinu*s hampers aquaculture production. Currently due to the negative impact of chemotherapy on human health and environment, there is an urgent need to discover both effective and safe antiparasiticide against *Ichthyophthirius multifilis*. In this study, anti-ich efficiency of aqueous leaves extract of *Moringa oleifera* (ALEMO) on *Clarias gariepinus* was evaluated for 1h. Experimental groups B-G excluding the control (group A) were challenged with 44,000 infective theronts and the percentage of theronts that successfully established infection (% PEI) in individual fish was quantified. Before and after infestation, gills and skin below the dorsal fin of the ich-infested fish were observed microscopically for *I. multifilis* abundance. After ALEMO exposure (1500mg/l-4500mg/l), ALEMO-treated fish showed lower infestation of *I. multifilis* in all investigated tissues than negative control (group B). The ich-infestation increased significantly in the negative group but the infestation in the ALEMO-exposed group and the positive control (fish cure) were significantly eliminated ($P<0.05$). Furthermore, the different concentrations of ALEMO (0.01g/100ml – 0.08g/100ml) showed a positive antiprotozoal efficacy within 24h in eliminating approximately 99% of the infective stage theront with estimated 95% confidence limit for concentration as 0.247g/100ml. The infection potential of theronts following 1h pre-treatment with aqueous leaves extract of *M. oleifera* showed slight re-infestation, prevalence and intensity with *I. multifilis*. Aqueous leaves extract of *Moringa oleifera* seems at present to be very effective and less costly in controlling *I. multifilis*.

OPTIMIZATION OF PIKEPERCH (*Sander lucioperca)* FIRST FEEDING WITH ROTIFERS *(Brachionus plicatilis)*

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First feeding is a critical stage for pikeperch (*Sander lucioperca* L.) larviculture, where severe losses occurred. Rotifers are widely used as first food for rearing of larvae in mariculture. The present series of trials aims to increase survival and larval fitness of pikeperch under controlled intensive conditions using the rotifer *Brachionus plicatilis* as a first diet.

The rotifer *B. plicatilis* tolerates wide range of salinities, but their transfer to freshwater conditions reduces rotifers availability for larvae. The study examined mobility of rotifers in low salinities (2, 4, 8 ‰). Clear plastic 500-ml beakers were used and filled with 200-ml water of prepared salinities. Rotifers were stocked into beakers with initial concentration (118 ind. ml^{-1}). Microalgae and aeration were not provided to the beakers. Rotifers were mixed in each beaker before sampling. Representative samples (1ml, three replicates) were randomly taken from the water column of each beaker with a pipette and transferred to a Sedgwick Rafter Chamber Cell to count under a microscope. After three hours of exposure to 4 and 8 ppt salinities, the study revealed over 60% and 44% at 2 ppt mobile rotifers.

The preliminary study examined the effects of rotifers density (2, 6, 10 and 20 ind. ml^{-1}) and a control (0 ind. ml^{-1}) on survival and growth of pikeperch larvae. Fish were stocked randomly into 2 L tanks at 100 larvae L^{-1} with salinity 5 ‰ at 16.5°C in four replicates. Pikeperch larvae at 5 DPH with mean total length 5.98 mm were fed rotifers three times a day. Residual counts of rotifers were made prior to each feeding. Microalgae *Nannochloropsis sp*. was added twice per day throughout the experiment. The mean survival rates of pikeperch larvae at 9 DPH was above 81% in groups 2, 6, 10 ind ml^{-1} and 78% in a group 20 ind ml^{-1}.There were no significant differences in survival between the feeding groups. The mean (±SD) wet body weight of pikeperch larvae with prey density at 20 ind. ml^{-1} was the highest (1.34±0.23) and significantly different compared to larvae in the treatments with 2 ind ml^{-1} and 10 ind ml^{-1}. The highest mean SGR at 9 DPH was 15.16 % with 20 ind. ml^{-1} and significantly different with a control 4.24%.

The study was supported by following projects: CENAKVA n. CZ.1.05/2.1.00/01.0024; CENAKVA II n. LO1205 under the NPU I program, project n. CZ.02.1.01./0.0/0.0/16_025/0007370; GAJU 060/2016/Z; NAZV QK1710310 and QK1820354.

ADDRESSING THE TRAGEDY OF THE AQUACULTURE COMMONS - ASSESSMENT OF THE GOVERNANCE OF SHARED WATERS AND SHARED RISKS FOR THE AQUACULTURE INDUSTRY

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Most assessment tools for aquaculture are focused on farm-level practices or generalized estimates of the environmental footprint of an industry. However, aquaculture farms do not operate in isolation; they are dependent on shared resources (land/coastal area, water, feed) and neighbouring farms are inherently interconnected. Operating in a shared environment necessitates coordinated management and regulatory regimes that extend beyond good farm level practices. This broader approach to management is commonly known as zonal aquaculture management. Adoption of zonal management in aquaculture makes an industry more resilient to shared environmental and economic risks (e.g. disease, poor water quality).

FishSource (www.fishsource.org) is a new tool that assesses the effectiveness of aquaculture governance and regulatory frameworks to manage shared risks across an aquaculture industry. In doing so, FishSource provides an indication of how and where zonal management approaches to aquaculture are being adopted. In order to understand how the broader aquaculture industry is managed and how shared natural resources are protected three key components are considered in parallel: 1) management systems that regulate industry growth and farm performance within scientifically determined limits; 2) the aquaculture industry's degree of coordination across multiple farms; and 3) the existence of monitoring, enforcement, and public reporting. The FishSource assessment framework addresses these components with respect to five principles:

1. Regulatory Framework: The regulatory system addresses risks to and from aquaculture through a zonal approach to siting, licensing, and production management.
2. Organized Producers Following a Code of Good Practice: The presence of an active producer organization representative of the whole industry and establishment of a Code of Good Practice.
3. Water Quality Management: The impact of aquaculture on the quality of public water resources is managed.
4. Disease Risk and Impact Reduction: Industry is protected from catastrophic losses through best practice disease management on farm and at the zone level.
5. Marine Feed Ingredient Management: The fishmeal and oil in aquaculture feed is sourced from well managed or improving fisheries.

There are currently 38 profiles on FishSource that cover multiple species and production areas. All assessments on FishSource are dependent exclusively on publicly available data, which is notably limited for most aquaculture production industries. Nonetheless, this tool presents a novel approach to understanding the performance of industry-level aquaculture management and helps to identify key steps that could be taken by various aquaculture industry stakeholders – from producers to buyers to governments – to reduce shared risks.

AN AQUACULTURE COMMONS SOLUTION: DEVELOPING AN AREA-BASED HEALTH MANAGEMENT APP FOR SHRIMP FARMING

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Aquaculture relies on common resources and shares common risks that require common management solutions. Health management is essential at the farm, zone and national levels in order to protect the industry and the natural resources it relies on. Although many geographies currently implement farm and national best practice, management at the zone level is often missing – in part because the tools to support this approach are not well developed. Area-based management requires data sharing from farms to a management entity in order to identify common causative factors and will provide an early warning system if data was available in real time.

There are an increasing number of farm management apps available across the industry, providing a range of services to enable individual farmers to move towards precision aquaculture. At present, there has been limited development of apps that could enable the management of wider risks and impacts shared by multiple farms in specific zones. Farmers in Asia use a variety of apps (if they use them at all), often supported by technicians from feed companies or processing plants. Any research needs to demonstrate the value of an area management tool as it is developed in order to provide confidence amongst all stakeholders to share data.

A multi-stakeholder project underway in Thailand is piloting the development of such an app. The project involves farmers, service providers, regulators, researchers and the market, who are keen to identify the factors that can reduce disease risk and improve productivity across the whole industry. Farmers themselves have been involved in designing the app and the data is managed locally by a shrimp club or cooperative to try to build greater trust n and understanding of the value of the process. Alongside the app development, the project aims to encourage farmers to access commercial diagnostic services through a competitive process where companies need to demonstrate added value to farmers. The current focus is shrimp, although the app should be applicable to other species. Early lessons learned will be presented.

As farmers and zone managers become more familiar with the value of data the industry should move more strongly towards precision aquaculture, thereby reducing risk and improving the opportunities to build a strong market for finance and insurance.

SALMON PRODUCTION COSTS AND IT'S EXPLAINING FACTORS: A COMPARISON BETWEEN THE FIVE MAJOR PRODUCING COUNTRIES

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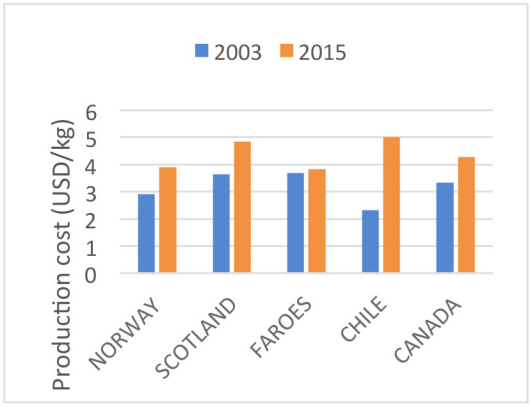
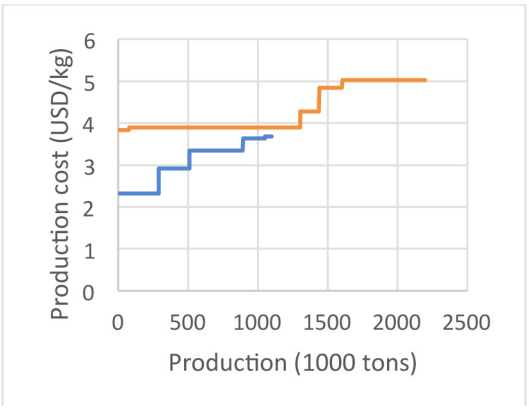
It is well known that productivity growth leading to reduced production cost and improved competitiveness is a key factor in the success of modern aquaculture (Asche, 2008; Kumar and Engle, 2015). There are few studies that compare the development in production cost in different countries. A main reason for this is that cost data is hard to come by, data comparable between different countries even harder. In this paper we have access to a unique data set on production cost for Atlantic salmon in the five largest salmon producing countries for the period 2003-2015. The data will allow us to compare production cost over time and to associate this with production growth and factors explaining the development in production cost. This is a particularly interesting period, as there have been substantial changes in production shares.

Research problem. What explains the different cost development for salmon-producing countries?

Results. The cost leader in 2003, Chile, is now the producer with the highest cost, while The Faroe Islands has seen the opposite development: from the highest to the lowest production costs (upper panel, costs in NOK). Several shocks influence the various producer countries differently. Both Chile and The Faroe Islands has seen serious disease outbreaks, in 2010 Chilean production was reduced to a third of the quantity produced in 2008 (Asche et al, 2009). Feed is the most important input factor with a cost share of over 50% (Asche and Oglend, 2015), and prices for the most important ingredients, fish meal and fish oil, are highly volatile and influenced by *El Ninos* (Asche, Oglend and Tveteras, 2013; Oglend, 2013; Ubliva 2015). Also longer trends like climate change (Hermansen and Heen, 2012) and regulatory system (Abate, Nielsen and Tveteras, 2016; Osmundsen, Almklov and Tveteras, 2017) have been shown to influence production costs, and with the geographical dispersion of the salmon producing countries, these trends have different impacts. The prevention and treatment of sea lice has become a major cost component (Iversen et al, 2015), and also influence countries differently. In the lower panel is shown the resulting supply curves for 2006 (lower curve) and 2015 (upper curve), with offered quantity at average costs for each country (here measured in USD). Even though production costs have risen steeply, the industry is still profitable. The paper also discusses the potential for further growth, as determined by both production cost, ecological potential, political climate and market access.





ECONOMIC EFFECTS OF SEA LICE - AN ILLUSTRATION FROM NORWEGIAN SALMON FARMING

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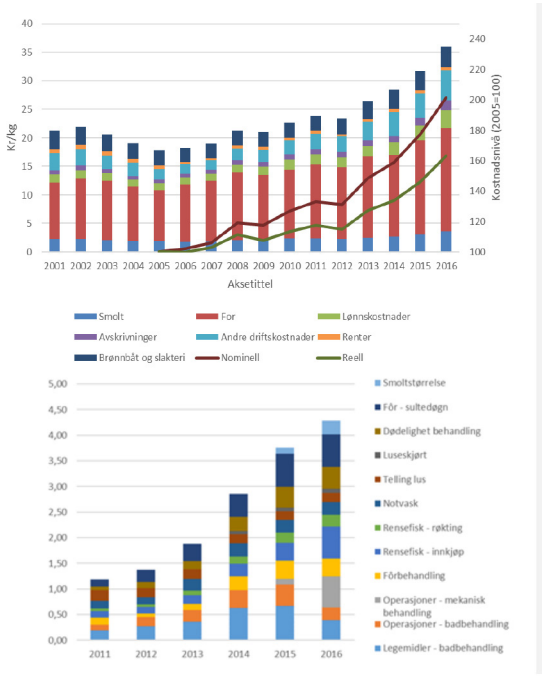
The production cost of salmon farming continue to rise. This paper describes the cost development for salmon farming in Norway, and examines the major drivers of production costs in salmon farming. From 2012 to 2016 production costs in the Norwegian aquaculture industry rose by 50 %. The costs of fighting sea lice have received by far the most attention, but increased feed cost has contributed even more to the cost increase.

Research problem: What explains the cost development for salmon farming?

Results. Salmon farming experienced cost reduction all through the 1980ies and 1990ies, through economies of scale, technological development, automation and control of the most important diseases (Iversen et al, 2015). As shown in the upper panel, costs was at their lowest in 2005. Even lower costs have been recorded in Chile and The Faroe Islands (Iversen et al 2016). From 2005 to 2016, though, cost rose by more than 100 % in nominal values, even adjusted for price increases, costs rose by more than 60 %. The two most important cost drivers are feed costs and costs related to salmon lice, both of which are thoroughly treated in the paper.

Feed is the most important input factor with a cost share of over 50%, and prices for the most important ingredients, fish meal and fish oil, are highly volatile and influenced by *El Ninos* (Asche, Oglend and Tveteras, 2013; Oglend, 2013; Ubliva 2015). Only in the two years from 2014 to 2016, feed costs rose from NOK 14 per kilo of slaughtered Atlantic salmon to NOK 18. The increase is due to both increased feed prices and higher use of feed per kilo produced. Since earlier reports of sea lice costs (like Costello, 2009), the cost of sea lice has increased sharply. Only over the last five years, costs rose from 1,2 NOK in 2011 to 4,3 NOK in 2016. Both prevention and intensive treatments have contributed to the increase. New methods of treatment and better prevention have lead to less of the expensive bath treatments in 2017, indicating that lice costs might have seen it's peak. Even though production costs have risen steeply, the industry is still profitable, due to a steady increase in demand and limited and moderate growth of production, with high prices as a result.



INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) BEST PRACTICES: STATE-OF-THE-ART IN THE ATLANTIC AREA

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According to FAO (2009), an IMTA system could be defined as: "the combination - in appropriate proportions - of different associated aquatic productions that both create a balanced and sustainable system for the environment (eg bioremediation), while creating stability and economic security (diversification = risk reduction) and a more favourable social acceptability".

As a circular economy paradigm, IMTA systems contribute to making aquaculture more sustainable and competitive. However, despite IMTA being encouraged by European Union (EU) policies, there still are technical, socio-economic, administrative and legal bottlenecks hampering its development to its full potential. To overcome these, the INTEGRATE project (INTERREG Atlantic Area 2014-2020 - EAPA_232/2016) supports meaningful cooperation between academia, the corporate sector and relevant authorities from five countries of the Atlantic Area (Spain, Portugal, France, Ireland and Scotland).

One particular aim of the INTEGRATE project is the systematisation of knowledge about IMTA to review, refine and consolidate results from previous projects on multi-trophic aquaculture with the goal of improving and making accessible existing knowledge related to IMTA, and key areas for innovation and development.

In this study, we carried out a wide exploration of the academic and grey literature without any expectation and/or a priori about the content of the articles. This allowed us to establish a categorical regrouping database about IMTA with the starting assumption that all cultivated aquatic organisms can be used within IMTA systems. Then, information derived from published reports, academic and grey literature were analyzed in order to elucidate technical, environmental/ecological, economic, social and regulatory best-practices at both regional and Atlantic Area level.

PEGASUS - PHYCOMORPH EUROPEAN GUIDELINES FOR SUSTAINABLE AQUACULTURE OF SEAWEEDS

Bertrand Jacquemin*, Michèle Barbier, Bénédicte Charrier, Rita Araujo, Susan Holdt and **Céline Rebours**

With contributions from Helena Abreu, Jaume Alberti, Annette Bruhn, Iona Campbell, Olivier de Clerck, Elizabeth Cottier-Cook, Alan Critchley, Aschwin Engelen, Jon Funderud, Claire Gachon, Alexander Golberg, Aleksander Handå, Anicia Hurtado, Kapikumer N. Ingle, Leila Ktari, Nagwa G. Mohammady, Frank Neumann, Sotiris Orfanidis, Shaojun Pang, César Peteiro, Pierre Ronan, Pierrick Stévant, Eric Tamigneaux, Klaas Timmermans and Thomas Wichard

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Seaweed resources play an increasingly important role in the European Blue Growth and Bioeconomy strategies. European production, mainly based on the harvesting of wild stocks is anticipated to boost with the increasing market interest in seaweed resources and the need to assure the environmental sustainability of future aquaculture. No homogeneous regulations exist across Europe, but there is an increasing interest at national levels to accompany industries in the development of seaweed aquaculture in a sustainable way. In the framework of the COST Action FA1406 PHYCOMORPH, a working group (WG) is currently drafting guidelines and recommendations for the future development of the seaweed aquaculture sector, taking into account the scientific, technical, environmental, legal and socio-economic dimensions.

The PEGASUS guidelines highlight the importance of seaweeds as a valuable biological and economical resource, and present and discuss the current status, needs and challenges linked to the development of a sustainable seaweed aquaculture sector. The recommendations are based on the current activities of the sector: a source for human needs with a socio-economic dimension and relevance to the UN Sustainable Development Goals, as well as the state-of-the-art of the cultivation and production processes. Challenges, bottlenecks and risks are identified and presented with a special focus on production issues regarding proliferation, breeding, choice of best cultivar, etc and on the risk of using non-indigenous and invasive species. The legislation and barriers for long-term sustainable production and industrial uses of seaweed biomass are also addressed, as are the Nutrition & Health Regulation required to ensure food security. The PEGASUS project identifies the fundamental research needed to unlock the potentials of seaweed aquaculture. The WG presents recommendations to overcome the identified knowledge gaps, bottlenecks, and risks, to provide scientific expertise to Policy makers (at European, national and regional levels) to help the development of frameworks for legislation and to adapt the ecosystem management framework. Moreover, advanced scientific knowledge will support SMEs and the algal industry to develop long-term sustainable seaweed aquaculture.

ENVIRONMENTAL ASSESSMENT OF AQUAPONICS: THE LETTUCE POINT OF VIEW

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Aquaponics System (AS) claims to be one of the solution for a more sustainable fish and vegetable production system. AS is an integrated system coupling a Recirculating Aquaculture System (RAS) to a Hydroponics System (HS) in which wastewater from RAS is the source of nutrient for HS. Thus, the aim of this study was to assess AS performance in the use of nutrient released from common carp (*Cyprinus carpio*) by lettuce production, compared to the similar lettuce production under HS condition, using a Life Cycle Assessment (LCA). The data were collected on the Ratho (Brindas, France) facilities, where both systems (AS and HS), designed at a commercial scale, are running under the same greenhouse.

LCA was carried out according to the standardized method (ISO 14040 and 14044). The only functional unit considered was 1 kg growth gain of lettuce produced. For AS, feed was the only source of nutrient for carp. Carp production was the only source of fertilizer for lettuce. Therefore, environmental impacts were allocated between carps and lettuce according to the fate of phosphorus from feed recovered by lettuce and carps. According to the midpoint CML baseline method, the impact indicators considered were acidification (AC), eutrophication (EU), climate change (CC), total cumulative energy demand (CED), net primary production use (NPPU), water dependence (WD) and land competition (LC).

For 1 kg of lettuce produced in AS, on farm energy use was the main contributor to WD (83%), CED (78%), GWP100 (70%) and AC (60%), whereas carp production was the main contributor to EU (50%) and NPPU (100%). According to LCA (Fig. 1), compared to HS using only chemical fertilizers, production of lettuce in the AS induced less impact in AC, EU, GWP100, LC and CED, but a direct benefit on water saving was not observed. Furthermore, if use of nutrient from formulated feed was of benefit to reduce impacts on climate change, it was not in regard of the NPPU (a part of fish meal and oil is now used to produce lettuce).

The results of this study seem to indicate that AS is able to decrease fish farming nutrient emissions, while decreasing most of the environmental impacts of HS lettuce production.

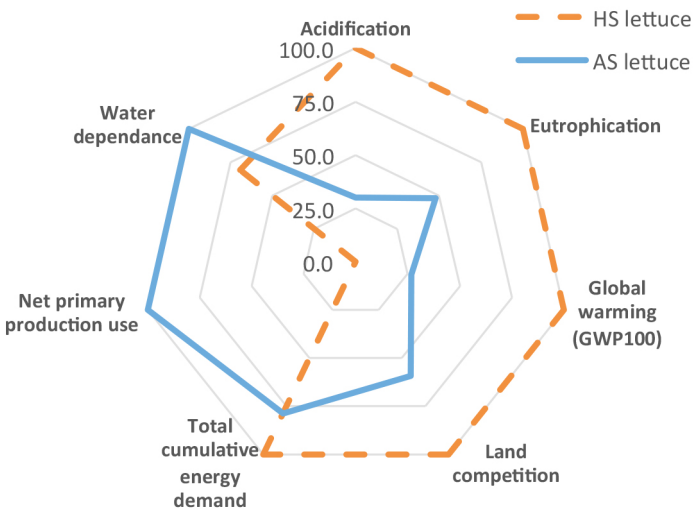

Figure 1: radial graph of comparative environmental assessment comparing lettuce production in aquaponics system (AS) with lettuce production in hydroponics system (HS), for 1 kg of lettuce produced. Values are expressed in percentage of the largest impact in each category and points closer to the center have less environmental impact.

MITOTIC AND MEIOTIC GYNOGENESIS AND TRIPLOIDIZATION OF THE BROOK TROUT (*Salvelinus fontinalis*) AND EVALUATION OF THE OCCURING MALFORMATIONS AFTER THOSE PROCESSES

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Introduction

Inducing development of offspring with genetic material of only one parent (gynogenesis or androgenesis) leads to produce a homozygotic Doubled Haploids (DHs) (mitotic gynogenesis and androgenesis) or heterozygotic (meiotic gynogenesis) larvae. Homozygotic individuals remain fertile and can be used to produce a genetically uniform clonal group of fish resulting a second round of the process (Komen and Thorgaard, 2007).

Inhibition of II polar body from the inseminated eggs results in development of the triploid embryos. Triploid fish growth proceeds at the slower rate than diploid individuals until reaching the sexual maturity of the diploids. Triploidy impairs gonadal development and growth of the triploid fish is not impeded by sexual maturation (Dunham, 2004).

In this study we have induced gynogenetic (mitotic and meiotic) and triploid development of the brook trout (*Salvelinus fontinalis*).

Materials and methods

Eggs from two brook trout females (SF♀) were collected and kept separately. Spermatozoa from two brown trout (ST♂) and one brook trout (SF♂) male were collected to the plastic containers. Sperm was diluted in the artificial seminal plasma. Sperm of the brook trout was irradiated with UV-C light for the 10 min. Irradiated sperm was used to activate eggs in the presence of the sperm activating medium (SAM). To recover diploidy in the gynogenetic embryos, high hydrostatic pressure (HHP) shock (9500 psi/5 min.) was performed 30 min. (meiotic gynogenesis) and 420 min. (mitotic gynogenesis) after insemination. Triploid fish were obtained by inseminating eggs with the diluted non-irradiated brook trout. HHP shock was performed 30 min after insemination. Survival rates of embryo and larvae, presence of malformations and only maternal inheritance of the offspring were evaluated.

Results

Larvae from the gynogenetic groups showed varied survival rates depending on the egg donor. Offspring of the SF1♀ ($SF1_{control}$ 98,2%, $G1_{meio}$ 42,2%) showed higher survival at the swimming stage than offspring of the SF2♀ ($SF2_{control}$ 29,8%, $G2_{meio}$ 1,1%). Overall, meiotic gynogenetic groups of larvae showed significantly higher survival than those from the mitotic groups (DHs) ($G1_{mito}$ 8,6%). No gynogenetic DHs were produced in eggs from of SF2♀ female.

Spinal deformations including kyphosis, scoliosis, lordosis and spiral larvae were observed among larvae from all groups. Most of the dead larvae from meiogynogenetic groups had scoliosis and kyphosis while dead DHs were c-shaped kyphotic larvae. Only few larvae from the control groups were deformed and showed scoliosis. Number of triploid and diploid larvae with body deformities was similar.

Homozygosity of DH larvae was confirmed with use of at least two pairs of microsatellite specific starters.

Discussion

Comparative analysis of survival and body deformities among triploids, meiogynogenetic and doubled haploids brook trout enabled verification whether genetic factors or side effects of the high hydrostatic pressure shock are responsible for the fish malformations and increased mortality among chromosome set manipulated specimens. Comparable number of malformed brook trout observed among fish from the triploid and the diploid control groups does not support hypotheses concerning HHP shock may be responsible for the body deformations in triploid fish. In turn, reduced survival and increased ratio of malformed DHs were presumably triggered by appearance of the recessive alleles.

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USE OF EGGS FROM DOUBLED HAPLOID (DH) RAINBOW TROUT AND UV IRRADIATED GRAYLING SPERM TO PRODUCE CLONAL FISH

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Introduction

Genetically uniform (homozygous) fish have great potential in aquaculture and biomedical research. To obtain homozygous individuals, traditional inbreeding programs requires mating of the siblings for at least 20 generations. Application of induced gynogenesis allows to obtain genetically uniform individuals in the second generation. Gynogenesis includes activation of eggs with the UV-irradiated homologous or heterologous sperm and diploidization of the maternal chromosomes using physical shock (temperature or high hydrostatic pressure) that blocks extrusion of the second polar body (meiogenesis) or inhibits the first cell cleavage (mitogenesis). Offspring provided in the course of mitogenesis are fully homozygous Doubled Haploids (DHs), which may be used as gamete donors for another round of gynogenesis in order to produce clonal fish lines Komen and Thorgaard (2007).

Material and Methods

Rainbow trout *Oncorhynchus mykiss* and European grayling *Thymallus thymallus* gamete donors came from the broodstocks kept in the Department of Salmonid Research, Inland Fisheries Institute in Olsztyn, Rutki, Poland. Mitotic gynogenesis was induced in April of 2012 to produce fully homozygous doubled haploid (DH) rainbow trout females. Eggs from six 4-year-old rainbow trout (RT♀) females were collected and kept separately. Spermatozoa from three 4-year-old rainbow trout (RT♂) and 3-year old grayling (T) males were collected to the separate plastic containers. Sperm was diluted in the rainbow trout seminal plasma and irradiated with UV-C light. Irradiated grayling and rainbow trout sperm was used to activate rainbow trout eggs in the presence of the sperm activating medium (SAM). Control groups contained eggs fertilized by non-irradiated sperm. To double maternal chromosomes, high hydrostatic pressure (HHP) shock (7500 psi/4 min.) was applied 350 min. after insemination. After four years of rearing, sexually matured gynogenetic females were examined (microsatellite DNA markers) to confirm their homozygosity. Four gynogenetic DH females (DH_1-DH_4) were chosen to be used as egg donors for the meiotic gynogenesis resulting in production of the clonal lines. Eggs from each female were activated with diluted in the artificial seminal plasma and irradiated (as above) grayling sperm. Part of the activated eggs were then exposed for HHP shock (9500 psi/3min.) applied 35 min. after insemination. Control haploid groups included activated eggs that were not subjected to HHP shock. Control diploid groups included eggs fertilized by non-irradiated sperm. Survival rates of embryos and larvae, presence of deformed speciemens and genetic uniformity of the offspring and egg donors were evaluated. 12 microsatellite markers from 10 linkage groups were studied.

Results

At the eyed stage, doubled haploid (DH) rainbow trout developing in eggs activated by UV-irradiated grayling (DH_G) and rainbow trout (DH_{RT}) spermatozoa showed 18.43% and 27.07% survival, respectively. At the swim-up stage, 11 (0.17%) gynogenotes that hatched from eggs activated by grayling spermatozoa and 44 (0.66%) gynogenotes hatched from eggs activated with rainbow trout spermatozoa were counted. After four years of rearing only four fully homozygous gynogenetic individuals (DH_1-DH_4) were found. However, only eggs DH_3 and DH_4 were successfully activated using UV-irradiated spermatozoa About 20% of the gynogenetic offspring of DH_3 and DH_4 females survived till hatching and formed two clonal lines: GC_3 and GC_4, respectively. Genetic identity of DH females and their gynogenetic offspring was confirmed. Many of the dead larvae collected after hatching were abnormal (20% GC_3 and 63% GC_4). Malformed larvae from clonal lines were emaciated with diverse skeletal deformities including scoliosis, abnormal body axis, kyphosis, lordosis, spiral or c-shaped larvae or varied head deformations. A non-specific malformations i. e. abdominal skin perforation and abdominal swelling were found in a few individuals.

Discussion

Meiogynogenetic reproduction of DH females resulted in production of two rainbow trout clonal lines. To assure inheritance of only maternal chromosomes grayling UV-irradiated sperm was used to activate rainbow trout eggs. To our knowledge, this is the first time when UV-irradiated heterologous spermatozoa have been successfully used to generate rainbow trout clonal lines. Expression of the recessive alleles in DH females may result in the delayed spawning time, disturbed gonadal development and production of low quality eggs. In the present study, eggs from only two DH females were able to develop into embryos after activation with the UV-irradiated spermatozoa. Increased ratio of the deformed larvae observed

(Continued on next page)

among fish from the clonal lines may be also related to reduced quality of eggs produced by DH females. Furthermore, fully homozygous clonal fish may be more prone to the viral and bacterial infections that clinical symptoms include, among others, reduced appetite and emaciation. Emaciation might also be caused by cephalic abnormalities including deformations of the jaw which impeded proper nourishing or underdevelopment of the intestines. Skeletal body deformities including scoliosis or lordosis rather appear due to the genetic causes. However, It is not excluded that some of the larvae body anomalies appeared in the course of the HHP treatment during induction of the gynogenetic development.

Conclusions

Eggs produced by rainbow trout DH females exhibited limited developmental competences after activation by UV-irradiated spermatozoa. Clones developing in such eggs had decreased survival and exhibited several body abnormalities.

Funding

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THE NEED TO CONTROL ILLEGAL CAVIAR TRADE: NEW INSIGHTS FROM SURVEY STUDIES

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There is a widespread believe among experts and law enforcement authorities that the trade in caviar is largely under control and has no longer a significant impact on wild sturgeon populations. This assessment is based on the fact that more than 90 % of caviar in legal international trade is derived from aquaculture and that all species of sturgeon and paddlefish are listed under CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), resulting in strict legal requirements. Additionally it is also a widely held believe that the highly endangered status of most natural sturgeon populations makes regular catches rather unlikely and thus their trade sporadic.

However, several findings indicate that illegal trade in caviar and other sturgeon products is still taking place at a noticeable level and that CITES regulations are not always met.

A caviar market survey in Romania and Bulgaria 2011-2012 included DNA analysis of 30 purchased samples, of which eight were traded without the mandatory CITES labelling on the primary container and five were sold as from wild sturgeons, four of which were from Beluga Sturgeon (*Huso huso*). Three samples were mislabelled and a fourth sample contained caviar from more than one species, which was not indicated on the CITES code and is only allowed for "pressed" caviar.

In several further cases in other EU countries, caviar could be found on sale without any CITES labels or - even more – with CITES labels that did not follow CITES requirements (e.g. not sealing the container or allowing other visual evidence of any opening).

These instances show that CITES requirements are not sufficiently implemented, thus weakening the effectivity of such regulations. It also shows that illegal caviar trade still occurs, and that demand for caviar from wild sturgeons still exists and this seems to create incentives to try to satisfy the market demand.

THE EFFECT OF HIGH UNSATURATED FATTY ACIDS ON ANTIOXIDANT STATUS IN *elovl2* KNOCKOUT ATLANTIC SALMON

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Background: Atlantic salmon fillet is a dietary source of indispensable long chain n-3 fatty acids (LC-n3FAs) for human and quality of this dietary resource affects on the stress oxidative scavenger system in human body. Since LC- n3 FAs are unsaturated and more vulnerable for oxidation, the activity of antioxidants is of interest to healthy fillet. The enzymes involved in this anabolism pathway are under control of dietary lipids, so we aimed to investigate the effect of knockout elovl2 (Fatty acid elongase-2) enzyme on antioxidant enzymes in muscle of Atlantic salmon fed by high unsaturated fatty acids (HUFA) compared to control in 12 weeks.

Methods and Materials: elovl2 knockout salmon eggs were produced by Cas9 microinjection method and screened to select the fully mutant juveniles. Two HUFA and LUFA diets were provided by Skretting Nutreco Co. Fresh water temperature and oxygen levels were checked during the trial. Knockout and wild type fish were divided in two groups to feed by LUFA and HUFA feeds. After 6 and 12 weeks, muscle tissues were dissected and kept in -80ºC until gene expression and enzyme activity analyses. Catalase (CAT) and Glutathione peroxidase (GPx) mRNA expressions were measured by RT-PCR and ABTS and antioxidant enzyme activities were determined by fluorimeter assays.

Results & Discussion: ABTS and CAT activities were higher in knockout group fed HUFA diets for 6 weeks; parallel to the mRNA expression of CAT and GPx. At the end of trial, this antioxidant activity was higher in wild-LUFA group for CAT and GPx mRNA levels. It may result from higher HUFA content of feedings and more susceptibility for oxidation reactions. The CAT and GPx enzymes activities among the groups were significantly changed after 6 weeks and 12 weeks. ABTS as a general marker and CAT and GPx as the specific marker of antioxidant activities showed a different trend of changes among the groups. *Elovl2* Knockout fish exposed to HUFA feed revealed an unexpected change due to lower ability of n-3 LCFAs synthesis and higher rate of HUFAs in diet.

Conclusion: The presence of LC n-3 FAs has a role in antioxidant status in muscle tissue of Atlantic salmon. Knockout elovl2 fish exposed to HUFAs showed higher antioxidant status in 6 weeks. The more synthesized LC-n3FAs can increase the need for antioxidant activity to prevent FA oxidation.

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UTILSING THE REFLEX ACTION MORTALITY PREDICTIVE (RAMP) MODEL AS A VITALITY MEASUREMENT IN CRUSTACEA AQUACULTURE

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The snow crab (*Chionoecetes opilio*) and king crab (*Paralithodes camtschaticus*) are two of the most important commercial crab species in the world. In Norway, the harvest of king crabs reached 2350 metric tonnes in 2017 and in 2016 approximately 5300 metric tonnes of snow crab were captured in Norwegian waters. Most of the king crabs caught in Norway are now exported alive (95%) and there is now a focus on exporting snow crabs alive rather than processing and selling as frozen products. This has resulted in the development of live holding systems for both crab species and the possibility of both long-term storage and quality enhancement in aquaculture systems (refered to as Capture Based Aquaculture in Norway). During this process the stress of capture, live transport and holding can result in significant mortalities if the animal is not cared for correctly. Despite this there is limited research on how to measure stress effectively in live crabs and particularly how to measure this in an industry friendly way. There is a very effective method available for predicting mortality in wild caught crabs (called the Reflex Action Mortality Predictor model or RAMP) which is based on observing a series of reflex actions and then assigning a resulting reflex score to each animal. In this presentation we describe a variation of this method for measuring crab vitality using reflex actions. Individuals can be assigned a vitality score as a measure of stress and vitality (for both snow and king crabs) when held in aquaculture facilities. This presentation will describe the results of three separate studies that have utilised this vitality index and discuss whether this is an effective, industry friendly method of measuring stress and vitality in live captive crabs held in aquaculture systems.

PERFORMANCE OF FIVE ULVA STRAINS: PRODUCTIVITY, CHEMICAL COMPOSITION AND NUTRIENT DYNAMICS AND SELECTION CRITERIA FOR FARMING PRACTICES AND ECOSYSTEM INTERACTIONS

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Introduction
Seaweeds are often mentioned as a promising product that can contribute to the growing global demand for food, feed and bio-based products. The increasing interest is reflected by their production, which has expanded at 8% annually in the past decade. The majority of seaweed production occurs in Asian countries, but the establishment of numerous research projects, shows the expanding interest of European countries in exploring the potential of seaweed cultivation.

Seaweed cultivation results in seaweed-ecosystem interactions. Seaweeds can, for example, be used as biofilters for removal of excess nutrients from land run-offs or fish farms. However, when densities exceed the carrying capacity, nutrient extraction may result in nutrient limitation with cascading effects to other trophic levels. Evaluating ecosystem interactions therefore warrants a good understanding of nutrient uptake and assimilation rates of seaweeds.

So far results published in literature present a large range in terms of growth rates, content and nutrient dynamics within seaweed species. Estimating the production potential and environmental interactions is therefore difficult. It is unknown to what extend the varying results can be attributed to environmental conditions and/or specific characteristics of the seaweed species used in each study, including genetic differences.

The aim of the current study was to investigate the differences in productivity, chemical composition and nutrient uptake for different Ulva spp. strains cultivated under standardised (environmental) conditions.

Material and Methods
Five Ulva spp. strains were cultivated in outdoor tanks (600L, n=5 per strain), from June till October 2017. Each strain was collected from a different location in the Eastern Scheldt area. Environmental conditions were standardised among all tanks, so differences in performance could be attributed to (genetic) origin of the strains. On a monthly basis the biomass of Ulva was reduced to 250 gram wet weight per tank, in order to prevent growth inhibition due to overstocking. Growth was measured bi-weekly, and monthly samples were collected for analysis of the chemical composition. Nutrient uptake rates were measured every month from August to October. Environmental conditions were measured continuously (light, temperature) or at regular intervals (nutrient concentrations).

Results and Discussion
Remarkable differences were observed between the five strains. Two strains showed very low growth and tanks/seaweed quickly became over grown with epiphytes. Growth of the remaining three Ulva spp strains also varied within the season. Similarly, chemical composition and nutrient uptake capacity varied between strains and within time.

Results will be presented on productivity, total nitrogen, carbon, amino acids, carbohydrate, fatty acid concentrations and specific leaf area (leaf area per unit leaf dry weight) of the Ulva strains. Two different methods for defining protein content will demonstrate the protein concentrations through time. The differences between strains highlight the importance for selection of strains in aquaculture settings as these might greatly influence production performance for commercial seaweed farms.

Differences in nutrient uptake between strains indicate that selection should be taken into account when quantifying seaweed-ecosystem interactions as some strains have significantly higher uptake rates than others. Relations between nutrient uptake, growth and chemical composition will be discussed.

DEVELOPMENT OF BENTHIC IMTA SYSTEMS BY COUPLING POLYCHAETE PRODUCTION TO SALMON FARMING: SYSTEM DEVELOPMENT, NUTRIENT DYNAMICS AND RISK OF MEDICINE RESIDUES

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Introduction

Significant production growth is predicted for the Norwegian salmonid industry, provided that key sustainability issues can be resolved. One bottleneck for expansion of salmonid farming is the release of fish wastes into surrounding marine ecosystems. This has fuelled interest in integrated multi-trophic aquaculture (IMTA), which offers an opportunity to recycle waste streams and simultaneously provide a new source for high quality marine proteins. Polychaetes are candidate species for incorporation in IMTA systems for conversion of the organic waste streams.

Polychaetes are naturally abundant in benthic habitats under fish farms and are important in environmental recovery by consuming and transforming the organic materials deposited from the fish (Dean, 2008). Opportunistic polychaetes commonly found underneath fish farms in Norway include *Ophryotrocha spp*. at hard bottom locations (R. Bannister pers comm) and *Capitella capitata* for farms in soft sediment areas (Kutti et al. 2007).

Salmon lice are a major problem in salmon farming, and a number of compounds are available to treat salmon lice, including hydrogen peroxide (H_2O_2). H_2O_2 has long been regarded as an environmental friendly extinguishing agent because it dissociates to water and oxygen. However, it has been shown that low concentrations could affect the physiology of aquatic animals, including polychaetes (Buchner et al. 1996, Abele-Oeschger et al. 1994). Potential negative effects of H_2O_2 on polychaetes, are important considerations for design of benthic IMTA systems, from a production and food safety perspective.

A series of field and laboratory experiments were performed to study the development and efficiency of benthic trays for polychaete production, to evaluate the nutrient turnover potential, and to define potential effects of hydrogen peroxide treatment on polychaete survival.

Material and Methods

Benthic trays were designed to stimulate natural polychaete production. Trays were deployed under a commercial salmon farm and allowed us to follow succession of polychaete abundances at different sites at the farm. Succession on trays was investigated by video surveys and species identification was done after retrieval of the frames.

Polychaetes were transported to the laboratory where growth, chemical composition, respiration rates and nutrient turnover of the most dominant species (*Capitella capitata*, *Ophryotrocha spp*. and *Malacoceros fuliginosus*) were measured. The tolerance of *Capitella capitata* and *Ophryotrocha spp*. to H_2O_2 exposure was evaluated by defining the lethal concentration (LC_{50}).

Results and Discussion

The broad range of results from the field and laboratory studies will be presented, and differences between polychaete species will be discussed in terms of nutrient turnover capacity and tolerance to hydrogen peroxide. The outcomes will be placed in the framework of (benthic) IMTA development, and will address issues such as system design (methodological challenges), IMTA efficiency (nutrient removal), risks of medicine residues in integrated systems, and the potential market of polychaetes produced in IMTA systems. Differences between coupled (open water) and decoupled (semi-enclosed systems) salmon-polychaete systems will be discussed.

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COST-BENEFIT ANALYSIS OF A BREEDING PROGRAM FOR ATLANTIC SALMON

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Breeding programs for Atlantic salmon are among the most advanced among aquaculture species. This study aims to quantify potential benefits of genetic improvement, which traits contribute most to increases in farm profit, and to evaluate who benefits from genetic improvement.

Cost-benefit analysis was performed for the breeding program the major salmon breeding company Salmobreed AS. Economic values for growth rate, feed intake rate, mortality rate and fillet yield were derived from a bio-economic model. The bio-economic model accounts for constraints on the number of smolts stocked per cage and quota on biomass at farm level. The economic value of sea lice resistance was derived using R_0 as breeding goal trait. Genetic gain per trait was predicted with SelAction (Rutten et al., 2002) and multiplied by economic values to predict the economic response to selection. Geneflow (Hill, 1974) was used to predict the increase in genetic level of the nucleus, the multiplier tier, and ultimately fish in production. Benefits of the breeding program were estimated from the sales of 120 million eggs per year, and the genetic level of the farmed population. Benefits were incurred by the whole supply chain including consumers, whereas costs were incurred by the breeding company only. Cost-benefit analysis was used to evaluate the profitability of the breeding program over time.

Costs of the breeding program were 4.5 million € per year. Improvement of growth rate and feed efficiency accounted for 95% of the economic response to selection. Improved resistance to sea lice increased farm profit by 7.5€/ton per generation, which was only 2.4% of the economic response. The benefits of the breeding program outweighed the costs after 5 years, and reached a net present value of 440 million euro in year 10. In the long run, the genetic potential of an egg is expected to increase by €0.33/egg per year. Only a minor proportion of the resulting increase in benefits is accrued by the breeding company.

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GROWTH PERFORMANCE OF AMAZON RIVER PRAWN REARED IN BFT

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The goal of this work was to evaluate the use of biofloc technology to reared Amazon River prawn (*Macrobrachium amazonicum*). One hundred Amazon River prawn juveniles, average weight 3.88 ± 0.36 g, were divided into two experimental units, 50 animals per each. Seven days before the stocking with prawn in experimental units (rectangular tanks, 0.72 m^2 x 0.35 m) with 250 L, water fertilization was carried out with a carbon source (sugar) and powdered diet to keep the carbon:nitrogen (C:N) ratio 10:1, resulting in an initial solids concentration of 100.0 mg L^{-1}. Seven days after prawn stocking, fertilization was maintained at 10:1 (C:N) to neutralize 40% of the feed nitrogen and to keep the ammonia below 1.0 mg L^{-1}. Calcium hydroxide was added when alkalinity fell below 30 mg .L^{-1} CaCO3, and when necessary, the dose was 10% of the daily ration. Prawns were fed two times per day (9:00 and 15:00), with commercial diet, with 3% of the prawn biomass. Biometric measurements were carried out weekly to check prawn growth and adjust the amount of feed offered. Dissolved oxygen and temperature were performed measured twice a day. Total suspended solids (TSS), settleable solids, pH, hardness and alkalinity were monitored twice a week. Ammonia (total ammonia nitrogen – TAN), nitrite-N, and nitrate-N were also monitored twice a week. Survival, final weight, week weight gain, specific growth rate, food conversion ratio and yield were all determined after six weeks.

Dissolved oxygen (6.1 ± 2.2 mg L^{-1}), temperature (27.1 ± 2.0 °C) and pH (7.1 ± 0.2)were suitable for species, however, for some weeks, ammonia nitrogen (2.4 ± 2.6 mg L^{-1}), and nitrite (4.3 ± 3.7 mg L^{-1}), were higher than the limits recommended for prawn. Nitrate (6,4 ± 5.8 mg L^{-1}), alkalinity (72.3 ± 13.6 mg $CaCO_3$ L^{-1}), hardness (88.7 ± 36.1 mg L^{-1}), settleable solids (19.6 ± 20.6 cm) and TSS (150.9 ± 85.8 mg L^{-1}) have not their lethal levels determined.

In conclusion it is possible to use BFT to maintain keep and rearing Amazon River Prawn (*Macrobrachium amazonicum*), however to improve the growth performance it is necessary to define nutritional requirements, as well improve the management techniques for this specie in BFT.

Table 1. Production data of Amazon River prawn juveniles reared in BFT.

Variables	**Mean ± S.D.**
Mean of final weight (g)	5.36 ± 0.71
Mean of final length (cm)	7.89 ± 1.04
Survival (%)	77.67 ± 5.85
Food conversion ratio	2.98 ± 0.14
Specific growth rate (% dia^{-1})	0.61 ± 0.04
Week weight gain (g $week^{-1}$)	0.29 ± 0.04
Yield (g m^{-3})	822.00 ± 17.75

SUSPENDED SOLIDS REMOVAL EFFICIENCY OF DIFFERENT MESH SIZE OF MICROSCREENS FILTRATION WITH FOAM FRACTIONATION ON SEAWATER RECIRCULATING SYSTEM FOR GROUPER FARMING

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It's difficult to remove fine particles accounted for 95% of total solids' counts smaller than 20μm in RAS. Foam fractionation is a simple and low-cost water treatment for removing fine particles. The aim of this study was to evaluate the solids removal efficiency of different mesh size of microscreens filtration following with foam fractionation, and to analyze particle size distribution (PSD) at each stage of filtration and foam fractionation. The water was introduced by the inlet pipe for four mesh size of microscreens (120, 90, 60 and 40μm) filtering to a buffer tank, then for foam fractionation and returned to RAS through outlet pipe. The PSD and concentration of suspended solids in inlet pipe, buffer tank and outlet pipe were measured, then removal efficiencies were calculated. The PSD can be converted by liner regression of log10 frequency versus log10 particle size into an integrative β-value.

The β-values (slope of regression) and removal efficiency of filtration (η_1) and fractionation (η_2) were shown in Table 1. (Values of the same column with different superscript letters are statistical significantly different, probability level α was 0.05). The β-values of buffer tank increased with microscreen mesh size minished. It demonstrated that larger particles will be broken into fine particles after filtration, and it get more serious with mesh size minished. The β-value of outlet pipe was relatively smaller than β-value of buffer tank for the same group. This suggested that fine particles were easier to be removed by foam fractionation than larger particles. Microscreen mesh size 40μm filtration with foam fractionation got the best water treatment effect. This study suggested that foam fractionation is an important supplement for removing fine particles.

TABLE 1. β-values (R^2>0.95), removal efficiency of filtration (η_1) and fractionation (η_2).

Mesh size	Particle size-frequency distribution β			Removal efficiency (%)	
	Inlet pipe	Buffer tank	Outlet pipe	η_1	η_2
120	2.86±0.09	2.89±0.11^{a}	2.76±0.01^{a}	8.01±5.73^{a}	11.10±5.60
90	2.94±0.03	2.96±0.02^{a}	2.85±0.01^{b}	9.70±2.17^{ab}	12.10±9.87
60	2.93±0.03	2.99±0.09^{a}	2.86±0.05^{b}	12.89±5.32^{ab}	9.93±2.30
40	2.88±0.10	3.20±0.13^{b}	3.09±0.05^{c}	20.18±8.02^{b}	14.75±7.10

EFFECT OF LYOPHILIZATE *Gammarus insensibilis* (CRUSTACEA:AMPHIPODA) IN GROWTH, CHEMICAL COMPOSITION AND SURVIVAL OF *Seriola dumerili*

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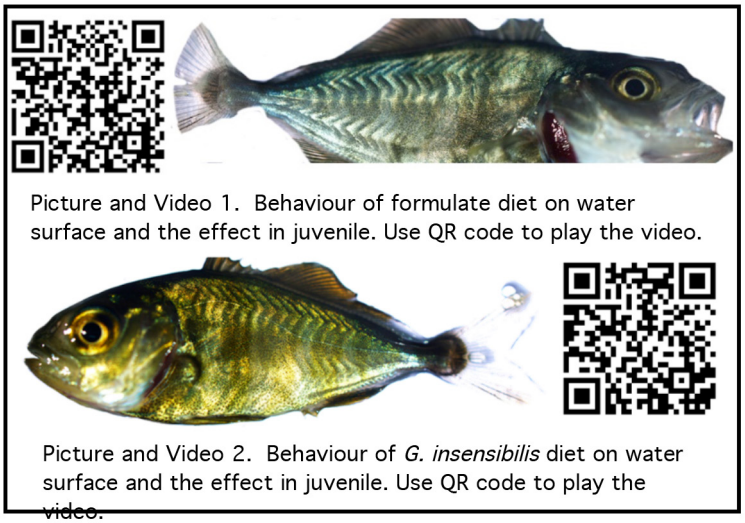
Three of the relevant areas to research in aquaculture: i) explore and investigate the potential of novel aquatic organisms as live feed, ii) The progress in 'Integrated Multi-Trophic Aquaculture' (IMTA) and iii) the aquaculture diversification in species highly appreciated by consumers.

In the present study, two experiments were done with *Seriola dumerili* larvae of 22 and 44 days after hatchery. They were feed with the amphipod *Gammarus insensibilis*, recollected of a terrestrial pond, and one formulated diet. Survival, biometry measures and chemical profile were measured.

In the first experiment, *G. insensiblis* treatment showed better survival, although the other parameters were no different. On the other hand, in the second experiment the survival was similar, the growth was better with formulated diet and *Gammarus insensibilis* treatment had the best chemical profile. *G. insensibilis* treatment showed higher values of ARA (5.53±0.18%) and DHA (19.07±0.19%), low level of TAG (2.18±0.5%) and a coloration similar (Picture 2) to that in wild juveniles *S. dumerili* (Picture 1). In addition, the feed behaviour of juveniles was different where the fishes feed with formulated diet (Video 1) presented an aggressive behaviour than the amphipod treatment (Video 2). The aim of this study is show the potential use of amphipod, such as *G. insensibilis* in the aquaculture rearing of *Seriola dumerili* larvae during two distinct stages of this development.



Picture and Video 1. Behaviour of formulate diet on water surface and the effect in juvenile. Use QR code to play the video.

Picture and Video 2. Behaviour of *G. insensibilis* diet on water surface and the effect in juvenile. Use QR code to play the video.

PROXIMATE COMPOSITION AND TRACE METALS CONCENTRATION OF *Macrobrachium vollenhovenii* (HERKLOTS, 1857) FROM BADAGRY CREEK, LAGOS NIGERIA

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Shellfishes have long been recognized as a valuable source of high quality nutrients in the human diet and also play significant role in income generation, employment creation and foreign exchange earnings of the country. Prawns and shrimps are known to consume sand and mud along with detritus during feeding, and the sediment act as sink for trace metals in the aquatic ecosystems. Hence, these metals can be taken up by these shell-fishes during feeding. These metals are essential for growth and development. The African river prawn, *Macrobrachium vollenhovenii* (Herklots, 1857), found in rivers, creeks and lagoons, and with a spread across the West African sub-region is one of the largest species of *Macrobrachium* known. Consequent upon its nutritional importance and wide spread, this study was carried out to assess the proximate composition and trace metals concentration of this prawn, in order to report the nutrient composition of this prawn from the public health point of view.

One hundred and sixty (160) samples of *Macrobrachium vollenhovenii* and water samples were collected over four months, July - August (rainy season) and February - March (dry season) from Badagry creek. The prawns were washed in flowing water to remove adhering dirt. Total length and body weight were recorded to the nearest 0.1cm and 0.01g respectively. Samples were oven dried at a temperature of 80°C for 72 hours to constant weight and milled separately to powder. The samples were analysed in triplicates. Temperature and pH were determined *in situ* while dissolved oxygen, salinity, total alkalinity and total hardness were determined by titration. The moisture, fat, protein, ash, carbohydrate and crude fibre contents were estimated. Trace metals concentrations were determined using the nitric acid digestion technique. Levels of metals (Fe, Mg and Zn) were expressed in mg/g dry weight. Data were analyzed using SPSS 20 statistical programme at five per cent level of significance. Analysis between seasons was done using the T-test method.

Length and weight of the prawns varied from 15.0cm-17.6cm and 72.76g-130.65g respectively. The mean values for protein, lipids, ash, crude fibre, moisture and carbohydrate were relatively high. Protein, ash, moisture and lipids were significantly different ($P<0.05$) between seasons. The Zn and Fe contents between the seasons were not significantly different ($P>0.05$), while Mg was significantly different ($P<0.05$). The values of these metals are within the recommendations set by the Food and Agriculture Organization and World Health Organization. Consequently, from the nutritional point of view, *Macrobrachium vollenhovenii* can be used as an alternative source of high quality protein, energy and mineral supply for human consumption and even animal feed formulation.

Fig. 1: *Macrobrachium vollenhovenii*

Table 1: Proximate composition of *Macrobrachium vollenhovenii* from Badagry Creek, Lagos, Nigeria

Parameters (%)	**Mean (Rainy Season)**	**Mean(Dry Season)**
Protein	52.18±1.11[a]	43±1.13[a]
Moisture	4.08±0.44[a]	31.25±5.23[b]
Ash	23.39±1.06[a]	12±1.11[b]
Lipids	9.65±0.65[a]	3.5±0.55[b]
Carbohydrate	1.58±0.07[a]	1.35±0.17[a]
Crude Fibre	9.15±0.42[a]	9.35±6.08[a]

Values with same superscript in the row are not significantly different ($p<0.05$).

Table 2: Trace metals concentration of *Macrobrachium vollenhovenii* from Badagry Creek, Lagos, Nigeria

Metals	**Rainy Season** (mg/100g)	**Dry Season** (mg/100g)
Zinc (Zn)	5.10±0.64[a]	5.16±0.27[a]
Magnesium (Mg)	20±1.02[a]	18±1.10[b]
Iron (Fe)	3.03±0.40[a]	2.59±0.39[a]

Values with same superscript in the row are not significantly different ($p<0.05$).

NUTRITIOUS POND CONCEPT, A PATHWAY TO SUSTAINABLE INTENSIFICATION OF AQUACULTURE SYSTEMS?

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One of the main concern and critics from the rapid raise of aquaculture is its dependence on capture fisheries for essential amino acids and unsaturated fatty acids. The industry has significantly reduced the amount of fishmeal and fish oil in manufactured pelleted feed. However, novel ingredients such as algae, insects or microbial protein are not (yet) available to small-scale farmers from developing countries where most of aquaculture production is sourced. One possibility to reduce the dependency on external high quality protein and essential fatty acids is to rely on the pond to provide these ingredients through the natural food web, and to design a feeding system based on it.

The Nutritious Pond system is based on ecological principal where by manipulating the composition of formulated pond diets the metabolic wastes resulting from nutrient inputs (feed, fertilizer, carbohydrates) become a balanced fertiliser stimulating the natural productivity of the pond. To achieve this objective, fundamental and applied research are combined to i) investigate biological process within the pond ecosystem and ii) determine the best nutrient ratio and carbohydrate type and format applied to the pond in different cultures of *P. vannamei* intensity. The novelty of the research also lies in the integration of the research outputs within a multi-stakeholder platform in Vietnam to design a new product adapted to local industry requirement, through a reflexive design approach.

Our research in controlled environment shows that the pond significantly contributed to the shrimp diet requirement for Highly Unsaturated Fatty Acids (HUFA). Raising carbon availability by addition of carbohydrate while reducing the feed load enhanced the contribution of natural foods to pond production and reduced nitrogen losses without reducing the yield. When transferred to on-farm context, fine-tuning of the system required testing different carbohydrate formats and application rates to fit with farmer requirements. On-farm testing resulted in productivity and economic gain, while using less feed. The effect on the pond ecology was significant for farmers with absence of disease, faster mineralisation of waste and more stable water quality parameters without using any additional probiotic.

The use of a reflexive design approach helps in integrating research finding in product development, while questioning issues from on farm testing driven experimental design. Beside promising results for a new feed system supporting sustainable intensification, our approach shows involving sector stakeholder in the research and design process not only helps to integrate research in local context, but feedback from stakeholders can also drive applied research and ultimately support the design of technology adapted to local needs.

VALUE CHAIN INNOVATION RESEARCH IN AQUACULTURE

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In the aquaculture literature, there is limited systematic knowledge of how innovation has been approached. The objective of this research is to analyse the different approaches to innovation used in aquaculture development with a specific focus on the Value Chain (VC) approach to identify gaps in aquaculture innovation research and discuss the potential complementarities between different approaches to innovation.

Using a systematic review methodology, one hundred publications were selected from the aquaculture literature covering the topic of aquaculture innovation. Within aquaculture innovation research, *Value Chain* (VC) studies remains limited with 10 peer review articles selected compared to *Transfer of Technology driven* approaches (61% of the selected studies).

Two main types of VC research can be distinguished: i) analysis of current and past regulatory frameworks and value chains to provide recommendations in the context of future challenges and ii) analysis of quality standards development in aquaculture value chains. Compared to other types approaches to innovation, such a *Transfer of Technology* or *Farming Systems*, VC research includes a wider range of actors (including consumers and politicy makers) and aims at analysing power relationships and the structure and the organisation of value chains. Most of this research was published after 2009, illustrating the recent interest of consumers and civil society for quality standards and food safety in the aquaculture sector, but also a driver towards greater transparency and equity along the different segments of global aquaculture value chain. In VC studies, institutions and policy are either embedded in, or central to, the analysis, and innovation is a process that can take place only with adequate institutional change, which differs from the approaches to innovation taken in the majority of innovation research in aquaculture.

However, the analysis found that the outcome of VC research has low practical applicability as it provides global recommendations that will require additional research to design concrete new regulations or organizational arrangements. For example, in-depth case study of pro-poor value chain analysis for better inclusion of the poor is absent. In addition, we found that ecological and technical dimensions are underemphasized, and thus this approach lacks insights at how farm level dynamics link to other parts of the value chain. Cross fertilisation integrating elements of *Farming System*, *Inclusive Innovation* or *New Product Development* approaches would help to support a farm level perspective and help to operationalise VC research to provide specific outcomes for poverty alleviation.

THE EFFECT OF FRESH AND FORMULATED DIET ON SOMATIC GROWTH, GONAD GROWTH, GONAD QUALITY AND SURVIVAL OF *Tripneustes gratilla* UNDER FARM CONDITION

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The study was conducted to investigate the effect of fresh and formulated diet on somatic growth (weight, diameter and height), gonad growth (gonadosomatic index), gonad quality (colour, texture and firmness) and survival of the collector sea urchin (*Tripneustes gratilla*) under farm condition for twelve weeks. The experimental diet consisted of fresh feeds; *Ulva*, a 50:50 mixture of fresh *Ulva* and *Gracilaria*, *Gracilaria* and formulated feed which contains 200 grams of dried *Ulva* per kg. There was no significant difference in the somatic growth (weight, diameter and height) of urchins fed both fresh and formulated diets. Urchins fed formulated diet were significantly higher at $p \leq 0.05$ for gonadosomatic index while those fed with *Ulva* were the least in terms of gonad growth. The gonad of urchins fed formulated feed was significantly lighter in colour $p \leq 0.05$. Also, none of the feeds was significantly different from each other $p \geq 0.05$ in redness and yellowness. There was no significant difference in the mortality rate of urchins fed both fresh and formulated diet $p \geq 0.05$. This study shows that the culture of sea urchin is viable under farm condition in South Africa. It also shows that formulated feed better contributes to gonad growth.

PISCINE ORTHOREOVIRUS SUBTYPE 1 (PRv1) FROM FARMED AND WILD SALMONIDS COLLECTED FROM NORTH AMERICAN WATERS: GENETIC DIVERSITY AND PHYLOGENETIC ANALYSIS

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Piscine orthoreovirus (PRv) is a double stranded non-enveloped RNA virus which commonly infects farmed and wild salmonids. There are 3 subtypes of PRV; PRv1 which infects Atlantic Salmon, species of Pacific salmon, species of trout and some non-salmonid finfish, PRv2 which infects Coho Salmon in Japan and PRv3 which infects Rainbow and Brown Trout in Europe. PRv1 is responsible, under some conditions, for the development of the disease Heart and Skeletal Muscle Inflammation (HSMI) in farmed Atlantic Salmon.

We examined the genetic diversity off and phylogenetic relationships between PRv1 from different host species and geographical areas over time. We are investigating: whether PRv1 has been spread globally by aquaculture and/or enhancement activities, whether particular genetic variants of PRv1 are associated with HSMI development, and whether molecular tools which will allow for tracking of PRv1 transmission between wild and farmed salmonids and non-salmonid reservoir hosts on the West Coast of North America can be developed. To this end we have conducted genome analysis on representative samples of PRv1 obtained from the East and West Coast of North America and compared these sequences to available whole genome sequences of Norwegian, Chilean and Canadian PRv1.

Gradient purified PRv1 or tissues infected with PRv1 were used. Ribosomal RNA-depleted stranded libraries were constructed and bi-directionally sequenced (Illumina HiSeq 2500 System) by the McGill University and Génome Québec Innovation Centre. Sequence reads were aligned to the PRv1 reference genome (KC715679) using 70-100 coverage threshold (CLC Bio software). Aligned sequences were concatenated to provide a draft genome of PRv1 representing partial sequences of the 10 segments. Phylogenetic analysis was performed on the concatenated sequences using both Maximum Likelihood (Mega v7) and Bayesian (BEAST v2). Bootstrap analysis (1,000 replicates) was used to validate tree topology.

Our preliminary phylogenetic analysis of concatenated genome sequences shows that PRv1 from the West Coast of North America clusters separately with high bootstrap credibility from PRv1 from Eastern Canada, Norway and Chile. Within this cluster a monophyletic group was evident for PRv1 from Atlantic Salmon. Interestingly, PRv1 from Eastern Canada clusters separately from PRv1 from Chile and Norway which form a separate monophyletic group. We are presently working to obtain additional PRv1 genome sequences from wild salmonids on the West Coast of North America to identify whether the genetic differences seen in PRv1 from wild Coho Salmon when compared to Atlantic Salmon are shared with other wild hosts.

THE GLOBAL SHRIMP FARMING INDUSTRY: STATUS, CHALLENGES AND PERSPECTIVES

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According to FAO, global production of farmed shrimp reached 4.05 MMT in 2011 and then increased to 4.17 MMT in 2012 (up 3.0 %), 4.30 MMT in 2013 (up 3.2 %), 4.68 MMT in 2014 (up 8.8 %), and 4.88 MMT in 2015 (up 4.2 %). In contrast, the GOAL surveys suggest that world production contracted from 3.87 MMT in 2012 to 3.49 MMT in 2013 (down 10 %), strongly bounced back to 4.30 MMT (up 23 %) in 2014 due to improving harvests in China, Vietnam, and Indonesia along with strong growth in India and Ecuador, to fall once again to 3.99 MMT (down 7 %) in 2015 due to disease problems in virtually all major countries in Asia. A minor recovery was estimated for 2016 which is expected to strengthen through 2019, when global shrimp farming production should reach 4.82 MMT (notice that this is slightly less than the global estimate of 4.88 MMT reported by FAO for 2015). GOAL forecasts rely on the expectation that major disease crises are averted in the near future.

At GAA, we recognize the main challenges to aquaculture growth include diseases and health management, aquafeeds and ingredients, environmental and social issues, marketplace, investment, leadership, consumer awareness and education. For example, the main viral and bacterial diseases have probably cost the industry over US$20 billion in the last three decades. And with the need to double production in the next two decades, significant increases in aquafeed production and expanded production of established as well as new, sustainable ingredients.

Industry trends include the need for more efficiency at every level of the production and marketing chain, industry consolidation, and creation of new markets, both internal and external. Increasing sustainable production will require more production from established and new species; expansion into new areas; improved domestication and genetic selection; new aquafeed ingredients; improved health management; new production technologies with increased control and better risk management and others. The industry also needs to attract more professional investors and better address the market, which will increasingly require more efficiency, quality control and traceability - through certification and sustainability of the entire production chain and sustainability.

CULTIVATION OF YELLOW PERCH, *Perca flavescens* (MICHILL 1814) FROM THE LARVAL STAGE TO THE MARKET SIZE IN A CLOSE RECIRCULATION SYSTEM

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In spite of number of published articles and research projects, tank culture of yellow perch has not yet been demonstrated on a commercial scale. The slow growth rates associated with poor water quality, low feeding levels or poor feed conversion have resulted in minimal success with the grow-out of significant quantities of yellow perch. Successfully raising the newly hatched fry to fingerlings in intensive aquaculture was identified as another major constraint to successful yellow perch aquaculture. The following article summarizes results from a three-year study with the goal of establishing optimal conditions and predispositions to successful commercial production of yellow perch under controlled environment of a closed recirculating system, that can result in improved survival and growth rates, and the subsequent grow-out of yellow perch to harvest size within commercially acceptable time frame. *P. flavescens* is a close relative to European perch, *P. fluviatilis,* and findings originated from this study can be applied to the cultivation of that species.

Constant temperature and a slightly elevated salinity were optimal for the development, growth and survival of yolk-sac larvae. Optimum growth temperature, estimated by maximizing the temperature-growth rate relationship function, was 13.9°C at 0.01044 mm/hour maximum growth rate. Constant light had a negative effect on growth and survival in this period. Two feeding schedules were successfully applied in the post larval period of cultivation. The first one started with a three days of feeding with rotifers as an initial live food, continued with newly hatched artemia nauplii. The second started with newly hatched artemia nauplii (INVE, AF strain) introduced 30 hours after the mouth opening, followed with 12 hour old nauplii 10 days after the initial feeding. Prolonged feeding with rotifers resulted in poor survival and reduced growth. In the period of the transition from live to artificial feed, a two week period of mixing enriched artemia nauplii with artificial feed starters gave the best results in survival and growth. Feeding activity, growth and survival of juvenile yellow was significantly better at 25°C than at the lower temperatures suggested by other authors. Perch proved to be very timid and skittish at lower temperatures (compared to sea bass, trout and tilapia grown in the same tank culture systems) reacting to any activity above and inside the tank. Daily feeding rate and the food conversion factor were also significantly better. Continuous feeding during the 16 hours of daylight resulted in better growth and survival than multiple daily feedings with same amount of feed. Conversion factor for the group fed continuously ranged from 0.8 at the beginning to 0.58 at the end of the experiment. For the fish fed four times per day conversion factor ranged from 0.68 to 0.47. The survival within the groups was not significantly different. In the controlled environment, at constant temperature, the growth cycle, from1g fry to market size fish of 170 g lasted 9 months in round tanks, and ten months in raceways. Monthly growth rate was 7.7% of the body weight in the first moth, and 2.1% in the end of the experiment. Constant elevated temperature and 16 hours photoperiod prevented normal gonadal development and sexual maturation in the first year. The weight of gonads was only 2.2% of total body weight.

EFFECT OF DIETARY PROTEIN TO ENERGY RATIO ON PERFORMANCE OF NILE TILAPIA AND FOOD WEB ENHANCEMENT IN SEMI INTENSIVE POND AQUACULTURE

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In (experimental) conditions where there is no natural food web, the optimal dietary digestible protein to digestible energy ratio (DP:DE) for tilapia ranges from 18 to 26 g.MJ^{-1}. In pond culture, which valorizes the potential of the natural food web, increasing the C:N ratio stimulates the food web productivity. This study assessed if reducing the dietary DP:DE ratio (i.e., increasing C:N input in the pond) below the optimal DP:DE ratio affects the productivity of tilapia kept under semi-intensive conditions, the food web dynamics and nitrogen balances at the pond level. 12 ponds, each divided into three compartments, were assigned to test the effect of two diets, which differed in DP:DE ratio (17 vs. 13 g.MJ^{-1}). Three feeding levels (no, low and high) were nested in each pond in a split plot design. Initial fish biomass was 1166 g.compartment^{-1} and the experiment lasted 60 days. Decreasing DP:DE ratio enhanced biomass gain and specific growth rate ($P<0.05$; 1195 vs. 986 g.compartment^{-1}; and 1.76 vs 1.55 %.d^{-1}). Body composition of tilapia was unaffected by diet and feeding level. Despite the difference in performance final fat content was equal at all treatments; being only 5%. Averaged over both diets, survival and feed conversion ratio increased with increasing feeding level ($P<0.05$). Diet composition did not alter any of water quality parameters and abundance and diversity related parameters of the food web. The total amount of N accumulated in tilapia N per pond was enhanced when dietary DP:DE ratio was decreased. The data on N gain and N balance at the pond level, suggested that the food web productivity was stimulated by the reduction in dietary DP:DE even below the assumed optimal levels reported in literature. It is hypothesized that the optimal dietary composition is dependent on the culture condition (i.e. extensive; semi-extensive versus intensive pond culture).

POPULATION STRUCTURE OF NILE TILAPIA *Oreochromis niloticus* STRAINS CULTURED IN TANZANIA USING SNPS MARKERS FROM ddRAD SEQUENCES

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Tanzania is the host of many different species of Tilapia. Nile Tilapia, *Oreochromis niloticus* is one of the most cultured species in Tanzania. Recently aquaculture production in the country has increased tremendously. Challenges such as availability of quality fingerlings is still facing the industry. Wild dependent of fingerlings from lakes and rivers for culture has led to the mixing up of Tilapia species to the extent that pure line of Nile Tilapia has become limited. For the future need of an optmum Tilapia breeding program in Tanzania, there is a necessity to understand the genetic structure of Tilapia species populations cultured in Tanzania. Seven populations from the government aquaculture centres namely TAFIRI(1), Ruhila (2),FETA(3), Lake Victoria(4), Karanga(5),Igunga (6) and Kunduchi(7) were examined by double- digest restriction site-associated DNA (ddRAD) sequencing. ddRAD technique sequence the regions around Res recognition sites using uses two restriction enzymes and produce a reduced representation of a genome. Multivariate method, DAPC was used to infer the genetic structure of Nile Tilapia species from 7 sampling sites. The scatterplot shows overlapping between 3, 4, 6, and 7 populations (Figure 1). In this study, we found that those populations which grouped together in the same cluster were the genetically closer ones and population 1 is genetically distinct.

FIGURE1. Scatterplot of individuals on the two principal components of DAPC. The graph represents the individuals as dots and the groups as inertia ellipses.

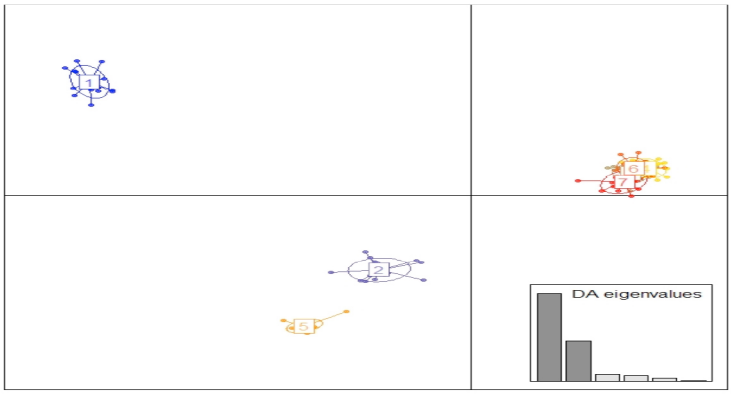
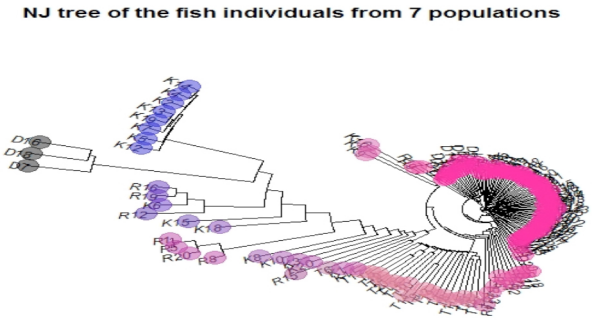


FIGURE 2. Neighbour-joining (NJ) tree showing bunches of related individuals.



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DNA were extracted from finclips of 140 fish individuals and ddRAD libraries were prepared and sequenced by Illumina sequencing technology. SNPs were obtained by aligning reads to a reference genome available at NCBI website. SNP calling variants resulted into filtered 2182 SNP loci.

For DAPC analysis, 40 PCA axes were retained corresponding to 97% of the variance and 6 discriminant functions were obtained. Clusters were defined a priori, according to the sampling populations. The scatterplot shows overlapping between 3, 4, 6, and 7 populations (Figure 1).

NJ tree showed branches of related isolates individuals. Individuals from FETA (F), Ruhila (R), Lake Victoria (LV) and Igunga (IG) were clustered together. Few individuals from Kunduchi (D) and Karanga (K) seems to be genetically distant from other individuals of the same population. All individuals of TAFIRI (T) population fell in the same group except T6 which seems to be far from the group ((Figure 2).

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OFFSHORE WIND FARMS AS POTENTIAL LOCATIONS FOR FLAT OYSTER (*Ostrea edulis*) RESTORATION IN THE DUTCH NORTH SEA

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Introduction

The "Dutch Energy Agreement" drives governments and industries to invest in renewable energy sources, of which offshore wind energy is one of the solutions to meet the agreed 16% target in 2023. Significant North Sea space is required to meet future goals, resulting in competition between current users, such as shipping, nature, fisheries, oil and gas, and the new wind parks. Currently the potential for multi-use functions of wind parks are worldwide under investigation. One of the options is nature-inclusive building, in which the design and construction of wind farms include the potential for co-design with inclusion of natural resources. Oyster bed restoration appears to be a viable option to support these ambitions in the Dutch North Sea, to simultaneously achieve biodiversity goals, restore ecosystem functions and enhance ecosystem services including future seafood production. For the recovery of flat oyster (*Ostrea edulis*) beds, knowledge is required about the conditions under which the active restoration of this species in the North Sea can be successfully implemented. A framework to determine suitability of wind parks for flat oyster restoration and recommendations for pilot studies are presented.

Materials and Methods

The conditions for the long-term development of a flat oyster bed are largely determined by four life-history processes: survival, growth, reproduction and recruitment. Tolerance ranges for abiotic and biotic environmental factors were derived from the literature to evaluate the suitability of already present and planned wind farm areas in the Dutch North Sea for flat oysters. The wind farms under study are within the limits for the factors salinity, water depth, water temperature, current velocity, food supply and oxygen concentration (Smaal et al, 2017). Selection criteria used are sea bed motion and bed shear stress, sediment composition and type of substrate, concentration of suspended particles and larval dispersal and retention. Information on the historical range of flat oysters in the North Sea (e.g., Olsen, 1883; Gercken & Schmidt, 2014; Smaal et al., 2015) was used as an extra 'habitat factor'. Bed shear stress and suspended sediment concentration was calculated with a model (van der Kaaij et al., 2017). Bed mobility data were derived from high-resolution multi-beam data and video images. For an estimate of potential oyster larvae dispersal from various locations and the chance of retention within a certain area, a particle dispersal model was used.

Results

The various sites experience different levels of sea bed shear stress. In addition, model simulations show that some wind farm sites qualify for potentially successful recruitment (Fig. 1). The objective of a pilot could be formulated as follows:

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Fig. 1. Simulated larval dispersal from the Borssele wind farm over a 10-day period. The legend presents relative concentration values.

conducting practical tests to establish (1) the extent to which flat oysters are able to survive, grow and reproduce on the chosen site, (2) whether the oyster bed is able to sustain itself through recruitment (larval production and sufficient substrate for settlement), and (3) the extent to which the oyster bed can serve as a habitat for other species.

Conclusion

Our analysis showed that a number of wind farms in the Dutch section of the North Sea are suitable for the development of flat oyster beds. This offers opportunities for multi-use in the form of nature-inclusive building. It can support the achievement biodiversity goals, restoration of ecosystem functions and enhancement of ecosystem services including future seafood production. To verify actual suitability of fixed wind parks for flat oyster restoration empirical tests are needed before large-scale restoration efforts are started. Pilots should focus on determination of survival, growth and reproduction of the oysters. In addition, expansion of the bed and self-sustainability and biodiversity of the newly established bed should be studied. For large scale development carrying capacity issues need to be taken into account. Combining oyster culture and oyster restoration is an option worth investigating. It could be very productive, as cultivation offers broodstock for larvae that can expand and maintain the natural bed and harvest adds to food security.

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NUTRIENT COMPOSITION AND PREDICTION OF IRON BIOAVAILABILITY OF FISH BY-PRODUCTS

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Introduction
Processing of fish in aquaculture generates by-products that are either used to produce low value products or are incinerated and discarded, thus increasing the energy, environmental and the financial costs of the process. Therefore, is important to evaluate the nutritional value of fish by-products.

For this reason we estimated the nutrient composition and bioactivity with regards to iron bioavailability of fish by-products (fins, skin, guts, bones, head, and gills).

Method/Design
A total of 36 meagers (6 big (1256,45 ± 232,32 g), 30 small (235,76 ± 38,45g)) and 60 sea breams (16 big (403,47 ± 72,92 g), 44 small (160,16 ± 30,79 g)) were obtained by HCMR. Institute in Crete, Greece. The fins were cut, lyophilized, homogenized and kept separately in sealed plastic boxes in deep freezer. Similar treatments were given to the skin, guts, bones, head, and gills. Nutrient composition analysis was conducted according to AOAC methods. Iron bioavailability was estimated with in vitro digestion methodology [1]. Each analysis was conducted in triplicate. The results are expressed as % per lyophilized sample.

Results
Protein content among the different samples was higher in skin (ranging from 76.2% to 43.2%), guts (59.6% to 26.9%) and gills (48.5% to 31.5%). Ash content was higher in fins (ranging from 49.1% to 45.8 for the different sample), bones (from 27.7% to 21.0%), and head (32.4% to 20.9%), with the lowest values measured in skin (15.1% to 4.4%) and guts (4.8% to 2.3%). Total lipid content was higher in guts (53.7% to 28.5%), gills (47.7% to 30.5%), head (44.8% to 37.5%) and lower in fins (7.3% to 5.17%). Differences were observed among the species. Predicted enhancement of iron bioavailability expressed as % ferrous and total dialyzable iron was higher in guts (26.3 ± 4.2% and 15.5±0.9%, respectively) and gills (11.4±6.5% and 25.0 ±7.0%, respectively).

Conclusions
These findings suggest that fish by-products have considerable nutrient composition and predicted iron bioavailability. Thus, fish by-products are exploitable.

References:
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NEW AND FLEXIBLE APPROACHES TO AQUACULTURE LICENSING

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Aquaculture is a major food production subsector receiving considerable attention as a way to fill the growing seafood supply gap, and it is a key component of the CFP and the Blue Growth agenda. Europe plans to help increase the sector's production and competitiveness and key priorities to facilitate the sustainable development of aquaculture include simplifying administrative procedures; ensuring access to space; enhancing competitiveness; and promoting a level playing field for operators. The complexity of the administrative processes necessary to obtain an aquaculture license is perceived as a barrier to development by many producers. The expected growth of European aquaculture would benefit from new regulatory and management approaches to improve the sustainability of the sector.

The European project TAPAS (http://tapas-h2020.eu/) aims to promote the sustainability of European aquaculture and to alleviate regulatory bottlenecks by providing effective tools for stakeholders at EU and a member state level. In an effort to do this a broad consultation process with stakeholders from across the sector was conducted. This involved a detailed questionnaire, which was widely circulated, supplemented with direct stakeholder interview and consultation. These consultations lead to the identification of the key bottlenecks to efficient licensing and regulation as well as to the identification of potential solutions and best practise which can help make a more efficient and effective regulatory process.

This presentation will detail the recommendations for new and flexible approaches to aquaculture licensing and regulation that emerged from this consultation process.

These recommendations, coupled with new approaches to aquaculture regulation, are currently available in a draft consultation document which is serving as a discussion document for close engagement and collaboration with industry and regulators. This discussion will highlight the most important stakeholder requirements and will ensure the acceptability and utility of the approaches recommended. These recommendations will be used to push the development of models and, modelling and decision frameworks, and for the refinement of existing tools and technologies.

The recommendations will form the basis for the development of the management tools, advice and guidance documents which will be key tools in the TAPAS Aquaculture Sustainability Toolbox and Decision Support System which will help support the development and implementation of spatial planning, enabling less costly, more transparent and efficient licensing.

SYNTHESIS OF ESCAPED FARMED SALMON IN NORWAY – GENETIC INTROGRESSION AND CONSEQUENCES FOR WILD SALMON POPULATIONS

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Genetic introgression of escaped farmed Atlantic salmon in wild salmon populations is currently on of the largest threats to Norwegian wild salmon populations. Here, our recent research on genetic introgression from escaped farmed salmon in wild salmon populations in Norway is summarised, with emphasis on how introgression is expected to affect life history traits, productivity, and viability of wild Atlantic salmon populations.

A set of SNP-markers collectively diagnostic in differentiating between wild and farmed Norwegian salmon, have been identified (Karlsson et al. 2011), and a standardized method for quantifying unidirectional genetic introgression (Karlsson et al. 2014) has been developed. From analyses of more than 20 000 individuals, we revealed significant farmed genetic introgression in 51 of 109 populations (47%) (Figure 1) (Karlsson et al. 2016).

Empirical data of proportions of farmed genome, sea-age, and size at capture from > 4000 individual adult salmon from 62 populations shows a wide spread change in important life-history traits from farmed genetic introgression, and that the effect depends on the phylogenetic origin of the wild populations (Bolstad et al. 2017) (Figure 2).

The average level of genetic introgression within a cohort was declining the fish grew older, suggesting a lower relative fitness in individuals with farmed ancestry, and a potential reduction in productivity (Figure 3).

Experiments in semi-natural streams show that wild salmon parr in sympatry with farmed salmon have lower survival than wild parr alone (Sundt-Hansen et al. 2015) (Figure 4).

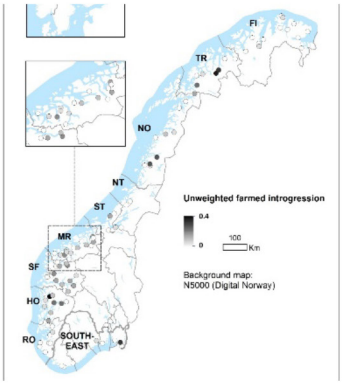

Figure 1. Estimated levels of farmed genetic introgression in 109 Norwegian wild salmon populations

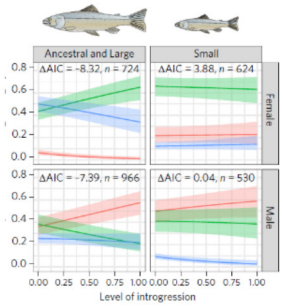

Figure 2. Effect of farmed introgression on sea age at maturation in wild salmon populations

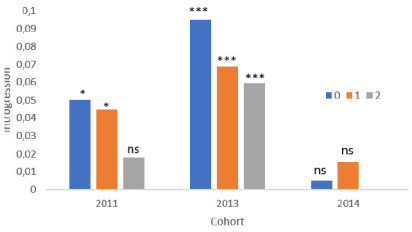

Figure 3. Genetic introgression at age 0+, 1+, and 2+ within cohorts

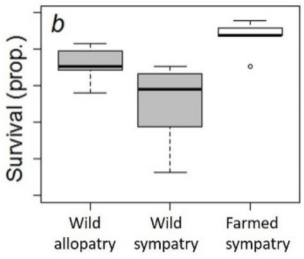

Figure 4. Survival of salmon wild parr in sympatry with farmed salmon (wild sympatry), wild parr alone (wild allopatry), and survival of farmed parr in sympatry with wild parr (farmed sympatry)

THE CONTRIBUTION OF AQUACULTURE TO POVERTY ALLEVIATION/FOOD SECURITY AMONG THE RURAL POOR IN THE FEDERAL CAPITAL TERRITORY (FCT) ABUJA, NIGERIA

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Nigeria has a huge potential for aquaculture development. However, the current domestic production of fish in Nigeria is 1.1 million metric tonnes with demand for fish consumption at 3.2 million metric tonnes per annum, creating a deficit of 2 million tonnes in supply of fish and fish products only being met by imports. The role of aquaculture in attaining household and national poverty alleviation, contribution to the conservation of natural resources and food security in Nigeria cannot be overstated. Aquaculture activities provides vital nutrition and a source of business that offers a profitable means of livelihood and promotes rural development and employment for both the local and urban dwellers alike.

To address the above challenges, this study determined among other things, the contribution and impact if any, aquaculture has on rural farmers and how this can further be improved or augmented if the farmers are empowered. The study examined measures put in place by the government to support farmers to increase their well-being and income, as well as improving and intensifying their protein dietary intake.

From the study, it came to light that aquaculture integration into other farming methods play a major role in the fight against food insecurity and reduce poverty through the production of more freshwater species of fish.

THE PERFECT RECIPE FOR CONTINUED SUCCESS – A CASE STUDY IN ENSURING A SUSTAINABLE SUPPLY OF SHELLFISH ACROSS THE GLOBE

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The UK coastal zone supports a high diversity of shellfish species which contribute over £250 million to the UK economy and their conservation and management is a high priority. Shellfish do not move far from where they settle and over their lifespan integrate bio-available chemicals from their environment into their tissues. This research sought to answer if a unique, site-specific chemical signature could be detected over time.

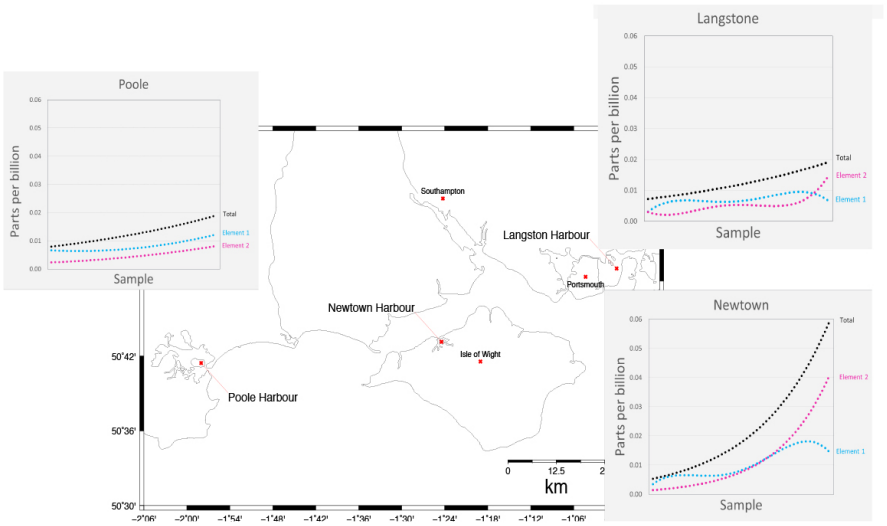
The results of this work will provide additional evidence for decision makers in conservation management and coastal planning; support the policing illegal harvesting of sensitive resources and provide certification for producers of high value, sustainably maintained stocks with a strong regional brand.

This research has developed an approach to identify where landed shellfish have been harvested that is**: Quick and simple; Cost effective; Robust and Persistent.**

Section 2: Methodology
A field trial using mussel (*Mytilus galloprovincialis*) spat from a single source beyond the study area, were grown in three south English harbours with a similar underlying geochemistry, but different surrounding land use. After a year of growth the chemical composition of the shells was determined for sixteen different trace elements encompassing common trace metals and some rare earth elements.

Section 3: Results
The field trial data provided the distribution and relative importance of each individual element as a discriminate for the growing locations of the mussels. The approach developed maximises the information from small data sets, yet can be refined as additional data becomes available.

Section 4: Conclusions
The field trial results indicate that the mussel chemical signatures have good potential to discriminate between harvest locations. In the future, these relationships might be applied as a proxy for mobile species, such as fish allowing a greater insight into their use of habitat. Ultimately, this would provide supporting evidence for ecological decision-making, and a sound basis for the protection of sensitive areas.



MOREFISH - ENHANCING PRODUCTION AND SUSTAINABILITY IN IRISH FRESHWATER AQUACULTURE

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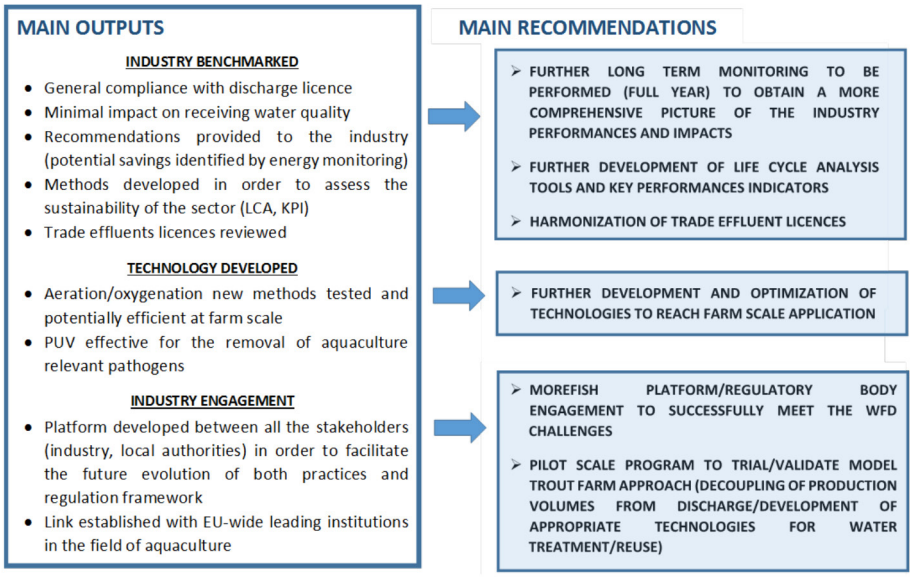
MOREFISH (2015-2017) was a multidisciplinary aquaculture project that developed and tested new innovative technologies and novel processes to improve production management and efficiencies at inland freshwater aquaculture sites in Ireland. This project addressed critically important needs identified by end-users including advanced aeration, efficient production management and benchmarking, and deployment of next-generation pulsed light disinfection technologies. These innovations will ultimately have key impacts including (i) enhancement of production efficiency and sustainability, (ii) reduction of environmental impacts of aquaculture production and (iii) improved fish health and reduced diseases/mortalities in rearing systems due to improved operating conditions. The project brought together a critical mass of engineering and scientific expertise, industry stakeholders and policy-makers, commercial operators and international experts to respond directly to pressing technical and policy gaps identified by this Department of Agriculture, Food and the Marine (DAFM) call that cites sustainable aquaculture as a priority with particular relevance to management and alleviation of finfish diseases through development of innovations. Specifically, MOREFISH targeted the development of beyond-state-of-the-art approaches to increase fish biomass yields, productivity and stocking densities, mitigate contamination and cross-infection, and reduce production costs and waste. The project partnered with a number of key pilot sites (commercial sites) to demonstrate key innovations. These pilot sites were strategically chosen to include key production facilities such as trout production and smolt for salmon production and to be representative of the whole Irish freshwater aquaculture industry. An overview of the main results from the MOREFISH project will be presented if this abstract was accepted.

The main outputs and recommendations from the project are presented on the figure below:



EFFECTS OF A SUSTAINABLE MIX OF FREE AMINO ACIDS ON FEED INTAKE IN WHITE SHRIMP *Litopenaeus vannamei* JUVENILE REARED IN AQUARIUM

Pierrick Kersanté*, Guillaume Le Reste, Luxsanawadee Soonngam, Romain Le Hen et Joël Duperray

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Due to the costs and the scarcity of marine raw materials, aquaculture feed formulators have to reduce their inclusion rates and to find alternative ingredients. Such a process is quite complex as both nutritional and functional factors have to be taken into account when selecting raw materials. Among functional factors feed palatability is one of the most important. Low levels of marine raw materials can lead to a decrease in Feed Intake (FI) in shrimps. Ingredients able to stimulate FI are therefore of primary importance. Single Amino Acids can be extracted from natural poultry keratin. The extensive acidic hydrolysis of this sustainable protein source simultaneously generates Mixes of free Amino Acids (MAA). These are among compounds known to stimulate FI in aquatic species. A trial was launched to assess the ability of MAA to influence this parameter in white shrimp, *Litopeaneus vannamei*.

A 35 days trial was carried out at the Neovia Nha Be research centre in South Vietnam. 432 healthy juveniles of *L. vannamei* with homogenous Initial Body Weight (IBW) were distributed in 24 aquariums (18 ind. per tank). Each aquarium was filled with 120 litres of brackish water. Aquariums were divided in three groups of eight tanks. Each group was fed a diet differing in MAA content: a control diet (no MAA), a diet containing 0.5% of MAA and a diet containing 1.0% of MAA (Control, MAA 0.5 and MAA 1.0 respectively). Feeds used were commercial feed on which a mix of water and MAA was sprayed. All feeds were analysed (see table 1). Animal were fed twice daily. Uneaten feed was collected to calculate Daily Feed Intake (DFI) and Feed Conversion Ratio (FCR). Mortality was checked daily. Individual weighting was performed at day 0 and day 35. Results were submitted to variance analysis and Duncan test.

Results are presented in table 2. MAA 0.5 and MAA 1.0 had no significant effects on survival and FCR. A significant positive effect was seen on Specific Growth Rate (SGR) and DFI. MAA 0.5 group had the higher SGR, followed by MAA 1.0 and control. DFI was equivalent in MAA 0.5 and MAA 1.0 groups.

This trial underlines the ability of MAA to influence FI and SGR when added on shrimp feeds. Results also suggest that a dosage of 5 kg/t is optimal in those conditions Based on its composition, it can be hypothesized that the effect on ingestion is linked to the high content of free amino acids in the product. Further research is needed to better understand how MAA can stimulate feed ingestion in crustaceans.

	Control	MAA 0.5	MAA 1.0
Protein (%)	42	42	41.7
Fat (%)	7.4	7.2	7.4
Ash (%)	10.9	10.9	11
Phosphorus (%)	1.48	1.5	1.47

Table 1 : feeds composition

	Control	MAA 0.5	MAA 1.0
SGR	2.00 ± 0.12^{a}	2.24 ± 0.16^{bc}	2.13 ± 0.13^{ab}
Survival (%)	88.1 ±6.8	93.8 ±4.6	83.3 ±15.1
FCR	2.38 ±0.15	2.27 ±0.10	2.43 ±0.25
DFI (g/ind/day)	0.353 ± 0.08^{a}	0.393 ± 0.10^{bc}	0.393 ± 0.09^{bc}

Table 2 : Growth performance, survival and daily feed intake in the 3 groups.

PROBIOTIC BACTERIA VARY IN THEIR ADHESION TO RAINBOW TROUT CELLS IN VITRO

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We investigated the adhesion properties of probiotic bacteria to rainbow trout gill cells *invitro*.

Cell tracker green labelled bacteria were statically incubated with the host cells without serum in 6-well plates at room temperature for one hour. Control samples included host cells incubated with unlabeled bacteria. Non-adhering bacteria were removed by centrifuging and washing the cells four times with 200μl DPBS with Ca^{++} and Mg^{++} at room temperature. After the final wash each sample was resuspended into 200μl DPBS and fixed by adding 200μl 4% paraformaldehyde before measuring by flow cytometry. The mean fluorescence intensity (MFI) of each test sample was divided by the MFI of bacteria in the region of interest (ROI) to determine the estimated number of associated bacteria per eukaryotic cell. *Salmonella enterica* Typhimurium (S), *Lactobacillus reuteri* (LR), *Enterococcus faecium* (EF), *Pediococcus acidilactici* (PA), *Paracoccus pantothrophus* (PP), *Bacillus subtilis* (BS) were tested in viable and heat inactivated (hi) form.

We report a novel procedure for quantifying bacterial attachment to eukaryotic cells. As a positive control *Salmonella enterica* Typhimurium was implemented that is known for its adhesion behavior to eukaryotic cells. A significant adhesion was observed with almost all tested strains only *Bacillus subtilis* did not adhere in a significant manner. Specificity of attachment was checked with heat inactivated strains.

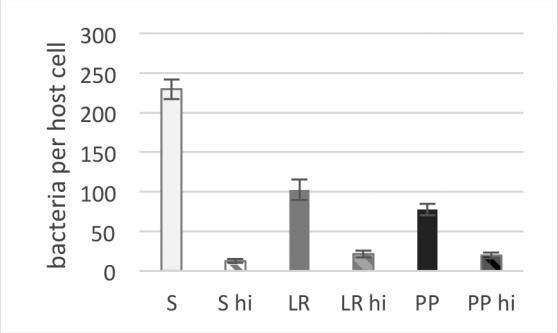

Figure 1: Viable, but not heat-inactivated (hi) *L. reuteri* (LR) and *P. pantotrophus* (PP) adhered well to RT-gill-W1 cells p3. *Salmonella* Typhimurium (S) was positive control.

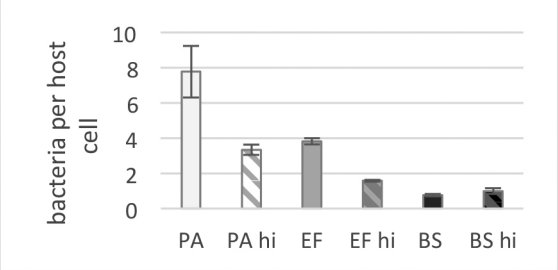

Figure 2: Viable *P. acidilactici* (PA) and *E. faecium* (EF) showed some adhesion to RT-gill-W1 cells p3, whereas *B. subtilis* (BS) did not.

INFLUENCE OF PROBIOTICS ON THE BACTERIAL BIOFILTER COMMUNITY IN RECIRCULATING AQUACULTURE SYSTEMS

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The aim of the study was the assessment of the impact of probiotic bacteria on the diversity of the microbial community of static bed filters in a recirculating aquaculture system.

Two independent 300 L recirculating aquaculture systems (RAS) were matured for two month until stocking with African catfish (*Clarias gariepinus*) at 2-4 g size at 25 fish/tank and restrictive feeding. At the same time probiotic treatment (AquaStar® Hatchery; *Bacillus subtilis, Enterococcus faecium, Pediococcus acidilactici, Lactobacillus reuteri*) started at 5 g/tank to one RAS once a week over the course of three months. The second tank served as control and was operated under the same conditions. Mechanical filters were cleaned twice a week. Temperature, oxygen, pH and turbidity was recorded daily; ammonia and nitrite every other day. Biofilters were sampled right before addition of the probiotic from both RAS and then after every 4 weeks until the experiment ended after 12 weeks. Total community DNA was extracted and used for metaprofiling analysis via 16S ribosomal RNA gene amplicon-sequencing on an Illumina MiSeq platform. Bioinformatic processing of data was performed using the QIIME pipeline, except for quality-filtering & OTU-clustering the USEARCH algorithm was applied. Taxonomies were assigned by using the RDP-classifier in combination with the GreenGenes 16S rRNA gene database. Microbial community profiles were generated, α - & β- diversity was determined and group significance statistics were done via QIIME.

No significant difference was detected between bacterial communities of both biofilters. This finding implies no interference in the native filter population of RAS by addition of probiotic bacteria to the tank. Furthermore the profiles did not change significantly over the sampling time-course, which nicely reflects the stability of the biological composition on the biofilters.

EFFECT OF SERVICE PROVISION MODEL FOR IMPROVING LIVELIHOODS OF SMALL HOLDER AND LANDLESS FARMERS OF BANGLADESH

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Bangladesh, the largest river delta in the world, depends largely on integrated and sustainable agriculture for its economic growth. Agriculture supports more than 75% of the population; 19% of them remain food-insecure (GHI 2013) whereas 31% still live below the poverty datum line (World Bank 2010). However, there are ample opportunities to harness the resources of the rural areas.

Improving food security and livelihoods (IFSL) project* is implemented from April 2015 to 2018 March aiming to help 0.127 million small holder and landless farmers of 4 districts (Khulna, Bagerhat, Gaibandha and Jamalpur) of Bangladesh. The project has forecast to promote value chain development, improve agricultural productivity and enhance the nutritional status of the beneficiaries along with increasing decision making capacity of women through local service provision model. The project deals with local service providers (LSP) so most of the development, extension, research programme are run in close coordination with them. Promoting and strengthening of LSP and SPA (association of LSPs) which act as a hinge between poor producers, private sector entities and government line agencies, helping poor to enter and successfully act in markets. SPAs support the producer group to organize into Micro and Small Enterprises (MSEs), to link with private sector.

At the end of the project, 86% households experienced a 31% rise in annual income, Landless labour increased cultivation from 31.22 to 66.72 decimal, Small holder farmers increased from 67.25 to 108.14 decimal, Local service providers increased from 59.46 to 80.42 decimal. Survey shows 96% of households are consuming three meals per day, 98.5% of households are consuming five out of the eight World Health Organization (WHO) recommended food groups. We have found that yield has increased by 32% in fish, 94% in poultry and 101% in tomato yields. After the project it is observed that most of the women of the family are engaged in making household decisions and market own produces.

The model seems effective for improving livelihoods of the rural people; hence it can be promoted for different geographical region to boost the rural people's life.

*The project is jointly implemented by United Purpose, Helvetas Swiss Intercooperation and WorldFish and funded by UKAID.

GROWTH POTENTIAL, BODY COMPOSITION AND STRESS RESPONSES IN *Pangasius hypophthalmus* WHEN CULTURED WITH INDIAN AND CHINESE CARPS

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Present study was focused to record the growth, body composition and stress responses in pangas (*Pangasius hypophthalmus*) when cultured with Indian major carps (*Labeo rohita* and *Cirrhinus mrigala*), Chinese carps (*Hypophthalmicthys molitrix, Ctenopharyngodon idella*) and *Cyprinus carpio*. The experiment was carried out in earthen ponds with an area of 0.03 ha each, for 90 days. There were three treatments and control groups, each having two replicates. Total 400 fish were stocked in polyculture system of different species at different ratios with *P. hypophthalmus*. The fish were weighed, measured at the time of stocking and after every fortnight 10 fish of each species were captured randomly by drag net for monitoring growth indices. The results indicated highest final weight, net weight gain and final length of *P. hypophthalmus* in T_1 compared to other species and higher than T_2, T_3 and control. The FCR and % SGR values of all species in T_3 were found comparatively better than T_1 and T_2 while among species the better FCR was recorded for *P. hypophthalmus*. In case of T_3, the highest % weight gain was observed for *P. hypophthalmus* (39.2%) and common carp (49.2%). Proximate analysis showed non-significant differences among different treatments. Similarly, stress responses were found in polyculture and monoculture with minimum variation. It is concluded that polyculture of pangasius with only Indian or with mixed culture of Indian and Chinese carp proved best combination with reference to weight gain and overall fish production.

COMPARATIVE STUDY ON GROWTH AND YIELD OF FAR EASTERN CATFISH AND RED LEAF LETTUCE GROWN IN AQUAPONICS SYSTEM WITH BIOFLOC TECHNOLOGY AND HYDROPONICS SYSTEMS

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A variety of production systems have been extensively studied to improve fish waste. The integration of intensive aquaculture with hydroponic vegetable production is commonly referred to as aquaponics. This study was designed to investigate the effects of various production systems [aquaponics system (AP) vs biofloc technology (BFT) system for fish, aquaponics system (AP) vs hydroponics system (HP) for red lettuce]. The aquaponics system consists of one fish tank (diameter 4.5m, height 0.7m), one sump tank (2×1×1m), plant bed with two-layer structure and two pumps to recirculate water. Microorganisms for BFT application were *bacillus* sp. and a small amount of alcohol and golden syrup was added as carbon source once a week for water quality management. Independent hydroponics system for red lettuce consists of automatic nutrient supply system, sump tank (0.5×1×1m) and plant bed with two-layer structure. Two groups of 2,200 fish (1,100 fish per group) of mean body weight of 12.7g were allotted to each of 2 tanks. Water temperature and dissolved oxygen levels were kept at 25.4±0.4°C to 28.6±0.1°C and over 6 mg O_2 L^{-1}, respectively for two production systems. Fish were fed with diet (moisture 8.9%, crude protein 49.0%, crude lipid 10.2%, crude ash 13.0%, crude fiber 2.2%) by hand at the rate of 2 to 6% of fish body weight per day for 7 weeks. Hydroponics cultivation was used as NFT (nutritional film tech) method and water electrical conductivity (EC) and pH levels were 0.9 to 1.3 dS/m and 6.55 to 7.11, respectively.

Table 1. Growth performance of far eastern catfish in two types of production systems for 7weeks.

Growth performance	AP group	BFT group
Initial fish No.	1,100	1,100
Final fish No.	1,007	1,041
Initial gross weight(kg)	14.02	14.12
Final gross weight(kg)	139.58	124.23
Initial average weight (g)	12.7±3.8^{ns}	12.6±2.4
4wk average weight (g)	41.1±6.3 ^{ns}	40.4±5.6
Final average weight (g)	138.1±8.5*	119.1±9.2
Gross feed intake (g/as-is)	156,330	152,920
WG (%)	987.4	845.2
FE (%)	88.66	79.58
SGR (%)	4.97	4.68
Survival rate (%)	91.54	94.63

Table 2. Red leaf lettuce growth in two types of production systems for 4weeks.

Growth factor	AP	HP
Leaf number	3.8	3.7
Leaf length (cm)	18.5	22.8
Leaf width (cm)	12.5	15.6
Total weight (g) per week	16.1	25.7
Dry weight rate (%)	4.87	3.97

COMPARATIVE STUDY ON GROWTH AND YIELD OF KOREAN BULLHEAD FISH AND FIVE LEAFY VEGETABLES GROWN IN AQUAPONICS SYSTEM WITH BIOFLOC TECHNOLOGY AND HYDROPONICS SYSTEMS

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A variety of production systems have been extensively studied to improve fish waste. The integration of intensive aquaculture with hydroponic vegetable production is commonly referred to as aquaponics. This study was designed to investigate the effects of various production systems [aquaponics system (AP) vs semi-recirculating aquaculture system (SR) for fish, aquaponics system (AP) vs hydroponics system (HP) for leafy vegetables]. The aquaponics system consists of one fish tank (diameter 4.5m, height 0.7m), one sump tank (2×1×1m), plant bed with two-layer structure and two pumps to recirculate water. Microorganisms for biofloc technology (BFT) application were *bacillus* sp. and a small amount of alcohol and golden syrup was added as carbon source once a week for water quality management. Independent hydroponics system for five leafy vegetables (red leaf lettuce, red sorrel, celery, beet, endive) consists of automatic nutrient supply system, sump tank (0.5×1×1m) and plant bed with two-layer structure. Two groups of 8,000 fish (4,000 fish per group) of mean body weight of 3.5g were allotted to each of 2 tanks. Water temperature and dissolved oxygen levels were kept at 25±0.5°C and over 6 mg O_2 L^{-1}, respectively for two production systems. Fish were fed with diet (moisture 5.4%, crude protein 50.4%, crude lipid 9.7%, crude ash 11.4%, crude fiber 3.0%) by semi-automatic feeder at the rate of 3 to 4% of fish body weight per day for 11 wk. Hydroponics cultivation system was compared to aquaponics as NFT (nutritional film tech) method and water electrical conductivity (EC), dissolved oxygen and pH levels were 1.2 dS/m, 10.7 mg/L and 7.1, respectively.

Table 1. Growth performance of Korean bullhead fish in two types of production systems for 11weeks.

Growth performance	AP group	SR group
Initial fish No.	4,000	4,000
Final fish No.	3,140	3,785
Initial gross weight(kg)	14.54	14.68
Final gross weight(kg)	56.05	54.25
Initial av. weight (g)	3.47±0.64^{ns}	3.56±0.75
4wk av. weight (g)	11.74±2.66^{ns}	9.46±1.88
Final av. weight (g)	17.85±5.20*	14.33±3.58
Feed intake (g/as-is)	57,649	58,693
WG (%)	414.41	302.53
FE (%)	72.00	67.41
SGR (%)	1.69	1.63
Survival rate (%)	78.50	94.62

Table 2. Five leafy vegetables growth in two types of production systems for 4weeks.

Species	Production system	Leaf No.	Total weight (g) per week
Lactuca sativa	AP	4.6	60
	HP	5.6	179.6
Rumex acetosa	AP	11.8	36
	HP	22.4	140.2
Apium graveolens	AP	6.4	27.2
	HP	6.2	77.2
Beta vulgaris	AP	4.6	26
	HP	5.4	31
Cichorium endiva	AP	5.8	54.6
	HP	10.6	172.2

FEMALE-SPECIFIC MARKERS IN PACIFIC ABALONE *Haliotis discus hannai* AND ITS CORRELATION WITH OCCURRENCE OF FEMALE ABALONES

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Development of the sex-specific markers in abalone provides valuable information for examining the productivity of the economically valuable marine invertebrate *Haliotis* abalones. In addition to zona pellucida domain 4 (ZP4) shown to be expressed in the female gonad, several genes including sperm protein (SP) and lysin (Lys) were tested for their exclusive expression. In order to examine the relationships between abalone growth and sex ratio, RT-PCR was carried out with two (small and large) size groups of Pacific abalone collected from various regions in Korea. Occurrence of the female and male abalones in a large size group determined by three sex-determination markers (ZP4, SP and Lys) reveals that the frequency of female was higher than that of male. Larger size of the shell length in female abalone of 3 years old than that of the mal indicated that female abalone may grow faster than male.

Macroscopic coloration of the gonad and their histological examinations have been used for indicating sexual maturity in abalones. To develop the sex-specific molecular markers, RT-PCR was carried out with various candidate gene including a female-specific ZP4gene. Genes encoding sperm protein (SP) sperm lysin (Lys), and two types of double-sex and mab-3 related transcription factor (DMRT) genes were tested for male-specific determination. Among five genes examined, three genes ZP4 (for female), SP, and Lys (for male) showed exclusive expression in female and male, respectively, gonads (Figure 1).

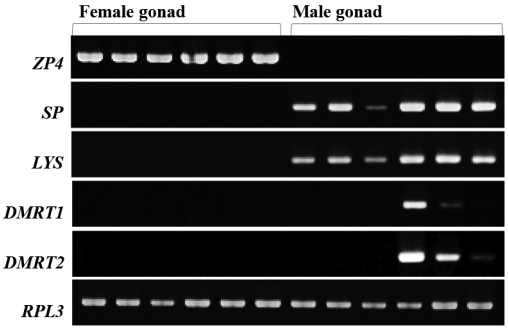

Figure 1. RT-PCR analysis used for determining expression of the markers.

To compare the frequency of female or male occurrence and its correlation with growth, abalones collected from 580 dpf were divided into two size groups (small and large). RT-PCR analysis using three sex-determination markers indicated the detection of one male and nine females from 10 large individuals. Similar results were obtained from experiments carried out with abalones at 700 dpf indicating 5 females and 3 males from 8 large abalones. These results indicated that the frequency of female in large group was higher than that of the male. Comparison of the shell length and approximately 10 mm difference in shell length of the female and male indicated its differential growth between female and male of Pacific abalone (Figure 2).

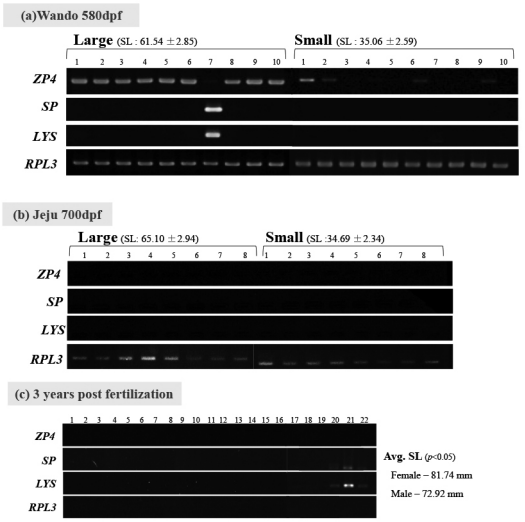

Figure 2. Detection of female- and male-specific markers in different size groups of Pacific abalones collected at 580 dpf (a), 700 dpf (b), and 3 years (c).

FISH MEAL REPLACEMENT WITH A MIXTURE OF WHEAT GLUTEN, SOY PROTEIN CONCENTRATE, POULTRY BY-PRODUCT MEAL AND TANKAGE MEAL IN DIETS FOR JUVENILE OLIVE FLOUNDER, *Paralichthys olivaceus*

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Fish meal (FM) replacement by alternative protein sources has long been of interest and will increasingly be important for the development of low-cost highly efficient aquafeeds. Therefore, this study was conducted to replace FM with a mixture of wheat gluten, soy protein concentrate, poultry by-product meal and tankage meal in diets for olive flounder. A FM based control diet was formulated to contain two kinds of FM, sardine and anchovy, by 65%. Four other diets were prepared to replace FM by 20%, 30%, 40% and 50% (FM20, FM30, FM40 and FM50) with the mixture. Juvenile flounder (BW, 5.41±0.01g) were distributed into 210L tanks at a density of 30 fish per tank with three replicates per dietary treatment. Fish were fed one of the diets to apparent satiation three times daily for 15 weeks. There was no significant difference in the weight gain (WG, %), specific growth rate (SGR, %), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival. Also, hematological parameters, hemoglobin (Hb, g/dL), hematocrit (Ht, %), aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, UL), total protein (TP, g/dL) and glucose (mg/dL), were not significantly different among all the dietary treatments. The results showed that the mixture of wheat gluten, soy protein concentrate, poultry by-product meal and tankage meal can replace FM up to 50% in diets for juvenile olive flounder..

Table 1. Growth performance, feed utilization, survival and hematological parameters of juvenile olive flounder fed the experimental diets for 15 weeks.

	Control	FM20	FM30	FM40	FM50
WG	1135±134	1214±114	1300±109	1090±41	1164±71
SGR	2.35±0.11	2.42±0.09	2.49±0.08	2.32±0.04	2.38±0.06
FCR	0.82±0.13	0.73±0.13	0.68±0.05	0.69±0.04	0.66±0.09
PER	2.10±0.34	2.31±0.42	2.49±0.18	2.42±0.15	2.56±0.39
Survival	88.0±0.0	88.0±17.0	98.7±2.3	94.7±6.1	93.3±6.1
Hb	4.55±0.50	4.63±1.02	4.23±0.31	4.17±0.23	4.27±0.38
Ht	20.8±0.9	20.8±0.3	19.9±2.2	20.5±2.4	20.9±0.2
AST	16.6±2.9	12.6±1.6	14.1±2.1	15.6±2.7	15.0±3.0
ALT	4.48±1.17	4.96±1.18	3.53±0.57	3.49±1.38	4.65±0.82
TP	7.83±0.31	7.62±0.13	7.66±0.47	7.61±0.34	8.33±0.24
Glucose	41.3±1.2	43.2±0.8	41.2±0.8	41.5±0.9	43.6±1.2

THREONINE REQUIREMENT OF JUVENILE OLIVE FLOUNDER, *Paralichthys olivaceus*

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A 14-week feeding trial was conducted to investigate threonine requirement of juvenile olive flounder using semi-purified diets containing 50% crude protein from fish meal and crystalline amino acids (CAA). Experimental diets were prepared to contain graded levels of 0.41, 0.78, 1.20, 1.76, 1.99 and 2.28%, respectively. Triplicate groups of olive flounder (BW, 23.2±0.4g) with each tank of 25 fish were fed the diets to apparent satiation three times a day. At the end of feeding trial, 0.41% group was significantly lower than all other groups in weight gain (WG, %), specific growth rate (SGR, %), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival. In whole-body analysis, ash content was significantly lower in 0.41% group compared to 1.76% group. In whole-body amino acids, threonine concentration of 0.41% group was approximately 10% lower than that of 1.76, 1.99 and 2.28% groups. The requirement of threonine in diets for olive flounder was estimated as 1.73% based on the weight gain.

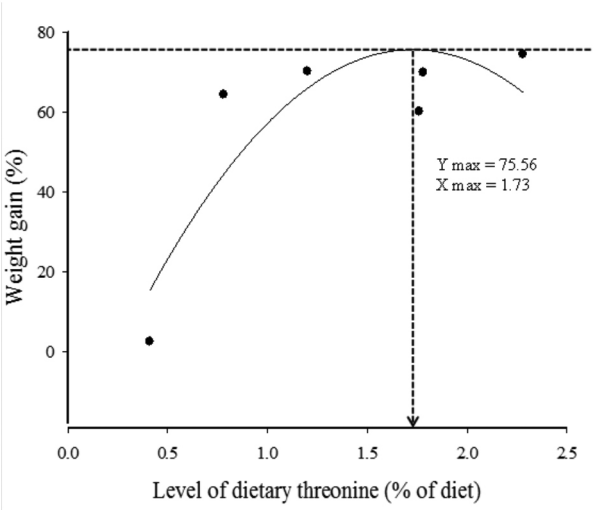

Figure 1. Polynomial regression of weight gain (%) against dietary threonine levels

Table 1. Growth performance, feed utilization and survival of the juvenile olive flounder fed the diets with graded levels of threonine for 14 weeks.

	Diets					
	0.41%	0.78%	1.20%	1.76%	1.99%	2.28%
WG	2.4±14.9^{a}	64.3±2.2^{b}	70.1±8.2^{b}	60.1±6.9^{b}	69.8±0.7^{b}	74.4±13.9^{b}
SGR	0.02±0.18^{a}	0.62±0.02^{b}	0.66±0.06^{b}	0.59±0.05^{b}	0.66±0.01^{b}	0.69±0.10^{b}
FCR	-1.12±7.22	0.84±0.02	0.74±0.13	0.86±0.05	0.69±0.06	0.73±0.17
PER	0.09±0.58^{a}	2.37±0.07^{b}	2.76±0.44^{b}	2.33±0.12^{b}	2.89±0.27^{b}	2.86±0.71^{b}
Survival	20.0±28.0^{a}	97.3±4.6^{b}	97.3±2.3^{b}	100±0^{b}	98.7±2.3^{b}	94.7±2.3^{b}

EFFECTS OF DIFFERENT DIETARY LEVELS OF FISH MEAL ON GROWTH PERFORMANCE FOR CATFISH *Clarias gariepinus*

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Catfish (*Clarias gariepinus*) is one of most important and widely cultivated fish in Indonesia because it grows fast, feeds on a large variety of agriculture by-products, and tolerates high concentrations of ammonia and nitrite. The decreased use of fish meal (FM) in fish diets can reduce the cost of feeds. Therefore, this study was conducted to investigate the different levels of fish meal inclusion in diets on the growth performance of catfish.

Triplicate groups of fish (average body weight, 2.0±0.01 g) were fed one of four experimental diets containing 12, 14 and 16% of fish meal and one commercial diet (designated as FM 12%, FM 14%, FM 16% and COM, respectively) for 11 weeks.

After the 11-week feeding trial, final body weight of fish fed FM 12% diet was not significantly different ($p<0.05$) from that of fish fed 16% and commercial diets, but significantly higher than that of fish fed 14% diet. However, the lowest feed conversion ratio was found in fish groups fed FM 16% ($p<0.05$). Average daily gain (ADG) showed the similar trend in final body weight. No significant differences were observed in survival among all the fish groups ($p>0.05$).

The results indicate that dietary supplementation of 12% fish meal is optimum inclusion level in order to reduce the cost of feeds for catfish.

Table 1. Growth performance of catfish fed the experimental diets for 11 weeks

	Diets			
	FM 12%	FM 14%	FM 16%	COM
IBW	2.0±0.00	2.0±0.01	2.0±0.01	2.0±0.01
FBW	$94.2±5.2^{bc}$	$86.2±1.7^{a}$	$99.9±3.3^{c}$	$91.6±2.95^{ab}$
FCR	$0.98±0.05^{a}$	$1.03±0.04^{a}$	$0.89±0.02^{b}$	$0.99±0.01^{a}$
ADG	$1.71±0.11^{bc}$	$1.57±0.03^{a}$	$1.84±0.07^{c}$	$1.67±0.06^{ab}$
SR	80.6±1.14	83.8±2.10	83.8±4.22	82.3±2.53

IBW= initial body weight (g), FBW= final body weight (g), FCR= feed conversion ratio, ADG= average daily gain (g/day), SR= survival rate (%).

GROWTH PERFORMANCE OF JUVENILE CATFISH *Clarias gariepinus* WITH DIFFERENT BRAND OF COMMERCIAL FEEDS IN INDONESIA

Sung-Sam Kim[1]*, Wahyudi Wahyudi[1], Angga Aditya Putra Nugraha[1], Mufti Islam Insani[1], Much Ali Adlan[1], Yanuar Su'bah Assaf[1], Lativa LM[1], Jeong-Dae Kim[2], Seung-Jun Shin[2]

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Fish nutritionist aims at producing a balanced commercial feed that promotes optimal fish growth and health. Good quality feed may provide maximum utilization of diets resulting in fast growth and high feed efficiency of the fish. On the contrary, poor quality feed leads to leaching of nutrients and decrease in feed efficiency. Therefore, it is important to standardize the quality of commercial feed for the target species in aquaculture for optimum production. This study was designed to compare the growth responses, feed efficiency and survival rate of catfish (*Clarias gariepinus*) fed different commercial feeds. Triplicate groups of fish (initial body weight, 1.51±0.04 g) were fed one of four commercial diets (A, B, C, and D) for 12 week. The proximate compositions of the commercial feed are shown in Table 1. After the 12-week feeding trial, final body weight of fish fed diet B was not significantly different from that of fish fed A and C diets, but significantly higher ($p<0.05$) than that of fish fed D diet. The highest feed conversion ratio was found in fish group fed diet D. No significant differences were observed in ADG and survival among all the fish groups ($p>0.05$). Present study showed that quality of commercial feeds was different depending on the brand. The findings in this study suggest that fish farmer should bear the quality of commercial feeds in mind when choosing them.

Table 1. Proximate composition of different commercial diets (% dry matter)

	Commercial Diets			
	A	B	C	D
DM	90.6	91.0	90.5	91.3
CP	40.4	36.4	37.0	36.2
CL	7.1	8.4	7.3	6.6
Fiber	3.1	3.6	3.4	3.2
Ash	11.3	10.5	9.9	10.7

Table 2. Growth performance of catfish fed the commercial diets for 12 weeks

	Commercial Diets			
	A	B	C	D
IBW[1]	1.49±0.03	1.53±0.07	1.51±0.01	1.51±0.01
FBW[2]	75.95±5.21^{ab}	80.59±5.26^{b}	74.92±4.61^{ab}	69.67±3.77^{a}
FCR[3]	0.96±0.05^{a}	0.98±0.05^{a}	0.97±0.01^{a}	1.05±0.00^{b}
ADG[4]	0.93±0.07	0.99±0.07	0.92±0.06	0.85±0.05
SR[5]	88.00±5.29	82.00±8.72	88.67±4.16	88.00±5.29

[1]IBW= initial body weight (g), [2]FBW= final body weight (g), [3]FCR= feed conversion ratio, [4]ADG= average daily gain (g/day), [5]SR= survival rate (%).

MOVEMENT CHARACTERISTICS OF SEA CUCUMBER, *Apostichopus japonicas* ON SURFACES OF SHELTER MATERIALS USING PARTICLE IMAGING VELOCIMETRY

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Movement characteristics of sea cucumber, Apostichopus japonicas on surfaces of varying degrees of roughness (a. PVC [0.68–0.9 mm], b. cement mortar [1.5–1.8 mm], and c. porous cement mortar [2–2.2 mm]) were classified using particle imaging velocimetry (PIV) and 2D velocity fields to identify the mechanisms of maneuverability. Six sea cucumbers were collected by a diver off Yeosu in South Korea with initial weights of 102 ± 10.2 g for large and 20.14 ± 2.3 g for small size groups. Experiments were conducted in an acrylic water tank (400 × 260 × 300; L × B × H) with a water temperature of 15°C. Sea cucumbers were acclimated for 7 days in a recirculation aquaculture system. Plates with different degrees of surface roughness, PVC, cement mortar, and porous cement mortar, were installed at the bottom of the tank. The experiment was replicated 10 times. Different velocities and forces were generated when the sea cucumbers moved to shelter materials of varying degrees of surface roughness by using PIV measurements (force: PVC > porous cement mortar > cement mortar). Sea cucumbers produced the greatest amount of force when moving over smooth surfaces like PVC. However, forces were minimized during movements on cement mortar (surface roughness 1.55–1.96 mm), suggesting that they move most efficiently along cement. Although sea cucumbers prefer rough materials, it is thought that a material on which they can attach all their tubercles, such as cement or stones rather than a porous cement material, may be the most suitable structural material.

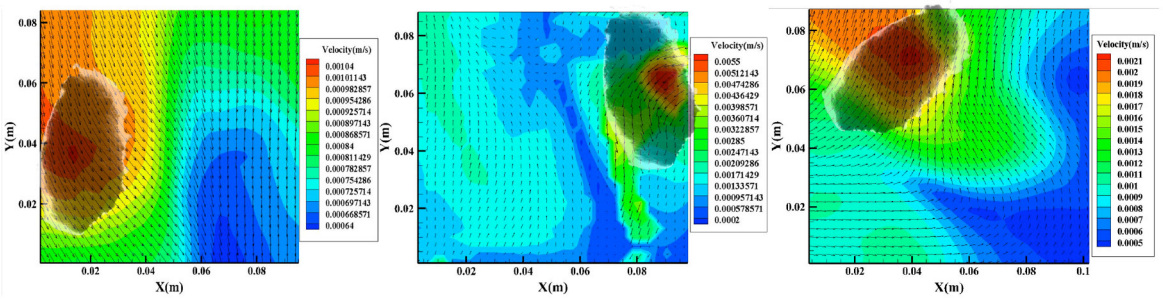

Fig. 1. Velocity distribution of large *A. japonicus* in shelters made of different structural materials using PIV: (a) PVC, (b) Cement mortar, and (c) Porous cement mortar.

INTERACTION BETWEEN *Ceratothoa oestoides* AND *Dicentrarchus labrax* IN AQUCULTURE (EASTERN ADRIATIC SEA, CROATIA)

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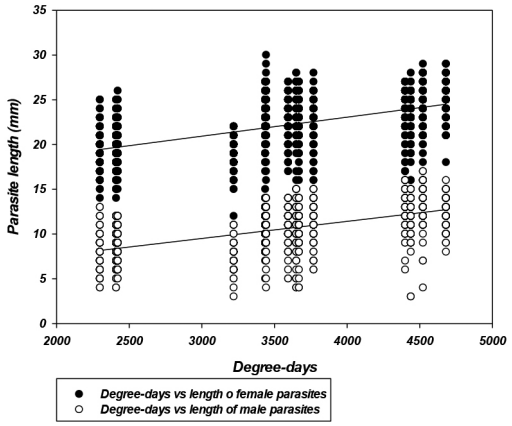
The cymothoid isopod (*Ceratothoa oestroides*, Risso 1916) is protandric hermaphroditic parasite. It is parasitizing in the buccal cavity and can cause significant production losses in cage reared sea bass (*Dicentrarchus labrax*). Biology of this parasite is often studied in laboratory conditions, while the literature data on the biology of this parasite in farming conditions are scarce. Therefore, the aim of this study is to collect data on the biology *C. oestroides* in the sea bass cage farming conditions in the Adriatic Sea.

The experiment was carried out at farm within 14 commercial net cages of 16 m diameter stocked with 200.000 fingerlings. Visual control of the entire population of fish in each cage was carried out consecutively between 2300 and 4700 degree-days (DD) of production. Total of 100 infected individuals were excluded at each sampling. Afterwards, biometric analyses were performed on infected fishes and on *C. oestroides* males and females. Sexual maturity and fecundity on *C. oestroides* females was determined. In addition, sea temperature were recorded daily.

Fish infected with *C. oestroides* were found in all cages. Overall, the percentage of infection ranged from 0.19% to 2.53%. Also, mature females were found in all cages, in range from 2% to 18%. Pearson correlation coefficient showed that there was a statistically significant and moderate positive correlation between DD and length of female parasites ($r = 0.512$, $p < 0.001$) and statistically significant and strong positive correlation between DD and length of male parasites ($r = 0.601$; $p < 0.001$; Figure 1). A statistically significant but weak positive correlation between the length of the female parasites and the number of pulli which it contains ($r = 0.375$, $p < 0.001$), as well as statistically significant strong positive correlation between the length of the fish and the length female ($r = 0.678$, $p < 0.001$) and male ($r = 0.607$, $p < 0.001$) parasites were also established. However, the correlation between DD and percentage of mature females was not established. The obtained data represent a further basis for considering the biology of this parasite in farm conditions in Adriatic Sea.

Figure 1. The relationship between the degree-days and length of females and males parasites.



MYCOTOXINS IN AQUAFEEDS: POST-HARVEST MEASURES FOR AQUAFEED PRODUCERS TO PREVENT CONTAMINATION IN THE FINISHED DIETS

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Mycotoxins are toxic, secondary metabolites produced by fungi under specific environmental conditions on almost all agricultural commodities worldwide. Nowadays, the higher inclusion of raw materials derived from plant sources in fish diets in addition to climate change has enhanced the risk of mycotoxin contamination in aquafeeds. The best strategy to prevent mycotoxins in aquafeeds is to avoid the inclusion of contaminated raw ingredients in the diets. Therefore, plant ingredients should be checked for mycotoxin contamination as soon as possible before the feed production starts. This study aimed to define a proper mycotoxin-monitoring plan for plant ingredients including a mycotoxin test procedure (sampling, sample preparation, and analysis) and the comparison with accept/reject limits.

A literature study was conducted in order to determine the accept/reject limits in complete feeds for different fish species. After combining the limits suggested within European Commission legislation and the information derived from peer-reviewed scientific articles, the action limits were defined. These limits were applied to raw ingredients using the maximum inclusion level that each ingredient can be found in the diets (Table 1). Batches of plant raw ingredients are sampled after their arrival to the factory according to the guidelines of European Commission for sampling mycotoxins in feedstuffs. A rapid test for mycotoxin detection in raw ingredients (NEOGEN Reveal Q+ kits) was used to give quantitative results for the following mycotoxins: Aflatoxin B1, Ochratoxin A, T-2/HT2, DON, Fumonisin B1 and Zearalenone. The results from the mycotoxin analysis are compared with the maximum acceptable limits as defined for the raw ingredients in order to accept or to reject batches. Finally, another applicable preventive measure is the inclusion of a mould inhibitor (Fylax Forte) in the recipes that aids in the prevention of fungal growth in the finished feeds during storage.

Preventive measures cannot however guarantee the absence of mycotoxins from the diets. The adverse effects of mycotoxins that may exist in the complete feeds can be eliminated with the supplementation of a mycotoxin binder (Mycosorb A+) within the diets that can prevent their absorption by animals.
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Table 1. Maximum acceptable mycotoxin limits in μg/kg (ppb) for plant ingredients commonly used in Coppens diets that can be applied to all target species.

	Wheat	Wheat gluten	Corn gluten	Soybean meal
Aflatoxin B1	18	51	101	50
DON	525	4044	3033	1500
Fumonisin B1	17501	50556	101112	50000
Ochratoxin A	175	506	1011	500
Zearalenone	525	1517	3034	1500
T-2 & HT2	350	1011	2022	1000

RESEARCH PROGRESS ON GENETIC EVALUATION OF THE GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* BREEDING POPULATION

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Farming of the giant freshwater prawn, *Macrobrachium rosenbergii*, is a rapidly growing industry, due to its high commercial value and tolerance for water quality changes. In China, a project aimed at establishing a selective breeding program to improve growth and survival for the cultured giant freshwater prawn was initiated in 2006. Genetic parameters and response to selection were estimated for harvest body weight using a fully pedigreed synthetic population from three introduced strains. The data included 118,922 progeny that were from 724 sires and 1218 dams in eleven generations with a nested mating structure. Variance components and genetic parameters were estimated using an animal model and the restricted maximum likelihood method. When data from all generations (G0-G10) were included in analysis, the heritability and common environmental effect estimates were low (0.065±0.010 and 0.053±0.004, respectively) and significantly different from zero (P<0.05). By G10 generation, the average coefficient of relationship between families was 4.98%. The response to selection in harvest body weight was estimated from the difference in the least squares means of body weight for the selection and control populations, while the predicted response was obtained from the difference in the mean breeding values between generations. After performing ten selections, the realised response was 41.46% and the harvest body weight of the nucleus breeding population was 60% higher than the wild population.

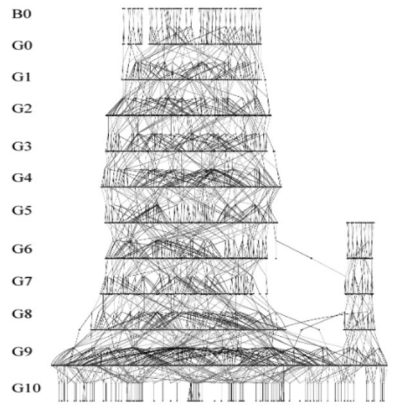

FIGURE 1 Pedigree of the nucleus breeding population

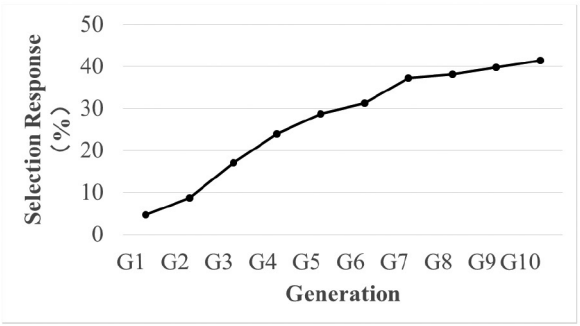

FIGURE 2 Cumulative selective response

OPTIMIZING CELL WALL DISRUPTION OF *Tetraselmis chuii* AND *Phaeodactylum tricornutum* BIOMASS FOR RELEASE OF NUTRIENTS AND BIOACTIVE COMPOUNDS FOR AQUAFEED AND FOOD APPLICATIONS (PRELIMINARY RESULTS)

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The aim of this study is to optimise cell wall disruption using bead milling, of *Tetraselmis chuii* and P*haeodactylum tricornutum,* for efficient release and preservation of the quality of nutrients. The microalgae biomass was provided by University of Bergen/Uni Research produced at the National Algae pilot Mongstad (NAM), Mongstad, Norway in a fed-batch process in four 800L photobioreactor systems (GemTube MK2-750 from LGem b.v). A Dyno-Mill Multi Lab (WAB; Willy A. Bachofen AG Maschinenfabrik, Muttenz, Switzerland), was used for the microalgae cell wall disruption experiments. For *Tetraselmis chuii* (*T. chuii*) a full factorial statistical design was chosen, whereas for the optimization study of cell wall disruption in *Phaeodactylum tricornutum* (*P. tricornutum*), a central composite design was used.

Cell wall disruption efficiency of *T. chuii* was affected by dry matter (DM), flow rate, and the interaction of flow rate with tip speed. In the case of *P. tricornutum* it was flow rate, tip speed, and the interaction of the two, that induced statistically significant effects (Figure 1). We achieved very high disruption efficiency (over 99%) for *T. chuii* biomass but lower for *P. tricornutum* (max close to 75%) for the same range of test factors, demonstrating the need for optimisation of cell wall disruption processing for each species. We saw higher lipid extraction efficiency by bead milling compared to non-processed biomass of species. Oxidative stability of the biomasses based on Oxipres analysis was reduced after cell wall disruption, whereas drying itself did not reduce the levels of highly unsaturated fatty acids such as EPA and DHA. Last, the cell wall disrupted microalgae biomasses contained slightly lower levels of the smallest compounds which may be due to standard analytical error or losses during the spray drying process.

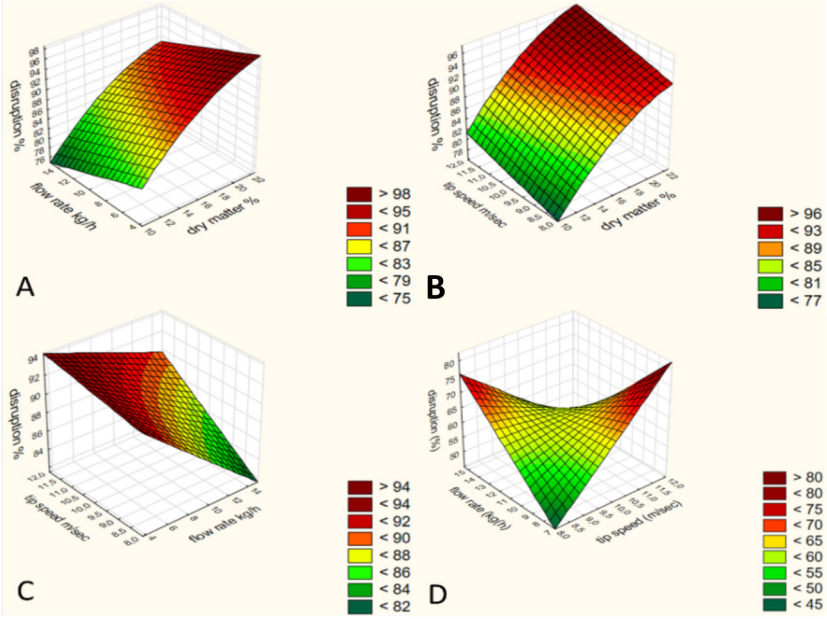

Figure 1. Response surface models for the prediction of cell wall disruption efficiency (D%) by bead milling in T. chuii and *P. tricornutum*: A. D% of *T. chuii* biomass as a response of flow rate and biomass dry matter content, B. tip speed and biomass DM and C. tip speed and flow rate; D. D% of *P. tricornutum* biomass as a response of flow rate and tip speed. For A B and C, the level of the constant design variable represents the centre point in the design.

ASSESSMENT OF THE PHYSIOLOGICAL STATUS OF RUSSIAN STURGEON OBTAINED WITH USING FROZEN-THAWED SPERM

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Nowadays interest in the fish sperm cryopreservation methods for the usage in fish farming is increased. However, at the moment these methods have not been widely applied in practice, perhaps because of the fish grower's fear that the obtained juveniles will differ from those traditionally obtained.

The aim of the work was to receive life-resistant Russian sturgeon (*Acipenser gueldenstaedtii* Brandt&Ratzeburg, 1833) offspring using cryopreserved sperm and to assess the offspring quality.

Low temperature preservation of male reproductive cells of sturgeon fish was conducted according to previously developed methods. Cryoprotection media consist of NaCl, KCl, $CaCl_2$, $NaHCO_3$, sucrose, mannitol, dimethyl sulfoxide (5%) and egg yolk (10%). Behavioural responses of the offspring were evaluated in the «open field» test, which was conducted by placing one individual (pre-larvae, larvae or juveniles) in a special installation, with a coordinate grid, where the number of the coordinate lines intersections by the object was registered. The fish were affected by the following stimulus: light, illumination 20 Lx, low-frequency signal (frequency 20 Hz), bright light (100 Lx), a high-frequency signal (300 Hz), vibroacoustic stimulus. The test was subjected to 30 individuals in the experimental and control groups at the 1st, 8th and 15th day after hatching.

The publication was prepared with the use of the Unique scientific installation "Modular installation-complex» of SSC RAS and Bioresource collections of rare and endangered species of SSC RAS No. 73602.

In case of the fertilization by a thawed sperm of Russian sturgeon in industrial conditions, in the experimental batch, the percentage of fertilization was 60%, in the control batch – 80%. In the analysis of morphometric indicators fish obtained by using cryopreserved sperm had an advantage in comparison with the traditionally obtained individuals.

No differences were revealed in the evaluation of the reactions of pre-larvae, larvae and juveniles, obtained by traditional technology and with the use of cryopreserved sperm.

The usage of cryopreserved sperm makes it possible to obtain resistant juveniles and can be recommended for using in artificial reproduction hatchery. This will allow obtaining genetically diverse offspring, will reduce the area and the cost of males maintaining, thereby allowing to increase the production of females stock.

Work performed under the Federal Program «Research and development on priority directions of scientific-technological complex of Russia for 2014-2020» on the project "Development of technical equipment, biotechnology, growing unconventional species of fish and invertebrates for the progress of aquaculture North-Western and southern Federal districts of Russia" (agreement № 14.607.21.0163 on 03.10.2016, at the unique identifier applied research (project) RFMEFI60716X0163), and Grant MK-115.2017.11.

APPLYING SOCIAL INDICATORS TO SUPPORT KNOWLEDGE TRANSFER FOR AQUACULTURE WITHIN THE "BLUE GROWTH STRATEGY"

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At the heart of one of the underlying challenges for implementing the Blue Growth strategy is how to build and implement the knowledge transfer mechanisms between different stakeholders and multiple levels. This is essential to foster a collaborative effort towards Blue Growth in Europe, whilst recognizing the contextual nature of marine resource use within the different Member States and different coastal regions. This has made operationalising Blue Growth difficult, since social interaction with marine resources has not been established out of the blue. Rather, each region inherits a long heritage of knowledge of ocean use that needs to be endorsed and transferred if sustainable aquaculture development under the Blue Growth strategy is to be fostered. Indeed, this is mirrored in the international problem that there is a gap in knowledge exchange between the aquaculture industry, policymakers trying to support aquaculture development and people who may oppose or depend on aquaculture for a job and/or food source. Using a social-ecological system thinking perspective we harnessed the assessment framework developed by the International Council for the Exploration of the Sea ICES Working Group on Social and Economic Dimensions of Aquaculture (WGSEDA), to explore a range of social indicators and metrics to understand how they could be mobilized to achieve measurable and applicable outcomes for assessing the social effects of the Blue Growth Strategy. First results show, that the placed-based and highly social-contextual nature of aquaculture production and the importance of the social construction of knowledge pertaining to certain aspects of aquaculture must be acknowledged, next to its potential to addressing and fostering global food security.

QTL MAPPING FOR BODY WEIGHT IN EARLY GROWTH STAGES OF COMMON DENTEX *Dentex dentex*

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Common dentex (*Dentex dentex*) is a species of great ecological interest with high commercial value for fisheries and promising perspectives in aquaculture. Early selection based on markers has the potential to speed up the prosses of genetic gain. In this study, 1981 SNP markers were generated using ddRAD sequencing of 133 individuals belonging to two full-sib families (79 and 51 offspring, respectively) with a common female parent. A total of 1911 SNPs were successfully mapped into 24 linkage groups (LODscore=7), with the Lep-MAP2 software, for the construction of the first genetic linkage map. Seventy of the originally genotyped markers were removed due to missing parental alleles and genotyping errors or because they could not be assigned to any linkage group. The sex-averaged linkage map has a total length of 1958,66 cM, with an average marker interval of 1,02 cM. The linkage map is relatively dense, but there is a big gap (45 cM) in linkage group 1, which may suggest that this LG comprises 2 different groups. This linkage map was then used to map quantitative trait loci (QTL).

For the QTL detection affecting early body weight, Variance Component Analysis (VCA) using maximum likelihood was applied on linkage groups with the QxPak 5.05 software. Nineteen QTL for early body weight were detected in 9 linkage groups. The proportion of phenotypic variance explained by the QTL ranged from 0.13% to 4.19% (chromosome wide scan). The two significant QTL in linkage group 17, explained almost half of the variance of all QTL detected (i.e. total QTL variance).

This first linkage map and QTL mapping will give insights for investigation of the genomic regions affecting growth traits. The results can be used for comparative genomics analysis with other Sparidae species. Further investigation of these results will also be made for QTL mapping of the other available morphometric features (total length, standard length and width).

LG	Number of QTL	Highest log likelihood ratio	Explained variation %
3	4	140.39	0.20
8	1	47.98	1.65
11	2	153.57	0.13
12	1	29.09	1.65
14	2	27.14	0.19
17	2	26.95	**4.19**
19	3	76.59	0.41
21	3	77.83	0.37
23	1	158.98	0.16
Total	**19**	-	**8.95**

Table 1. The number of QTL for early body weight detected in each linkage group and the proportion of phenotypic variance explained by the QTL.

Body Weight (g)

Statistics / *Groups*	Mean ± SD	Median	Range		Offspring N.
			min	max	
Family 1	1.82 ± 0.59	2.01	0.62	2.89	79
Family 2	1.84 ± 0.65	1.84	0.77	3.01	51
Total	1.83 ± 0.61	1.99	0,62	3.01	**130**

Table 2. *Statistics for body weight in early stages of the 130 offspring in the two families.*

RESISTANCE TO *Flavobacterium psychrophilum* IN ISOGENIC LINES OF RAINBOW TROUT: DOES THE INFECTION ROUTE MATTER?

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Flavobacteriosis is a bacterial disease of adult (Bacterial Cold-Water Disease, BCWD) or young (Rainbow Trout Fry Syndrome, RTFS) rainbow trout caused by *Flavobacterium psychrophilum* (*Fp*). The disease causes substantial mortality in young fish, resulting in major economic losses worldwide. Due to the lack of efficient commercial vaccine, the only way to fight the disease is to use antibiotics, which raises environmental concerns. Selective breeding for fish naturally resistant to BCWD/RTFS is a promising solution, but a reliable test to evaluate resistance of fish is needed to carry out selection. Experimental infectious challenges with *Fp* are usually performed using intramuscular (IM) or intraperitoneal injection as a route of infection which is easy to manage but not representative of natural infection since it bypasses physical and immune barriers likely to contribute to host defense (e.g. mucus, skin…). Bath infection (BA) is expected to be closer to the natural infection but is more difficult to manage in practice. No data is available on the existence of potential interactions between the route of infection and the fish genotype (i.e., underlying resistance mechanisms). We therefore compared the effect of the route of infection by *Fp* (IM *vs* BA) on the survival of a set of isogenic rainbow trout lines.

Fish from fifteen homozygous isogenic lines, previously established as described in Quillet *et al*. (2007), were screened for resistance to *Fp* using IM or BA as route of infection. For the IM challenge, 20 to 40 individuals per line (mean weight 5g) were injected (15 CFU/fish) with strain FRGDSA 1882/11. For each replicate, fish from the different lines were gathered in a single 300 L tank. For the BA challenge, fish from each line were distributed into two 10 L aquaria (50 fish per line, mean weight 2.4g) and infected by immersion during 24h in a bacterial suspension in water maintained at 10°C under vigorous aeration (2 replicated challenges, 15 aquaria each). Bacterial concentration in aquarium water ranged from 4.10^5 to 2.10^6 CFU/ml at the beginning and after 24h of immersion, respectively. For both types of challenge, dead fish were removed twice a day during 4 weeks. The statistical analysis of kinetics of mortality was carried out with the Survival Kit software (Mezaros *et al*, 2013).

Whatever the type of challenge (IM and BA), fish lines exhibited significant differences in survival rate and kinetics of mortality. Therefore, both routes of infection revealed genetic variability among lines and could be used for selection. When lines were classified according to resistance, there was a significant re-ranking among challenges. Interestingly, several lines that were classified as highly susceptible when subjected to a IM challenge showed a marked increase in relative resistance when challenged using the BA route. This supports the hypothesis that BA and IM challenges trigger different defense mechanisms and that external barriers (e.g. skin or mucus) may share a significant role in the resistance of some lines. Thus, using BA or IM experimental challenge in a breeding program will likely select fish with different resistance mechanisms.

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Quillet, E., Dorson, M., Le Guillou, S., Benmansour, A. & Boudinot, P. (2007):Wide range of susceptibility to rhabdoviruses in homozygous clones of rainbow trout. *Fish Shellfish Immunol*, Vol: 22 Iss: 5 pp: 510-519

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AN OVERVIEW OF AQUACULTURE IN INDONESIA

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Indonesian fisheries production in 2016 reached 23.51 million tons (Mt) with a contribution of 6.83 Mt (29.06%) of capture fisheries and 16.68 Mt of aquaculture production (70.94%). Indonesian aquaculture production in 2016 increased by 5.35% compared to 2015 (CDSI, 2017) and is dominated by aquatic plants (11,269,341 ton y-1) 70%, (especially seaweed) and by inland waters production. The main aquatic commodities producecd in brackish water are shrimp (602,000 tons y-1) and milkfish *Chanos chanos* (625,300 tons y-1), while the main freshwater fish commodities are *Oreochromis niloticus* (1,039,000 tons y-1), *Clarias gariepinus* (719,600 tons y-1), *Cyprinus carpio* (461,100 tons y-1) and *Pangasianodon hypophthalmus* (339,100 tons y-1) [FishStatJ 2016-2018]. The only large-scale native species is *Osphronemus goramy* (113,400 tons y-1), which has been traditionally reared for decades on Java Island.

One of the Indonesian goverment programs is to increase the aquaculture production which will support the welfare of the fish farmer community, however, such an increase has to face the following requirements and/or limitations : limited provision and distribution of superior broodstock and quality seeds; increasing the demand for safe products, both foreign and domestic markets; feed efficiency; quality of the aquatic environment which decreases as a result of agricultural and domestic wastes; threat of disease, from local and foreign origin; limited facilities and infrastructures of aquaculture fisheries, mainly related to the condition of waterways, production roads, electricity grids (DGA, 2015). To address these problem, Indonesian goverment has made several policies such as developing a program of local feed self–suffiency known as "GERPARI" and giving Indonesia national registration for the feed produced; establishing the national broodstock center program and composing the standards in producing superior seeds; providing a good aquaculture practice certificate to the community hatchery unit so that the seeds produced are healthy and uniform; conducting training related to the financial management and providing free land certificates as grants to obtain soft loans from the banks.

The results of the implementation of the program are expected to increase the availability of aquaculture products and their value and to expand the widespread readiness of the community for business and employment opportunities in the field of aquaculture which will be characterized by an increase in the number of new labor and business groups.

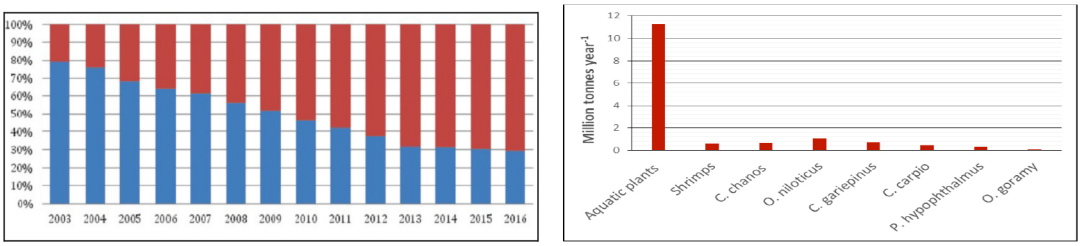

Figure 1. Contribution percentage of capture fisheries (■) and aquaculture production (■) and the main aquatic produced (Million Tonnes Year^{-1}) in Indonesia (Source : DCSI, MMAF. 2017 and FishStatJ, FAO. 2016-2018).

HISTOPATHOLOGICAL CHANGES IN THE SKIN OF *Heteropneustes fossilis* CAUSED BY ARSENIC

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The investigation of histological changes in the organs of fish is an accurate way to assess the effects of chemical toxicity. Hence, the study was undertaken to examine the effect of different sub lethal concentrations of arsenic on the histology of skin of Indian catfish *Heteropneustes fossilis*.

Live specimens of adult *H. fossilis* (body weight 38–47 gm) were procured from the local market and acclimatized to laboratory conditions for two weeks in 50L plastic tanks each containing 15 fishes. Wire nets were used to cover the tanks in order to prevent the jumping out of the fishes. During acclimatization, the fishes were provided with commercial dry fish feed pellets *ad libitum* at approximately 2%–3% of body weight of the fish/day. Fishes were divided into three groups. Groups I and II were exposed to sub lethal concentrations of arsenic i.e. 4 and 8 ppm, prepared from the stock solution of arsenic (Merck, Germany) and group III was maintained in arsenic free water to serve as control for 3,6 and 15 days. Each group was maintained in triplicate.

Histo-pathological Analysis: Small pieces of skins were taken and immediately fixed in 10% neutral buffered formalin after 3,6, and 15 days from each group i.e. group I, II and III. Fixed tissues were processed routinely for paraffin embedding. Embedded tissues were cut into sections of 5-7μ thickness and then stained with Harris' hematoxylin and eosin stains and studied under microscope.

The skin from exposed fishes showed morphological and histological alterations. Externally the skin showed hyper and hypo-pigmentation and there are damages in epidermal layer. Histo-pathologically it showed hyperplasia of mucus producing cells, epidermal and dermal poly-morph dermal necrosis and aggregation of nuclear leukocyte infiltrations, areas of melanin pigments. Fishes from the control group showed no histopathological changes. It was also observed that the damages were more in fishes exposed to higher concentrations of arsenic.

In conclusion, the present study showed that histo-pathological changes in the skin of the experimental fishes were due to arsenic toxicity. Also, the results showed that the degree of damage to the tissues was proportional to the concentration of the arsenic.

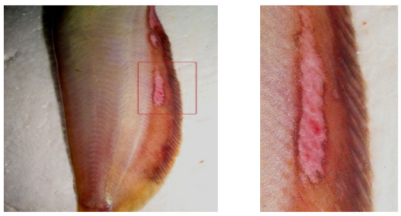

Fig 1: Damaged skin of *H. Fossilis*

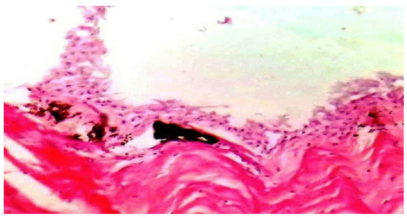

Fig 2: Microscopic view of skin

NUTRITION, A LIMITING FACTOR FOR PERFORMANCE, WEIGHT GAIN, TILAPIA STRAINS HIGH IN SUB-SAHARAN AFRICA. CASE OF PEASANT FARMING IN THE WEST OF THE DRC

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Tilapia is one of the main fish in Africa (Carlos Lietar, FAO, 1984) However, despite the potential of exploitation this fish, breeding seems not yet mastered in sub-Saharan Africa. The West of the DRC specifically in the provinces of Congo-Central ex Bas-Congo, Kwango and Kwilu (from the dismemberment of the former province of Bandundu) tilapia is the main species farmed, but with low weights average at harvest, 48 g to 6 months at the farmer and 80 g to the Kiyaka Station. Compared with the frozen tilapia sold in the DRC and imported from China or Thailand with an average weight of 250g, the following questions would find answers:

The low weight of Tilapia to the West of the DRC would be due to a genetic degeneration of the local strain.

The low weight of Tilapia to the West of the DRC would be due to bad breeding system

To answer the question of whether the low weight of tilapia to the West of the DRC was due to a genetic degeneration or simply the wrong system of breeding, we have introduced a new strain of tilapia that we compared the performance of gain weight in the same conditions with the local strain.

Both strains were fed on basis of the internal are the leaves of banana trees combined with cassava and Lucaena in the respective proportions of 70, 25 and 5% with a complement of 25 kg/ha of concentrated feed (18% PD) and 50 kg/ha of the dung chickens every 10 days, and we have achieved the middleweight of the order of 83,34. 149,12 and 182,28 g respectively at 3, 6 and 9 months with the local strain.

As the introduced strain, we have obtained the results from 85,94 g to three months, to 6 months 150,18 g and 218,22 g at 9 months.

Statistical analysis (Z-test) has shown that there is no significant difference between the local strain and the strain introduced in what concerns gain weight at different ages.

At the end of our study it is clear that nutrition would be the main factor limiting the performance of strains of tilapia as a result, the growth of fish farming in sub-Saharan Africa will leave the management of nutritional standards of fish breeding.

Photos1: Tilapia 9 months raised to the Station of Gimbi, INERA-DRC. The local strain on the left and the introduced right.

WATER SUPPLEMENTATION OF A MIXTURE OF MELISSA EXTRACT (*Melissa officinalis*) AND MAGNESIUM TO REDUCE PHYSIOLOGICAL STRESS REACTIONS OF SALMONS (*Salmo salar*)

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Well-boat transport and loading are very stressful for fish such as salmon. It is suspected that the low recapture rates and survival of the hatchery-reared salmon smolts were, in part, caused by the handling and transport of the smolts prior to the release. Cortisol, lactate and glucose blood levels are physiological parameters of stress. A study was carried out on smolt Salmon to assess the effect of a mixture of Melissa officinalis and magnesium, in order to reduce stress.

In order to evaluate the effect of *Melissa officinalis* combined with magnesium (Durelax® liquid), 250 ppm were directly put in aquarium of smolt and compared with a negative control. Measures were made at T0, T+2h, T+4h and T+6h. The manipulations required for the measurements of stress parameters were source of stress for the sampled fish.

Two smolts of each condition were taken and cortisol, glucose and lactate level were analyzed.

A decrease of different stress responses of salmon smolts treated with the mixture of *Melissa officinalis* and magnesium was observed. The concentrations of cortisol, glucose and lactate were lower for the fish in the aquarium that contained 250 ppm of the mixture.

It has been shown that stress has many impact on salmon: decrease of the immunity, of the feed behavior and on the locomotor activity. This may impact the performances and can induce growth decrease and FCR increase. Care should be taken during commercial boat transports and *Melissa officinalis* combined with magnesium could be used to calm salmon and decrease stress reaction on the well-boat.

Figure 1: evolution of the blood glucose

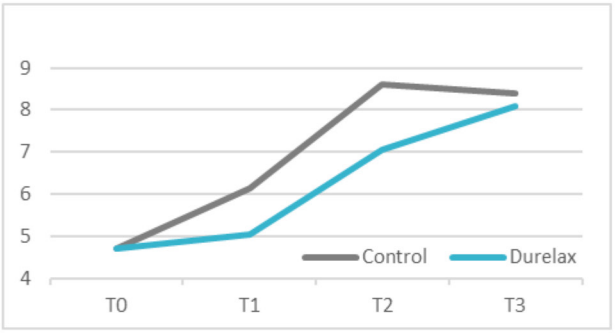


Figure 2: evolution of the blood cortisol

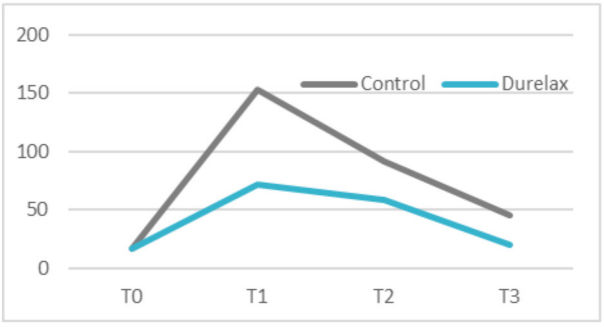
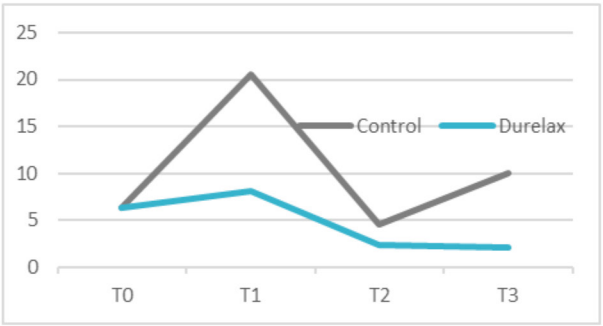


Figure 3: evolution of the blood lactate



EMERGENCE OF THE HEART AND SKELETAL MUSCLE INFLAMMATION SYNDROME IN FRANCE

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A new pathology affects salmonids in France since a few years : heart and skeletal muscle inflammation. It is associated with a group of viruses named Piscine orthoreovirus (PRV), with at least three viral species (PRV1, PRV2 and PRV3).

In France, the first cases were identified in 2014 in a cohort of sea-water reared Atlantic salmons (*Salmo salar*), which presented a high mortality level. The presence of PRV was diagnosed by real-time PCR. In 2015, unusal mortalities were reported in fresh-water farmed brown trouts (*Salmo trutta*) on another site. Several affected fish presented macroscopic lesions of pericarditis and petechiae in the adipose tissue. From this last outbreak, several fish were sampled and analysed by histology. Lesions compatible with those associated with HSMI were revealed (fig.1). Samples were sent to the french Reference National Laboratory : a PRV 3 was detected by conventional PCR and sequenced. In 2016, 2017 and 2018, further five outbreaks possibly caused by HSMI and all associated with the presence of PRV were identified (table 1). Noticeably, farmed rainbow trout and wild Atlantic salmons were also affected. PRV1a and PRV1b were identified in wild and farmed salmons respectively.

In total, 3 species of salmonids were affected by HSMI in 3 different sites in France. They were all associated with one of 3 different PRV genotypes, which suggested distinct viral origins. High similarities with other genotypes described in other countries suggest that international exchanges of contaminated fish occurred.

It would be important to know if the virus is endemic or only present in some sites. An epidemiological study would be necessary to review the associated risk of HSMI.

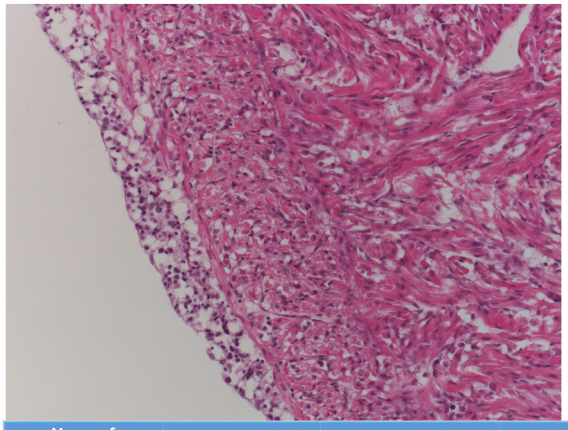

Fig 1. Diffuse mononuclear pericarditis. HESx200

Year of diagnosis (HSMI and test PRV)	Species	Origin	Genotype
2014	*Salmo salar*	Farm	nd
2015	*Salmo trutta*	Farm	PRV3
2016	*Salmo salar*	Farm	PRV1b
2017	*Salmo salar*	Farm	nd
2017	*Salmo salar*	Wild (maintained 1 year)	PRV1a
2017	*Oncorhynchus mykiss*	Farm	PRV3
2018	*Oncorhynchus mykiss*	Farm	nd

Table 1.

BENTHIC ECOSYSTEM RESPONSE TO ORGANIC ENRICHMENT IN RELATION WITH MUSSEL BIODEPOSITION

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Interactions between bivalve aquaculture and the environment are complex. In suspended mussel culture, great quantities of biodeposits may fall from culture structures and impact sediment properties (*eg* sulfide level, organic matter content) and local benthic communities. In turn, changes in community structure may affect biogeochemical cycling due to the different functional role of species. Although several indicators (mainly sediment properties and community structure) have been largely used to determine the influence of mussel aquaculture on the benthic environment, it is less clear the extent to which benthic functional diversity is affected and may contributes to affect ecosystem functioning. Moreover, little attention has focused on dose-response relationships.

This study evaluated the dose-response relationship between mussel biodeposition and benthic conditions over a wide range of deposition levels. Twenty-four mesocosms were deployed in îles-de-la-Madeleine (Qc, Canada) in July 2017 that created various levels of mussel deposition. Measurements were taken in October/November 2017 to determine the processes by which bottom ecosystem characteristics and functioning was impacted.

Sediment characteristics [redox potential, sulfides, %OM, particle size, bacteria abundance] indicated non-linear effects of biodeposition. Multivariate community structure (taxonomic and functional) and nutrient fluxes varied among treatments. High abundances of upward conveyors and surface/sub-surface deposit feeders were associated with the highest mussel densities and with higher nitrogen fluxes.

We suggest that a “reset” of benthic biogeochemical conditions occurs at the end of summer whereas benthic community changes appear to persist over longer time scale. These findings highlight the importance of temporal monitoring of the environmental effects of aquaculture. This study also suggests the benefit of deposit feeders and active sediment reworkers for bioremediation of aquaculture wastes.

DEMONSTRATING THE SUSTAINABILITY AND RAISING AWARENESS FOR THE POSSIBILITY OF CRUSTACEAN PRODUCTION IN RAS SYSTEMS IN POMERANIA, POLAND

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Aim of the study is to evaluate the potential of crustacean production in RAS in Poland: The aim will be achieved through: laboratory study focused on a mini-scale RAS white leg shrimp *Litopenaeus vannamei* cultivation and the experiments on growth and nutritional value of the shrimp, and socio-economic study regarding consumer perception and identification of scientific market knowledge.

Laboratory study - Growth and nutritional value of *Litopenaeus vannamei* from the small-scale laboratory culture

Two RAS min-systems, each consisting of three main units: culture shrimp tanks 500 (liters),) filters (mechanical and biological filters, mechanical protein skimming with ozone, UV sterilization, submersible pumps) with a control system (electronic devices) and water preparation tank (250 liters), were installed in the laboratory of the University of Gdansk in the end of June 2017. Both systems have closed water flow, and the only difference is biological filtration: a trickle filter, and fluidized moving filtration media. In the following weeks, the system was tested and developed. Shrimps larvae were ordered at post-larval stages PL12 (ca. 1100 specimens) from a hatchery in Florida, US.

During trail 1 (November 2017 – March 2018) around 550 shrimps were placed in each RAS (T = 17 °C, S = 33 PSU). They were gradually (3 weeks) acclimatized to a higher temperature (25 °C) and lower salinity (29 PSU). During the whole experiment they were fed six time per day with the known portion of commercial feed (0.8, 1.2 and 1.5 mm pellets, Scretting, Norway). Water parameters such as temperature, salinity, pH and redox potential were also controlled six times a day, as were the behavior, mortality and number of molts. Ammonia, nitrite, nitrate, phosphates and silicates were determined three times a week. Weight (± 0.001 g) and length (± 1 mm) of randomly collected shrimps (10 from each tank) were determined every second week. The whole experiment was carried out for 16 weeks. After this time, the shrimp were stunned (i.e. rendered unconscious) by chilling in ice water. Some of them were transferred to the laboratory of National Marine Fisheries Research Institute for the studies of sensory attributes (color, smell, taste, texture) and weight loss in flesh during freezing. Remaining shrimps were frozen to other analyzes, i.e. elemental composition (carbon, hydrogen and nitrogen contents) and calorific value which will be performed at the University of Gdansk and nutritional value and level of chemical contaminants, which will be performed by a project partner – National Marine Fisheries Research Institute. Trail 2 is planned for August – December 2018

Technological and business setup recommendation as well as decision – support model input will be provided based on socio-economic studies (based on questionnaires on public perception and market analysis of shrimps on the Polish market), biological, chemical, technical, technological and economic data obtained from the laboratory study, and stakeholders and workshops/summer school participants' feedback and expectations.

TROPHIC INTERACTIONS AND CONNECTIVITY STRUCTURE SPATIAL PATTERNS OF PACIFIC OYSTER RECRUITMENT IN A HEAVILY SHELLFISH FARMED MEDITERRANEAN LAGOON

F. Lagarde*, A. Fiandrino, M. Ubertini, R. Tremblay, S. Mortreux, C. Chiantella, B. Bec, C. Roques, D. Bonnet, I. Bernard, H. Cochet, G. Messiaen, M. Richard, E. Roque D'orbcastel, S. Pouvreau, C. Lett.

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We hypothesize that top-down control remove the structure of pediveligers supply in the ISFZ. More broadly, the biological processes of oyster recruitment in Thau appears to encompass connectivity, early settlement mortality, metamorphosis delays and survival in the local context of the ecological restoration of a Mediterranean lagoon under process of oligotrophication.

Thau Lagoon (Fig. 1) is a major shellfish site in the Northwestern Mediterranean partly structured by an intensive filter-feeders exploitation. Lagarde *et al*. (2017) studied the temporal pattern of Pacific oyster (*Crassostrea gigas*) recruitment, highlighting the importance of trophic contributions. As a complement, we focus here on the spatial pattern of recruitment. First, *in situ* larvae and settlers abundances were related to hydrodynamic connectivity between farmed oyster stocks and larval sampling sites quantified with the Model for Applications at Regional Scales (MARS-3D).

The structuring of pediveliger supply was correlated with connectivity outside shellfish farming zones (OSFZ) while no structure appeared inside (ISFZ) (Fig. 2). We also found that trophic intakes differed between both zones (Fig. 3), with filter-feeders top-down control ISFZ (higher abundances of picoeukaryote) *vs* bottom-up control OSFZ (higher nano and microphytoplankton biomasses).

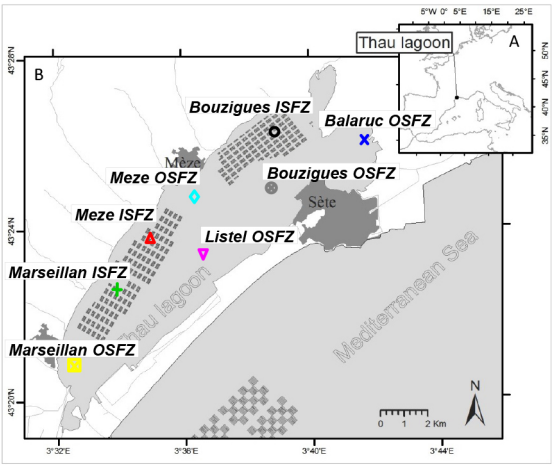

Fig. 1: Sampling sites located in Thau lagoon. Areas with grey boxes: bivalve farms.

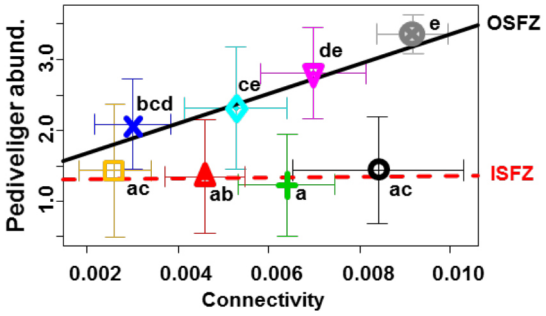

Fig. 2: Mean ± SE of simulated connectivity and observed pediveligers abundances (log_{10} $plate^{-1}$) for the different sampling sites of Fig. 1. Letters differentiate group levels in Tukey Contrasts Multiple Comparisons of Means. Lines show regressions for OSFZ (plain black) and ISFZ (dash red) respectively.

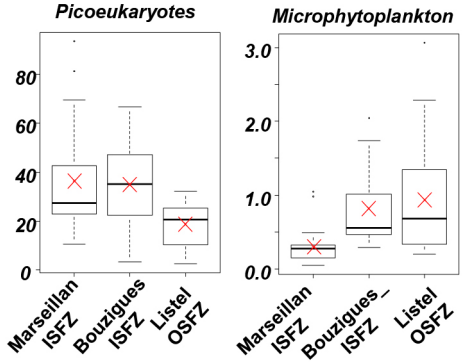

Fig. 3: Picoeukaryote abundances (10^6 cell l^{-1}) and microphytoplankton biomass (µg l^{-1}) that influenced significantly ($p<0.05$) oyster settlement and recruitment in contrasted ISFZ vs. OSFZ sampling sites. Red crosses indicate means.

THE STUDY OF WATER AND SEDIMENT QUALITIES, AND PRODUCTIONS OF GREEN-HOUSE GASES IN WHITE SHRIMP *Litopenaeus vannamei* CULTURE

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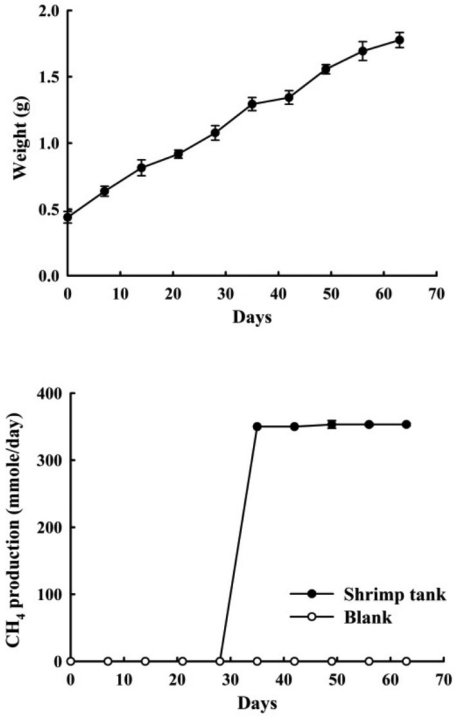
This study investigated quality of water and sediment, and also sediment gas productions (CH_4□N_2O) in culture of white shrimp (*Litopenaeus vannamei*). The stocking density of white shrimp was 103 individuals/m^2 and culture period was 63 days. The total length of shrimp increased from 2.6 to 8.2 cm and weight increased from 0.4 to 1.8 g in average from day 0 to day 63. The growth of shrimp significantly slowed after day35. The levels of NH_4^+-N, NO_2^--N, and NO_3^--N in water were significantly higher in the shrimp tanks than the blank at day 7, 14, and 42,, respectively, but levels in sediment in the shrimp tanks were higher than the blank at day 28, 35, and 28. It represents slower accumulation of nutrient ions in the sediments than in the water. Both contents of organic matter and total nitrogen in the sediments of shrimp tanks were significantly higher than the blank after day 21, and the total carbon was after day14.

The oxidation-reduction potential (ORP) of sediments indicated that the sediment decreased to highly reduced conditions (<-100 mV) after day 28 in the shrimp tank. Both productions of both N_2O and CH_4 in the sediments of shrimp tank were significantly higher than the blank after day 35 and positively correlation with the organic matter, carbon and nitrogen contents in the sediment, but negatively correlation with the ORP levels. The N_2O productions in the sediment of shrimp tank increased from 9.83 μM/g/day at first to 9.97 μM/g/day at day 63, and were significantly higher than the blank after day 35. The CH_4 productions were first detected in the sediment of shrimp tanks at day 35 and average levels were 18.08 μM/g/day at day 35, then to 18.25 μM/g/day at day 63, while no productions of CH_4 in the blank. In conclusion, the results indicated that growth of shrimp were negatively correlated to the nitrogenous ions in water and reduction levels in sediment.



IN-DEPTH GENOMIC CHARACTERIZATION OF A UNIQUE COLLECTION OF RAINBOW TROUT ISOGENIC LINES

Delphine Lallias*, Mekki Boussaha, Maria Bernard, Céline Ciobotaru, Nicolas Dechamp, Mathilde Dupont-Nivet and Edwige Quillet

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A unique collection of 17 isogenic homozygous rainbow trout lines has been established and maintained in INRA fish experimental facilities. These lines exhibit a wide range of phenotypes for most of the traits screened for. Such isogenic lines have many advantages in experimental approaches, including homozygosity, within-line genetic homogeneity, the availability of the same 'individual' genetic background at different places and times or the possibility to carry out investigations at different levels (cell, tissue, whole individual) for a given genetic background. In particular, these lines are being used to investigate the molecular bases of complex traits: lines with contrasted phenotypes are used for functional and genetic analyses (such as transcriptomics or QTL mapping).

Genomic characterization of isogenic lines is pivotal to realize the whole benefit of this material in integrative approaches aimed at dissecting complex traits. Having access to the genomic variability among lines is essential in expression or QTL studies in order to identify polymorphism(s) responsible for the phenotypes of interest. So far, the main information has come from RAD-tag genotyping, resulting in no more than a few thousand SNPs per line, which limits the accuracy of investigations at whole genome level or in targeted areas.

The objective of this study was to carry out in-depth genomic characterization of the trout isogenic lines, by investigating both small genomic variations (SNPs and InDels) and structural variants (SVs). SVs are defined as genomic alterations that affect large DNA segments ≥ 50 nucleotides, thereby causing modifications in either DNA quantity (insertions, deletions and duplications) or DNA structure (inversions). Although SVs have received increasing interest in many species and were shown to be associated with several diseases and phenotypes, they are poorly documented in fish.

All isogenic lines (one or two individuals per line) have been resequenced at a depth of coverage ranging from 10X to 32X, on an Illumina HiSeq X-Ten platform, in paired-end 2x150 bp configuration. Analysis of small genomic variants was performed according to the GATK Best Practices. The identification and characterization of SVs was done by using 4 different tools corresponding to three distinct but complementary approaches: i) Pindel and Delly (split-read approach); ii) BreakDancer (paired-end approach); iii) CNVnator (depth of coverage approach). After SVs annotation, a subset of SVs with potential relevant biological effects will be validated experimentally.

Funding: CRB-Anim (Biological Resource Centers for Domestic Animals)

ANALYSIS OF GENETIC VARIABILITY OF GLOBAL DNA METHYLATION IN RESPONSE TO AN EARLY TEMPERATURE STRESS IN RAINBOW TROUT

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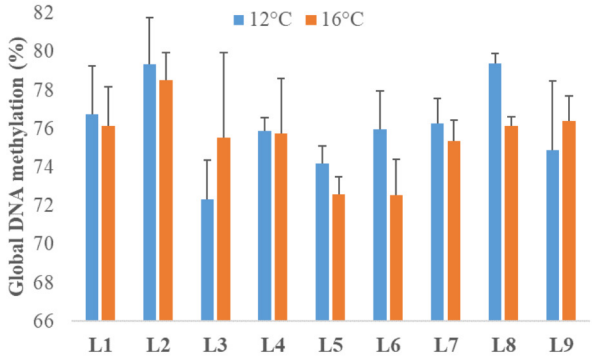
We aim to investigate the potential role of epigenetic marks in the expression of phenotypes and their variability in fish, in particular genetic variability of epigenetic marks in response to an environmental stress. In this context, rainbow trout isogenic lines are the material of choice. Within each line, all fish have the same genome i.e. there is no genetic variability. This allows the comparison of epigenetic marks among several individuals with the same genotype. The environmental stress chosen here is temperature because several studies have reported genetic determinism of thermotolerance. Moreover, rainbow trout isogenic lines have recently been characterized for their response to temperature and the existence of a high between-line variability was shown. The aim of this study is to contribute to the understanding of why certain lines are more tolerant to temperature stress than others, by investigating the implication of epigenetic mechanisms in the variability of the response to temperature.

The first objective of this study was to test whether temperature regime experienced during early development leads to epigenetic modifications within and between lines. Nine rainbow trout isogenic lines were chosen. For each line, half of the eggs were incubated at standard temperature (12°C) and the other half at high temperature (16°C), from eyed-stage to hatching. At eyed-stage just before hatching, analysis of global DNA methylation with LUMA (LUminometric Methylation Assay) revealed significant differences between lines but little or no effect of incubation temperature (Figure). At hatching, no effect of incubation temperature was observed on mortality or malformation rates. After 4 months of rearing at 12°C, mean body weight of batches incubated at 12°C or 16°C was similar.

The second objective of this study was to test whether early temperature regime impacts response to a stress experienced later in life. After tagging fish individually at a mean body weight of 3-4 g, the different batches were grouped into 9 tanks to perform acute temperature stress on 5-month-old juvenile fish. The response was measured in terms of time to equilibrium loss and Upper Thermal Tolerance (UTT). Globally, there was little significant effect of early exposure to high temperatures on the response to a late temperature stress, but tendencies seemed to appear. Thus, in the future, the impact of a longer exposure to high temperatures during early development will be tested.

Funding: This study was carried out within AQUAEXCEL[2020] funded by European Union's Horizon 2020 research and innovation programme under grant agreement No 652831.



UNSUSPECTED *OsHV-1* GENOMIC DIVERSITY AT INTER AND INTRA-HOST LEVEL

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Ostreid herpesvirus 1 (OsHV-1) is a major pathogen affecting *Crassostrea gigas* production, as well as some other edible mollusk species in France and in the world (Australia, New Zealand, Sweden, etc.).

The aim of this study is to characterize the simple and complex nucleotide polymorphisms of various OsHV-1 viral population on infected individuals collected worldwide, in order to determine the existing phylogeny (using genome-wide dataset) between OsHV-1 specimens and to determine the inter and intra-host diversity to get better insights how disease is built during infection.

First results show that OsHV-1 samples from France and New Zealand infecting *C. gigas* did not cluster together, meaning that New-Zealand OsHV1 viral population is another structural variant compare to OsHV-1 µVar. In addition, we confirm the proximity of AVNV to OsHV-1 µVar-like cluster that suggest a recent host shift from *C. gigas to C. farreri*. The genome-wide study of simple and complex polymorphism suggests that some genomic regions are deleted in several specimens or accumulate a high level of substitution. This non random pattern of polymorphism suggests that some genomic regions are under selective process. Contrary to a common belief, we found variants within all infected individuals. The biological interpretation of these observations is discussed in detail.

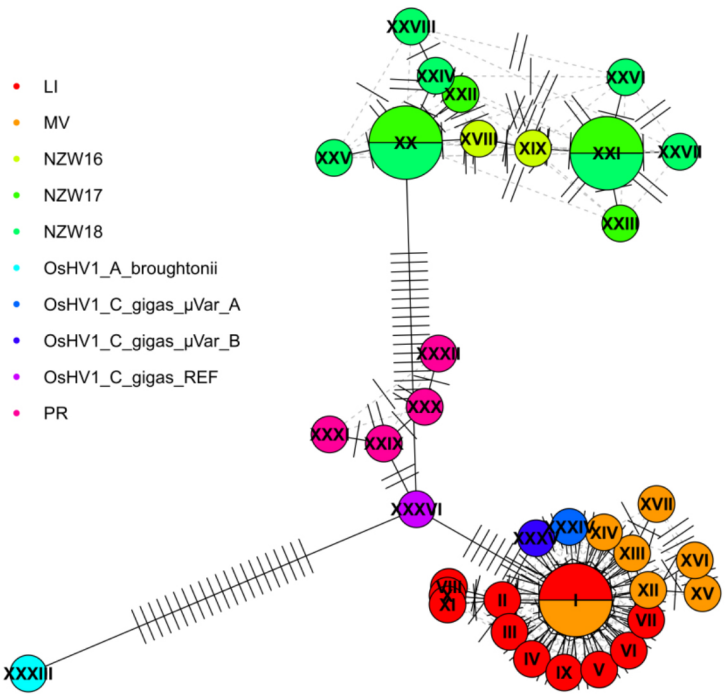

Figure 1: Minimum spanning network for all reconstituted viral haplotypes. Roman number indicates the haplotype's ID. Colors indicate the specimens where a given haplotype have been found. Some haplotypes are shared amongst specimens. Finer crossing between circles symbolize the number of mutations that differentiate haplotypes.

THE PACIFIC AND THE PORTUGUESE CUPPED OYSTERS, A PAIR OF SEMI-ISOLATED SPECIES AS GENOMIC RESOURCES FOR SHELLFISH AQUACULTURE

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The existence of marine semi-isolated species pairs evolving in the "grey zone" of the speciation continuum provides interesting opportunities to contribute to some highly debated questions in the field of speciation genomics. Because genetic divergence does not easily maintain in the face of gene flow in the absence of reproductive barriers, high gene flow species, such as broadcast-spawning marine invertebrates with highly dispersive pelagic larvae, offer valuable study systems for disentangling the mechanisms at play during speciation.

In this context we have focused our interest on the Pacific cupped oyster, *Crassostrea gigas*, and the Portuguese cupped oyster, *C. angulata*, representing worldwide important resources for shellfish aquaculture. They are parapatrically distributed in their native range in the north-western Pacific and introduced recently in Europe. Furthermore, whether *C. gigas* and *C. angulata* truly represent biological species, semi-isolated species of populations of the same species also remains unclear. Therefore, thanks to new genomic resources we (1) tested the existence of genetic barriers between *C. angulata* and *C. gigas* by searching for genomic regions that remain differentiated in the presence of gene flow, (2) developed a dedicated SNP panel allowing to discriminate both genomic backgrounds and their hybrids (Figure 1), and (3) investigated more thoroughly the distribution of both genomic backgrounds in Europe and their level of recent introgressions more particularly in Portugal and France.

Our results suggest that the environmental transition caused by the co-introduction of the two species in Europe did not affect the genomic architecture of partial reproductive isolation, and that these semi-species are still evolving in the so-called "speciation grey zone". Furthermore the recent reciprocal introgressions observed in contact zones in Portugal and France highlight the need to better characterize and potentially conserve those genomic resources of importance for shellfish aquaculture.

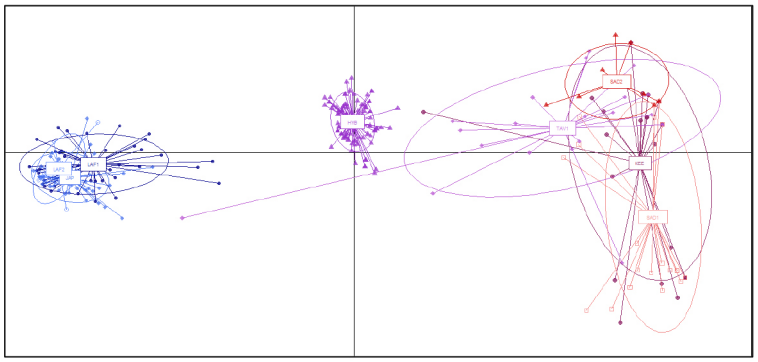

Figure 1. Discriminant analysis performed with 15 SNPs markers on 8 populations from European and Asian *C. gigas* populations (in blue on the left), European and Asian *C. angulata* populations (in pink on the right) and experimental hybrids (in purple in the middle).

GERM STEM CELLS TRANSPLANTATION IN FISH: AN INNOVATIVE BIOTECHNOLOGY FOR THE FAITHFULL REGENERATION OF CRYOPRESERVED GENETIC RESSOURCES Collected FROM SELECTED LINEs of AGRONOMIC INTEREST

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Interactions between the nuclear and mitochondrial genomes are important for animal performance traits. The mitochondrial genome is transmitted to the offspring by the female only, through its accumulation into the oocytes. Unfortunately, fish oocytes and embryos cannot be cryopreserved, which results in the absence of an appropriate procedure allowing the conform regeneration of selected fish lines. The present study was aimed to set up a standard and practical biotechnology based on germ stem cell (GSC) grafting that could be easily implemented in fish farms to conserve and regenerate the whole genetic characteristics (mitochondrial and nuclear genomes) of original and/or selected populations in fish.

Highly purified germ stem cell (GSC) and total testicular cell fractions were obtained from sex-reversed females (named neomales) belonging to a wild type (i.e. black skin) homozygous isogenic trout line. The cell fractions were injected independently in the abdominal cavity of triploid trout embryos homozygous for the dominant “golden” mutation (yellow skin). Male and female triploid trout embryos become sterile unless their gonads are colonized by diploid transplanted germ cells. Using diagnostic genetic markers, we showed that the percentage of successfully transplanted male and female recipients was high (about 80%) and similar whatever the cell fraction. Interestingly, we observed that grafted females ovulated during the egg-laying season of the recipient fish line (November instead of January for the donor fish line). Egg production from 2 years old grafted females reached normal values (2200 eggs/kg body weight) for both cell fractions, but egg quality indicators (eggs size and percentage of hatched embryos) tended to be improved after using the total testicular cell fraction. In contrast, milt production and sperm counts of precocious one year old males were highly variable regardless of the GSC fraction used but remained sufficient to fertilize thousands of eggs. Genotyping showed that milt contained spermatozoa derived from donor GSC only. Progenies were generated using eggs and milt collected from grafted female and male recipients, respectively. As expected, all fry were genetically identical to the GSC donor fish line with a black colored skin and a female genetic sex.

In conclusion, this study demonstrates that total testicular cells can be transplanted into triploid recipient trout embryos to efficiently and faithfully regenerate valuable genetic resources in farmed fish.

Acknowledgements: This study was supported by the AquaExcel^{2020} UE project.

DIETARY LYSINE REQUIREMENT FOR JUVENILE TOTOABA, *Totoaba macdonaldi*

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A 12-week feeding experiment was conducted to determine the lysine requirement for *Totoaba macdonaldi* juveniles. Five isonitrogenous and isocaloric diets, with 420 g kg^{-1} crude protein (CP) and 21 MJ kg^{-1}, were formulated to contain increasing levels of dietary lysine. After HPLC analysis the actual lysine dietary levels were found to be; 16, 17.7, 22, 25.1, and 27.8 g kg^{-1}, dry matter basis). Each diet was randomly assigned to triplicate groups of 10 fish and fed to apparent satiation by hand, three times a day for 84 days. Growth in terms of weight and length, survival and feed utilization (as feed conversion rate, protein efficiency ratio, and protein productive value) were used as response variables. Lysine digestibility of diets was estimated using acid-insoluble ash as an internal marker. Results revealed that final weight and weight gain increased with increasing levels of lysine in the diet, from 16 up to 22.0 g kg^{-1} of the dry matter. Thermal growth coefficient was the response variable used to estimate the lysine requirement and using a quadratic broken-line model gave the highest fit (R^2 = 0.82). The model estimated that highest growth is obtained with a dietary lysine level of 19.3 g kg^{-1} DM or 4.6 % of dietary crude protein (equivalent to 16.4 g kg^{-1} of digestible lysine). Using the results here obtained, estimates of other amino acid requirements were calculated using the quantified lysine requirement and totoaba whole-body amino acid concentrations, which can now be used to formulate practical diets for juvenile totoaba, summing for the further development of the commercial culture of this promising species.

GUT MICROBIOTA OF MIGRATING WILD RABBIT FISH (*Siganus guttatus*) LARVAE HAVE LOW SPATIAL AND TEMPORAL VARIABILITY

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We investigated the gut microbiota of rabbit fish larvae at 3 locations in Vietnam (Thuan An: northern, Quang Nam: intermediate, Binh Dinh: southern sampling site) (Figure 1A) over a three-year period. This is the first study on the gut microbiota of migrating rabbit fish larvae. The Clostridiales order was the most predominant in the gut (Figure 1B), and location-by-location alpha diversity showed significant differences in Chao-1, Hill number 1 and evenness.

Analysis of beta diversity indicated that the location, not year, had an effect on the composition of the microbiota. Statistical analysis showed that for 2014 the gut microbiota was different from the other years for Thuan An and Quang Nam. In 2014 the gut microbiota of fish from Quang Nam was different from Binh Dinh, in 2015 the gut microbiota was different for all locations, and in 2016 Thuan An was different from the other locations (Figure 1C). There seems to be a time-dependent trend in the North-South axis for the gut microbiota.

We found limited variation in the gut microbiota geographically and in time, and strong indications for a core microbiome.

This suggest that at this life stage the gut microbiota is under strong selection due to a combination of fish-microbe and microbe-microbe interactions.

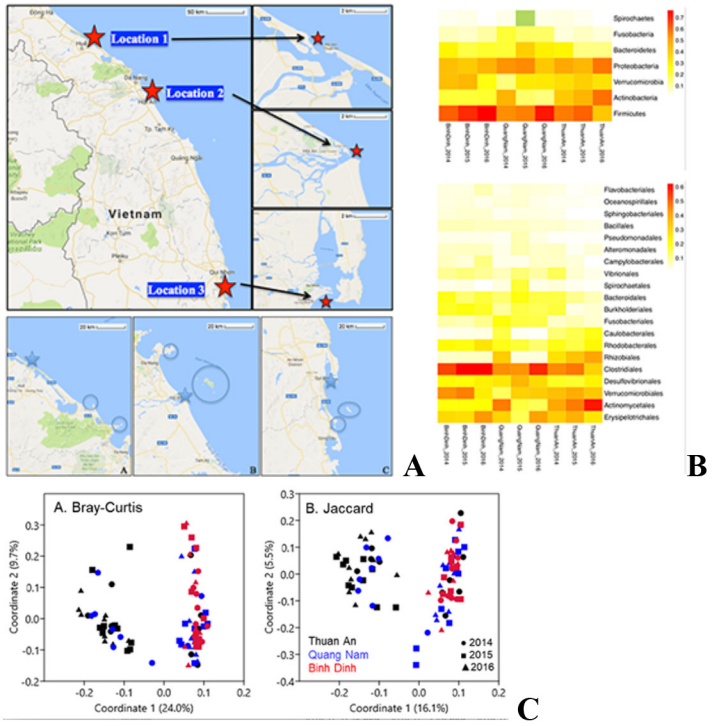

Figure 1. A. Sampling locations of wild rabbit fish over a three-year period; Location 1: ThuanAn; 2: QuangNam; 3: BinhDinh. **B.** Heat map showing the square root transformed relative abundance at phylum (upper figure) and order (lower figure) levels of the gut microbiome. **C.** PCoA ordination based on Bray-Curtis and Jaccard similarities for rabbit fish gut microbiota from 3 locations over 3 years.

FORMALIN USE IN FRESHWATER FISH FARMING

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Formalin is used in animal health as an inactivating agent in the manufacture of vaccines but also for its biocidal properties for the disinfection of premises and livestock equipment. It is also used to prevent and treat diseases, especially in aquatic production for ectoparasitic flagellates, however few data are available on its usage. Formaldehyde classification as carcinogen in the European regulation since 2014 now requires the search for substitutes.

As a part of a study on health practices, a representative sample of 101 freshwater fish farms was investigated in France in 2014. A questionnaire on the veterinary products used in connection with the housing structure, husbandry practices and sanitary status was completed during an on-farm visit.

Sixty four percent of the farms used formalin, mainly as a preventive or curative measure against parasites (87% of uses), with the objective of maintaining the parasite population at an acceptable level to the fish. The use of formalin was significantly associated with a higher frequency of parasitic diseases reporting. *Ichtyobodo necator* infections were the first reason reported for the use (39%) of formalin. This parasite is very widely encountered in freshwater salmonid farms and is responsible for cutaneous and branchial lesions causing frequent bacterial superinfections. Formalin was more commonly used on mixed farms with nursery and on-growing stages (71% of users in this category) than on farms specialized in on-growing (52% of users in this category). Fish farms more often rear young fish, from eggs to frying stages. Rainbow trout fry syndrom reports were also more common in user fish farms. Wearing protective equipment for formalin handling was limited and mostly consisted in wearing gloves (69%).

In specific sanitary conditions the use of formalin is deemed to be essential, due to the lack of effective alternative. To secure its use, protective measures must be defined and adopted to minimize exposure of the workers.

Tenacibaculum INFECTIONS IN MARINE FINFISH AQUACULTURE: RECENT EVOLUTION IN CLINICAL ASPECTS AND STRAIN IDENTIFICATION – IMPACT ON PROPHYLACTIC METHODS

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Tenacibaculum infections (previously named flexibacteriosis) represent one of the first bacterial condition being reported in marine aquaculture in a large range of species including flat fish (sole, turbot) and finfish (seabass, seabream). In some marine areas or in recirculating systems, they severely threaten production with mortalities rising over 30%.

These pathologies are generally affecting fish from nursery phase to pregrown fish up to 80-100gr average weight. Prevalence and intensity of the infections vary in relation with environmental parameters and zootechnical rearing conditions. Severity of clinical symptoms and mortality rates are influenced by fish size, water quality parameters and temperature, rearing factors such as handling, mechanical lesions or stress and interfering parasitic infestations. Those infections are initially external, affecting the dermal and epidermal layers. Lesions usually develop as co-infections, becoming ulcerative. Diagnosis is done on fresh smears from scrapping of the lesions by light microscopic observation directly or after quick staining (MGG/RAL555). *Tenacibaculum maritimum* is observed as long, gliding Gram negative bacteria.

For the past five years, clinical symptoms observed are more diversified, including deeper ulcerative lesions. Correlatively, increasing failure in treatments are reported. Monitoring and research programs such as the FUI Pathotrackfish have confirmed that *T. maritimum* actually represents only 50% of the strains isolated from clinical cases (Table.1). Analytical methods recently applied to fish pathogens identification based on proteomic fingerprinting using high-throughput mass spectrometry MALDI-TOF BRUCKER spectrometry or genomics (MLST, WGS) confirm the presence or the emergence of different pathogenic species, two of which are largely dominant: *T. maritimum* and *T. discolor*. In most cases, co-infections by at least two *Tenacibaculum* strains are noticed, which may explain the evolution of clinical aspects, different pathogenicity patterns and poor treatment efficacy.

The use of specific media for cultivation and *in vitro* antibiotic sensitivity testing, as well as swabs with adapted transport media, allow a better isolation and identification of these strains. This is a key point to apply efficient therapeutic treatments. This accurate identification of *Tenacibaculum* strains opens the field for preparation of monovalent or multivalent auto-vaccines adapted to each situation. Those vaccines are now tested in the field.

Table1. Identification of the 313 isolates of *Tenacibaculum* isolated from clinical cases during the FUI Pathotrackfish by mass spectrometry.

Species identification	Number	%
Tenacibaculum maritimum	172	54.9%
Tenacibaculum discolor	110	35.2%
Tenacibaculum mesophylum	9	2.8%
Tenacibaculum gallaicum	6	1.9%
Tenacibaculum aestuarii	5	1.6%
Tenacibaculum dicentrarchi	1	0.3%
Tenacibaculum soleae	1	0.3%
Tenacibaculum litoreum	1	0.3%
Tenacibaculum sp.	8	2.6%

PHYLOGENETIC COMPARATIVE STUDY AND POPULATION STRUCTURE TROUGH GENOMIC ANALYSIS OF *Streptococcus agalactiae* STRAINS ISOLATED FROM AFRICAN TILAPIA FISH FARMS

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Tilapia (*Oreochromis niloticus* L.) aquaculture is an important source of protein, income and employment in Africa and worldwide. In the African continent, the estimated total tilapia production in 2016 was 280,817t worth 487,053 million USD with the major producers being Egypt and Nigeria, Ghana being estimated in fifth position in 2016 (FAO).

In Ghana, the high demand of aquaculture products due to the rapid population growth have resulted on the thriving of tilapia fish farming. Tilapia aquaculture was estimated to be 52,074t in 2016 (FAO) and is mainly produced on commercial lines (mainly cages) around the Lake Volta. In Ivory Coast tilapia production was estimated to be 2,840t in 2016 (FAO) and is also focused on lakes.

High mortality rates during the production cycle due to diseases are one of the major constraints to the sustainability and growth of tilapia farming. Bacterial diseases such a *Streptococcus agalactiae* and *Streptococcus iniae* are considered the most important ones for the tilapia industry.

In the present study, eleven *Streptococcus agalactiae* isolates were recovered from diseased Nile tilapia during streptococcosis outbreaks in tilapia on-growing farms in Ghana and Ivory Coast. Initially, the isolates where identified and characterised based on proteomic fingerprinting using high-throughput mass spectrometry, Maldi-Tof Brucker. For the genomic sequencing of this isolates, a single colony of the bacteria was culture on COS media and genomic DNA was obtained from the cultured bacteria using a QIAGEN QIAmp mini Kit. Whole Genome Sequencing and bioinformatics analysis where afterwards performed to inferred the phylogenetic relationships and population structure. The phylogenetic relationship between the isolates as well as with already published ones were inferred using Maximum-likelihood and Bayesian analysis.

In the light of the results obtained, the epidemiological implications to apply control measures to avoid the spread of specific bacterial lineages as well as the monitoring of vaccination programs and vaccine development (since the efficacy of *S. agalactiae* vaccine to Nile tilapia seems to be linked to strain specificity) are discussed and presented.

EVALUATION OF THE ORGANIC MATTER QUALITY OF FRENCH OYSTERS BEDS AND THE SHRIMP'S, *Penaeus japonicus*, QUALITY AFTER ASSECS WITH OR WITHOUT THE APPLICATION OF MARCEL MEZY'S TECHNOLOGIES® MICROBIAL ECOSYSTEM

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Marcel Mezy's Technologies® (MMT's) is a unique fertilization process based on the combined actions of microorganisms. This process allows achieving a future approach of farming including soils health, profitability and increased quality of productions. The microbial complex bonifies the soil, enabling a quick humus soil formation and thus the accumulation of various trace elements, needful for the plant's growth. For the last few years, polyculture of *Penaeus japonicus* and *Crassostrea gigas* in oyster beds is implemented by French oyster farmers. Both species benefits of this approach: shrimps feed at night, putting in suspension phytoplankton filtered by oysters the next day. The density of shrimps in oyster beds is one to two shrimps per m^2 remaining an extensive farming. Nevertheless, every year the soil quality decreases, affecting the natural phytoplankton and zooplankton production in the pond. This reduction in natural food source oblige farmers to feed shrimps with artificial feeds. Using the MMT's during dry-out periods when emptying the oyster beds, should allow invigorating the natural soil production and consequently the trophic production in the ponds.

A three weeks dry-out period is implemented in April 2017 to four oyster beds, with respectively 2 beds of 500 m^2 and 2 beds of 1 000 m^2. Two of them are fertilized with MMT's at 100 kg/ha. The remaining 2 beds are held as usual and just dried, being negative controls. In May, shrimps are introduced in the four oysters beds at a density of 1 shrimp/m^2. After a complete 6 months production cycle, shrimps are harvested from end of September to beginning of November. For the assay, shrimps are not fed , to evaluate the efficiency of the microbial ecosystem on the natural production in the oyster beds. Three samples are done to monitor the soil evolution: during the initial dry-out period, before the introduction of the shrimps and at the end of the production cycle when ponds are drained. Analysis are performed at MEZAGRI's laboratory to evaluate humic matter, ammonium and nitrates levels. At the end of the assay, the total biomass and average weight of the shrimps from each oyster bed are recorded. Quality parameters (potassium, vitamins, proteins and omega 3 levels) of the shrimps are also measured.

An increase of humic matter, ammonium and nitrates is observed in the fertilized ponds during the dry-out period. Assimilable trace elements are gathered on humic acid and slowly released in water column during the rearing period, inducing an increase of the food supply chain production (phytoplankton, zooplankton, annelids…) in the MMT's oyster beds. After 6 months breeding, shrimps from the MMT's fertilized begs are colorful. They show a higher average weigh, higher vitamins proteins and omega 3 levels than shrimps from the controls beds.. The results observed during this initial assay held in 2017 need to be confirmed by further assays in 2018 which will be implemented by oysters farmers.

DIRECT AND TRANSGENERATIONAL EFFECTS OF GLYPHOSATE, ALONE OR CO-FORMULATED IN TWO COMMERCIAL PRODUCTS, ON THE ANTI-INFECTIOUS DEFENSES OF JUVENILES RAINBOW TROUT *Oncorhynchus mykiss*

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Glyphosate, the herbicide active substance (AS) of Roundup products widely used in agriculture, is one of the more common chemical compounds measured in freshwater and generate a high media interest all around the world. Whereas the physico-chemical properties confer to this AS a fairly favorable environmental safety profile, recent studies have reported some deleterious effects in aquatic organisms after a glyphosate or Glyphosate Based Herbicide (GBH) exposure. Actually, some adjuvants have been suspected to be more toxic than the AS, so the major point of this project lies in a comparative assessment of the potential impacts of a simple (AS alone) versus a complex (GBH) exposure in rainbow trout (RT) *Oncorhynchus mykiss*. Moreover the direct and the transgenerational potential effects of the products are investigated on i) genitors assessing their general health (immune system and reproductive capacity and ii) the offspring (F1 and F2 generations) of contaminated genitors, evaluating the early development and anti-infectious defenses of juveniles.

In November 2017, 48 genitors were daily exposed to an environmentally relevant dose of glyphosate (around $1\mu g.l^{-1}$) from AS alone or two GBH (i.e. Roundup Innovert® and Viaglif Jardin®) during 10 days before to give one first offspring born in December 2017 (F1). Animal experiments will be conducted on all generations during three years to simulate chronic chemical exposure observed *in situ*, until F1 generation would engender the next generation (F2). After four months exposure period to AS alone and two GBH, offspring have been challenged by bath containing 10^4 $TCID_{50}$ mL^{-1} of Infectious Haematopoietic Necrosis virus (IHNv). Ten conditions were tested according to the herbicide exposure, infected or not with virus. Mortalities were daily recorded and organs were collected from dead fish for virological examination. Fish samples were made 96h and 6 weeks after infection to analyze specific and non-specific immune parameters. Otherwise, another part of the offspring (F1) was acutely exposed to $100\mu g.l^{-1}$ of glyphosate during 96h to maximize the physiological response of RT by the different pollutants and allow us to develop innovative molecular and cellular tools and define relevant biomarkers to follow glyphosate and GBH effects.

Thanks to the high level of our experimental design (i.e. direct and transgenerational exposure to glyphosate alone and GBH in acute and chronic exposure, infected or not with virus), this study will be useful to evaluate global effects of glyphosate alone or co-formulated in the health of RT. In addition, quantification of the AS and possible metabolites of glyphosate in fish flesh will make it possible to estimate the risk to the consumer in order to better protect the environment and human population's health.

EFFECTS OF A SUSTAINABLE MIX OF FREE AMINO ACIDS ON GROWTH PERFORMANCE AND FEED UTILIZATION OF WHITE SHRIMP *Litopenaeus vannamei* JUVENILE REARED IN POND

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Single amino acids (such as L-Cystine) used in food supplements and in feed formulations, can be extracted from natural poultry keratin. The extensive acidic hydrolysis of this sustainable protein source simultaneously generates Mixes of free Amino Acids (MAA) and small peptides. Some of them are interesting candidates for aquaculture feed industry due to high protein and free amino acids content (50.4% and 47.3% respectively in this trial). Free amino acids (FAA) are easily and rapidly digested. This characteristic can influence nutrients utilization by the animal. Moreover, FAA are also known for their potentialy advantageous high water solubility. A trial was launched to evaluate the effect of including MAA in a commercial diet on white shrimp growth parameters.

A 38 days trial was carried out at the Neovia Nha Be research centre in South Vietnam. 2 560 *L. vannamei* were distributed in 16 cages (160 ind. per cage) divided in 2 groups (8 replications). Cages used were hapa-like structure containing 2.5m^3 of water. All cages were located in one pond. Shrimps Initial Body Weight (IBW) was homogenous. Each group was fed a different diet. Control diet was adapted to local shrimp farming conditions and fed to the control group. A dose of 0.5% of amino acids mix was added to the control diet (MAA 0.5% diet) and fed to the second group. Both feeds were manufactured in a feed plant. Table 1 gives details on both feeds. Animal were fed twice daily in feeding trays. Uneaten feed was collected and weighted to calculate Daily Feed Intake (DFI) and Feed Conversion Ratio (FCR). Mortality was checked daily. Ten animals in each cage were weighted at the beginning and at the end of the trial.

Results obtained are presented in the table 2. MAA 0.5% diets had no significant impact on survival and daily feed intake. Adding 0.5% of amino acids mix enhanced Specific Growth Rate (SGR) and weight gain. It also results in a significantly lower FCR.

Based on those results we can conclude that amino acids mix is a good candidate for shrimp feed formulation. Its incorporation in the feed allows better growth performance. It also has a capacity to decrease the FCR. Its high content in free amino acids can influence the kinetic of availability of such compounds in the haemolymph. A new set of trial should be undertaken to measure its ability to contribute to the amino acids digestion in shrimp and potentially stimulate feed intake in relation with the solubility level of these small molecules.

	Control	MAA 0.5%
Protein (%)	43.52	42.8
Lipid (%)	7.76	7.4
Ash (%)	12.95	12.43
Fiber (%)	1.71	1.87
Phosphorus (%)	1.79	1.78

Table 1 : feeds composition

Treatment	Control	MAA 0.5%
Weight gain (% over IBW)	227.9 ± 8.3 [a]	250.1 ± 12.3 [b]
SGR	3.21 ± 0.07 a	3.39 ± 0.10 b
Survival (%)	88.4 ± 5.6	84.8 ± 13.9
FCR	2.09 ± 0.20 b	1.82 ± 0.21 a
DFI (g/ind/day)	0.75 ± 0.07	0.72 ± 0.09

Table 2 : growth parameters of shrimp fed the control and MAA 0.5% feeds for 38 days

DEFINING UNITS WITH HIGH POTENTIAL FOR AQUACULTURE WITHIN SPECIES: TOWARDS AN INTEGRATION OF INTRASPECIFIC DIFFERENTIATION IN FISH DOMESTICATION PROGRAMS

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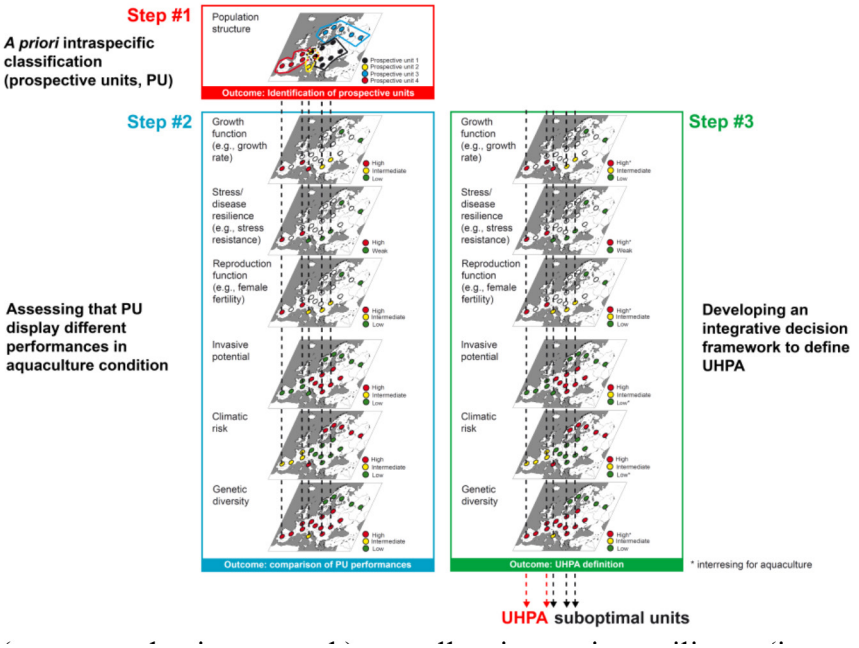
Fostering the sustainable development of agriculture (e.g., terrestrial agriculture and aquaculture) relies partly on the production diversification through new species domestication. However, domestication programs usually consider species as a unity, disregarding intraspecific geographic differentiation. Therefore, they overlook potential individualistic responses of differentiated allopatric populations to the domestication constraints. Yet, such a differentiation can shape genetic, phenotypic, and ecologic specificities, which impact the ability to live in captive environment or the potential socio-economic attractiveness of a particular population. Therefore, integrating intraspecific differentiation information in domestication process could facilitate new domestication programs and enhance the agriculture sustainable development. Here, we develop a three-step integrative approach based on evaluation of traits important for species exploitation (see figure).

The first step addresses the need of *a priori* definition of differentiated populations within a species of concern prior to evaluating the specific potential of populations for new domestication programs. This allows classifying wild populations according to their differentiations (based on genetic and possibly, known phenotypic variation) defining so called prospective units. The second step is the core of the multi-trait comparison approach. For each prospective unit, traits involved in different biological functions (e.g., reproduction, growth) as well as in species resilience (i.e. to stresses and disturbances commonly observed in targeted production environment) are evaluated in standardized conditions. Moreover, we also integrate key traits to face today's and tomorrow's challenges of agriculture (e.g., climate change, invasive species, international trade regulations). The third step is an interpretation of second step results taking into account demands of the industrial producers, the consumers, and other stakeholders. The aim of this last step is to highlight population groups that can meet production requirements, profitability targets, and regulatory requirements. Such population groups are subsequently considered as UHPA (Units with High Potential for Agriculture/Aquaculture). When alternative production developments are considered, the UHPA can be specific to a particular targeted production environment. The approach is here exemplified by an ongoing research on freshwater species such as the European perch (*Perca fluviatilis*). Potential interests, limitations, and future prospect of the approach for aquaculture are discussed taking into account success story and failure observed in terrestrial species.



MONITORING OF MICROPLASTIC DYNAMICS AND ANAYSIS OF ECOTOXICOLOGYCAL AND PHYSIOLOGICAL EFFECTS IN MARINE MYSID

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Mysids are one of the most important food items for numerous aquariums, fisheries, and even for human. Mysids are relatively small-sized crustaceans that are commonly found in most aquatic environments, such as brackish, estuarine, coastal, and oceanic environments. For more than 20 years, they have served as an ecotoxicology model taxonomic group because of its ease of culturing and handling in the laboratory, wide geographical distribution, short life-span, and physiological sensitivity to various environmental factors. In this study, juvenile and adult marine mysids (*Neomysis awatschensis*; Crustacea; Mysidae) were exposed to different sizes of microplastics, and the bioconcentration dynamics and responses of ecotoxicological and physiological responses were measured during the exposure and additional depuration periods. Microplastics bioconcentrated by age- and size-specifically and the levels reduced gradually during the depuration phase. We measured morphological growth parameters and quantified the hormone ecdysterone (20-hydroxyecdysone: 20E), which controls molting in mysids. The lengths of the whole body, antennal scale, exopod, endopod, and telson were significantly smaller microplastics-exposed juvenile mysids than control group. However, no significant modulation in the levels of 20E was observed by microplatics exposure in juvenile. After exposure to different sizes of microplastics, a series of parameters of antioxidant defenses system were significantly modulated during exposure and early depuration periods in juvenile mysids, while adult mysids showed no significant change. Our results suggest that microplastics could affect mysid growth and the significances are strongly associated with microplastics' sizes in *N. awathchesis*.

REPLACEMENT OF DIETARY FISH OIL WITH ALTERNATIVE LIPID SOURCES IN A PRACTICAL DIET FOR MANDARIN FISH *Siniperca scherzeri*

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Mandarin fish is considered a promising target species for freshwater aquaculture in the Asian countries due to its high consumer's preference, excellent taste, high market value, rapid growth, and high resistance against disease. However, despite the commercial importance of mandarin fish to Asian freshwater fish production, no commercial formulated feed is yet available for this species, and studies on nutrient requirements are rather limited. Indeed, there is no information available on the use of alternative lipid source in the practical diets of this important fish species. A 12-week feeding trial was, therefore, designed to evaluate the effect of total replacement of fish oil (FO) with lard, soybean and linseed oil on growth, feed utilization, body composition and hematological parameters of mandarin fish juveniles. Four practical diets were formulated in the present study. The control diet contained 60 g FO kg^{-1} (100% FO) and three test diets were prepared by replacing FO with lard, soybean and linseed oils (designated as Lard, SO, and LO, respectively). Each diet was randomly allocated to triplicate groups of 20 fish (initial average weight of 1.8 ± 0.5 g $fish^{-1}$). Fish fed the SO diet exhibited significantly lower final body weight than those fed the FO diet. The highest values of feed efficiency and protein efficiency ratio were recorded for fish fed Lard and SO diets which were significantly higher than those obtained for the FO group. Nevertheless, complete replacement of FO with the three tested alternative oils had no remarkable impact on hematological indices, fillet and whole-body composition. These results of the present study clearly demonstrate that the complete replacement of FO in juvenile mandarin fish diets is a technical reality. These findings are useful in dietary formulation to reduce feed costs without compromising mandarin fish growth.

FIRST ISOLATION, GENOMIC CHARACTERIZATION, AND PATHOGENESIS OF SPRING VIREMIA OF CARP VIRUS ISOLATED FROM COMMON CARP *Cyprinus carpio carpio* AND LARGEMOUTH BASS *Micropterus salmoides* IN SOUTH KOREA

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Spring viremia of carp virus (SVCV) is single strand RNA virus belonging to the genus *Sprivivirus* within the family *Rhabdoviridae* and listed in World Organization for Animal Health (OIE). The genome of SVCV consist of 5 genes (N, P, M, G, and L). In this study, we analyzed the complete genome of SVCV isolates from common carp and largemouth bass in South Korea. The genome sequences of SVCV isolates were amplified using reverse transcription-PCR (RT-PCR) and cloned into the pMD20-t vector (TaKaRa) according to the manufacturer's instructions. Thereafter, nucleotide sequences of SVCV isolates from Korea were confirmed by sequencing. The complete genome sequences of SVCV isolates were analyzed using CLC Main Workbench software (Version 7.7.1, Qiagen). The phylogenetic trees were constructed using neighbor-joining methods and 1000 bootstrap replicates in MEGA (Version 7.0.20). To demonstrate pathogenesis of SVC isolated from Korea, we performed challenge experiment with different temperature and infection methods. ADC-SVC2016-1 was isolated from common carp and ADC-SVC2016-3 was isolated from the largemouth bass. The nucleotide sequences of ADC-SVC2016-1 and ADC-SVC2016-3 were completely matched. The genome size of Korea isolates was 11,034bp. The phylogenetic tree showed SVC isolates from Korea were clustered in Asia clade of SVCV. After challenge experiment with different temperature, SVC isolate from Korea induced high cumulative percent mortality (80%) at 15°C, while the mortality at 25°C was 5%. In challenge experiment with different infection methods, Intra-peritoneal injection of SVCV (ADC-SVC2016-1) showed 80% of mortality in common carp, while immersion method of SVCV exhibited moderate mortality (45%) in common carp. In cohabitation, infected fish showed 80% of mortality, while the recipient fish did not show any mortality in common carp. These results were contributed to the epidemiology of SVCV in South Korea.

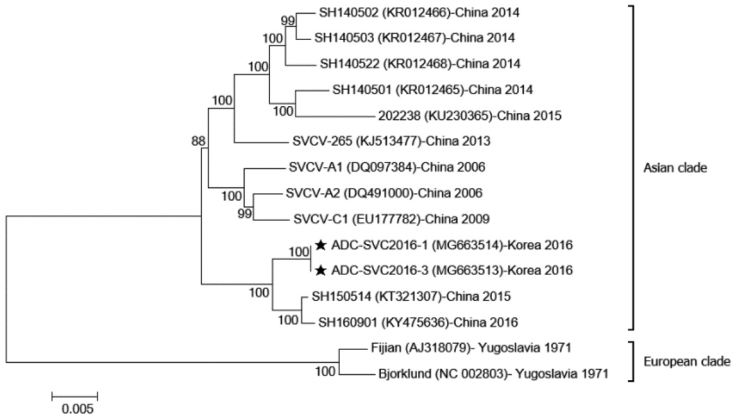

Figure 1. Phylogenetic analysis of the complete genome of SVC isolated from South Korea

OPPORTUNISTIC SPECIES AND SEASONAL VARIATION OF EPIPHYTIC FORAMINIFERAL ASSEMBLAGES IN ABALONE FARM NETS

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Abstract: Monitoring of dissolved oxygen (DO), temperature, and epiphytic foraminifera on seagrass in the nets of abalone farm at three areas (Haenam, Pyeongildo, Soando) with different sea water movement, respectively, were conducted between April 2016 and January 2017 to identify the opportunistic species, seasonal variation, and relationships with DO and temperature of epiphytic foraminiferal assemblages under environmental conditions affected by the biodeposits discharged from abalone farming. The results showed that the highest frequency of abundance of epiphytic foraminifera occurred in September (22.96 °C), although the temperature was highest in August (24.37 °C); this may be related to the consistently stable high temperatures during this time of (22–23) °C. There was no direct relationship between DO and epiphytic foraminiferal assemblages. *Rosalina bradyi* (frequency: 78.6 %; species diversity: 0.96; distributed across three areas in July) was found to be an opportunistic species for nutrient enrichment discharged from abalone farming. The difference of frequency of abundance among the three areas may reflect the spatial heterogeneity of biomass caused by the weak current speed.

Acknowledgements This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT & Future Planning (NRF-2017R1A2B1006247).

PHYSIOLOGICAL ANALYSIS AFTER CO_2 EXPOSURE FROM HATCHING STAGE IN RAINBOW TROUT

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Reduction of chronic stress consequences is of major relevance in aquaculture due to its supposed impact on fish health and welfare. Among important stressors, chronic exposure to high-CO2 has been shown to affect fish growth, feed conversion efficiency and nephrocalcinosis (Fivelstad 2013). Several studies are performed to measure the effect of CO_2-induced ocean acidification on marine system, however less is known about CO_2 in freshwater (Ou et al 2015). The aim of the present study was to assess in freshwater rainbow trout the effects of a chronic exposure to moderate CO_2 level on fish health status and coping ability through a multivariate analysis covering endocrine and physiological parameters. Fish health status and coping ability was evaluated before and after a 24hours-challenge.

Experiment was conducted in INRA PEIMA experimental infrastructure. At hatching stage, fish were divided in 6 flow-through tanks. All tanks used running spring water with different CO_2 concentrations (more or less degassed): control group: 3 tanks with 0-3mg/l CO_2 (pH~6.6) and CO_2 group: 3 tanks with 8-15mg/l (pH~6) (figure). O_2 was 10- 11mg/l. Six month later, part of fish was sampled and the other part was exposed for 24 hours to bad water quality by reducing water renewal and increasing density.

Growth performance was followed during 6 months. Blood and various tissues including gill, head-kidney, pituitary and brain were collected. Gill functions were assessed by measuring blood parameters (ion levels, pH, hematocrit, complement and lysozyme activities) and gene expression levels in gill tissues. In the same fish, we measured mRNA levels of major genes regulating HPI (hypothalamo-pituitary-interrenal) axis activity in head-kidney, pituitary and brain. In addition, before and after 24h-challenge, responsiveness of the HPI axis was also assessed on sub-groups of control or hypercapnia group by submitting trout to acute netting and handling stress (duration: 4 minutes) with a 1-hour recovering period in control conditions. Then, blood was collected for further analysis of plasma cortisol levels.

Measurements of the various parameters of the biological functions as described above are presently in progress. Preliminary results show a clear effect of CO_2 on growth. Furthermore, gill responses to a 24h-challenge are different between control and CO_2 groups. Overall, these analyses will give us a comprehensive description of the health and welfare status of trout exposed to our CO_2 condition.

Study funded by EU-Horizon 2020 (AQUAEXCEL 2020 project)

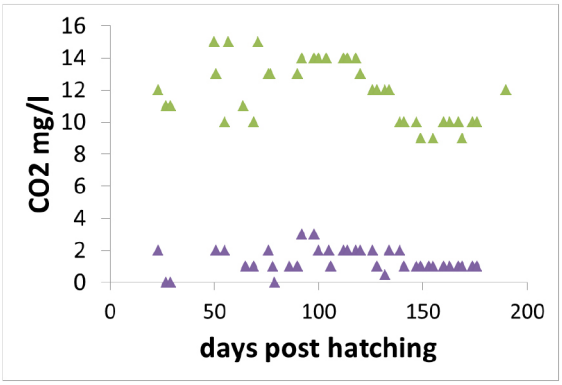

Figure: Evolution of CO_2 in water in control tanks (violet) and CO2 tanks (green) during experiment.

PERFORMANCE AND WELFARE IN BALLAN WRASSE *Labrus berggylta* AND LUMPFISH *Cyclopterus lumpus* JUVNILES REARED AT DIFFERENT LIGHT REGIMES

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Salmon producers consider cleanerfish in salmon cages as an important non-medical method to control number of salmon lice. The two species being farmed at the moment, lumpfish *Cyclopterus lumpus* and ballan wrasse *Labrus berggylta* differ both anatomically and physiologically. Knowledge about requirements of each species is important for the production of healthy and robust fish. Light might affect performance and welfare of fish. The present study investigated effects of different coloured light and of amount of light on performance and welfare in lumpfish and ballan wrasse. Two consecutively experiments with identical setups using 150 l tanks were done with lumpfish and Ballan wrasse respectively (Table 1).

LED lamps with different wavelengths were placed over the tanks, and amount of light was adjusted to 1, 5 or 20 micromol by dimming the lights and additional shading using layers of opalescent plastic. Mean start weight was 5.6 grams for lumpfish and 4 grams for Ballan wrasse. Survival, fish weight, external welfare indicators, cortisol in plasma, glucose and lactate in full blood were recorded at start and end of the experiment. The lumpfish experiment lasted five weeks (mean weight 43.4 g) and Ballan wrasse experiment eleven weeks (mean weight 25 g).

There were no significant differences between treatments in survival or growth. Occurrence of cataract was significantly affected by wavelength in both species. Green light (520 nml) resulted in the highest prevalence of cataract in both species. Green light also resulted in a significantly higher prevalence of skin damage in Ballan wrasse compared to blue or white light, but not in lumpfish. Plasma cortisol in Ballan wrasse was lowest at blue light, and highest at white light. For lumpfish, the cortisol level was highest at green light. Glucose in blood was highest at green light in Ballan wrasse, but not affected by either light colour or intensity in lumpfish. The results suggest green light (520 nm) had a negative impact and blue light (420 nm) a positive effect on welfare indicators in both species. The results also showed that amount of light influenced fish welfare less than light spectre.

Table 1. Experimental setup. Two replicate tanks per combination.

	Amount of light (micromol)		
Colour/wavelength	1	5	20
Blue/450 nm	xx	xx	xx
Green/520 nm	xx	xx	xx
Full specter white	xx	xx	xx

BIOPRESERVATION, A NATURAL PRESERVATION TECHNOLOGY USING SELECTED MICROORGANISMS TO GUARANTY SAFETY AND QUALITY OF SEAFOOD PRODUCTS

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Apart from those that are sterilized, most foods are naturally contaminated by microorganisms (yeasts, molds or bacteria). During the storage, even at chilled temperatures, some of them can grow and deteriorate the sensory quality of the products, leading to waste and economic losses. Other microorganisms can also be pathogenic for human. But in some cases, some of them can play a beneficial role, inhibiting the development of those undesirable microorganisms and being themselves safe and neutral.

Biopreservation is the controlled reproduction of this naturally occurring phenomenon. It consists in selecting those beneficial microorganisms and introducing them at high level in food to guaranty its safety and quality all along the shelf-life. It is a soft-technology alternative to the use of additives or damaging decontamination processes. Lactic acid bacteria are good candidates for biopreservation because (i) they have many natural antibacterial properties (ii) they are part of the common flora of many food; (iii) they are recognized as non-hazardous to human health; (iv) they benefit from a healthy image conveyed to consumers by dairy products.

A variety of seafood are highly perishable and may constitute a hazard for human health : products eaten raw (sushi, carpaccio, tartar), lightly preserved (smoked, pickled, salted) or ready-to-eat (peeled cooked crustaceans, fish salads) and convenience food (fish or shellfish packed under vacuum or modified atmosphere).

This talk presents the strategy of protective bacteria' selection, the regulation context and examples of application in seafood developed at Ifremer, especially for prevention of risk associated to the lethal bacteria *Listeria monocytogenes* (Figure 1), for histamine-producing bacteria and for spoiling bacteria.

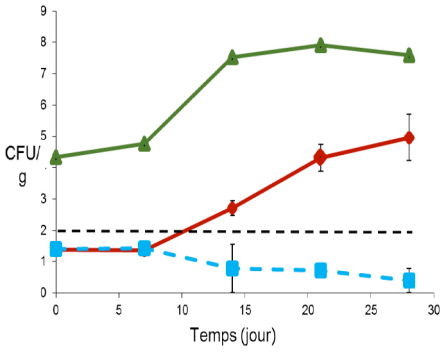

Figure 1 : Growth of *Caronobacterium divergens* V41 (full-line, triangle), *Listeria monocytogenes* (full line, diamond) et *L. monocytogenes* (dotted line, square) in presence of *C. divergens* V41 in cold-smoked salmon stored for 1 week at 4°C and then 3 weeks at 8°C

β-GLUCANS ADMINISTRATION INDUCES METABOLIC CHANGES AND DIFFERENTIAL SURVIVAL RATES AFTER A BACTERIAL OR VIRAL INFECTION IN TURBOT *Scophthalmus maximus*

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The innate immune response is capable to ward off pathogens and remember previous infection using different mechanisms, this kind ***of*** immune reaction ***has*** been called "trained immunity". An epigenetic reprogramming in cells of the innate immune system and changes in cellular metabolism have been observed during training with some immunostimulants like β-glucans or during viral infections. We hypothesize that β-glucans could induce metabolic changes used for the host to fight against pathogen.

Accordingly, we evaluated in turbot changes in parameters related to metabolism which could affect to the survival after a previous treatment with β-glucans and subsequent administration of Viral Hemorrhagic Septicemia Virus (VHSV) or bacteria (*Aeromonas salmonicida* subsp. *salmonicida*).

The results obtained supporting that β-glucans, VHSV and *A. salmonicida* induce changes in metabolites, ATP and mRNA expression of enzimes related with the glucose and fatty acid metabolism.

Changes observed were different between the viral and bacterial infection after the previous β-glucans treatment (Fig. 1). β-glucans appear to have a great therapeutic potential and could induce trained immunity against bacterial disease but not against the viral disease that seems to take advantage of the β-glucans metabolic alterations.

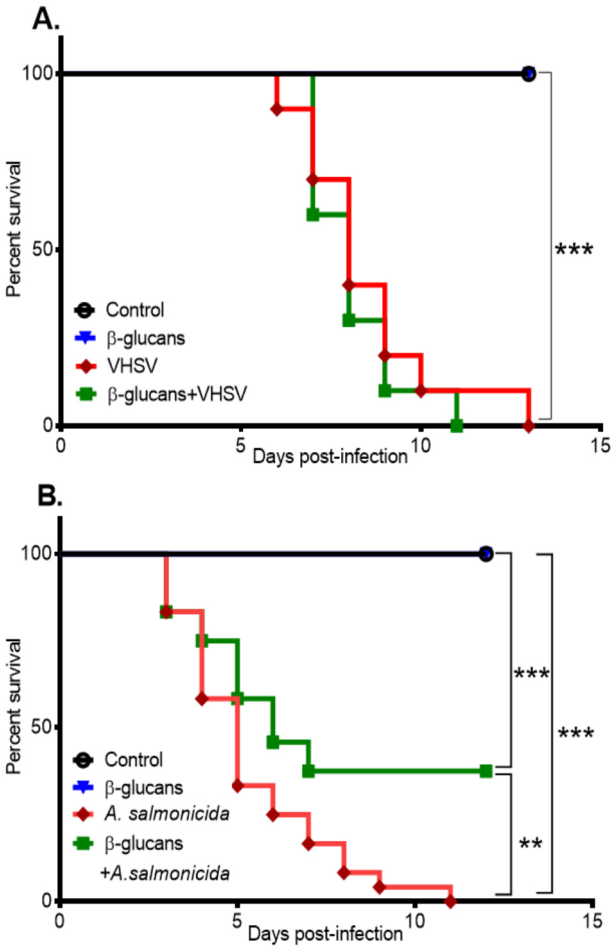

Fig. 1. Survival rates of fish. Fish were intraperitoneally injected with β-glucans and after one week with VHSV (A) and with *A. salmonicida* (B). Kaplan-Meier cumulative survival curves were analyzed for statistical significance with a log-Rank (Mantel-Cox) test.

CHRONIC EXPOSURE TO LOW ENVIRONMENTAL CONCENTRATIONS AND LEGAL AQUACULTURE DOSES OF ANTIBIOTICS CAUSE SYSTEMIC ADVERSE EFFECTS IN NILE TILAPIA (*Oreochromis niloticus*) AND PROVOKE DIFFERENTIAL HUMAN HEALTH RISK

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Antibiotics used globally to treat human and animal diseases exist ubiquitously in the environment at low doses because of misuse, overdose and poor absorption after ingestion, coupled with their high-water solubility and degradation resistance. However, the systemic chronic effects of exposure to low environmental concentrations of antibiotics (LECAs) and legal aquaculture doses of antibiotics (LADAs) in fish and their human health risk are currently unknown. We investigated the *in vivo* chronic effects of exposure to LECAs and LADAs using oxytetracycline (OTC) and sulfamethoxazole (SMZ) in Nile tilapia, (*Oreochromis niloticus*) and their human health risk. Twenty *O. niloticus* weighing 27.73 ± 0.81 g were exposed to water containing LECAs (OTC at 420 ng/L and SMZ at 260 ng/L) and diets supplemented with LADAs (OTC 80 mg/kg/day and SMZ 100 mg/kg/day) for twelve weeks. General physiological functions, metabolic activities, intestinal and hepatic health were systemically evaluated. The possible human health risks of the consumption of the experimental Nile tilapia fillets in adults and children were assessed by using risk quotient. After exposure, we observed retarded growth performance accompanied by reduced nutrients digestibility, feed efficiency, organ indices, and lipid body composition in treated fish. Antibiotics distorted intestinal morphological features subsequently induced microbiota dysbiosis and suppressed intestinal tight junction proteins. Exposure of fish to LECAs and LADAs induced oxidative stress, suppressed innate immunity, stimulated inflammatory and detoxification responses, concomitantly inhibited antioxidant capacity and caused lipid peroxidation in intestine and liver organs. Both LECAs and LADAs enhanced gluconeogenesis, inhibited lipogenesis and fatty acid beta oxidation in intestine and liver organs. The exposure of fish to LECAs and LADAs induced anaerobic glycolytic pathway and affected intestinal fat catabolism in intestine while halted aerobic glycolysis, increased hepatic fat catabolism, and induced DNA damage in liver. The hazard risk quotient in children for fish treated with OTCD was > 1 indicating human health risk. Overall, both LECAs and LADAs impair general physiological functions, nutritional metabolism, and compromise fish immune system. Consumption of fish fed with legal OTC provoke health risk in children. Global stringent prohibition policy for use of antibiotics in aquaculture production and strategies to limit their release into the environment are urgently required to protect human health.

EFFECT OF PERACETIC ACID ADDITION ON GEOSMIN AND 2-METHYL ISOBORNEOL LEVELS IN RAINBOW TROUT *Oncorhynchus mykiss* RAISED IN RECIRCULATING AQUACULTURE SYSTEM (RAS)

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An indoor pilot-scale recirculating aquaculture system (RAS) consists of ten 500 L units, separate water treatment and quality control systems. Recirculating water is treated in mechanical and biological units, including waste feed collector, swirl separator and drum filter (60 μm mesh size), two up-flow fixed-bed and two moving bed bioreactors (4 x 150 L). Dissolved carbon dioxide is removed from the water by packed aeration tower with pH adjustment at 6.16.8 by adding NaOH solution to compensate for the alkalinity loss due to nitrification.

In this experiment, tank units were randomly divided into four groups with juvenile rainbow trout and employed for four months. In the beginning, there were 50 fish in each tank, increasing in weight to 411 ± 43 g during the experiment. PAA was applied to the pump sumps 2.2 mg PAA L^{-1} solution per day for 0, 1, 2 or 4 times per week.

Geosmin (GSM) and 2-methyl isoborneol (MIB) were extracted from the sample matrix by headspace solid phase extraction (HSSPME). 1 g of fish fillet was placed in a 10 mL HS vial with saturated NaCl (aq) solution. The method of standard addition was used to construct calibration curves for quantification of GSM and MIB. Sealed sample vial was placed in a water bath at 60 °C. A needle and a DVB/CAR/ PDMS fiber were kept for 30 minutes to complete the extraction before introducing the fiber directly into the gas chromatograph-mass spectrometry (GC-MS).

Separation and quantification of GSM and MIB in circulation water and in rainbow trout flesh was performed with GC-MS equipment by Agilent 6890 series/5973 N GC/MSD system with a Phenomenex Zebron ZB-5MSi capillary column (30 m x 0.25 mm x 0.25 μm). The temperature of the oven started at 45 °C for 3 min. and increased 30 °C min^{-1} to achieve 300 °C (total time 14.5 min). Selected ion monitoring (SIM) mode was used for the detection of GSM and MIB with base peak areas of *m/z* 95 and *m/z* 112 were used for the quantification of GSM and MIB.

The concentrations in rainbow trout fillet (Figure 1) range on average between <LOD and 10.7 ng g^{-1} for GSM and <LOD and 9.3 ng g^{-1} for MIB. Generally, the average concentrations of both GSM and MIB decrease with increasing number of PAA additions per week but were insufficient to entirely inhibit the formation of off-flavor compounds. Additionally, the fish had developed an unusually thick layer of mucus and slime on the skin surface in units of PAA addition. This may have been due to PAA-induced irritation of fish skin and changes in the circulating water conditions.

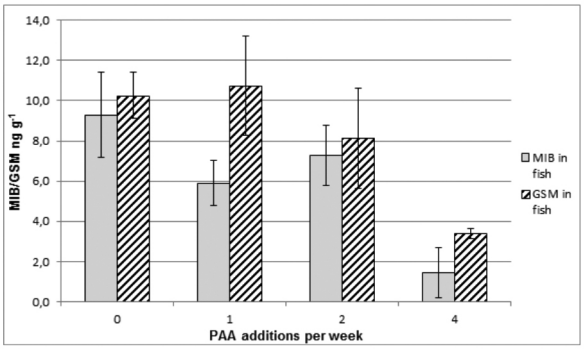

Figure 1. Concentrations of GSM and MIB (ng g^{-1}) in rainbow trout fillet after addition of PAA solution 0, 1, 2, or 4 times per week.

SUSTAINABLE INTENSIFICATION OF AQUACULTURE-LESSONS FROM VALUE CHAIN ACTORS

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Efforts towards sustainable intensification (SI) of aquaculture have mainly been focused on grow-out production system design and management. This paper looks at how progress towards the main tenets of SI has been achieved by actors elsewhere in the value chain. Examples of both market-led and local-to-production incentives are used to illustrate how the private sector has progressed towards more sustainable outcomes. Barriers to innovation and the role of external agents are also examined in this assessment based on on-going fieldwork in Europe, Asia and Africa. In particular the impacts of certifiers and standards are assessed in the context of trends towards consolidation and/or specialisation in the sector. The relative importance of local and introduced technologies in progressing SI are investigated and the functions of collaborative learning reviewed; the increasing multipolarity of world trade in aquaculture in terms of inputs and outputs makes opportunities particularly dynamic. The perspectives of both upstream and downstream actors in value chains, particularly producers and suppliers of juveniles, nutritional and health inputs, those involved in co-product value addition and marketing are critical to this analysis. Priorities for key research, development and policy actions are discussed.

RIBONUCLEASES 1 HAVE ANTIMICROBIAL FUNCTION BOTH *IN VITRO* AND *IN VIVO* IN BLUNT SNOUT BREAM *Megalobrama amblycephala*

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Ribonuclease 1 (RNase1) is a vertebrate-specific enzyme that mainly performs digestive activity in herbivorous mammals. Our previous study has shown that *M. amblycephala* RNase1 (*Ma*-RNase1) exhibited both digestive activity and potent antimicrobial activity in blunt snout bream. In order to explore its antimicrobial activity *in vitro*, bacterial viability assay was used. The results showed that *Ma*-RNase1 rapidly killed Gram-negative and Gram-positive bacteria at micromolar concentrations and in a dose-dependent manner. To better understand the regulatory effects of *Ma*-RNase1, its protective effects and immune defense were investigated via an *in vivo Aeromonas hydrophila* infection model. After 72 h, *A. hydrophila* infection obviously produced marked tissues damage and apoptosis in gut and liver, while RNase1 pre-treatment effectively counteracted *A. hydrophila* induced injury. Quantitative real-time PCR and immunoblot analysis indicated that RNase1 mRNA and protein were up-regulated in the kidney and gut during infection. Furthermore, *A. hydrophila* infection significantly induced *tnf-α* and *il-1b* mRNA expression in liver, but not in the RNase1 pre-treated group. In addition, a significant increase in immune related genes (*nf-κb* and *tlr4*) were found in liver, kidney and gut in *A. hydrophila* infected fish, while an obvious decrease in *myd88* and *tlr4* were found in liver, spleen, kidney and gut in RNase1 pre-treatment group. Collectively, these data suggest that *Ma*-RNase1 have antimicrobial function both in *vitro* and in *vivo* and RNase1 pre-treatment effectively counteracted *A. hydrophila* induced injury and appeared potent protective effects and immune defense in blunt snout bream.

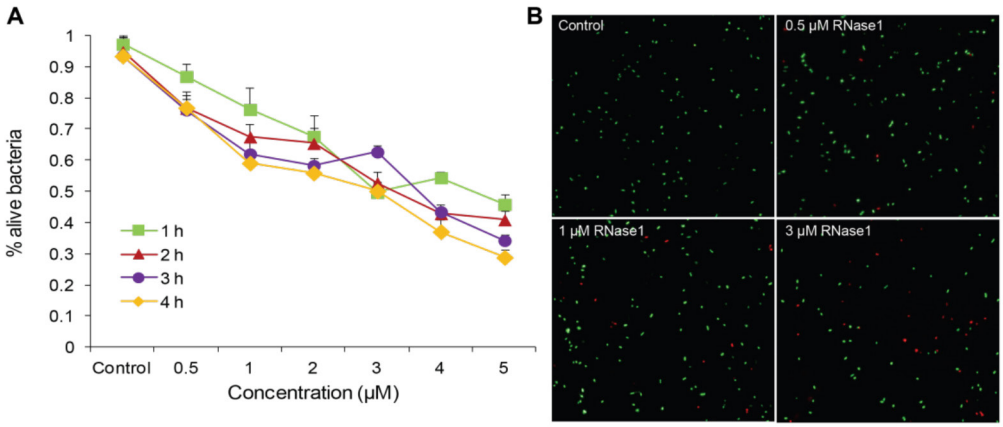

Figure. *Ma*-RNase1 rapidly killed Gram-negtive *A. hydrophila* at micromolar concentrations

EXPRESSION PATTERNS OF DNA METHYLTRANSFERASE GENES DURING GAMETOGENESIS AND EARLY DEVELOPMENT IN RAINBOW TROUT *Oncorhynchus mykiss*

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DNA methylation is one of the most extensively studied epigenetic mechanisms that modulate gene expression and cellular differentiation. It plays critical roles during gametogenesis and early development of vertebrates. Previous studies in mammals demonstrated that DNA methylation is accomplished through the activities of DNA methyltransferases (Dnmts). Dnmt1 is associated with the maintenance of methylation status, whereas Dnmt3a and Dnmt3b are essential for *de novo* methylation. The present study aimed at investigating the dynamic transcription pattern of *dnmt* genes during gametogenesis and early development for the first time in rainbow trout (*Oncorhynchus mykiss*), an economically important fish species.

Due to the fact that the teleost experienced a third round of whole genome duplication (WGD) apart from the two round of WGD that occurred at the root of the vertebrate lineage, the evolutionary of *dnmt* genes remains unclear. Indeed, there are three common fates for the duplicated genes after WGD: loss one of the duplicate gene, sub- or neo-functionalisation. In the present study, two ohnologs of *dnmt1* (*dnmt1a* and *dnmt1b*) and eight paralogs of *dnmt3* (including 3 paralogs of *dnmt3a*: *dnmt3aa*, *dnmt3ab1*, *dnmt3ab2*, and 5 paralogs of dnmt3b: *dnmt3ba1*, *dnmt3ba2*, *dnmt3bba1*, *dnmt3bba2* and *dnmt3bbb*) were identified in rainbow trout. Spatial expression patterns of *dnmt* genes were analysed using the PhyloFish database to identify the main expression territories of these genes. Results showed that, both *dnmt1* ohnologs were preferentially expressed in unfertilised eggs. *dnmt3a* paralogs were highly expressed in brain, whereas *dnmt3ba1*, *dnmt3ba2*, *dnmt3bbb* were predominantly expressed in skin, head kidney and ovary, respectively, which could be possibly linked to a sub- or neo-functionalisation after WDG.

The dynamic expression patterns of *dnmt* genes were analysed in trout gonads during gametogenesis (between two spawn). Our results showed that there was a remarkable increase in the mRNA levels of *dnmt1a* and three of the five *dnmt3b* paralogs in oocytes compared with ovaries in females. In males, we observed the highest mRNA level of *dnmt1a* together with the lowest mRNA levels of *dnmt3ab1* and *dnmt3bba1* at the late-spermatogenesis stage. Besides, mRNA levels of *dnmt* genes were also analysed during early development of trout from stage 0 (oocyte) to stage 36 (alevin). All *dnmt* genes displayed a similar expression pattern, with no or low mRNA levels from stage 0 up to 15, followed by a sharp increase at the setting up of the most of the vital organs (stage 22/23) and then decreased after hatching. By contrast, there was relatively high transcript abundance of *dnmt3bbb* from the beginning of embryogenesis, suggesting its functionally importance during early development.

In conclusion, the dynamic expression patterns of *dnmt* genes during gametogenesis and early development suggested that epigenetic mechanisms actively participated in these physiological processes in trout. The distinct transcription patterns of *dnmt3b* paralogs indicated that they may subject to sub- or neo-functionalisation after WDG.

SELECTIVE BREEDING OF SWIMMING CRAB *Portunus trituberculatus* IN CHINA

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The swimming crab, *Portunus trituberculatus* (Crustacea: Decapoda: Brachyura) is widely distributed in the coastal waters of Asia-pacific countries. The farming of *P. trituberculatus* has been developing rapidly in China over the last decades and the highest yield of 100,000 tons with 40,000-ha farmed area. However, the swimming crab harvests have declined drastically with frequent outbreaks of disease. Moreover, the wild fishery ground and fishery season of this crab are almost disappeared in many regions since 1990s. In order to enrich this resource, scientists at the Yellow Sea Fisheries Research Institute (YSFRI) worked to select and breed a new variety of *P. trituberculatus* with improved growth performance since 2005. In 2010, they have produced a fast-growing population called "Huangxuan No.1" after five generations of selection. Further new species selective-breeding research is ongoing.

The goal of the breeding program is to develop a faster-growing cultured crab (*P. trituberculatus*) through population selection strategy. In 2005, a mating design is applied. The base selective population was established after finishing the combining ability test and genetic structure analysis. Each generation selective intensity was around 5% to select the bigger ones. After mating, the females migrate to the pond indoor for overwintering. Females extrude a brood of fertilized eggs with stored sperm, then moved to the cultivate pond indoors. The released larvae, termed zoea, are advected to indoor farming pond where they go through four zoea-stage larvae, one megalopa and juvenile stage. At juvenile II stage stage, randomly sampled animals were moved to the ponds outdoor separately. The ponds were separated into several parts with net.

Till date, the new variety "Huangxuan No. 1" for fast-growthing of *P. trituberculatus* was selected successfully in 2010 after five generation selection from four geographical population, and the body weight increased 20.12% and survival rate increased 32.00%, respectively, compared with the unselected population.

EFFECT OF SALINITY ON Na^+/K^+-ATPase IN PACIFIC ABALONE

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Na^+/K^+-ATPase (NKA) belongs to the P-type ATPase family, which is located in the cell membrane and is distributed in diverse tissues and cells. The main function of the NKA is to regulate the equilibrium of osmotic pressure. To better understand the role of NKA in the osmoregulation, the full length cDNAs of NKA α subunit and β subunit were firstly cloned and characterized from Pacific abalone *Haliotis discus hannai* in the current study.

Sudden salinity change stimulation (low salinity seawater (LSW) or high salinity seawater (HSW)) lead to the bunji-change of ion concentration (Na^+ and K^+) in hemolymph, however, the relative stability of ion concentration in tissue revealed that Pacific abalone has strong osmotic pressure regulation ability when faced with the salinity change. Meanwhile, the expression and activity of the NKA was significantly decreased (LSW) or increased (HSW) at the ion concentration re-establishing stages, which was consistent with the coordinated regulation of ion concentration in hemolymph (Figure 1). Moreover, a highly significant positive correlation between cAMP concentrations and the NKA mRNA expression (NKA activity) was observed in mantle and gill (Figure 2). Therefore, the sudden salinity change may affect the NKA transcription activation, translation and enzyme activity via cAMP mediated pathway.

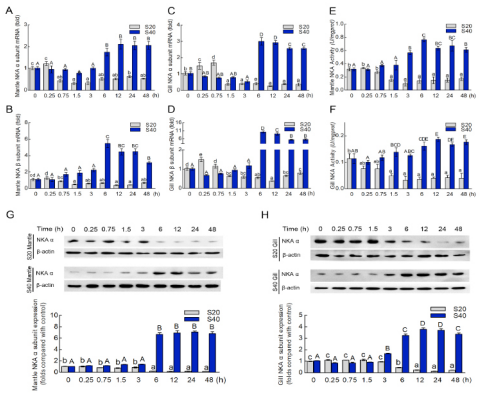

Figure 1 Analysis of NKA expression and activity.

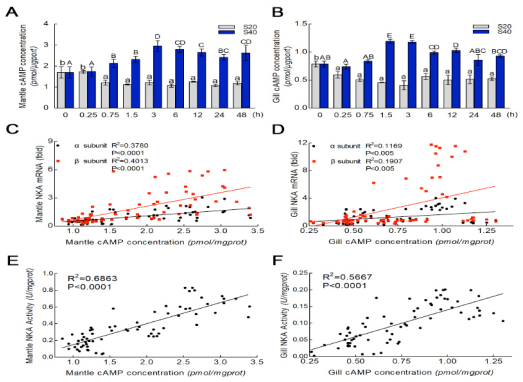

Figure 2 Correlation between cAMP concentrations and NKA.

MOLECULAR CHARACTERIZATION AND IMMUNE RESPONSES OF DIM 1 LIKE PROTEIN (DLP) FROM BIG-BELLY SEAHORSE (*Hippocampus abdominalis*)

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DLP (Dim 1 Like Protein) is a protein which shares similar amino acid sequence identity to Dim 1 (Dimethyl adenosine transferase 1) protein. DLP is implicated in pre-mRNA splicing and the S/G2 transition in cell cycle progression as same as Dim 1 protein. RNA splicing carried out by a multi-component complex known as the spliceosome, composed of more than 300 distinct proteins and five small nuclear ribonucleoproteins (snRNPs). Five snRNPs (U1, U2, U5, and U4/U6) are highly conserved RNA/protein complexes involved in splicing processes. Normally, DLP is located in the cell nucleus and shown to interact with the U5 in spliceosome during pre-mRNA splicing. Further, it pre-assembles with U4/U6 to form a [U4/U6: U5] tri-snRNP complex which required for promoting pre-mRNA splicing. Moreover, Dim 1 has a thioredoxin fold but does not contain the thiol di-sulfide redox active universal CXXC motif. Therefore, DLP is related to thioredoxin super family. In this study, DLP was selected from big-belly seahorse for the molecular characterization of relevance to their immune responses. According to the in-silico analysis performed, evolutionary relationship identified as DLP belongs to the thioredoxin superfamily. DLP consisted of 149 amino acids with 17 kDa and 6.5 isoelectric point (pI). Pairwise alignment with DLP with other orthologs showed that highest sequence similarity 97.3% and identity 98.7% with *Hippocampus* species.

Quantitative real-time PCR (qPCR) results revealed the highest tissue-specific immune expression in the ovary followed by skin and blood and lowest expression in the spleen among fourteen different tissues from healthy seahorses. As these tissues actively participating in the pre-mRNA splicing process intensive expression can be observed in all tissues. Seahorses subjected to immune stimulation with lipopolysaccharides (LPS), polyinosinic:polycytidylic acid (Poly I:C), *Edwardsiella tarda* and *Streptococcus iniae* to observe the transcriptional pattern in the DLP. Blood tissue showed downregulation up to 12 hours postinfection and upregulation occurred after 12 hours. In response to the immune stimulation, T cells alternative splicing happens and activated the immune response. All most all the protein-coding genes contain non-coding sequences of genes called introns, which must be completed and precisely removed to yield matured functional mRNAs. Therefore, RNA splicing consists of a process of removing introns and joining exons as a prerequisite for the expression of many eukaryotic genes. The mRNA splicing plays a major role in improving complexity and diversity of proteins in eukaryotes by producing different types of mRNA using pre-mRNA to defend against harmful invaders and immune stimulants. Altogether the DLP gene can be identified as an immunologically important gene in seahorses.

THE EFFECT OF DURATION OF SPAWNING INDUCTION AND FEED ENRICHMENT WITH ARA-OIL ON THE REPRODUCTIVE PERFORMANCE OF VIRGIN ZANDER (*Sander lucioperca* L.) BREEDERS IN FULLY CONTROLED CONDITIONS

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The issue of spawning induction in zander in fully controlled conditions has been an issue of recent interest. Nevertheless, most of these studies did not evaluate the spawning performance of breeders. From other side, only limited number of researches have been dealing with broodstock nutrition and even those mainly focusing on the usage of forage fish. Nevertheless, studies on perch (*Perca fluviatilis*) revealed the level of arachidonic acid (ARA) in dry feed to be rather strong modifying factor of the reproductive success. Therefore, the aim of our study was to evaluate the readiness of virgin breeders for artificial reproduction with respect to the duration of spawning induction as well as the effect of feed enrichment with ARA rich oil on the reproductive performance of virgin zander breeders reared exclusively in recirculation aquaculture system (RAS).

Two-year-old virgin zanders were distributed in two 3 m^3 tanks inside the experimental RAS. Each tank was stocked with 36 individuals which were fed with either unmodified Coppens SteCo Repro (CONTROL) or enriched by vacuum diffusion with 40% ARA concentrated oil (ARA). In the course of two months, temperature was gradually decreased from 22 to 9 °C which was further on kept stable between 9 and 10 °C. Oocyte size was monitored by monthly biopsy starting from 3.5 months since the start of spawning induction. Five different pairs of fish from each feeding group were hormonally induced on three different occasions with one-month time-frame between each (1st, 2nd and 3rd). Reproductive success was evaluated in each occasion as well as the fatty acid composition of the stripped eggs. Additionally, preliminary evaluation of the larviculture success was performed after 3rd propagation.

Most of the evaluated reproductive performance parameters were in favor of CONTROL group, with single statistically significant difference in embryo survival 3rd (42.6 ± 19.1 % and 11.5 ± 18.9 %, $p = 0.048$). However, higher share of spermiating males was found in ARA group in 1st (80% vs. 40%). In terms of duration of spawning induction, longer induction positively affected most of the parameters in CONTROL (embryo survival 28.7 ± 13.1% and 42.6 ± 19.1%), however it had negative effect on the ARA (embryo survival 22.9 ± 8.7 % and 11.5 ± 18.9 %) in second 2nd and 3rd, respectively. Nevertheless, in both groups higher share of females' post-spawning mortality was observed in the last propagation trial (60%), compared to second (20%). Larval survival did not differ between the groups (13.2 ± 5.0 % in ARA and 11.4 ± 1.9 % in CONTROL) neither did the size of month old juveniles (43.8 ± 8.6 mg in ARA and 43.0 ± 6.3 mg in CONTROL).

The aim to improve the reproductive performance of fish by feed enrichment with ARA-oil to the level similar to eicosapentaenoic acid (EPA) failed. Previous studies on the perch nutrition revealed EPA : ARA ≈ 1 to be optimal in terms of reproduction success. In case of the present study, either the ARA ratio in the feed was not optimal for zander or the problem lays some other altered feed properties connected with the enrichment. With respect to spawning duration examination, it seems that longer duration can lead to improved success of spawning, however the breeders' post-injection mortality might increase. Finally, results of the present study require additional studies related to both induction schedule and broodstock nutrition.

THE INFLUENCE OF DIET ON GONAD QUALITY IN THE SEA URCHIN *Paracentrotus lividus*

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In the present study, we tested three diets (a seaweed, a manufactured feed, and the same manufactured feed supplemented with pigments) on adult *Paracentrotus lividus* in order to determine which one brings the largest gonads and/or the marketable colour.

Adult *P. lividus* were fed *ad libitum* during three months with a basal diet (B, Veronesi, Verona, Italy), a supplemented diet (A) or *Ulva lactuca* (U). The A diet was B diet supplemented with 100 mg/kg of astaxanthin (Veronesi, Verona, Italy). Gonad colour, gonadosomatic index (GSI), wet feed intake (WFI), absorption efficiency (AE) and food conversion ratio (FCR) were compared among each treatment.

The B and A diets brought higher lightness (L*) and lower redness (a*) than the U diet; statistically, A diet had higher L* than U diet, and a* values were not different among diets, although urchins fed with the B diet had lower values than samples at the initial time (T_0). Yellowness (b*) was similar among diets and T_0 (Table 1). In terms of gonad growth, the two manufactured diets brought higher GSI; A diet showed significantly higher GSI than U diet and T_0 (Table 1). WFI, AE and FCR were higher in urchins fed U diet (Table 1).

Our results are in agreement with the results by Cyrus et al. (2014); GSI is higher in sea urchins fed with manufactured diets, which are more efficient in the conversion of the ingested feed into units of gonad tissue. The high intake of *U. lactuca* can be due to its low protein content and digestibility (Fernandez and Boudouresque, 2000). Astaxanthin promoted gonad growth, as reported previously by Peng et al. (2012), also fostering a brighter and yellower gonad colour. However, none of the tested diets contributed to improve the red component in gonad colour. Therefore, a more effective feeding protocol to enhance gonad colour remains to be identified.

Our experiment confirms that manufactured diets represent a promising cost-effective feed for aquaculture purposes, enabling a rapid and considerable gonad growth with a minimum amount of feed consumed. Further research on how to improve gonad pigmentation through diet will contribute to an optimal formulation of artificial diets for sea urchin culturing.

TABLE 1. Mean L*, a *, b*, GSI, WFI, AE and FCR assessed at the start (T_0) and at the end of the trial, for the three treatments (U, B, A). Different superscripts in the same row indicate significant differences.

	T_0	U	B	A
L*	31.8^{c}	41.0^{bc}	50.5^{ab}	52.2^{a}
a*	20.0^{a}	16.2^{ab}	13.2^{b}	14.5^{ab}
b*	21.7	22.4	24.6	26.5
GSI (%)	1.0^{c}	2.7^{bc}	8.5^{ab}	10.3^{a}
WFI (g)	-	79.7^{a}	19.7^{b}	20.2^{b}
AE (%)	-	65.9^{a}	59.2^{a}	46.8^{b}
FCR	-	25.0^{a}	3.2^{b}	2.3^{b}

PRELIMINARY RESULTS OF QUANTITATIVE PCR OF *Haplosporidium pinnae* n. sp. AFFECTING PEN SHELL *Pinna nobilis*

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The Pen Shell (*Pinna nobilis*) has been a protected species since 1992 Annex II of the Barcelona Convention (SPA/BD Protocol 1995), Annex IV of the EU Habitats Directive (EU Habitats Directive 2007), and Spanish Catalog of Threatened Species (Category: Vulnerable, Royal Decree 139/2011) (Vazquez Luis et al., 2017 *Front Mar Sci* 4:220). However, an outbreak caused by an haplosporidan parasite has recently been reported associated with mass mortalities of *P. nobilis* in western Mediterranean (Darriba, 2017 *J Invertebr Pathol* 148:14-19). The characterization of the parasite by histology, TEM, SEM and SSU rDNA sequence confirmed the causal agent as a new species and the name *Haplosporidium pinnae* was suggested (Catanese et al., 2018 *submitted*). In autumn 2016, worrying high mortality rates reaching up to 100% in the center and southernmost coasts of the Spanish Mediterranean Sea including Balearic Islands were reported (Vazquez Luis et al., 2017). In summer 2017, other Mediterranean countries reported on the critical situation in their *P. nobilis* populations. As a consequence, Spanish authorities has changed the status of the species from vulnerable to critically endangered and approved emergency actions for maintaining 215 *P. nobilis* specimens in 3 Spanish aquaculture centers, 50 of which arrived at the IFAPA Agua del Pino (Southeast of Spain) research center in December 2017. Unfortunately, after several weeks in the IFAPA facilities, some pen shells showed warning unspecific signs of illness: mantle retraction, gaping, slow closing, slow response to touch and reopening of the valves after a short time, resulting finally in death.

Thus, the focus of this study was to develop species-specific conventional PCR (cPCR) and quantitative PCR (qPCR) techniques to diagnose *H. pinnae*. This research was carried out by designing a pair of primers to target a specific DNA fragment. Amplification was confirmed by electrophoresis in agarose gel and sequencing. Two standard curves were calculated using serial dilutions of the plasmid and plasmid diluted with DNA of pen shell; the efficiency (E) was from the slope of the standard curve following formula (Ptaffl, 2001 *Nucleic Acids Res* 29:e45) $E=10^{-1/slope}-1$.

Preliminary results show that only one melting temperature peak was observed with a mean 84.10.01 C. The limit of detection of the qPCR assay was 100ag which is equivalent to <10 *H. pinnae* DNA copies / ng of plasmid DNA. The *H. pinnae* standard curve displayed an amplification efficiency of 102 % and linearity (R^2) of 0.994. When plasmids with DNA of pen shell were tested similar results were obtained (E = 103% and R^2 = 0.992). This study could provide a useful tool to assess the conservation of *P. nobilis*. However, the results shown in this summary are preliminary, therefore we must continue working on the development of fast, specific and effective diagnostic techniques.

Acknowledge: This research has been funded by the project MAPAMA ref. 28-5310 within LIFE IPE INTERMARES (LIFE15 IPE ES 012) and the agreement IFAPA-UCV 014/2018. M. López-Sanmartín contract is co-financed by the State Plan for Scientific and Technical Research and Innovation 2013-2016, MINECO, ref. PTA2015-11709-I. G. Catanese was supported by a research contract from DOC INIA CCAA program.

EFFECT OF ENCAPSULATED ESSENTIAL OILS EMBEDDED IN THE ICE USED DURING STUNNING AND SLAUGHTERING, AND IN COLD STORAGE, ON FRESH FARMED SEA BREAM (*Sparus aurata* L) SHELF LIFE

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In previous works it has been proven that the traditional method of farmed fish slaughtering using hypothermia in water and crushed ice slurry, and without stunning, results in excessive stress in the animal. As a consequence, the quality of the fish suffers and shelf life shortens when kept refrigerated on ice. To improve animal welfare, we have developed a new method for farmed fish stunning and slaughtering that reduces stress. It is based on encapsulation technology of clove essential oil (CEO) in β-cyclodextrins (β-CDs), and ice manufacturing that contains these CEO-β-CDs embedded in the ice crystals. This ice with CEO-β-CDs has a certain anaesthetic effect on the fish, which suffers less stress and less stunning time at slaughtering. On the other hand, our research has also shown that an adequate combination of essential oils (EO), encapsulated in β-CDs and embedded in the ice, when used in fish cold storage obtains a significant lengthening of the shelf life of the fish. Therefore, in this research work the influence of the successive use of these two technologies is analysed.

At industrial level, CEO-βCDs included in the ice were evaluated in farmed gilthead seabream (*Sparus aurata* L.) at slaughtering, using crushed ice in sea water as killing method. Then, the fish was cold stored at 3 ºC with ice toping in polystyrene boxes, using a combination of EOs-β-CDs embedded in the ice crystals. Fish stunned by traditional method had higher cortisol levels in plasma immediately after slaughtering. Conversely, fish stunned and slaughtered using crushed ice containing CEO-β-CDs had low cortisol and glucose levels in plasma immediately after slaughtering (Fig. 1). The fish anaesthetized with ice including CEO-β-CDs, and then stored in polystyrene boxes with ice toping including EOs-β-CDs has a longer shelf life than fish with traditional slaughtering and ice storing, with increments of shelf life greater than 25-30% respecting to farmed fish killed and stored with traditional procedures (Fig. 2).

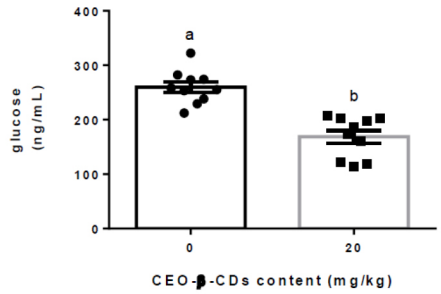

Figure 1. Glucose levels in plasma of fish stunned and slaughtered with sea wáter and ice containing CEO-β-CDs.

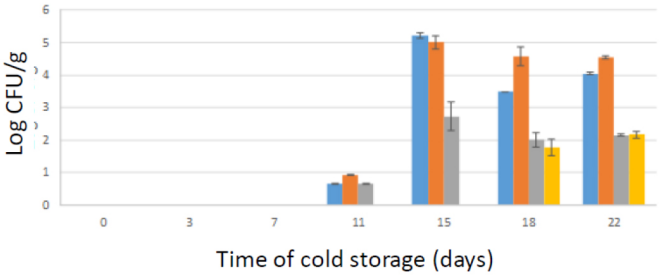

Figure 2. Lactic-acid bacteria in fish traditionally killed and ice stored (bar 1, starting from the left), and in fish stunned using sea water and ice containing CEO-β-CDs, and stored in ice including EOs-β-CDs (bar 4).

TRANSCRIPTOMIC AND PHYSIOLOGICAL RESPONSE OF *Crassostrea virginica* EXPOSED TO WATER-ACCOMMODATED FRACTION (WAF) OF PETROLEUM

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Crassostrea virginica is a species of economic importance, representative of the coastal lagoons of the Gulf of Mexico. Oyster aquaculture production in Mexico represents 46%, and it occupies the third place in volume. The fishery production of the oyster in 2014 was 53,757 tons, with the states of Veracruz, Tabasco, and Tamaulipas representing around 87.5% of the total volume. Also, considerable efforts are being made in these states to increase the aquaculture production of *C. virginica*. However, the increase in oil exploration and production represents a risk of potential oil spills with possible adverse effects on the aquaculture zones and ecosystem. Based on above, the objective of the present study was to assess the impact of exposure to WAF on the transcriptomic and physiological response of *C. virginica* through RNA-Seq and respirometry.

Oysters were sampled at Morales Lagoon (Tamaulipas, Mexico) and they were acclimated to temperature and salinity at the UTMART laboratory. Then, a 3-week oil exposure trial was carried out using a crude light oil (35° API). The experimental design consisted of a completely randomized design, and it included four treatments and three replicates (n=30). The treatments consisted of exposure to different concentrations of oil: control, 50, 100 and 200 µg/L. Light oil added to the experimental units was prepared based on Singer et al. (2000). Each week, samples from digestive gland were obtained to asses the oil bioaccumulation, and others were stored in RNA-Later to -80°C to RNA extraction. The assessment of the metabolic respiration of oysters was carried out using respirometric chambers per triplicate for each treatment.

The results showed oil accumulation in oyster tissues, directly proportional to the oil concentration (Fig. 1). Also, the analysis of the transcriptome showed that there was an effect of both the concentration of hydrocarbon and the exposure time (Fig. 2). Differential expression analysis (Fold change > 2 and FDR < 0.05) with edgeR and DESeq2 showed that the highest number of over-expressed genes were found in weeks 1 and 3 for 100 and 200 µg/L. Although, oxygen consumption results showed no significant differences between treatments (P > 0.05), there was a significant effect of oil exposure on the differential expression on genes.

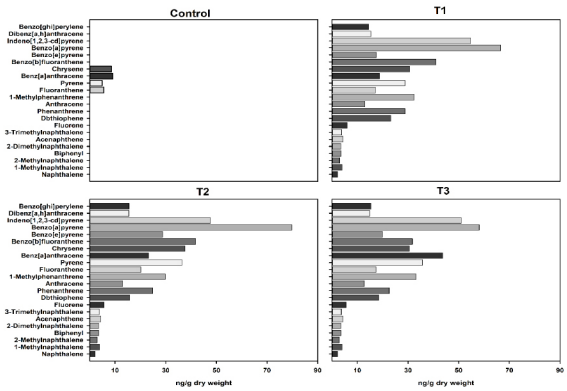

Fig. 1. PAH's detected in the soft tissues of *C. virginica*. T1-T3: Week 1-3 at 200 g/L.

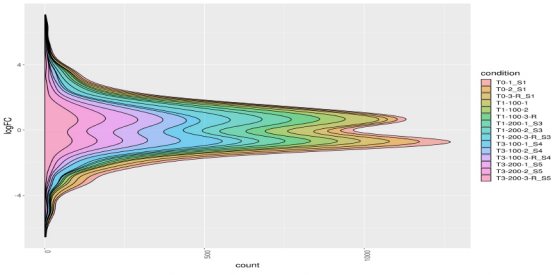

Fig. 2. Density plot of gene expression in the oil exposure trial. FC: Fold change.

DETECTION OF *Marteilia cochillia* IN *Cerastoderma edule* FROM HUELVA COAST (ANDALUSIA, SW SPAIN)

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Marteilia cochillia Carrasco et al., 2013 (*Aquaculture* 412-413: 223-230) is a new emerging parasite of *Cerastoderma edule* associated with mass mortalities of this species from the Ebro Delta (Catalonia, NE Spain) and cockle fishery collapse in the Ría de Arousa (Galicia, NW Spain) (Villalba et al., 2014 *Dis Aquat Organ* 109: 55-80). Recently, Carballal et at., 2016 (*Dis Aquat Organ* 122: 137-152) have shown histological evidence that *C. glaucum* is resistant to *M. cochillia* resulting significantly less infected than *C. edule* in the same areas.

Histopathological analysis of samplings from the coast of Huelva (Andalusia, SW Spain) in the spring of 2015, revealed the presence of *Marteilia* sp. in cockles from Carreras river (3/30) and Piedras river (1/30). PCR analysis and sequencing of DNA from paraffin sections of a heavy infected sample from Carreras river confirmed the presence of *M. cochillia*. Therefore, during 2016 (April, July, October) to 2017 (January and May) new samplings from natural beds of cockle species were carried out to determine the prevalence of *M. cochillia* in Huelva coast. The specimens were processed for classical histology, and samples of gill, mantle and digestive gland were preserved for DNA analysis. The differentiation between *C. edule*/*glaucum* was performed by PCR analysis according to Freire et al., 2011 (*Eur Food Res Technol* 232:83–86). Molecular diagnosis of *Marteilia* was carried out by amplifying and sequencing different fragments of the ribosomal locus.

All sampled cockles turned out to be *C. edule*. The histological results showed equal *Marteilia* prevalences of 1.3 % in Carreras river (2/150) and Piedras river (2/150). Different sequences obtained using species-specific PCR confirmed the identity of *M. cochillia*.

In conclusion, *M. cochillia* is detected for the first time affecting *C.edule* from Gulf of Cádiz (SW Spain) with very low prevalence.

Acknowledge: This work was supported by the "Ministerio de Economía y Competitividad (MINECO), Plan Nacional I+D" and the European FEDER funds through project AFROBIV (CGL2014–53557-P). M. López-Sanmartín has a contract co-financed by the State Plan for Scientific and Technical Research and Innovation 2013-2016, MINECO, ref. PTA2015-11709-I. M. Pérez-Miguel has a FPI PhD MINECO contract to MPM ref. BES-2015-072703.

PRELIMINARY RESULTS OF QUANTITATIVE PCR OF *Haplosporidium pinnae* n. sp. AFFECTING PEN SHELL *Pinna nobilis*

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The Pen Shell (*Pinna nobilis*) has been a protected species since 1992 Annex II of the Barcelona Convention (SPA/BD Protocol 1995), Annex IV of the EU Habitats Directive (EU Habitats Directive 2007), and Spanish Catalog of Threatened Species (Category: Vulnerable, Royal Decree 139/2011) (Vazquez Luis et al., 2017 *Front Mar Sci* 4:220). However, an outbreak caused by an haplosporidan parasite has recently been reported associated with mass mortalities of *P. nobilis* in western Mediterranean (Darriba, 2017 *J Invertebr Pathol* 148:14-19). The characterization of the parasite by histology, TEM, SEM and SSU rDNA sequence confirmed the causal agent as a new species and the name *Haplosporidium pinnae* was suggested (Catanese et al., 2018 *submitted*). In autumn 2016, worrying high mortality rates reaching up to 100% in the center and southernmost coasts of the Spanish Mediterranean Sea including Balearic Islands were reported (Vazquez Luis et al., 2017). In summer 2017, other Mediterranean countries reported on the critical situation in their *P. nobilis* populations. As a consequence, Spanish authorities has changed the status of the species from vulnerable to critically endangered and approved emergency actions for maintaining 215 *P. nobilis* specimens in 3 Spanish aquaculture centers, 50 of which arrived at the IFAPA Agua del Pino (Southeast of Spain) research center in December 2017. Unfortunately, after several weeks in the IFAPA facilities, some pen shells showed warning unspecific signs of illness: mantle retraction, gaping, slow closing, slow response to touch and reopening of the valves after a short time, resulting finally in death.

Thus, the focus of this study was to develop species-specific conventional PCR (cPCR) and quantitative PCR (qPCR) techniques to diagnose *H. pinnae*. This research was carried out by designing a pair of primers to target a specific DNA fragment. Amplification was confirmed by electrophoresis in agarose gel and sequencing. Two standard curves were calculated using serial dilutions of the plasmid and plasmid diluted with DNA of pen shell; the efficiency (E) was from the slope of the standard curve following formula (Ptaffl, 2001 *Nucleic Acids Res* 29:e45) $E=10^{-1/slope}-1$.

Preliminary results show that only one melting temperature peak was observed with a mean 84.1±0.01 ◦C. The limit of detection of the qPCR assay was 100ag which is equivalent to <10 *H. pinnae* DNA copies / ng of plasmid DNA. The *H. pinnae* standard curve displayed an amplification efficiency of 102 % and linearity (R^2) of 0.994. When plasmids with DNA of pen shell were tested similar results were obtained (E = 103% and R^2 = 0.992). This study could provide a useful tool to assess the conservation of *P. nobilis*. However, the results shown in this summary are preliminary, therefore we must continue working on the development of fast, specific and effective diagnostic techniques.

Acknowledge: This research has been funded by the project MAPAMA ref. 28-5310 within LIFE IPE INTERMARES (LIFE15 IPE ES 012) and the agreement IFAPA-UCV 014/2018. M. López-Sanmartín contract is co-financed by the State Plan for Scientific and Technical Research and Innovation 2013-2016, MINECO, ref. PTA2015-11709-I. G. Catanese was supported by a research contract from DOC INIA CCAA program.

INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) APPLIED TO THE SHRIMP REARING IN A BIOFLOC SYSTEM

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This study aimed to evaluate the performance of an integrated multi-trophic aquaculture system applied to biofloc shrimp rearing.

The IMTA system was composed by a shrimp (*Litopenaeus vannamei*) rearing tank (800 L), a tilapia (*Oreochromis niloticus*) rearing tank (90 L) and by floating rafts for the salicornia (*Sarcocornia ambigua*) culture. A submerged pump constantly took the water from the shrimp tank to tilapia tank. Them, by gravity, the water flow through the salicornia floating rafts returning to the shrimp tank. The shrimp, tilapia and salicornia stock densities were 312 shrimp m^{-3}, 445 tilapia m^{-3}, and 106 plants m^{-2} respectively. The same experimental units were used in the control system without the salicornia. The species and the system performances were evaluated. The initial weight was 4.09 ± 0.05 g, 1.16 ± 0.04 g and 1.17 ± 0.35 g for shrimp, fish and salicornia respectively. The shrimps were fed according to the feed table, and the fishes were fed with 1% of the fish biomass, stimulating tilapia to use biofloc as a food source. Results show no difference between average performance from shrimp and tilapia in both treatments (Table 1). Only IMTA total biomass was significantly higher than control system.

These results demonstrate a yield increase of up to 17.4 % by integrating *L. vannamei*, *O. niloticus* and *S. ambingua* in a biofloc system.

Table 1: *Liopenaeus vannamei*, *Oreochromis niloticus* and *Sarcocornia ambigua* performance in an integrated biofloc culture system for 57 days.

	IMTA	Control	p
Shrimp performance			
Mean final weight (g)	14.6 ± 0.4	14.1 ± 0.2	0.0906
Survival (%)	88.0 ± 4.0	89.3 ± 2.4	0.6083
FCR	1.7 ± 0.1	1.7 ± 0.1	0.6535
Growth rate (g week $^{-1}$)	1.5 ± 0.1	1.4 ± 0.0	0.1313
Final biomass (kg)	3.2 ± 1.5	3.1 ± 0.9	0.5886
Yield (kg m^{-3})	4.0 ± 0.2	3.9 ± 0.1	0.5886
Tilapia performance			
Mean final weight (g)	11.4 ± 1,2	11.5 ± 1.1	0.8589
Survival (%)	91.3 ± 3.2	87.5 ± 7.4	0.4453
FCR	0.15 ± 0.02	0.16 ± 0.01	0.6535
SGR (% day $^{-1}$)	4.41 ± 0.17	4.39 ± 0.20	0.8943
Final biomass (kg)	0.42 ± 0.06	0.40 ± 0.03	0.5737
Yield (kg m^{-3})	4.65 ± 0.65	4.49 ± 0.32	0.6737
Salicornia performance			
Mean final weight (g)	23.0 ± 6.26		
Survival (%)	92.19 ± 4.03		
Final biomass (kg)	0.68 ± 0.20		
Yield (kg m^{-2})	2.27 ± 0.67		
Shrimp plus tilapia plus salicornia			
Total final biomass (kg)	4.30 ± 0.34[a]	3.56 ± 0.08[c]	0.0051
Total yield (kg m^{-3})	4.83 ± 0.38[a]	3.99 ± 0.09[b]	0.0051

Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the Test T ($p < 0.05$).

IMPROVEMENT OF MOLECULAR DIAGNOSTIC TOOLS FOR A BETTER CONTROL OF VIRAL FISH PATHOGENS

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Viral pathogens highly impacting for fish health and international trade are regulated in the European Union (Directive 2006/88/EC) and submitted to an organized surveillance which aim is to prevent diffusion inside the EU and rapidly control outbreaks. Official diagnostic methods used to detect these regulated pathogens were historically based on a cell culture phase followed by a characterization step. EU Decision 2015/1554, applicable on the 1st of April 2016, enriches the panel of official detection tools giving the possibility to directly apply a molecular technique of real-time Reverse-Transcription Polymerase Chain Reaction (RT-qPCR). This incrementation of European regulation is in line with the evolution of the diagnostic practices observed in recent years in the field of fish pathology and presages of a more and more frequent use of molecular tools.

This work aimed to develop and validate a qualitative RT-qPCR method specific to Infectious Hemorrhagic Necrosis virus (IHNV). Protocol proposed by Purcell *et al*. (2013) was modified and optimized to allow a "one-step" analysis from a mixture of organs or culture supernatants. Positive and negative RT-qPCR controls as well as a positive exogenous process control (IHNV contaminated organs) were integrated to check the extraction and amplification steps. To minimize the risk of false negatives, the method also included the addition of a RNA bacteriophage in defined quantity in each sample to analyze, allowing the validation of the extraction step individually (Ninove *et al*. 2011).

The analytical and diagnostic performance of our RT-qPCR were assessed using samples obtained from cell culture or experimental fish contaminations. All genotypes of IHNV (E, M, U, J, L) were efficiently detected but not viruses like Viral Septicemia Haemoragic virus (VHSV), Infectious Necrosis Pancreatic virus (IPNV), Perch rhabdovirus, Eel Virus European X (EVEX), Pike Fry Rhabdovirus (PFR), Infectious Salmon Anemia virus (ISAV), … In our conditions, diagnostic sensibility and specificity of 100% as well as repeatability of 96.7% were obtained. A proficiency test organized among 6 European diagnostic laboratories has demonstrated a high compliance rate of 100%. The standard operational procedure (SOP) was submitted to public consultation in France and the method will be used by the official surveillance laboratories in a close future. A similar method specific to VHSV is under development and validation. These molecular methods are very specifics, with short time to results (24-48h) compare to cell culture analysis, especially for negative samples. Nevertheless, cell culture still represents a powerful tool for prospective analysis, allowing large spectrum detection. The enrichment of validated methods of diagnostic makes it possible to continuously improve the quality of the surveillance networks and to limit as much as possible the risk of epidemics within the EU.

PIKE, A "DISCREET VECTOR" OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS (VHSV) AND INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) ?

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Viral Hemorrhagic Septicemia virus (VHSV) is a novirhabdovirus listed at the European level (Directive 2006/88) responsible for severe disease in several freshwater fish species, particularly rainbow trout (*Oncorhynchus mykiss*). Since 2011, several isolations of VHSV were reported in France, particularly in 2 fish farms located in an area rich in ponds containing a large diversity of white fish but also pikes (*Esox Lucius*), a species susceptible to VHSV (OIE 2017). The most recent outbreak occurred at the beginning of spring 2016, with a significant increase of mortality on rainbow trout when water temperature grew up. VHSV could be detected and characterized by Sanger sequencing on full Glycoprotein gene. The isolates detected in 2016 in the 2 fish farms were identical (100% identity on 1524 nucleotides), and clustered in the same branch than previous isolates from the same farms. However, some differences not consistent with the estimated VHSV evolution rate (between 1.74x10E-3 and 6.01x10E-4 for genotype Ia isolates ; Einer-Jensen *et al*., 2004) suggest that several strains would circulate in the area. Epidemiological and laboratory investigations were engaged to identify the putative origins for viral dissemination in this specific hydro-geographical context.

Possible importation of infected fish was checked, and confrontation of French isolates sequences with German and Italian databases allowed showing high but not total identity with German isolates. Analysis of a potential carriage by wild fish allowed identifying pikes infected by both VHSV and Infectious Hematopoietic Necrosis virus (IHNV) in 1 out of the 9 sampled ponds in the incriminated area. Genetic characterization showed that the VSHV isolates belonged to Ia genotype and shared a high nucleotide identity (respectively 99.5% and 99% for complete G gene) with 2014 and 2016 isolates from rainbow trout. The pike IHNV clustered in European genotype but seemed quite different from recent isolates circulating on the French territory, with a maximum of 97.8% identity with an isolate identified in 1994. Both these VHSV and IHNV isolates were shown to be highly virulent for rainbow trout through experimental contamination, with at least 85% of mortality after bath infection. No mortality was observed for pike infected by the same route but interestingly, VHSV or IHNV could be isolated from rainbow trout fingerlings added to pike tanks for feeding after several days of contact.

Pike would represent a silent vector for the 2 notifiable viruses VHSV and IHNV and could contribute to their persistence in adapted hydro-geographic areas, contributing to recurrent infection events in salmonid farms. Complementary *in vivo* investigations should be carried out in order to deeply investigate the susceptibility of pike to VHSV and IHNV isolates and better characterize the level of risk associated to a potential virus release.

GROWTH PERFORMANCE OF SEA URCHIN *Paracentrotus lividus* FED DIETS WITH INCREASING PROTEIN LEVELS

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Sea urchins' roe (or gonads) are highly appreciated worldwide as a gourmet product called *uni* comparable to caviar. The establishment of commercial culture of sea urchins requires the development of feasible, cost effective, high quality formulated dry feeds able to provide the nutritional requirements to healthy somatic growth and to produce roe of high quality and market value. The protein requirements of *Paracentrotus lividus* has not been evaluated and is of major importance to formulate balanced feeds. This study aimed to evaluate the growth performance of *P. lividus* sea urchins fed with diets of different protein levels.

Six experimental practical diets were formulated with increasing protein content: 20 – 25 – 30 – 35 – 40 and 45 % DM. Fat content was kept constant (7%) and all diets were extruded. Triplicate groups of sea urchins *P. lividus* (initial weight: 16.9 ± 4.7 g) were fed each diet for 107 days. At the end of the experimental trial, the urchins' growth performance and feed intake were evaluated. Whole body proximal composition, nutrients gain and retention were determined.

The sea urchins final weight averaged 23.1 ± 1.5 g, and SGR 0.3 ± 0.05 % day^{-1}, with no significant differences between treatments. Protein intake was similar between treatments (1.30 ± 0.22 g/Kg of ABW/day), but feed conversion ratio (FCR) decreased concomitantly with increasing dietary protein and was lowest in P40 and P45 diets (Figure 1). All diets resulted in a 5% increase in sea urchins' gonad index, reaching 8.2 ± 1.4% by the end of the trial. Whole body proximate composition was similar between treatments, with exception of gross energy that was highest in sea urchins fed diet P20%. Fat gain of sea urchins fed diet P20% was similar to those fed P30%, but significantly higher than other treatments. The present results show that sea urchins tend to adjust feed intake to match their protein requirements. Considering FCR results, diet P30 seems the most adequate for sea urchin.

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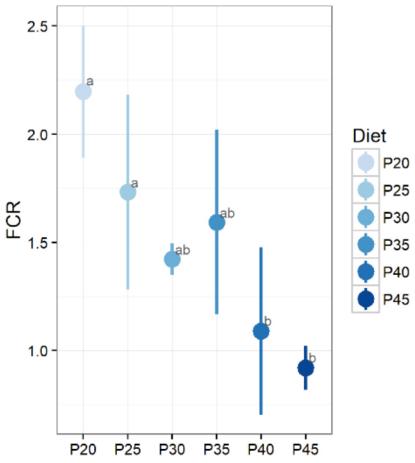

Figure 1 – Feed conversion ratio of the sea urchins fed diets with increasing protein levels. P20 to P45 indicate the protein level of the different diets. Different superscripts indicate statistical significant differences ($p < 0.05$).

GROWOUT AND FUCOIDAN YIELD OF NEW ZEALAND NATIVE FUCOID BROWN SEAWEED *Hormosira banksii* USING PACIFIC OYSTER FARM INFRASTRUCTURE

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Culturing seaweed with bivalves and fishes has been shown to be an effective method of bioremediation by removing nutrients released by the cultured animals from the seawater. Cultured seaweeds have known uses as food, industrial raw materials, as well as for pharmaceuticals and cosmeceuticals, including the use of fucoidan recovered from fucoid seaweeds. In New Zealand, little known about the potential for the aquaculture of native seaweeds. The feasibility of producing fucoidan by co-culturing a native fucoid seaweed, *Hormosira banksii*, with Pacific oysters using existing oyster farm infrastructure in the Mahurangi Harbour, New Zealand, was examined in this study.

An initial experiment aimed to see if the source populations (N = 6) of *H. banksii* affected seaweed growth, and fucoidan yield when grown out in oyster culture baskets on an intertidal oyster farm. Seaweed for starting cultures were sourced from six populations from within a 10 km radius of the oyster farm and cultured in oyster baskets for 200 days. At each source population, the *H. banksii* was growing on either rocky or muddy mangrove substrate. A subsequent growout experiment was carried out with macroalgae from two of the six populations showing the most favourable growth and highest fucoidan yield in the initial experiment.

Assessment of the thalli morphology and fucoidan content showed marked differences among the populations for *H. banksii*. Fucoidan yield was highly variable among the source populations, ranging from (2.2 – 7.0 % of dry weight, *SE* = 0.38) which is within the range of other commercial fucoidan species. The wet mass of cultured seaweed from all six source populations declined over the experimental period of culture, but the rate of decline was ($F = 11.37$, $p < 0.001$, $\eta^2 = 0.76$). Post-growout fucoidan yield did not differ significantly from seaweed harvested from the wild ($F = 2.18$, $p = 0.11$, $\eta^2 = 0.41$). Despite the prospects for fucoidan yield, the lack of growth of *H. banksii* when cultured in suspended baskets on an oyster farm indicates that more conducive culture conditions may need to be identified for this seaweed species before commercial culture can be progressed.

different among locations on the oyster farm ($F = 36.13$, $p < 0.001$, $\eta^2 = 0.91$). Location, growth substrate and source population of seaweed were found to influence fucoid yield after 200 days

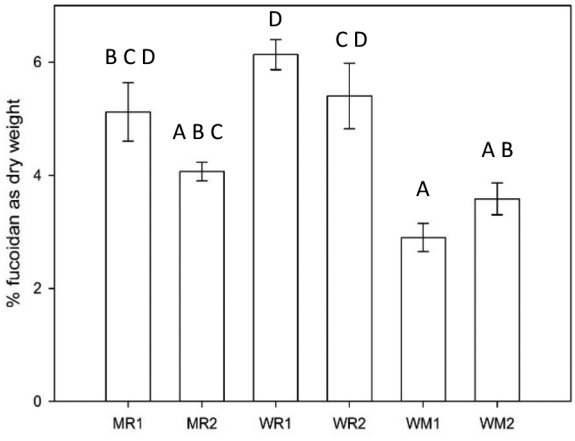

Figure 1. Mean fucoidan content as a percentage of dry weight of *H. banksii* sampled from six source populations. MR = Mahurangi Harbour Rocks, WR = Whangateau Harbour Rocks, WM = Whangateau Harbour Mangrove. Different superscripts above bars denote significant difference at $p < 0.05$. Error bars indicate standard error.

ENCOUNTERING THE THERMAL PREFERENCE OF MARAENA WHITEFISH (*Coregonus maraena*) REARED IN AQUACULTURE

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Introduction

As a local specialty in the German Baltic Sea area the demand for Maraena whitefish (*Coregonus maraena*) is increasing. Due to declining catches a stocking program for the wild population in the Pomeranian Bight was established but catches would not meet the demand. Therefor an aquaculture program was established with the Mecklenburg-Vorpommern Research Centre for Aquaculture and Fisheries. After investigating protocols for reproduction, seasonal and out-of-season, and rearing and first-feeding of the larvae a more detailed temperature protocol for rearing and fattening was needed. So the study aimed at finding the thermal preference of Maraena whitefish during different developmental stages.

Materials & Methods

As Fry (1947) stated is there "what may be termed a final preferendum, that is a temperature around which all individuals will ultimately congregate, regardless of their thermal experience before being placed in the gradient." To find that final preferendum, a trough was divided into seven compartments. One end was heated and the other end was cooled leading to a stable temperature gradient from 8.6°C to 19.9°C (Figure 1). Tested were three sizes of Maraena whitefish, juveniles with 19.5 ± 5.0g (n=6*10), sub adults with 127.5 ± 5.5g (n=6*10), and adults with 237.1 ± 24.2g (n=6*6). All animals were reared at 18.3 ± 0.2°C at least four weeks in advance of the preference trials. For the experiment the fish were placed in the gradient trough at ambient temperature. Three cameras, installed above the setup and enabling observation of all fish at all times, were synchronized and took a picture every five minutes. For the final temperature preferendum the last three hours until the 45th hour were analyzed.

For data analyses ANOVA was performed. Post-hoc comparisons were done through Tukey-HSD-test (homogeneous variances) and Dunnet T3-test (inhomogeneous variances). For all tests the level of significance was set to 5%. All tests were done by IBM SPSS Statistics 24.

Results

For all three tested size classes a rather low temperature sector was chosen by Maraena whitefish. The fish chose an average temperature of 11.3-14.3°C. Smaller fish were found at a temperature of 11.3±1.0°C. This was a significantly lower temperature ($p<0.05$) than the preferred temperature of both bigger groups. The middle sized class chose an average temperature of 14.0±0.3°C whereas the biggest fish were found at an average temperature of 14.3±1.1°C, which was not a significant difference. At the same time the bigger fish showed a wider distribution throughout the experimental trough than the smaller fish which were primarily found in the second and third coldest compartment (Figure 2).

Conclusion

This was the first time that the thermal preference for Maraena whitefish was investigated. It was shown that smaller fish prefer colder temperatures than bigger or at the same time older fish. The final preferendum was rather cold for *Coregonus maraena*. The determined temperatures will lead to a better production protocol in intensive aquaculture. These are an important hint to optimal growth temperatures and the most effective production temperatures.

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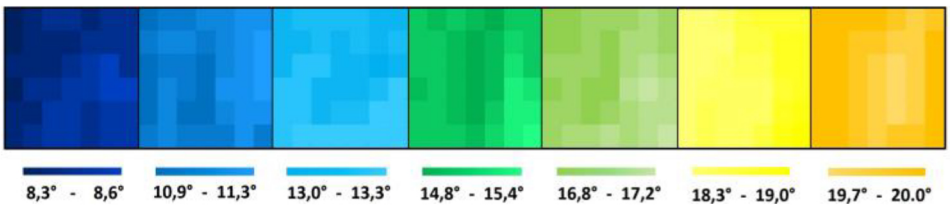

Figure 1 Temperature distribution between and in the compartments of the experimental trough. Temperatures are shown in °C.

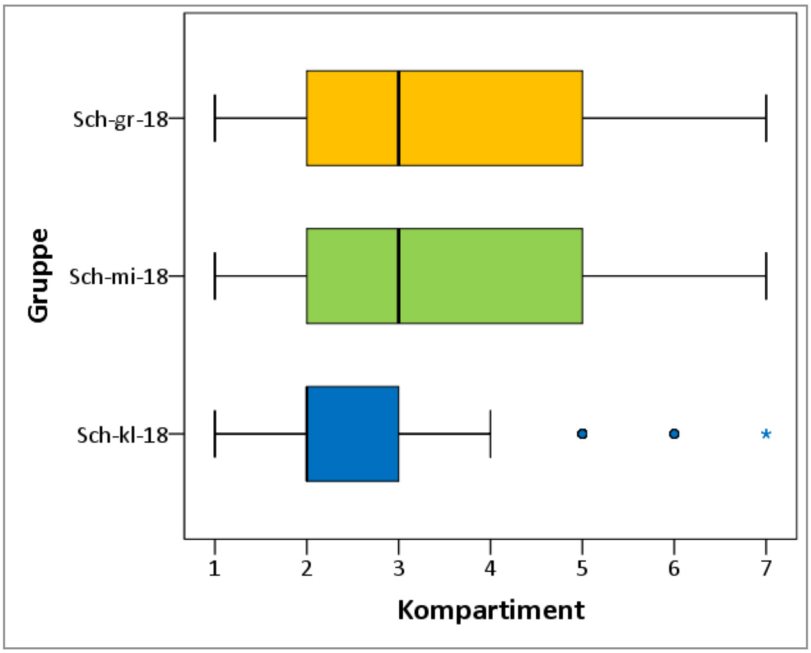

Figure 2 Distribution in the experimental trough for the three different size classes (Sch-gr-18: ~237g, Sch-mi-18: ~128g, Sch-kl-18: ~20g) after 45h. N=2220 observations for small and middle-sized fish, N=1332 observations for big fish

THE COURSE OF GROWTH, FEED INTAKE AND FEED EFFICENCY OF DIFFERENT TURBOT (*Scophthalmus maximus*) STRAINS IN RAS

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As feed is the major factor of costs in aquaculture production, traits such as feed intake (FI), feed efficiency (FE) and daily gain (DG) are becoming key issues of trait specific breeding programs. For most livestock, the rate of growth is strongly correlated to FI. FE, FI and DG are strongly related to each other. Therefore, it is possible to shift the growth curve to a more economic one by manipulation of FI. To do so, the limits and mathematical relations between these traits must be known, in order to manipulate the feed intake, either by feeding management or by breeding.
We fitted the flexible nonlinear model:

$$y \quad a \cdot \exp(\quad b \cdot x \quad c/x)$$

to long-term data of DG, FI and FE of different strains of communally reared turbot from a recirculating aquaculture system (RAS). We modelled each trait as a function of actual body size. We used the coefficient of determination (R^2) to display the suitability of the model. Split of growth characteristics between the strains was determined via a deviation bound set at a 2.5 % level. The curves of each trait were analyzed via shape, location of the point of inflection (POI), minimum and maximum.

We observed a major change in growth characteristics with a POI between 60 – 110 g weight, 15.7 - 18.6 cm length, respectively (Figure 01). The relationships between FI and FE to actual size were the same for both strains although the magnitude of the curves diverged (Figure 02). Diversion of the growth curves, related to sexual dimorphism, occurred similar in both strains at 460 - 500 g body weight.

Growth and growth-related traits are not linear nor isometric in turbot. Our results verify distinct life-stages with precise boundaries, which cause changes in trait characteristics. Accordingly, findings from experiments with juvenile fish are intransferable to other life-stages. The biological processes related to growth are still the same in different breeding strains.

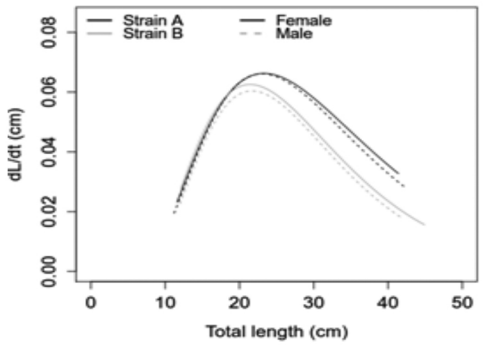

Figure 01: Daily gain as a function of actual body length in different turbot strains

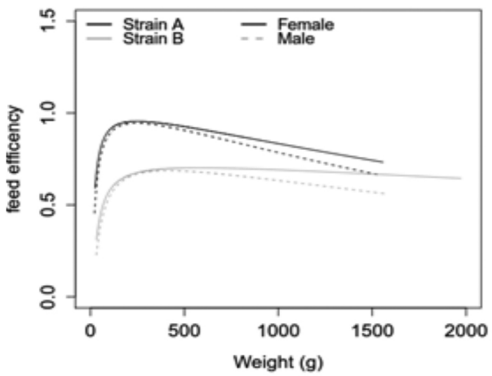

Figure 02: Feed efficiency as a function of actual body weight in different turbot strains

IMMUNE-RELATED GENE EXPRESSION IN *Litopenaeus vannamei* FED *Aloe vera*

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Worldwide shrimp production has decreased mainly due to viral and bacterial diseases. Therefore, in the last years, the use of natural products such as medicinal plants have become relevant to reduce mortalities. In a recent work, *Litopenaeus vannamei* was protected by *Aloe vera* from the infection with WSSV and *Vibrio parahaemolyticus*. Therefore, immune-related gene expression was investigated in *L. vannamei* fed with *A. vera*.

Shrimp weighing 2.9 g ± 0.8 g were fed with 1 g *A. vera*/kg feed. Six shrimp were sampled at 0, 6, 12, 24, 48, and 72 h for gene expression. Samples of time zero were taken just before feeding with aloe. After feeding with aloe during 24 h, uneaten food and waste material were removed and then shrimp were fed only with commercial feed. Time series expression of genes was measured by quantitative real-time PCR in hemocytes (translationally controlled tumor protein [TCTP], superoxide dismutase [cMnSOD], and penaeidin4) and hepatopancreas (heat shock protein 70 [HSP70]). Reference genes for hemocytes (40S–S24, β-actin, and ubiquitin) and hepatopancreas (β-actin, EF1α, and ubiquitin).

Dietary *A. vera* significantly down-regulated the expression of TCTP in hemocytes. Conversely, the mRNA expression of cMnSOD and penaeidin4 genes was significantly up-regulated. The mRNA expression of TCTP gene was significantly down-regulated ($P < 0.05$) at 12, 24, 48, and 72 h after feeding with *A. vera* (Fig. 1). The mRNA expression of cMnSOD gene was significantly up-regulated ($P < 0.05$) at 24, 48, and 72 h, but the reduction of its expression to the control level (0 h) was not observed in the time studied (Fig. 1). The mRNA expression of penaeidin4 was significantly up-regulated ($P < 0.05$) at 6 h and 12 h and the reduction of its expression to the control level (0 h) was observed from 24 h on (Fig. 1).

The dietary treatment significantly ($P < 0.05$) up-regulated the expression of HSP70 in the hepatopancreas at 48 h and 72 h, but the reduction of its expression to the control level (0 h) was not observed in the time studied (Fig. 1).This is the first report showing the effect of *A. vera* on the modulation of shrimp immune response.

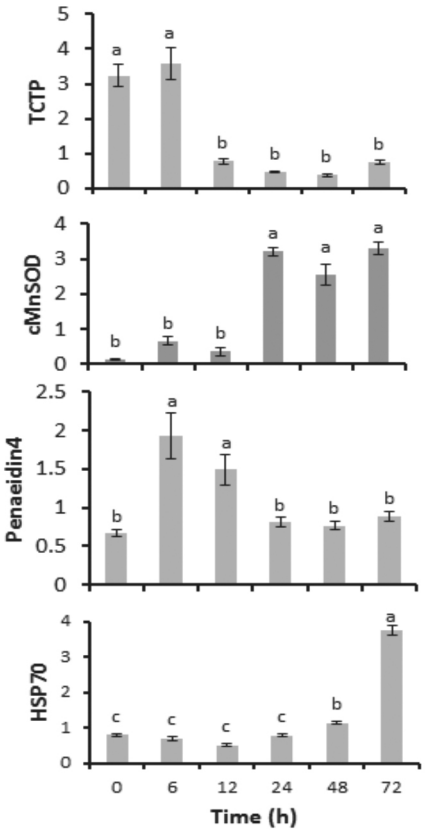

Figure 1. Relative expression of immune-related genes in *L. vannamei* fed with *A. vera*. Results are mean ± SE. Different letters indicate significant differences ($P < 0.05$).

THE BLOOD DISORDER IN *Osphronemus guramy*: IS IT POSSIBLY CAUSED BY MEGALOCYTIVIRUS INFECTION?

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Fish farming of *Osphronemous guramy* has faced a tremendous increase in all areas of Indonesia in the last years. Despite relevance of this production, the disease's knowledge for this fish is scattered and not systematic. Among the frequent pathogens are bacteria belonging to *Mycobacterium* spp. In 2016 until nowadays, a virus belonging to the Megalocytivirus family has been evidenced. Mortality of guramy was reported during the transition from summer to rainy season (November to December) with clinical symptoms of bleeding throughout the body and erosion of tail caudal, guramy mortality ranges from 50 to 80%. A descriptive survey of diseases in guramy farms was undertaken on representative farms (randomly chosen) in several areas of West Java and Central Java such as Banjanegara, Banyumas and Ciamis districts. Collecting data from each district were from 2 to 3 guramy farms.

The total number of white blood cells from diseased guramy were increased but the number of red blood cells was almost always decreased. There were many very immature and enlarge white blood cells were often present in blood samples examined under a microscope (Figure 1).The large numbers of white blood cells that have a very immature appearance (blasts) may indicate acute leukemia (cancer of the white blood cells) while this condition also have enlarge white blood cells that usually happened in chronic leukemia. The spread of Megalocytivirus must be minimized due to high mortality of infected fishes and lack of technology in development of rapid, sensitive and specialized viral diagnostic tests for early detection. It is recommended that further studies such as risk factors (i.e. age, kind of pond, rearing practices) which can increase the impacts of disease, should be carried out so that the farmers can prevent and manage disease outbreaks.

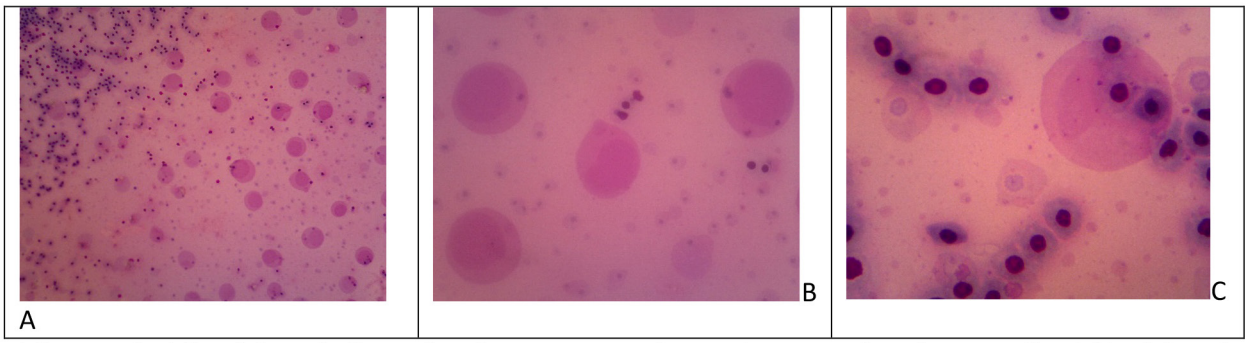

Figure 1. The blood disorder in guramy, immature and enlarge of white blood cells: the magnification microscope of (A) 100x; (B) 400x; (C) 1000x

HIGH-RESOLUTION LINKAGE MAPPING AIDED BY GENOME SURVEY AND TRANSCRIPTOME SEQUENCING IN *Portunus trituberculatus*: APPLICATIONS IN GROWTH-RELATED QTL AND GENE IDENTIFICATION

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A high-resolution genetic linkage map is an essential tool for decoding genetics and genomics in non-model organisms. In this study, linkage mapping was constructed for *Portunus trituberculatus* using specific-length amplified fragment sequencing (SLAF-seq). A high-resolution genetic linkage map with 10,963 markers was obtained, as far as we know, this has never been achieved in any other crustacean. The linkage map covered 98.85% of the whole genome with a mean marker interval of 0.51 cM.

A genome survey and transcriptome sequencing enabled 2,378 explicit annotated markers to be anchored to the map. Quantitative trait locus (QTL) mapping revealed 12 growth-related QTLs with a high mean *PVE* value of 23.7. Nine genes identified from the growth-related QTL region were considered important growth-related candidate genes. In particular, RE1-silencing transcription factor and RNA-directed DNA polymerase genes encoded nonsynonymous amino acids, which suggests a potential influence in growth regulation.

We have demonstrated that high-resolution linkage mapping aided by genome survey and transcriptome sequencing could serve as an important platform for QTL mapping and the identification of trait-related genes.

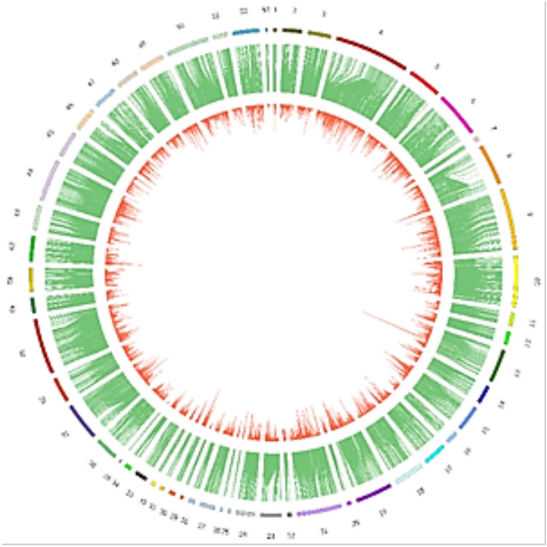

Fig Integration of linkage map, genomic scaffolds and transcripts. Outer ring, the linkage group; Intermediate ring, contigs or scaffolds of genome assembly aligned with markers from the linkage map; Inner ring, transcript sequences of transcriptome aligned with markers of the linkage map.

MICROBIOME OF ULVA GROWN IN ABALONE EFFLUENT WATER: IS THEIR A DISEASE RISK ASSOCIATED WITH ITS USE AS AN AQUAFEED?

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Fresh Ulva or addition of dried Ulva to aquafeeds has proven successful for a variety of aquacultured marine animals; enhancing feed consumption, growth, product quality and health. In South Africa, production of Ulva in land-based systems with abalone has occurred successfully since 2002. However, despite the obvious benefits of growing Ulva in integrated systems for water bioremediation and recirculation as well as its use as an aquafeed component, production is still limited to a small number of farms. Biosecurity of integrated systems remains one of the main constraints preventing wider adoption of this technology, since Ulva grown in effluent water may be considered to be a disease risk when fed back to animals on the farm. To better understand potential disease risks, we studied the microbial communities associated with the seawater and Ulva obtained from raceway systems supplied with abalone (*Haliotis midae*) effluent water (EW) or natural seawater that had been fertilized (FSW). Water samples were collected at the inlet and outlet of each system. A culture (traditional plate count) and non-culture (NextGen sequencing of the 16S rRNA gene) based approach was adopted. The culture based approach utilised three selective media for the isolation and quantification of bacteria; namely tryptic toy agar (TSA), thiosulfate-citrate-bile-sucrose (TCBS) agar (Vibrio selective), and Ulvan agar (primary polysaccharide of Ulva used as the sole carbohydrate source). We demonstrated a significant reduction in the total number of culturable bacteria between the inlet and the outlet of each system. There was also significantly less culturable *Vibrio* spp. associated with Ulva compared with the surrounding seawater for both the EW and FSW systems. These findings were supported by NextGen amplicon sequencing data and a principle co-ordinate analysis revealing significant differences in the microbial community profile of Ulva and seawater within and between systems. This study suggests a selectiveness of Ulva for preferred bacterial taxa and potential health and environmental benefits for the use of Ulva in integrated systems.

OPPOSITE EFFECTS ON THE EUROPEAN SEABASS (*Dicentrarchus labrax*) IMMUNE RESPONSE AND DISEASE RESISTANCE

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Methionine is an example of an amino acid (AA) with recognized roles in the immune system, DNA methylation, polyamines biosynthesis, and in processes involved in the control of inflammation. Tryptophan participates in an array of physiological mechanisms of the neuroendocrine-immune network and plays a critical role in macrophages and lymphocytes function. Thus, a potential immunomodulatory role of both AA during infection is envisaged and deserves further attention. In this context, two identical trials were performed to evaluate the effects of methionine or tryptophan supplementation on the European seabass inflammatory response and disease resistance to *Photobacterium damselae piscicida* (*Phdp*) after a 4 weeks feeding period. A control diet (CTRL) was formulated to meet the seabass AA requirements, and four CTRL-based diets were supplemented with DL-Methionine at 0.5% and 1% (MET 0.5 and MET 1, respectively) or L-Tryptophan at 0.35% or 0.39% (TRP 0.35 and TRP 0.39, respectively) of feed weight. Methionine supplementation translated into an enhanced immune response to infection, as observed by improved cellular and humoral defences as well as modulation of key immune-related genes. This enhancement of immunity resulted in increased survival of fish fed methionine-supplemented diets and ultimately suggest a positive effect of methionine administration in a dose-dependent manner. Tryptophan supplementation led to a reduction of peripheral blood lymphocytes, monocytes and peritoneal macrophages numbers and down-regulation of several immune-related genes in response to the *Phdp* insult. As a result, fish fed TRP 0.39 presented the highest mortality whereas fish fed TRP 0.35 and CTRL showed similar disease resistance. Therefore, data point out deleterious immunomodulatory effects of TRP 0.39, under the present experimental setup. This study brings relevant insights about the opposite roles of individual AA on the seabass immune system, and their possible application as nutritional additives for fish health management should be carefully considered.

DOMESTICATED SPF SHRIMP AQUACULTURE ACTIVITIES IN BANGLADESH

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Bangladesh has the pond area >275,000 ha, the right conditions like soils, salinity and climate. Black tiger production is 330kg/ha/year and fresh water prawn is 671kg/ha/year [in traditional farming system] and in semi intensive farm it is 2 to 3 tons/ha/year which is very low production compare to other country. Cause of low production is disease free shrimp larvae, slow growth of animals in pond and diseases infestation in the grow-out pond. Almost all PLs come from wild brood stock. 30-80% contaminated with WSSV. To make the shrimp aquaculture is sustainable and environment friendly Bangladesh introduces domesticated SPF shrimp in the shrimp hatchery and farming sector.

Bangladesh is importing SPF PPL [Parents post larvae] from MOANA Nuclear Breeding Center Hawaii in three batches per year at three month interval. After quarantine PPLs are reared in indoor rearing tank for one month and then transferred to outdoor grow out pond for minimum nine month to make them adult. In grow-out pond we are using specialized feed which has high range of animal protein. After nine month they get ready for maturation activities. Only selected good animals are shifted to maturation facilities and feed them for 21 to 30 days. In this time PCR tested virus free raw/wet and formulated maturation feed are provided to the animals. After the suitable time we go for maturation following different techniques. Bangladesh is now developing shrimp gonad as well as producing nauplii to produce post larvae from ablated/ Non ablated animals, with artificial insemination and natural mating.

SPF Shrimp larvae are produced from SPF nauplii for shrimp farm. From nauplii to shrimp larvae survival percentage is 60 to 85 %. Because of its clean character no need to use any non allowed inputs. UV sterilized conditioned water are used the all production system. Three types of algae like thalasiossira, chaetoceros and skeletonema are used as live feed. All shrimp larvae go to farmer after quality testing.

Those domesticated SPF shrimp larvae are stocking in the extensive/traditional and semi intensive farm with strict bio-security condition. Though due to insufficient supply of domesticated SPF shrimp larvae shrimp production is remarkably increased in the farm where stocking those shrimp larvae. In traditional or semi-intensive farm production is now 650kg/ ha/year and in semi-intensive farm it is 6 to 7 tons/ha/year.
Special features of Domesticated SPF Shrimp activities in Bangladesh.

All production activities is confirmed without non allowed inputs & Environment friendly. Gonad development procedure is followed by ablation method and without ablation with environmental manipulation. Traceable shrimp larvae and exportable shrimp production.

Challenge of this activities in Bangladesh:

Environmental parameters fluctuation and Natural digester is the main challenge.

STATUS UPDATE ON MALODOR FORMATION AND ACCUMULATION SOURCES IN AQUACULTURE

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Most studies have considered microbiota as the primary source of off-flavors in farmed fish. The microbial by-products geosmin and 2-methylisoborneol are believed to be the main cause of earthy/musty malodor patterns in aquaculture. However, other compounds that might potentially contribute to the earthy/musty off-odor notes were barely discussed. Furthermore, there is a lack of information about the possible contribution of fish feeds to aquaculture malodor. For this reason, comprehensive analyses were accomplished to investigate the influence of both water quality and aroma composition of commercial fish feeds on the sensory properties and flavor contents of rainbow trout.

To this aim, several aquaculture water and fish samples were collected during different seasons (spring and summer) of two consecutive years from three different earthen-ponds farms. The water of these farms is sourced from two different water river systems. The commercial feeds that are used in these farms were also collected. Odorants of water, fish meat, and fish feeds samples were extracted using solvent assisted flavor evaporation (SAFE); subsequently, extracts were analyzed by multi-dimensional gas chromatography-mass spectrometry/olfactometry (MD-GC-MS/O) using columns with different polarities (DB-FFAP and DB-5). Furthermore, the differences between the aroma profiles were explored using comparative aroma extract dilution analyses (cAEDA) and aroma profile analyses.

In this study, several odorants were described for the first time as potential off-odorants in aquaculture. These include, amongst others, skatole (fecal-like), 4-ethyloctanoic acid (goat-like) and *p*-cresol (horse stable-like). Furthermore, beside geosmin and 2-methylisoborneol, we could identify 10 other flavor compounds with the odor impressions earthy/musty in water and fish samples. The study has also confirmed the potential impact of fish feeds on off-odor accumulation in cultured fish. It showed that compounds such as skatole, 4-ethyloctanoic acid, indole, and cresols may originate from fish feeds. It is also suggested that feeds might be the primary source of fatty acid-derived volatile aldehydes that have previously been reported in aquaculture water and fish. Furthermore, compounds like geosmin and 3-isopropyl-2-methoxypyrazine (IPMP) were identified in fish feed samples. Based on these findings, feeds can now also be suspected as being potential sources of compounds that have previously been thought of as being exclusively accumulated from microbial sources, e.g. cyanobacteria. These findings call for further investigations on the complex mechanisms of off-flavor formation in aquaculture systems and fish with the aim of improving the quality of aquaculture products.

INTER-TISSUE RELATIVE EXPRESSION OF TRANSFERRIN REVEALS THE POTENTIALS OF *Tilapia zillii* AS A BIOINDICATOR FOR WATER POLLUTION

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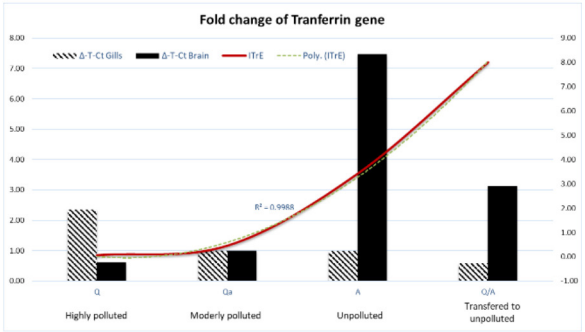
Egyptian fish ranked as the 7th producer worldwide. Most of the aquaculture activities are centered in the Nile Delta Region. This region characterized by dense human population and high agriculture activity, and the contamination of water with industrial wastes is highly possible. Three major water sources in Egypt were categorized for sampling as highly polluted closed source (Q: Birket Qarun, 80 km southwest of Cairo), polluted open source (Qa: El-Qanater El-Khayreya fishing area, Qalyubia Governorate) and unpolluted open source (N: Nasser Lake, Aswan). The locations are considered a suitable habitat and fishing sources for tilapiine species, one relevant species persistent in the three water sources is the redbelly tilapia (*Tilapia zillii*, syn. *Coptodon zillii*). The fish was used as a model of study to show its potential as a bioindicator to water contamination and pollution by human practices, by measuring the inter-tissue differential expression of iron-binding metalloprotein (Transferrin) among the three water locations. An average number of 60 fish/location were captured alive and held in glass ponds filled with source water from each location and left for adaptation prior to transferrin quantification. Up to 10 fish samples from the polluted sources were transferred to glass tanks filled with water from the unpolluted one for five days (Qa/A). Total RNA was extracted from both gills and brain tissues and quantified using nanodrop device, total cDNA was synthesized and quantified in triplicate by real-time PCR using commercialized kits. The acquired Ct values were used to calculate the fold-change for each sample compared to b-actin as a house keeping gene for each tissue/sample. Outliers were excluded, and inliers were used to estimate average Ct value of each case. The fold change of transferrin was calculated for each tissue (Δ-T-Ct = b-actin-Ct – Trans-Ct); and then standardized by subtraction from Δ-T-Ct estimated for Qa (where Δ-T-Ct is equal in both tissue). The transferrin inter-tissue relative expression (ITrE) = 2^ - (Δ-T-Ct gills / Δ-T-Ct brain) was calculated for each location. Within each sample, the average Ct value of transferrin was higher than the b-actin for Qa, A and Qa/A samples but not Q samples, which reflect an over expression response of transferrin from the polluted and unpolluted water sources only and vice versa. In brain the Δ-T-Ct was reversely proportional to the pollution indicator in contrast to Δ-T-Ct in gills, which shows a suitable case to estimate a relative expression estimate that based on the fold change of transferrin among both tissues. Thus, the ITrE was found to increase by the decrease of pollution, and it fit the polynomial model ($R^2 = 0.99$) among the four categories. In conclusion, the study showed that, *T. zillii* can survive in different water sources with varied water pollution levels. Measuring the fold-change of transferrin expression in gills relative to brain tissues can be used as an indicator of the water pollution while reversing the effect validated the indicator for water-quality measures suitable for human consumption or aquaculture.



DEVELOPING LAND-BASED INTEGRATED MARINE AQUACULTURE SYSTEMS FOR SUSTAINABLE SEAFOOD PRODUCTION

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Future expansion of environmentally friendly and sustainable aquaculture is needed to meet the ever-increasing demand for safe and sustainable seafood. Conventional aquaculture is challenged by high water demand, pollutant discharge, and problems related to biosecurity and disease. Some of these barriers can be addressed by land-based recirculating aquaculture systems (RAS). However, marine RAS face obstacles that need to be addressed, such as high energy demands, biosecurity issues, discharges of saline water and organic salty solids, and the lack of a standardized RAS filtration system.

The need to expand marine fish production and improve the economic viability and sustainability of RAS led to the development of a zero-discharge, integrated marine aquaculture (aquaponic) system (IAS). The prototype, commercial-scale system design incorporated solids filtration, a moving bed bioreactor for nitrification, a sand filter for solids removal and denitrification, ultra-violet light sterilization for water quality, and hydroponic plant beds. Water treatment capacity, nutrient cycling, and biomass production were evaluated in the prototype IAS producing red drum (*Sciaenops ocellatus*), edible halophytes (sea purslane, *Sesuvium portulacastrum*, and saltwort, *Batis maritima*), and organic solids for three production cycles.

Extensive analysis of solids, organic matter, and nutrients (nitrogen and phosphorous) in water and plant biomass was used to develop detailed mass balances for the system. Simultaneous operation of the moving bed bioreactor and plant beds resulted in high ammonia removal rates, allowing the system to support a high fish biomass density (38.8 kg/m^3 in year 1, 48.8 kg/m^3 in year 2, 45.2 kg/m^3 in year 3). This study demonstrates that marine IAS is an effective way to sustainably produce marine fish, edible halophytes, and fertilizer. Addition of biological filtration and a denitrifying sand filter was shown to benefit high-density fish production in situations where there are space limitations or niche markets for plants.

EFFECT OF AERATION ON THE DIGESTIVE SYSTEM OF ATLANTIC SEABASS (*Dicentrarchus labrax* L.) CULTURED IN SEA CAGES

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Oxygen concentration in seawater can be an important environmental factor which may influence appetite, feed utilization and stress level especially at high water temperature during summer and early autumn in Mediterranean fish farms. An experiment was run in the frame of EU funded research project for the benefit of SME, AirX-" Oxygenation by efficient air diffusion system for aquaculture farms (cages and earthen ponds) ". Two cages with approximately 15,000 European seabass of average weight of 450 g were cultured from 13th of August to 15th of September 2014 at the commercial fish farm Zervas/Kyriazis A/S in Vorios Evoikos, Greece. In this trial an AirX diffusor system constructed by Oxyvision A/S, Norway was set at 3.5 m depth in one of the cages and seawater was thereby oxygenated for 6 hours each day by injection of air. Optical oxygen sensors (Hach Lange HQ40D) monitored oxygen concentration in the two cages every 30 min. Samples were taken from gut, liver and pyloric ceca of the fish in both cages before the experiment both for histology analysis and for determination of expression of genes related to lipid digestion of the fish at the 27th of August and at the 18th of September. Tissues were embedded in Technovit 7100, and sections of 5 μm were stained with Polychrome I and II (methylene blue/azure II and basic fuchsin) (Benett et al., 1976). Gene expression of lipase and phospholipase in liver tissue and pyloric ceca were carried out by qPCR using β-actin and EF1a as control genes.
Histological analysis showed that fat deposition was significantly higher in liver of fish in aerated cage while numbers of goblets cells in gut and pyloric ceca increased significantly during the experiment.

Feed conversion rate was lower in aerated cage and the data support that aeration induced increased appetite and improved digestive capacity of the fish compared with control cage.

References

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HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN BLOOD OF PIKEPERCH (*Sander lucioperca*) THROUGHOUT SEMI-CONTROLLED REPRODUCTION

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Manipulation during intensive farming often leads to unpredictable reproductive performance, which is one of the most limiting factor of effective pikeperch aquaculture. Furthermore, stress response is a generically costly process resulting in fish mortality, poor reproduction performance and in addition, it may inhibit spawning.

In this study blood plasma of 21 males and 21 females of pikeperch broodstock was analyzed for investigation of stress response throughout semi-controlled reproduction in recirculation aquaculture system (RAS). In total, seven experimental groups, each included 3 males and 3 females, were sampled subsequently: (1) before hormonal induction; (2) after hormonal injection; (3) exactly after spawning; (4) 24 and (5) 48 hours after spawning - female and male were kept separately; (6) 24 and (7) 48 hours after spawning - both sexes were kept together. Two ml of blood were drawn from the caudal vein of fish using an 18 G x 1 ½ heparin containing syringes. To avoid potential influence of handling and sampling on measured parameters each fish was sampled only once. The hematological profile included: an erythrocyte count (RBC), hematocrit (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and leukocyte count (LEUCO). Biochemical profile included indexes of cortisol (CORT), calcium (Ca2+), glucose (GLU) and lactate (LACT) in blood plasma.

Throughout reproduction females had higher level of RBC, Hb, LEUKO, Ca2+, LACT and cortisol in blood plasma. Among fish sampled after spawning occurrence, males had higher index of Ca2+ in comparison to fish sampled before application of hormones. Females removed from males exactly after spawning had higher number of erythrocytes and indexes of Ca2+ in blood, while levels of GLU and LACT were higher in fish kept together. Males kept alone in the tank had less leukocytes in plasma, without differences in other measured parameters.

Spawning affected physiology and immune response of both sexes. Results showed that females were more influenced by reproduction and had stronger physiological reaction on handling, manipulation and keeping with male after spawning. Separating of sexes significantly decreased stress response of females and influenced immunity of males. Obtained results could be used for optimization of reproduction protocol of pikeperch species.

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TRIGGERING OF PROTEIN PHOSPHORYLATION IN RAINBOW TROUT *Oncorhynchus mykiss* EPITHELIAL CELL LINE RTGILL-W1 BY VIRAL AND BACTERIAL PAMPS

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Dynamic modification of cell proteins is a key strategy for the quick response of cells to external stimuli. Phosphorylation is the best characterised post-translational modification playing a role in a wide range of biological processes such as the rapid signalling of the innate immune response, a mechanism well characterised in mammals. In order to study protein phosphorylation on a proteome-wide scale different mass spectrometry based approaches are available. In this study, the phosphoproteome of RTgill-W1, trout gill epithelial cells after viral and bacterial PAMP stimulation was analysed.

RTgill-W1 cells were grown onto 6 well plate and transwells. Cells were stimulated with poly(I:C) and MDP at 10µgmL^{-1} for 30 minutes. Protein concentration in cell lysates was measured by BCA assay and 8 µg of protein was run in 1D SDS-PAGE to check the quality of proteins. Protein cell lysates were then digested with trypsin. Enrichment of phosphopeptides was achieved by using titanium dioxide (TiO_2) resin spin tips (High-Select TiO_2, Thermo Scientific). Samples were analysed using a LC-MS/MS Orbitrap Velos FTMS. Data were processed by uploading raw spectra into Thermo Proteome Discoverer 1.4. Peptide and protein identification was performed with SEQUEST algorithm. An in house compiled database containing proteins from the latest version of the UniProt SwissProt database was compiled to include only *Oncorhynchus mykiss* entries.

A total of 422 phosphopeptides and 317 phosphoproteins were identified. From these, 288 phosphopetides and 203 phosphoproteins were unique in poly(I:C) stimulated cells (Figure 1). A total of 560 phosphorylation sites were detected where 9.79% sites were serine phosphorylated followed by Threonine (6.78%) and Tyrosine (1.4%). Phosphoproteins identified covered a wide range of functions from transcription, translation to intracellular trafficking and cytoskeleton.

This study allows for the first time, an untargeted view of the key signalling pathways that are quickly activated in response to bacterial and viral PAMPs in fish gill cells.

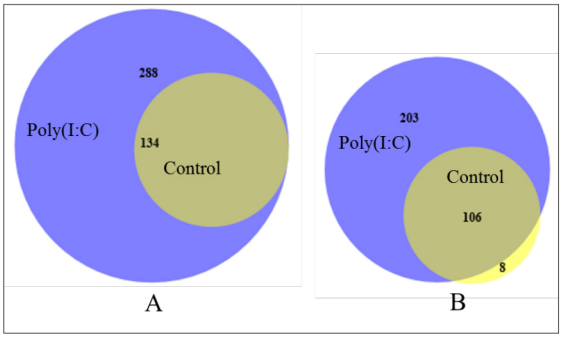

Figure 1: Venn diagram showing the number of phosphopeptides (A) and phosphoproteins (B) identified in control and poly(I:C) stimulated cells

NEW APPROACH TO A QUANTITATIVE METHOD TO ESTIMATION OF THE GAMETOGENIC CONDITIONS IN THE EDIBLE SEA URCHIN *Paracentrotus lividus*, BY IMAGE ANALYSIS

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Image analysis is a powerful tool used in different science fields such as medicine, computer, engineer, life and nature. This instrument gives information present in pictures using mathematical algorithms corroborate scientifically. In natural sciences is common using the image analysis in histological samples, but it is less used as tool to obtain gonadal information.

The typical gonad analysis in marine animal are: gonadal index using weights and maturation or condition stage by visual analysis of histological slides. This last one is based on a previously information filed by some researchers and it is a subjective interpretation, producing in many cases mistakes results or sub classifications of the gonadal condition stages. Theses interpretations can be disregards because depend of the research, except when it is necessary to corroborate if there are some changes in the structures, cell quantities, etc., like in the aquaculture or ecotoxicological researches.

The histological dyes, like haematoxylin-eosin (HE), show specific and differenced information about a specific tissue such as gonadal mass of some marine invertebrates like sea urchin. The aim was implementing a new quantitative method for image analysis of HE histological slides using the pixel colour variations in different maturation stages by software tools such as Color Deconvolution and CellProfiler. Fig. 1 show the new method.

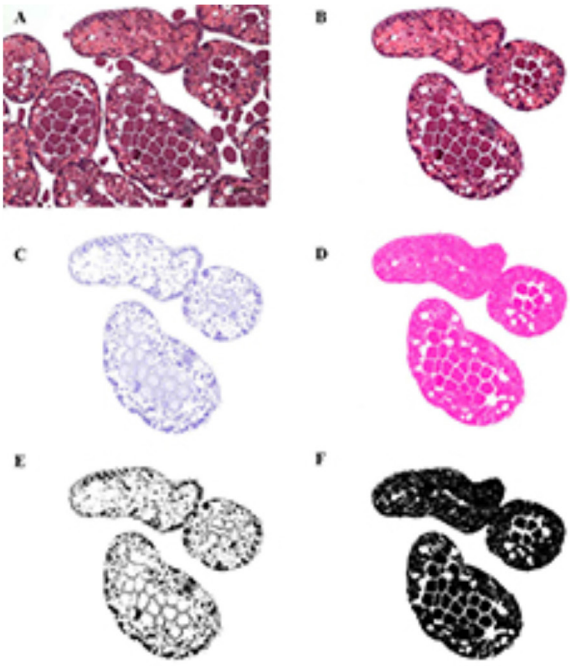

Fig. 1. Image processing. The picture shows: the original image (A), without incomplete follicles (B) after the cleaning process, the selected layers generated by Color Deconvolution plugin, violet layer (C) and rose layer (D), finally, the binary images by layer (E, F).

BIOFILM AS TOOL TO EVALUATE ORGANIC AND CHEMICAL DISPERSION FROM MARINE FISH FARMS

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Introduction

Marine biofilms are organized communities of mixed micro-organisms, typically surrounded by a matrix of extrapolymeric substances (EPS) which facilitates the attachment of the community to any surface (Characklis and Marshall, 1990). Diatoms and bacteria constitute the major components of biofilms occurring in the marine environment. The moment a clean surface is submerged in the sea, biofilm-forming micro-organisms rapidly colonize and form highly complex, dynamic three-dimensional (3D) surface structures (Davey and O'Toole, 2000). Using these communities on artificial surfaces facilitates the direct comparison between sites without confounding environmental and physical variables (Webster and Negri, 2006). The analysis of biofilms in test substrates enables medium term (days) rather than momentary states of the studied ecosystem (Brummer et al., 2003). The aim of this work was to: (1) quantify fish farm particulate organic carbon, particulate organic nitrogen and total phosphorus, and (2) to correlate them with the structural, trophic and element accumulation changes in the biofilm community at an offshore fish farming zone from Mediterranean.

Materials and methods

The Western Mediterranean case study of the TAPAS Project Horizon 2020, offshore Fish Farming Zone of San Pedro del Pinatar (Murcia, Spain), is one of the most aquaculture intensive areas in Spain of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) production, with an average production of around 11.000 tonnes per year. The fish farming is exclusively carried out in open sea cages. The changes in the biofilm community due to organic matter enrichment and trace elements contamination derived from fish farming were studied. The biofilm biomass and chlorophyll *a* were quantified along an environmental gradient of fish farm wastes. Total organic carbon (TOC), total organic nitrogen (TON), total phosphorus (TP), trace elements and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were measured. The field assays were performed using glass slides as the artificial substrate for biofilm community development. Glass slides were supported by slide holders. The slide holders, in turn, were maintained 3 m below the water surface by an anchoring system and a buoy. Particulate sedimentation rates were measured by means of sedimentation traps composed of four attached cylinders (100 cm height and 12 cm diameter). Each cylinder had a funnel at the bottom, which guided the particulate matter into a 250 ml polyethylene tube. Biofilm slides and sediment traps were deployed from fish cages located at the edge of the fish farms facility along horizontal transects at 0, 25, 75, 175, and 650 m from the fish cages.

Results

Our results indicate that organic pollution influenced carbon and nutrient accumulation of biofilms as it has been previously reported (Sanz-Lazaro et al., 2011). PCA analysis grouped stations following the environmental gradients, indicating that the accumulation pattern of trace elements in biofilm was consistent along the fish farm influence. Cu, Zn and Cd were the main metals released to the environment due to fish farm, which had similar accumulation dynamics as TOC, TON and TP along the environmental gradients. Concentration of these chemicals in biofilms showed higher values for samples close to the fish cages. The isotopic signatures clearly differentiated the biofilm communities along transects. Diatom abundance, biomass, species richness and H´ showed a very similar trend with distance, greatly increasing close the fish farm and stabilizing further from the fish farm. The nMDS plot of diatom assemblages showed an environmental gradient between sampling stations, and that the within-station similarity among samples increased with distance from the fish farm. the regression model showed that the community structure descriptors reached their asymptotic point further from the fish farm than the POC, PON and TP sedimentation rates.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 678396

INTESTINAL MICROBIOTA PROFILE CHANGES FOLLOWING SEAWATER TRANSFER IN ATLANTIC SALMON *Salmo salar*

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Atlantic salmon (*Salmo salar*) are well known for their complex life cycle, spending their juvenile years in freshwater followed by migration to marine feeding grounds, only to return to their natal rivers to spawn. Both aquatic environments are characterised by a variety of chemical and biological differences. Therefore, Atlantic salmon need to undergo drastic physiological changes to adapt to a multitude of environmental factors.

Among the required changes, osmoregulatory adaptions is the most crucial, allowing the fish to acclimatise to the increased salinity in seawater. Functional adaptations of organs, such as gills, kidney and intestine are required to regulate loss of water and excess salt burden. Although the role of gills and kidney as osmoregulatory organs is well established, less is known about the involvement of the intestine. Apart from changes in body physiology, salmon also have to respond efficiently to novel pathogens, microbes and dietary items encountered in the marine environment.

The importance of a balanced interaction of organisms with their microbiota has been well established in a variety of mammals, including humans. However, microbiota study of fish has only picked up in recent years and less is know how changes in internal and external factors may impact on the community structure of beneficial, harmless and potentially pathogenic bacterial species.

As the intestine of Atlantic salmon is in constant contact with the external environment, we hypothesised that the intestinal microbiota would change from freshwater- to seawater-acclimatised fish. To this end we compared the intestinal microbiota harboured in the distal digesta of Atlantic salmon freshwater fish (FW) kept in a commercial Scottish freshwater hatchery with that of their full-siblings after seawater acclimatisation (SW) by a 16S rRNA (V3-V4) high-throughput sequencing approach.

We found that microbial richness and diversity was significantly higher in FW compared to SW. Using Metastats analysis, a higher number of Operational Taxonomic Units (OTUs) unique to FW compared to SW was found, plus an additional 238 shared OTUs, where abundance was significantly higher in FW. A core microbiota of 19 OTUs was identified in 100% of all fish, which indicates that certain microbes are maintained to fulfil minimal functions within the gut. The uniqueness of the respective microbial profiles can also be correlated with significant differences in KEGG pathways including lipid and amino acid metabolism.

GENERATION OF SALMONID INTERFERON ATTENUATED CELL LINES BY GENE EDITING USING CRISPR/CAS9 APPROACH

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Outbreaks of viral diseases in fish farms are a major factor of economic losses for the aquaculture of salmonids. Research into antiviral vaccines is therefore in high demand. However, high quantities of virus products are required for vaccine design and testing. Currently this is limited by the low yield of viral particles production in the available fish cell lines.

In the last years, gene editing with the CRISPR/Cas9 system has been applied successfully to edit cell lines and whole organisms in animals and plants. Recently, an edited fish somatic cell line was established in Chinook salmon (*Oncorhynchus tshawytscha*) embryonic cells (CHSE). A stable cell line, termed CHSE-EC, expressing the enhanced green fluorescent protein (EGFP) and Cas9 was isolated (Dehler et al., 2016). The EGFP target was then disrupted by transfection with a targeted guide RNA, which introduced mutations through double-strand breaks with an efficiency of 35%.

To build on this proof of concept, we targeted genes in the interferon pathway to break the antiviral response and produce stable cell lines which allow increased virus production. Guide RNAs were designed against key transcription factors in the interferon signalling pathway and transfected into CHSE-EC cells. Single-cell sorting into 48 well plates was achieved with fluorescence-activated cell sorting (FACS), selecting for cells that were EGFP-negative. Within 2 weeks a confluent monolayer was obtained in the wells and clonal cell lines were transferred to 25 cm^2 flasks. Gene knock-out on both haplotypes was confirmed by sequencing of EGFP-deficient clones. Preliminary transcriptome analysis in these cell lines stimulated with recombinant interferon type I suggest that the interferon signalling pathway has been disrupted and cells may now be more susceptible to virus invasion, which we are currently investigating.

Apart from establishing a workflow of CRISPR/Cas9 enabled gene editing of immune gene targets, the engineered cell lines may be of interest for vaccine research and production.

FISH WELFARE: REDUCING THE NUMBER OF FISH IN EXPERIMENTS WITH SHOALING SPECIES

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Experimental studies with fish and small-scale fish monitoring set-ups demand the integration of ethical principles and the legal framework (EC, 2010, *OJEU*, 33-79) concerning the 3 Rs (Russell & Burch (1959) *The Principles of Humane Experimental Technique*. London: Methuen & Co): Replacement (avoid or replace the use of animals), Reduction (use fewer animals, or obtain more information from the same number) and Refinement (elimination or minimization of potential pain, suffering or distress). The present study was approved by the Ethical Committee of the University of the Basque Country UPV/EHU for Animal Welfare No. CEBA/285/2013MG and intends to contribute to the Reduction "R" by identifying the lowest number of fish (European seabass) that could be used for monitoring and/or experimental purposes. This was done by quantifying the predictability of the trajectory followed by the shoal's centroid (given by its Shannon entropy, SE) as a function of the number of individuals. We performed one experiment starting with 50 fish and lowering the number to 25, 13 and 1 fish, and another one starting with 1 fish and adding 1 new fish daily for 5 days (5 fish the last day). Every day, the fish were recorded for 1h and the SE values were calculated for 3 arbitrary basal (B) periods of 3.5 min (corresponding to shoaling), and for a 3.5 min period in response (R) to an event which was a hit in the tank (to provoke a schooling response). The coefficient of variation (CV) of the SE was largest for 1 fish (37% and 18% for the daily averaged B-SE and R-SE respectively) and decreased concomitantly with the number of fish (10% for the B-SE of 25 fish systems and 6% and 2% for the B-SE of 13 and 50 fish, respectively). The SE of the systems kept a power relationship with the number of fish (R^2=0.93 for B and R^2=0.92 for R; Figure 1). Thus, 5 to 13 fish should be the lowest number to use in experiments with shoaling species in order to reach a compromise between acceptable variability (about 10%) in the data and reduction in the number of fish. This may be the first scientific work intended to estimate the lowest number of shoaling fish to be used for experimental purposes. It will be be published in Frontiers in Physiology.

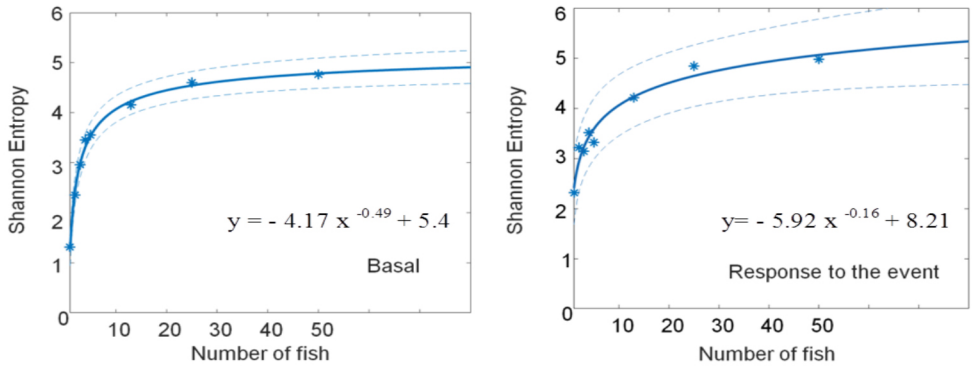

Figure 1. Curve fitting of the SE as a function of the fish number. The basal state and the response to the event are shown with the 95% confidence bounds.

GENETIC ARCHITECTURE OF SEX DETERMINATION IN TURBOT *Scophthalmus maximus*

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Turbot (*Scophthalmus maximus*) is a flatfish with increasing aquaculture value in Europe and China. This species shows extreme sexual growth dimorphism, females growing faster and becoming sexually mature later than males, hence the interest of industry in producing all-female populations. Sex determination (SD) of turbot shows a major genetic component, the main quantitative trait loci (QTL) being located at linkage group (LG) 5, but minor QTLs and temperature influence have also been reported. Available data suggest a ZZ/ZW system of recent evolutionary origin, since recombination is not restricted and no genetic divergence at this region is observed males and females. In this study, we carried out a GWAS analysis of SD in this species using 18,165 SNPs in a large set of 36 families. Although, previous findings were confirmed (major SD region at LG5), ~30% of families showed a different pattern suggesting association with other LGs or environmental influence. Standard SD LG5 families were used to narrow down to 531 kb the region where the master gene is putatively located. This region was deeply analyzed by re-sequencing ZZ and WW individuals to look for a diagnostic difference between sexes related to SD. Furthermore, candidate genes were scrutinized for structural differences and their expression profiles studied along the critical SD period. All data support that SD in this species meets to a complex trait with the major locus being recently recruited.

A CLOSER LOOK AT TURBOT GENOME REVEALS A GENETIC COMPONENT OF PARASITE RESISTANCE: NEW TOOLS FOR SELECTION

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Developing genomic tools for cultured species is fundamental for improving production traits. Genetic breeding programmes are underway for turbot (*Scophthalmus maximus*) since 1992, and in addition to growth rate, there is an increasing interest for achieving more robust broodstock resistant to diseases. In this study, a genotyping by sequencing approach (2bRAD-seq) was used to identify and genotype 18,125 SNPs in a sample of 1,439 turbot from 36 families coming from a disease transmission experiment specifically designed for disentangling the different components of host response to infection by *Philasterides dicentrarchi*. This parasite is responsible of scuticociliatosis, a disease that results in severe economic losses for the aquaculture industry. SNP data from full-sib families was used to construct a high-density genetic map that allowed anchoring most of the turbot genome scaffolds into an improved assembly (96.5% coverage), reflecting the 22 chromosomes of its karyotype. Physical and genetic maps were compared to assess the recombination rate pattern across the genome and between individuals and sexes. Males displayed shorter maps, mainly due to a lower recombination around centromeres. The presence of genomic reorganizations (i.e. inversions) at specific chromosomes was suggested when comparing individual maps. The results from the disease transmission experiment indicated that susceptibility explains a larger proportion of genetic variation in resilience than tolerance. Heritability for susceptibility was moderate, and within the range found for similar traits in aquaculture species. Genome-wide association analyses allowed the identification of a candidate QTL region for resilience to scuticociliatosis that explained 33% of the total genetic variance, and had an effect of about five days of increased survival. Twelve loci located in this region belonged to the same linkage block and were put forward for validation in a new set of challenged families for future application in MAS programs. Finally, a genome-wide comparison between turbot, Japanese flounder and tongue sole enabled to integrate the genomes of the three species facilitating the transfer of information for improving selection in other important flatfish. In summary, this study entails an important advance in turbot genomics and shows the potential applicability of genomic tools for breeding programmes in this species.

SEX DETERMINISM IN MOLLUSCS
FROM FUNDAMENTAL RESEARCH TO THE CONTROL OF SEX IN AQUACULTURE

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Molluscs include a richness of species with a great diversity of life cycles and of sexual strategies supported by various sex-determining mechanisms. Therefore, Molluscs offer a unique opportunity for addressing questions about the underlying mechanisms of sex. This is also a phylum poorly known concerning these aspects. Yet, Molluscs have to be taken into account on the science chessboard in order to give an accurate view of sex determination in the Animal Kingdom and of its evolution. At last, Molluscs include species of economic and nutritional importance, which makes knowledge on their sex determination highly necessary to provide useful tools for the control of their sex in aquaculture.

This presentation will provide an overview on the current knowledge amongst Molluscs, on (1) the different sexual systems (gonochorism, simultaneous hermaphroditism, sequential hermaphroditism), (2) the sex-determining mechanisms (sex chromosomes, oligo/polygenic, mitochondrial DNA transmission…), (3) the influence of environmental factors (food, temperature, exogenous steroids, pollutants) and the sensitive time-window. Some husbandry practices for controlling the sex of Molluscs in aquaculture will then be discussed, in view of what is done in fish aquaculture.

A special emphasis will be made on oysters such as *Crassostrea gigas*, whose production now accounts for more than 70% of overall French shellfish aquaculture turnover and which is particularly interesting for its high phenotypic sex plasticity. The genetic and environmental sex-determining mechanisms underlying this plasticity will be illustrated by recent results on the gene network, on a long-term follow-up study of sex-ratios and with an environmental conditioning. These promising results will participate in unlocking some of the mysteries around the sex determination of Molluscs.

EFFECT OF INCREASING TOTAL DAILY IRRADIANCE IN *Ulva ohnoi* PHOTOINHIBITION CULTURED IN AN INDOOR IMTA-RAS SYSTEM

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Photoinhibition is the light-induced reduction in the photosynthetic capacity of a photosynthetic organism which takes places when they are exposed to high irradiances that exceed their light energy requirement, and includes photoprotection and photodamage. In indoor systems seaweed are brought from full light to full darkness. In seaweeds exposed to low light, such as in indoor systems, the damage can be reversible, but increasing the duration of exposure photodamage can be irreversible.

The objective of this work is to analyze in an indoor system the photodamage and the variation on photosynthetic capacity of *Ulva ohnoi* cultured under different total daily irradiances.

U. ohnoi tanks were integrated in a recirculating system (RAS) with two tanks of sole (*Solea senegalensis*) and a nitrifying biofilter, and were cultured at 0.8 and 1.6 kg m^{-2} with bottom agitation. Photosynthetic parameters were measured daily before lights were on with a pulse-amplitude modulation fluorometer (PAM-2100, Walz, Germany). Parameters measured were: F_0, Fm and maximum quantum yield of photosystem II.

Clear differences were observed in F_0, which increase in both tanks at the highest TDI (Fig. 1a and 1b week 4), and moreover, did not decrease after lowering the lighting hours (week 5). The increase in F_0 indicates damage to PS II, and results also indicate that photodamage is irreversible, since F_0 did not decrease when TDI was decreased.

Acknowledgements: This work was funded by Spanish Ministerio de Economia y Competitividad (AGL2013-41868-R).

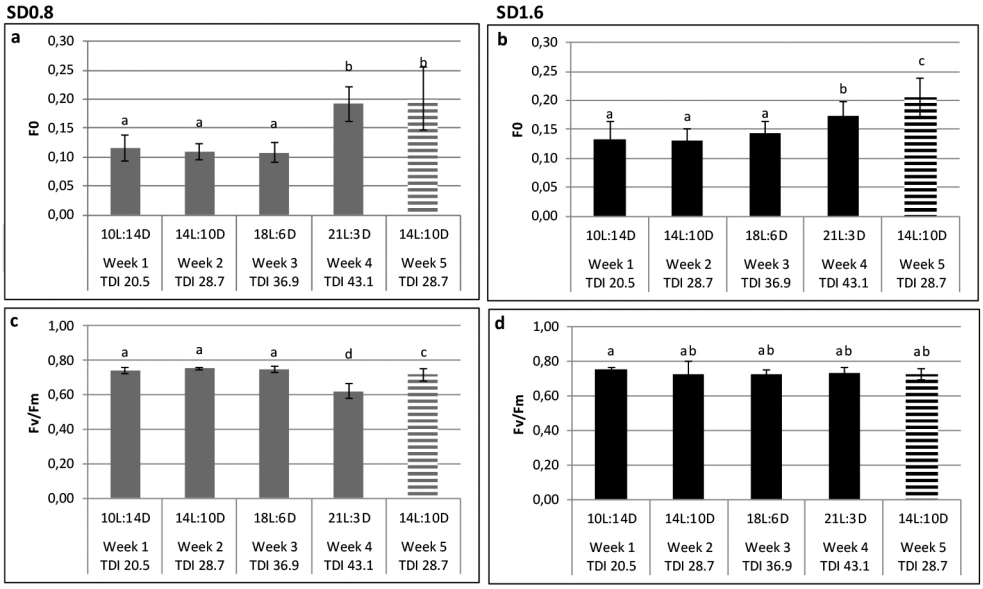

Figure 1: F_0 and Fv/Fm average before lights on under different TDI (mol m^{-2} d^{-1}).

Clear differences were observed in F_0, which increase in both tanks at the highest TDI (Fig. 1a and 1b week 4), and moreover, did not decrease after lowering the lighting hours (week 5). The increase in F_0 indicates damage to PS II, and results also indicate that photodamage is irreversible, since F_0 did not decrease when TDI was decreased.

BACTERIAL DEGRADATION OF *Ulva lactuca* POLYSACCHARIDES IN THE DIGESTIVE TRACT OF THE SEA URCHIN *Tripneaustes gratilla*

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The marine algae *Ulva lactuca* reveals high potential as source for bioethanol generation mainly due to its fast growth and high content of carbohydrates. However, efficient degradation of *Ulva* cell wall polysaccharides, like ulvan, is required. Marine grazer like the sea urchin *Tripneaustes gratilla* may be a potential source of unique bacteria and enzymes for ulvan degradation into simple monosaccharides. In the current study, *Tripneaustes gratilla* was fed with either *Ulva lactuca*, from an integrated multi-trophic aquaculture system in Eilat, Israel, or with seaweed- free pellets. A meta-analysis of 16S rRNA gene sequence with the ilumina Miseq platform was used following with sequences clustering into operational taxonomic units (OTUs) at the 0.97 similarity level. To compare the bacterial diversity between treatments, Shannon and Simpson indices were calculated from the OTUs. *In vitro* trials aimed enrichment and isolation of bacteria that are capable with Ulva and ulvan decomposition. Bacterial OTUs revealed 19 classes that differed in their frequency distribution in response to different diets or sample origin (fig. 1). *Clostridia*, *Flavobacteriia* and *Mollicutes* dominated the digestive tract of sea urchins fed with *Ulva lactuca* while prevalence of *Marinilabiaceae* and *Caldicoprobacter* was high in sea urchins that fed with the seaweed- free pellets. Bacterial community richness and diversity were significantly higher in the intestine region comparing to samples from esophagus and stomach. Both richness and diversity indexes were higher in sea urchins fed with *Ulva lactuca*. *In vitro* enrichment of crude gut samples in the presence of ulvan revealed bacterial candidates that are capable with ulvan degradation when provided as sole carbon and energy source.

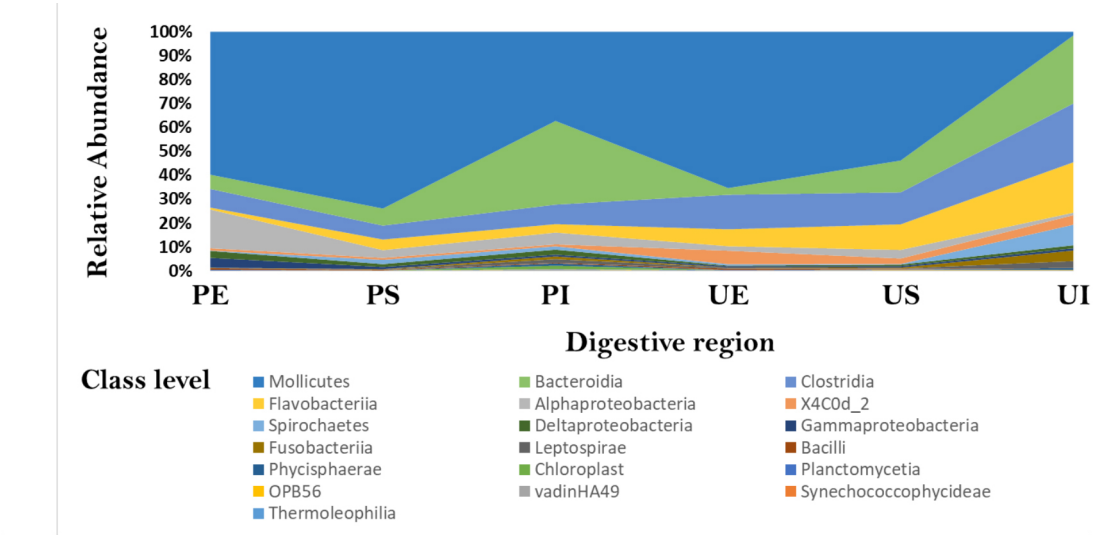

Figure. 1. Relative abundance (in percentages) of different bacteria classes in samples from the digestive tract of *T. gratilla* when fed with *U. lactuca* (U) and seaweed- free pellets (P). The second letter stands for the different regions in the sea urchin digestive tract: Esophagus (E), Stomach (S) or Intestine (I). Bacterial OTUs with ≥97% sequence similarity clustered into classes using the Greengenes version 13.8 database.

GENE EXPRESSION IN HEPATOPANCREAS AND TAIL MUSCLE ACROSS MOULT STAGES OF THE REDCLAW CRAYFISH *Cherax quadricarinatus*

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Moulting is an essential biological process in crustaceans which allows for growth and development and has a significant impact on their physiology and feeding behaviour. Previously studies have reported that gene expression patterns can be influenced by moult stage. Few studies, however, have focused on genes involved in fatty acid (FA) desaturation and elongation, and none have done this in the context of the redclaw crayfish *Cherax quadricarinatus*, an emerging species for aquaculture. Understanding the expression of genes involved in FA biosynthesis across the moult stages of *C. quadricarinatus* will provide tangible benefits for aquaculture production of this species and contribute towards our general understanding of the moult cycle on gene expression. The aim of this study was to analyse the expression of genes involved in FA biosynthesis in hepatopancreas (HP) and tail muscle (TM) across three different moult stages to confirm if the moult stage affected the expression of these genes in these tissues.

Sub-adult *C. quadricarinatus* (14.5 ± 1.5 g; n = 50) were maintained in tanks at 27.4 ± 1.0 ^{0}C with dissolved O_2 > 6.0 mg/L, pH 7.5 ± 0.1 and total NH_4^+ < 0.25 mg/L. All animals were fed with a control diet (45% protein, 8% lipid), once a day *ad libitum*, for one week after acclimatisation. Crayfish were harvested at stages of pre-moult (P), moult (M) and intermoult (I) according to their moult mineralization index. Four genes were identified by preliminary transcriptome analysis and reverse transcriptase-quantitative PCR subsequently focused on these gene families: *delta-6 fatty acyl desaturase* (*D6FAD*), *delta-9 acyl-CoA desaturase* (*D9FAD*), *fatty acid elongase protein* (*ELOVL*) and *elongation of very long chain fatty acids protein 6* (*ELOVL6*).

Figure 1 shows that moult stage does not appear to influence the expression of the targeted genes. We observed that most target genes studied have higher expression in HP than TM with *D9FAD* having the highest expression level. This information is important to understand the influence of the moult cycle on the expression of the genes involved in the biosynthesis of FA in *C. quadricarinatus*.

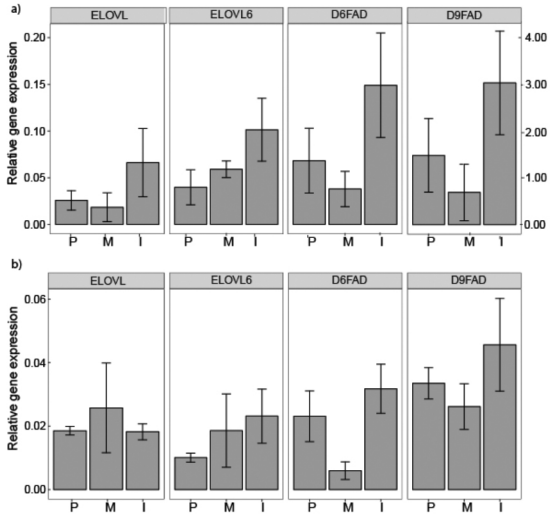

Figure 1. Relative expression of the genes involved in fatty acid synthesis: *ELOVL, ELOVL6, D6FAD* and *D9FAD* across three moult stages in (a) hepatopancreas (y-axis is in a different scale for *D9FAD*) and (b) tail muscle, normalised by 18S. Values are normalised expression ratios, corresponding to an average of samples (n = 5) with standard error (S. E.).

NUTRIENT AND ENERGY DIGESTIBILITY IN EUROPEAN SEA BASS *Dicentrarchus labrax* FED DIETS CONTAINING FIVE DIFFERENT INSECT MEALS

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Insects are a new and promising alternative protein source for aquafeeds. This study aims to evaluate and compare the nutrient and energy digestibility of diets in which 30% of fish meal was replaced by proteins from the insects *Tenebrio molitor* (TM), *Hermetia illucens* (HI), *Musca domestica* (MD), *Zophobas morio* (ZM) or *Alphitobius diaperinus* (AD).

For this experiment, 18 groups of 15 fish (35.1±8g) were placed into 18 indoor 250l cylindroconical tanks equipped with a settling column. Diets (Table I) were designed to be isoproteic and isoenergetic and to meet the nutrient requirements of sea bass. The apparent digestibility coefficients (ADC_x) were determined using 1% celite® as an inert marker.

Table II shows the apparent digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP-N*6.25), crude fat (CF-Folch), acid detergent fiber (ADF) and energy (GE-calorimetry) of the experimental diets. Statistical differences were observed between the diets for all the nutrients except for crude fat. The diets containing *Musca domestica* and *Tenebrio molitor* showed overall digestibility similar to each other and to the one of fish meal. Despite the poor digestibility of the ZM diet in terms of dry matter, organic matter and energy, the protein and fat digestibility were similar to fish meal. Acid detergent fiber is the fraction of the diet containing chitin from the insects but also lignin from plant ingredients. ADC_{ADF} was significantly higher in FM, ZM and AD diets. Some studies have shown that chitin can interfere with fat digestibility, however in the present study no differences were found in ADC_{CF}.

In conclusion, our results indicate that the inclusion of *Musca domestica* and *Tenebrio molitor* meal had no adverse effects on the digestibility of the diets and that fish meal can be successfully substituted by those insects in 30% in the diets of European sea bass.

Table I: Composition of the experimental diets (%)

	FM	TM	HI	MD	ZM	AD
Fish meal	65	45.5	45.5	45.5	45.5	45.5
Insect meal	0	19.5	19.5	19.5	19.5	19.5
Fish oil	10	6	9.7	6.2	1.3	5.3
Wheat	16.4	17.2	15.2	17.5	19.3	19.0
Wheat Gluten meal	6.9	8.4	6.8	9.1	11.3	7.8
Vitamin & Mineral mix	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.5	1	0.9	0.3	0.9	0.9
Lysine	0	1.2	1.2	0.7	1	0.8
Celite	1	1	1	1	1	1
Composition (% of dry matter)						
Crude protein	60.3	60.1	59.5	59.5	59.1	60.0
Crude fat	19.2	19.2	17.0	17.7	15.2	12.6
Ash	10.8	9.9	10.8	10.3	9.0	9.6
ADF	8.8	4.8	9.9	9.5	6.4	6.9
GE (kcal/g)	5.5	5.5	5.4	5.4	5.4	5.3

Table II: Apparent digestibility coefficients of nutrients and energy of diets in which 30% of fish meal was substituted with different insect meals. In the row different letters denote statistical significant difference ($p<0.05$), (Mean ± standard deviation, n=3).

ADC	FM	TM	HI	MD	ZM	AD
DM	77.38 ± 0.60^{a}	77.39 ± 0.65^{a}	73.11 ± 1.13^{b}	76.56 ± 1.29^{ab}	68.53 ± 2.54^{c}	75.18 ± 0.88^{ab}
OM	83.77 ± 0.49^{a}	82.43 ± 0.42^{ab}	79.91 ± 1.09^{b}	82.22 ± 1.23^{ab}	75.99 ± 2.18^{c}	82.38 ± 0.54^{ab}
CP	91.63 ± 0.25^{a}	92.27 ± 0.29^{a}	89.61 ± 0.90^{b}	91.76 ± 0.17^{a}	92.45 ± 0.54^{a}	92.72 ± 0.15^{a}
CF	92.21 ± 0.42	92.36 ± 0.19	91.21 ± 0.52	92.20 ± 1.58	87.36 ± 3.50	89.68 ± 2.14
ADF	82.17 ± 0.88^{a}	49.80 ± 2.68^{c}	61.34 ± 1.20^{d}	70.32 ± 1.58^{b}	76.02 ± 4.85^{ab}	75.71 ± 1.30^{ab}
GE	88.49 ± 0.46^{a}	86.94 ± 0.36^{ab}	85.82 ± 1.02^{b}	87.27 ± 0.84^{ab}	79.84 ± 1.74^{c}	85.50 ± 0.56^{b}

(Continued on next page)

Acknowledgements
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CARP EDEMA VIRUS DISEASE OF KOI *Cyprinus carpio koi* IN THE CZECH REPUBLIC

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Carp edema virus (**CEV**) belonging to the family Poxviridae causes disease, known as koi sleepy disease (**KSD**), which is characterized by extremely lethargic behaviour, overproduction of mucus on the skin and gills, enophthalmia, gill damage and generalized edema. The disease is associated with mortality rates up to 80 % in koi and common carp (*Cyprinus carpio*) aquaculture, resulting in serious economic losses in several European and Asian countries and in the United States.

In this study, we report the first data supporting the presence of CEV in koi in the Czech Republic.

Koi carp were collected from 5 different locations in the Czech Republic during the years 2013 - 2017 (Table 1). Based on the clinical signs, lethargic behaviour and increased mortality, the fish were examined for the presence of the koi herpes virus (CyHV-3) infection, spring viremia of carp virus (SVCV) infection and CEV infection.

All koi showed negative PCR results for CyHV-3 and SVCV detection. The presence of CEV in all tested samples of koi was confirmed by PCR followed by sequence analysis. All but one CEV isolate were found to be virus variants from genogroup IIa, which is mainly detected in diseased koi. In one case, we found a virus variant from genogroup I, which often occurs in common carp. This suggests that koi infected with CEV from genogroup I might recently have had contact with carp suffering from clinical KSD. The results indicate that the prevalence and spread of this disease should be carefully monitored in order to minimize the economic risk to both carp and koi industries.

Table 1. Samples of koi (*Cyprinus carpio koi*) used in this study and examined for the presence of carp edema virus (CEV) using the nested PCR method of CEFAS (Matras et al. 2016). PCR for koi herpes virus (CyHV-3) was performed as previously established by Gilad et al. (2004). The method described by Koutná et al. (2003) was used to determine whether spring viremia of carp virus (SVCV) was present.

Sample	**Year**	**Stage**	**Sampling location**	**Clinical signs incl. death**	**CEV (Nested PCR)**	**CEV Genogroup**	**CyHV-3 (qPCR), SVCV (cell culture, cPCR)**
CZ-1845	2013	adult (> 1 kg)	Garden pond	Y	-/+	I	-,-
CZ-1895	2014	adult (> 1 kg)	Garden pond	Y	+/+	IIa	-,-
CZ-2029	2017	adult (> 1 kg)	Garden pond	Y	-/+	IIa	-,-
CZ-2032	2017	juveniles (10 – 15 cm)	Retail shop	Y	+/+	IIa	-,-
CZ-2066	2017	adult (> 1 kg)	Garden pond	Y	+/+	IIa	-,-

AQUACULTURE PROMISING OBJECTS & NEW TECHNICAL MEANS

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Aquaculture is the only reliable source for increasing food production. The rapid development of areas related to artificial cultivation of aquatic bioresources started in the 1970-80s. Since then, the total volume of annual fish production has increased by almost 10 times. This rapid growth is due to the development of technologies for the industrial cultivation of valuable fishing objects, which provide very acceptable economic indices. It should be noted that the cost of artificial production of one ton of fish products per unit of protein is 2.6 times less than the cost of cattle meat, 2.4 times – for pigs, and 1.5 times – for poultry. The cost of caught fish is higher than the cost of meat.

The required intensive development of aquaculture is possible only in case of its industrialization and integration of relevant scientific and technological solutions into it, both in terms of reproduction, feeding, and maintenance, and processing. Industrial cultivation of fish should be based on the same principles as the cultivation of broiler chicken at industrial poultry farms. Only such an approach will allow getting away from artisanal farms and developing a modern high-tech fishing industry.

SSC RAS has extensive experience in the development of biotechnologies and new technical means for aquaculture in Russia. A specialized complex, which is a system of modules, one of which is a unique modular installation, which allows adjusting the parameters of the aquatic environment to simulate various conditions, has been developed at SSC RAS for experimental studies. The possible introduction of so-iuy mullet (haarder), pike-perch, pike, and vimba into the aquaculture of South Russia has been determined based on the study of biological and adaptive characteristics of objects. Methods of intensive cultivation of so-iuy mullet and its adaptation to artificial conditions of the environment should provide a high yield of products per unit of useful area (80%) and high survival rates of the object at all stages of production cycle, as well as reduce production costs when compared to existing technologies (by 10-20%).

New technical means and biotechnological methods of cultivation of non-traditional aquaculture objects (as much in line with the world developments as possible) are developed. A new technical means “A Spawning Device for Pike-perch” allows approaching the natural spawning conditions of pike-perch as a result of technical engineering devices’ application, such as the system of temperature control, hydrochemical regime, water level, and removable artificial nests. The device (developed to increase the efficiency of spawning and growing the resistant offspring of pike-perch) with high technical indices of aquatic environment parameters’ regulation by goal indices increases the yield of viable larvae by 10-15% in comparison with the world analogues and the yield of resistant juveniles – by 20%.

The study is within Agreement No. 14.607.21.0163 of 03.10.2016 (UI RFMEFI60716X0163).

INFLUENCE OF THE MICROBIOLOGICAL PREPARATION ON AGRICULTURAL CROPS PRODUCED TOGETHER WITH THE HYDROBIONTS VIA AQUAPONIC METHOD IN THE FLOOR-TYPE SYSTEMS

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Nowadays, in the energy conservation era and when environment preservation is one of global priorities, the development of new aquaculture and agriculture technologies plays vital role. Development of vertical farms and multi-storey complexes is a promising area of global scientific and technological development. Intensification of the production of traditional types of food supply are also addressed through the development of aquatic organisms production in artificial environments, aquaponics in particular (hydroponic plant cultivation, in which nutrients come from waste products of fish). Above mentioned technologies are gaining momentum in Russia, and can be used for increasing the supply of remote northern cities with fresh vegetables and fruits, and other high-quality food supply.

At the current stage of aquaculture development, there is a certain need for high level of optimization of fish production in water recirculation systems (WRS).

Experimental scientific research on the joint cultivation of hydrobionts and plants took place in a aquacomplex of the scientific and expeditionary base "Kagalnik" of Southern Scientific Center of Russian Academy of Sciences for several years. During the research, an experimental integrated system for the joint cultivation of hydrobionts and crops was developed. Optimal parameters of the aquatic environment were defined in line with the biological needs of all types involved into the cultivation process. A selection of specific hydrobionts and crops were chosen for best growing results in water recirculation system.

During the aquatic plant experiment, spinach and lettuce were grown together with tilapia. Plant seeds were pre-soaked in a culture liquid of *Serratia ficaria* TR3 with a cell titre of 10^9 CFU / ml. The experiment was carried out in two variants - seeds were treated with a bacterial suspension and with regular water. 20 plants were used In each variant of the experiment. After cultivation, sprouted seedlings were transplanted into an aquaponics plant.

Nitrates level in grown salad leafs was monitored at the final stage of the experiment both in the experimental and control groups. Data given in Figure 1 shows significantly reduced level of nitrates in plants that were treated with culture liquid of *Serratia ficaria* TR3, which means that such treatment can be used to grow bio-safe cultures.

Cultivation of aquaculture objects and green plants in an artificially-constructed system of a floor type is an environmentally friendly production. This is a vital criteria in the prevailing environmental conditions.

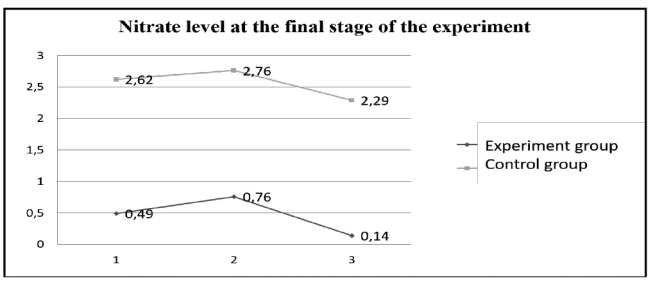

Figure 1. Nitrate level in salad leafs

BIOFOULING ON CULTIVATED KELP: REGIONAL AND LATITUDINAL VARIATION IN NORWAY

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Biofouling on cultivated kelp by epibionts usually has a negative impact on the quality and quantity of kelp biomass. Therefore, kelp is harvested before the onset of epibionts, and the growing season and time for harvesting in high latitudes is often limited to late spring or early summer. Little is known about the variation between cultivation sites with regard to species composition and the timing of biofouling on cultivated kelp. This variation was studied through two field studies. In the first study, the kelp *Saccharina latissima* was cultivated at three sites in Troms, northern Norway (at 69 °N). In late August 2014 the kelp fronds were harvested and the fouling was analysed. The biofouling varied widely between the three locations, both in species composition and area fouled (Fig. 1). The frond area covered by epibionts varied between 9.0 ± 2.5 % at the fjord site, 13.2 ± 3.8 % at the inshore site, to 61.4 ± 9.1 % at the semi off-shore site (all at 8 m depth). Species composition of the biofouling community also varied between sites, with the dominant taxa being hydroids at the most protected and bryozoans at the most exposed site. In the second study, *S. latissima* was cultivated at ten sites located from 58 °N to 69 °N. Local genetic material was pre-cultivated at the same lab, transferred to the study sites at the same time and cultivated in similar rig set-ups in the sea. Growth, content and biofouling were examined each second to 4th week throughout the whole growth season of 2017. The preliminary results from this study show that the timing of initial colonisation of biofouling, rate of cover and species composition are highly variable along the Norwegian coast, and that there is a prolonged growth season further north due to later biofouling settlement.

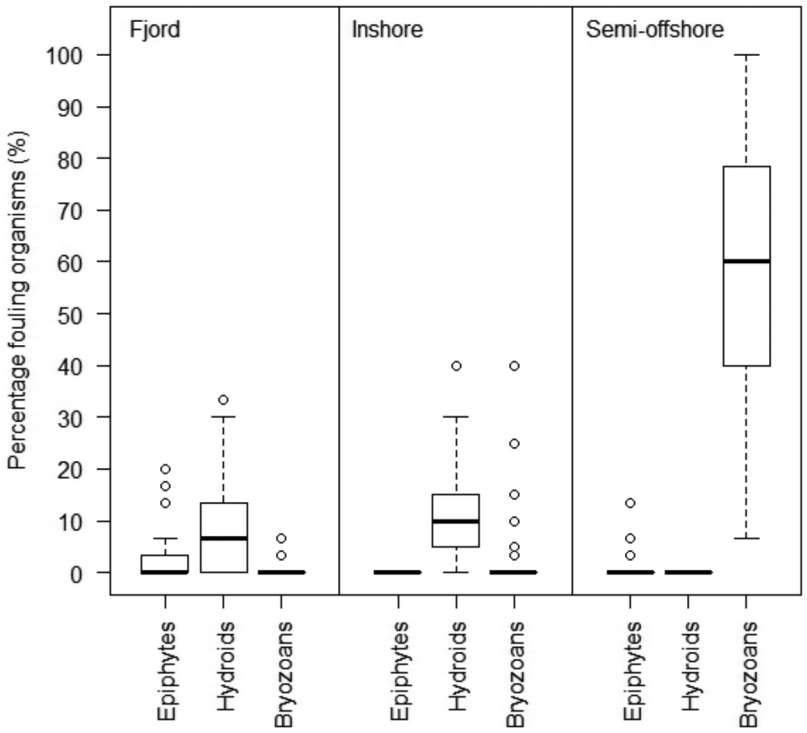

Fig. 1: The biofouling cover (%) separated by type at the three sites in Troms

THE WELFARE OF SALMONIDS REARED FOR RESOCKING

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Salmon *(Salmo salar L.)* and sea trout *(Salmo trutta L.)* are the main fish species in Latvia which are reared with an aim to maintain their population in natural water bodies, and to compensate the loss in fish population caused by cascades of hydro power plants and other anthropogenic factors. Salmon and sea trout smolts are released longtermly in Daugava, Gauja and Venta River estuaries according to the National Fish Restocking Programme. As it is important to have their return, strong and viable smolts to be released in rivers, therefore before the release their health and welfare were scrutinized.

Fish health was evaluated in five fish farms with flow-through rearing system. Several indirect (temperature, oxygen, flow rate etc.) and direct (weight, fork length, condition factor, fin necrosis) welfare indicators were measured. Direct indicators were sampled for 100 randomly chosen salmon or sea trout parr in every farm.

Throughout the complete rearing process parr were ensured to have lighting according to the season. Water temperature varied from 0.1ºC in winter to 26.3ºC in summer. Oxygen level varied from 7.9 mg/L to 11.7 mg/ L. Stream velocity in Latvian rivers inhabited by salmon is 0.5-2.0 m/s^{-1}. For salmon and sea trout to be able to adapt to life in river it is important to ensure similar velocity in the fisheries. However, unfortunately velocity in fish farms varies from 0.06 ±0.02 m/s to 0.13±0.06 m/s. Rearing density varies from 11.1 kg/m^3 to 53.0 kg/m^3, and condition factor varies from 0.81±0.08 to 1.2±0.15. There is a correlation ($r=0.65$; $\alpha= 0.01$, $n=15$) between those factors, and it is clearly visible that condition factor increase with an increased rearing density. It means that in higher density fish move less and have more feed: fish gain weight and are shorter. The high rearing density favours development of fin necrosis ($r=0.23$; $\alpha= 0.01$, $n=500$).

Fish health is influenced by manifold factors, site-specific for every river and every fish farm, nevertheless our study reveals that in all flow-through fish farms nearly all main growing conditions are adequate to rear salmonids adapted for natural environment changes (fluctuations of temperature, seasonal changes of physical, chemical and biological water content), but exception is too low stream velocity and high fish density maintained in rearing basins. If salmonids would be kept at density 22 kg/m^3 the Condition factor would fit 0.95 that correspond to Condition Factor for natural fish. To diminish fin necrosis and condition factor, rearing density has to be decreased till 22kg/m^3 and stream velocity increased at least till 0.5 m/s.

GAINING BETTER INSIGHT INTO THE REPRODUCTIVE PHYSIOLOGY AND EARLY LIFE DEVELOPMENT OF BONEFISH (*Albula vulpes*)

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Bonefish (*Albula vulpes*) are a valuable fishery resource of tropical and subtropical ecosystems worldwide. Despite their importance, there is a limited information on bonefish life history and ecology. The present study aims to describe, for the first time, the oocytes development, their lipid characteristics, and reproductive hormones in wild bonefish during the reproductive season in different tidal flat pre-spawn aggregations (PSA) locations in Grand Bahama Island, Bahamas. Bonefish likely follow a group-synchronous ovarian development and produce lipid-rich eggs [total lipid (TL) content was > 26% of the wet mass (WM)]. The major lipid class was a neutral lipid, the wax esters-steryl esters (WE-SE; >48% of TL), suggesting their use to support buoyancy and/or energetic development. Fish reproductive state was evaluated using histological analysis of the oocytes and determination of sex hormone levels of 17β-estradiol (E2) and testosterone (T) in the plasma. Levels of 17β-estradiol were low in individuals sampled outside of the spawning season relative to fish sampled during spawning months. Testosterone levels did not change as female bonefish entered the spawning season. Within the spawning season, bonefish are commonly found along flats, or PSA. Levels of 17β-estradiol did not differ between PSA and flats fish, however, testosterone levels were significantly higher in fish from the PSA. Our results bring useful information concerning the reproductive physiology of bonefish indicating that as bonefish are transition to the PSA from flats habitats, vitellogenesis is still occurring and PSA may serve as a final staging location for Bonefish. These results not only serve as a benchmark for determining the nutrient requirements, and hormonal status of broodstock to produce high quality eggs from bonefish captive broodstock but also will help establish a meaningful management practices for this species.

SEA URCHIN *Paracentrotus lividus* PRODUCTION AT AQUACULTURE RESEARCH STATION (PORTUGAL)

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The sea urchin is a marine resource whose value and demand presents a growing trend, primarily due to the interest of the international markets. The interest comes mainly from their gonads (roe) and reach very high prices due to its value as a product of gourmet cuisine. Due to the growing demand and consequent capture a decrease of the natural stocks has been observed. The sustainable consumption of this resource requires the development of aquaculture production that satisfy the international market. Production trials of sea urchins (*Paracentrotus lividus*) at IPMA started using breeders captured off the Algarve coast in 2016 and conditioned at Aquaculture Research Station (EPPO) facilities, fed with macroalgae *Ulva* spp. and corn grain (*Zea mays*). Spawning induction was obtained by injection of magnesium chloride 0.5 M in the celomic space by the sea urchins peristomial membrane. Fertilization and embryonic development were accompanied by microscope observation. During the planctonic stage the sea urchins larvae were fed with microalgae Genus *Isochrysis* and diatoms Genus *Chaetoceros* and *Skeletonoma*. A survival rate of 30% was observed at 15DAH (days after hatch). At age 27DAH metamorphosis and fixation was verified. Reached the bentonic stage the sea urchins were fed with the same mixture of microalgae and macroalgae *Ulva* spp (fig. 1). After 4 months of age sea urchins were fed exclusively with macroalgae and macroalgae and corn. At 7 months of age (220DAH) sea urchins fed with macroalgae showed a higher somatic growth (test diameter) (fig. 2), however individuals fed with seaweed and corn had a better GSI (gonado-somatic index).

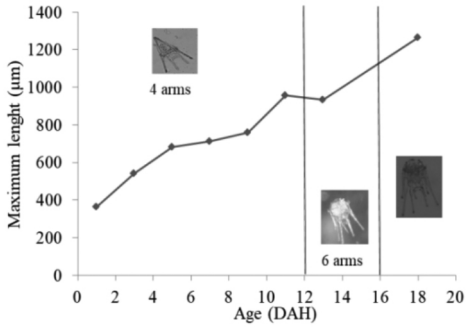

Figure 1- Growth of cultivated *P. lividus* larvae.

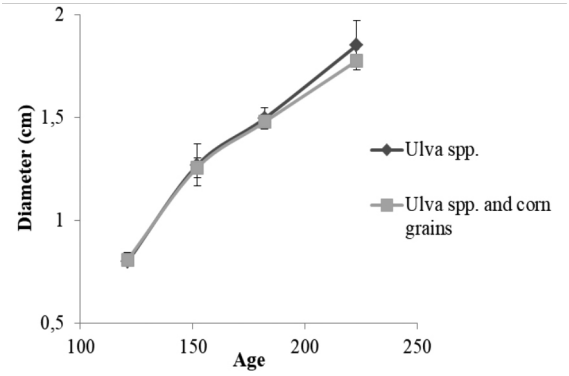

Figure 2- Growth of cultivated *P. lividus* juveniles with two feeding regimes.

THE EFFECT OF FISH MEAL REPLACEMENT WITH INSECT MEAL ON GROWTH PERFORMACE AND SURVIVAL RATE OF ZEBRAFISH (*Danio rerio*)

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Introduction

Zebrafish (*Danio rerio*) is a very popular Cyprinid tropical aquarium fish and is used as a research model in many studies because of its small adult size, the continuous breeding cycle, the high hatching rate and the large numbers of eggs per brood. In addition, it is easy to manage and can be produced and kept in captivity for a long period (Ulloal et al. 2014). The aim of the present study was to investigate the effect of fishmeal replacement with insect meal (10%, 20% and 30% replacement) on growth and feed utilization of zebrafish (*Danio rerio*).

Material and Methods

A total number of 120 juvenile zebrafish (*Danio rerio*),(3.4±0.03 cm mean total length and 0.32±0.01 g mean total weight) were obtained from ovigorous female that were kept in captivity for more than 100 days in 27ºC.The juvenile zebrafish were distributed in twelve 40L aquariums as described in Vlahos et al. (2013) and Mente et al. (2016). The fish were divided in four treatments, three replicates per treatment with a stocking density of 10 fish/aquarium and were fed with four iso-nitrogenous and iso-energetics experimental diets three times per day, 5% of their body weight for a period of 30 days. In the experimental formulated diets,the fishmeal protein was replaced by an insect meal(H. Illuscens) at 10%, 20% and 30% respectively.At the end of the experiment fish were anaesthetized with 2- phenoxyethanol (0.1 mL/L) and their final weight and length was measured. Data were analyzed using the one-way ANOVA test. Tukey'stest was used to determine the significantdifferences between the means of all groupsat $P<0.05$.

Result and Discussions

Survival rate for all treatments ranged between 90% (FM diet) and 100% (IM10 diet) and 93.3% (IM20 and IM30 diets). The results showed that 30% replacement of fishmeal by insect meal did not affect zebrafish growth rates. Henry et al. (2015) showed that insect meal can be used as an ingredient in fish diets up to 36% without causing negative effects on fish growth in trout*Oncorhynchus mykiss*, as well as on food efficiency, although levels of insect mealhigher than 12% lead to unsuccessful growth results for turbot (Kroeckel et al. 2012).

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Bonamia ostreae DETECTION IN SEAWATER: AN APPROACH TO ESTIMATE PARASITE SURVIVAL OUTSIDE ITS BIVALVE HOST *Ostrea edulis*

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Ostrea edulis is the native European flat oyster species. Since the late 1960-1970's, it has been affected by two epizootic diseases: marteiliosis (1968) and bonamiosis (1979), caused by the protozoan parasites *Marteilia refringens* and *Bonamia ostreae* respectively. These diseases still cause significant mortality and production volumes are very low. Nevertheless, there is a renewed interest for this species by oyster farmers, mainly because of its patrimonial, economic and ecological interests. In order to better understand the mechanisms of the disease transmission, we have developed a real-time PCR-based approach allowing early detection of *B. ostreae* DNA in seawater. Additionally, this methodology has been used to estimate the survival of this parasite outside its host.

This approach is based on three main steps: filtration of seawater on a 1 μm mesh size polycarbonate membrane, DNA extraction from the membrane filters using the DNeasy © PowerWater © Kit (Qiagen, Inc.) and real-time PCR analysis. The real-time PCR assay targets a 199 bp fragment coding for the SSU 18S rRNA of *Bonamia sp*. Detection and quantification limits were established using 10 in 10 dilution range, obtained from a purified *Bonamia ostreae* suspension. The different dilutions of the parasite suspension were applied on polycarbonate membranes, then processed as previously described. Obtained results allowed detecting and quantifying up to 10 and 30 parasites per membrane filter, respectively.

This approach has been successfully used to estimate the survival of the parasite in seawater after its shedding from oysters. Obtained results showed that *B. ostreae* DNA could be repeatedly detected until 72 h after the parasite is shed from oysters (Figure 1). This result is close to the conclusion of Arzul et al. (2009), who estimated an average viability of 30 % after 48 h of incubation at 15°C in 0.22 μm filtered natural seawater, based on the measure of esterase activity by flow cytometry.

Complementary, a similar approach is being developed to detect *B. ostreae* RNA. Such information will be useful not only to detect parasite presence but also to assess the status (alive or dead) of the parasite in different compartments including seawater.

Arzul I, Gagnaire B, Bond C, Chollet B and others (2009) Effects of temperature and salinity on the survival of *Bonamia ostreae*, a parasite infecting flat oysters *Ostrea edulis*. Dis Aquat Org 85:67-75. https://doi.org/10.3354/dao02047

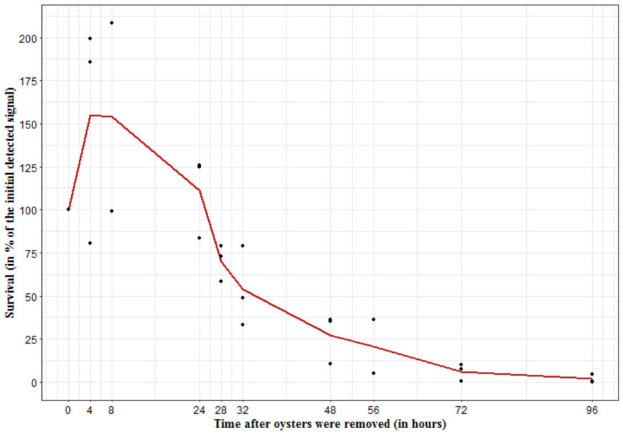

Figure 1: Temporal evolution of the detection of *B. ostreae* DNA after shedding

EFFECT OF DIFFERENT TEMPERATURE AND TIME APPLICATION IN ULTRASONIC WATER BATH ON THE LIPID QUALITY OF *Schizochytrium sp.*

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Microalgae have high content of protein, lipids, vitamins, minerals, carbohydrates, pigments, which are potentially used in many areas such as food, feed, cosmetics, fertilizer and biodiesel are also a great raw material because of their high-volume reproduction per unit area.

Even though microalgae are used in many industries, lipid production from the algae is highly challenging due to the difficulty of breaking their cell walls. Hence, various methods such as ultrasonic water bath, vortex, thermal shock, osmotic shock are applied to increase the lipid yield.

In this study, it was aimed to determine the effect of ultrasonic water bath exposure(40kHz) on spray dried powder form of *Schizochytrium sp*. (MarinBio, Turkey) lipid quality at different times (10-20-30 min) and temperatures (20-40-60°C). Free fatty acid (FFA), peroxide value (PV) and fatty acid composition analysis were performed to determine the quality of algae lipid obtained in the study.

Dominant saturated and polyunsaturated fatty acids were found as palmitic acid (C16:0) and DHA (C22:6) respectively. Total saturated fatty acids (SFAs) were between 52.81% -69.73% and the total unsaturated fatty acids (PUFAs) were 29.00%-44.99%. The total mono unsaturated fatty acid (MUFAs) values were found to be very low.

Increases in temperature led to increases in the amounts of EPA and DHA, which are extremely important for human health. The increase in time seemed not to cause a significant change on omega-3 fatty acids. It also has been determined that increase in temperature and time increases the amount of FFA and decreases the amount of PV.

EFFECTS OF REPLACING FISHMEAL BY *Chlorella vulgaris* AND FISH OIL BY *Nannochloropsis gaditana* AND *Schizochytrium* sp. BLEND ON GROWTH AND FEED EFFICIENCY OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Replacement of fishmeal and fish oil in fish diets is still a major issue for aquaculture. Some microalgal species seem to be suitable alternatives due to their high protein content and richness in EPA and DHA. The aim of this study was to evaluate the effects of replacing fishmeal protein by *Chlorella vulgaris* meal and fish oil by a blend of *Nannochloropsis gaditana* and *Schizochytrium* sp. on growth and feed utilization of *Sparus aurata*.

Materials and Methods

S. aurata juveniles of 1.10±0.01 g initial mean weight were distributed in triplicates to 18 seawater aquariums and fed six 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 was replaced by a blend of *N. gaditana* and *Schizochytrium* sp. at 50% (SN50) and 100% (SN100). Fish were fed to satiation two times a day for 12 weeks.

Results and discussion

All CM-fed groups had similar (P>0.05) feed intake, SGR, FCR and PER with the control (Table 1) indicating that partial replacement of fishmeal protein by *C. vulgaris* meal up to 30% was successful. *C. vulgaris* meal has been successfully used to replace FM protein in diets of other fish species such as *Paralichthys olivaceus* (up to 15%, Rahimnejad & Lee 2016) and *Oreochromis niloticus* (up to 50%, Badwy *et al*. 2008) and of crustaceans such as *Macrobrachium rosenbergii* postlarvae (up to 50%, Radhakrishnan *et al*., 2015).

Furthermore, both SN-fed groups had a better, although not significant, growth and feed utilization performance compared to control group (Table 1), indicating that total fish oil replacement by *N. gaditana* and *Schizochytrium* sp. blend was successful. Other studies with *S. aurata* indicated that fish oil replacement by a blend of 5 microalgal species (Eryalçın & Yıldız 2015) and by *Schizochytrium* sp. or *Crypthecodinium cohnii* (Ganuza *et al*., 2008) was also successful. The study suggests that *C. vulgaris*, *N. gaditana* and *Schizochytrium* sp. are promising alternatives for fishmeal and fish oil replacement in the diet of *S. aurata*.

Acknowledgements

Scholarship for post-graduate studies (PhD) was awarded by Onassis Foundation. The study was funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation 2014-2020, Greek Ministry of Economy and Development.

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

	Fishmeal replacement				Fish oil replacement	
	CM10	CM20	CM30	Control	SN50	SN100
Feed intake (g fish^{-1})	16.5±1.5	15.6±3.4	15.1±2.8	14.0±2.1	17.6±4.1	17.6±2.5
Final body weight (g)	16.0±2.3	15.9±4.8	13.2±2.9	12.4±2.3	16.5±5.0	17.0±3.0
SGR (%/day)	3.18±0.16	3.14±0.40	2.93±0.27	2.87±0.22	3.18±0.41	3.24±0.21
FCR	1.11±0.07	1.09±0.14	1.26±0.08	1.25±0.07	1.17±0.15	1.12±0.06
PER	1.72±0.10	1.79±0.22	1.53±0.10	1.52±0.08	1.66±0.20	1.71±0.09

THE EFFECTS OF FISH FARM STRUCTURES ON NEAR-FIELD WATER CURRENTS: A CASE STUDY FROM FRØYA

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Impacts of open circular finfish cages on the local current field are investigated by field measurements and a 3-dimensional hydrodynamic model. The relationships between the current flow pattern and characteristics at site scale are examined at a mariculture site ("Rataren") north of Frøya, Central Norway (Fig. 1, left). This is a region where the current pattern is rather complex due to irregular bathymetry, skerries, islands, the influence of the Norwegian coastal current and strong wind conditions. The numerical model was set up in 32 m horizontal resolution evaluated against independent observations of the current from an array of ADCPs (Acoustic Doppler Current Profiler) and CTD measurements deployed approximately four cage diameters (200m) from the farms (Fig. 1, right). Two different field campaigns were performed with 1) small fish size (200 g – 400 g) and 2) large fish size (3 kg – 5 kg). The influence of the fish cages on the current is analyzed by calculations of current speed reduction at 10 m depth from one ADCP upstream of the cages to an ADCP downstream of the cages. If such impact occurs, it was expected that a noticeable increase in current flow reduction should have been detected between situations with small fish and large fish in both main directions of the flow. The results indicate that whereas there is such current reduction in one of the main directions, there is no such reduction in the other direction. A drag correction term was introduced in the model in order to parametrize the effects of the cages on the current field. Numerically, there was an effect, but not on a scale greater than a few cage diameters. The main conclusion from this study is that there is no obvious impact of the large fish on the current field at the scale investigated here.

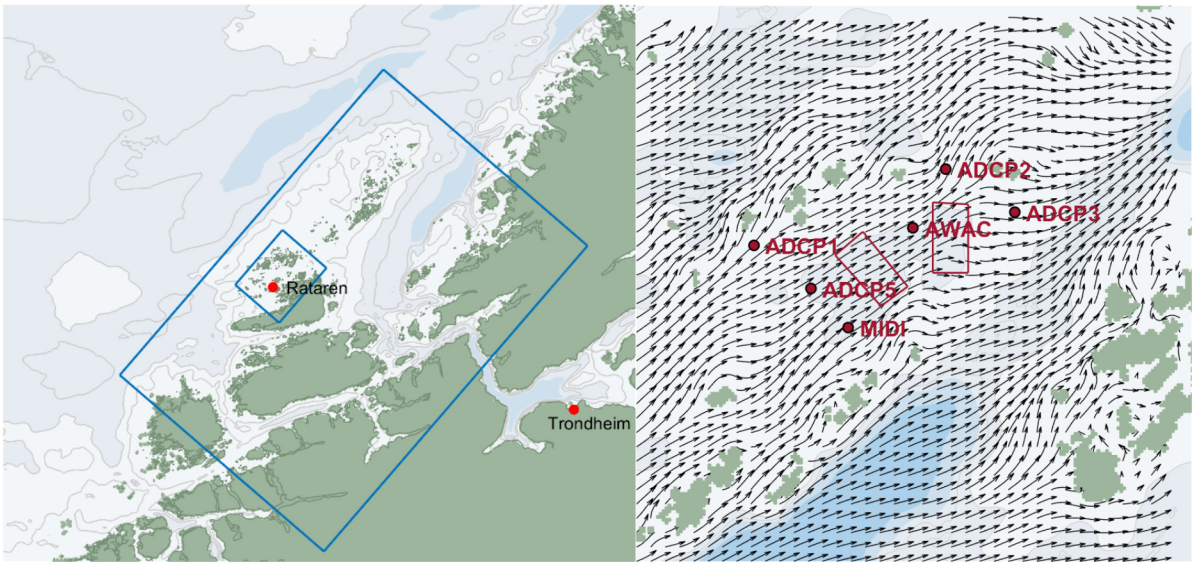

Figure 1. Left: The coast of Central Norway, indicating the location of the farm at Rataren (63.78° N; 8.52° E), the city of Trondheim, and hydrodynamical model domains of 160 and 32 m horizontal resolution. Right: detail of the model domain in 32 m resolution showing the farm layout and setup of the field campaigns. The arrows indicate simulated mean surface current field in April.

BIOCHEMICAL COMPOSITION AND FATTY ACID PROFILE OF SEA BASS *Dicentrarchus labrax* CO-CULTURED WITH *Ulva* sp. IN RECIRCULATING AQUACULTURE SYSTEM

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Studies on marine Integrated Multi-Trophic Systems (IMTA) have shown that seaweed filtration has the potential to improve the efficiency of Recirculating Aquaculture System (RAS). This study presents the co-cultivation of European sea bass (*Dicentrarchus labrax*) and *Ulva* sp. in indoor RAS with biofilm filtration in an effort to improve the quality and growth of sea bass using an alternative diet based on vegetable oils.

The experiment was performed at the Laboratory of Applied Hydrobiology and lasted for 12 weeks. The RAS consisted of 2 units: the first (U) was used for the co-culture of *Ulva* and sea bass and the second was used as a control (C) without *Ulva*. Each unit comprised of two levels with 3 tanks each, the upper one for sea water with or without seaweed and the lower for fish (CF groups without *Ulva*, UF groups with *Ulva*). Seawater flowed from tanks of the upper level to the lower level into the tanks containing the fish. Rearing conditions were similar in both RAS units. The daily feed consumption per fish was estimated. Dietary fish oil was replaced by a mixture of vegetable oils (50% rapeseed oil: 50% palm oil). Proximate analyses for moisture, ash, protein, and fat (% wet weight), as well as fatty acid analysis (% total fatty acids) were performed for feed and for fish at the beginning and at the end of the rearing period.

The average initial (35.42 ±0.476 g) as well the final body weight (83.73± 1.270 g) did not differ among fish groups showing similar specific growth rate (SGR). In addition, Feed Conversion Ratio (FCR) had no statistically significant difference between CF and UF groups. However, final Condition Factor and feed consumption (g/fish) were significantly higher in UF compared to CF groups. Although protein content was significantly higher in UF than in CF groups, Protein Efficiency Ratio (PER) and Productive Protein Value (PPV) were similar in both groups. Moisture was significantly ($P<0,005$) higher in CF than in UF groups, whereas ash and fat were significantly higher in UF than in CF groups. Fatty acid profile analysis showed significant differences in the content (% total fatty acids) of total saturated and total monounsaturated; the former being elevated in CF groups whereas the latter in UF groups. The content (% total fatty acids) in total polyunsaturated fatty acids was similar in both groups.

The replacement of fish oil by the introduced mix of vegetable oils showed satisfactory levels of SGR (1.02 ± 0.010) and FCR (1.08 ± 0.019) for sea bass. Trying to elucidate the role of *Ulva* in IMTA, we can assume that *Ulva* has an effect on sea bass (a) growth: the Condition Factor is higher in UF and (b) quality: the protein content is elevated in UF groups as well as the quantity of EPA and DHA (% wet weight) due to the increased fat content. The differences observed, could be due to the fact that in *Ulva*-seabass RAS the fish could be fed on *Ulva* organic matter derived from seaweed decomposition, hence the co-cultivation of sea bass and *Ulva* could result in fish with a higher nutritional value.

BLUE CAREER CENTER OF EASTERN MEDITERRANEAN AND BLACK SEA: PROGRESS IN MENTOR PROJECT

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The "Blue Career Centre of Eastern Mediterranean and Black Sea (MENTOR)" is a two-year project co-funded by the European Maritime and Fisheries Fund in the framework of the EASME call for Blue Careers in Europe. It involves seven partners from Cyprus (UCY: University of Cyprus, coordinator; MARINEM: Maritime Institute of Eastern Mediterranean; CCCI: Cyprus Chamber of Commerce & Industry), Greece (NTUA: National Technical University of Athens; AUA: Agricultural University of Athens), Bulgaria (MCB: Marine Cluster Bulgaria) and Romania (CMU: Constanta Maritime University) (see http://www.bluecareers.org).

The general objective of the project is to set up a regional platform - the Blue Career Centre - to promote the dialogue between business stakeholders, education & training institutions, research organizations, policy makers and the civic society. This would allow them to jointly develop and carry out measures to close the skill gap, tackle unemployment and make "Blue Careers" more attractive to young people. Four marine and maritime economic activities have been selected as of strategic importance in the Eastern Mediterranean and Black Sea regions: Maritime Transport, Marine Aquaculture, Cruise Tourism, and Offshore Oil & Gas.

The secretariat of the BCC MENTOR was registered in January 2018 by MarInEM in Cyprus. The successful operation of the first Blue Career Centre is expected to set an example and a model for all other sub-basins. A network of Blue Career Centers may eventually be established, bringing together stakeholders of various European marine and maritime clusters in a common effort to achieve Blue Growth from Mediterranean and beyond.

So far, four MENTOR Career Fairs were held: 1) in Constanta, Romania, hosted by CMU; 2) in Athens, Greece, hosted by NTUA; 3) in Limassol & Larnaca, Cyprus, hosted by Mar.In.E.M., CCCI, the Cyprus University of Technology and the Cyprus Maritime Academy; 4) in Varna, Bulgaria, hosted by MCB. The Consortium partners developed a "Career Guidance Framework" aiming to map the needs of the selected MEAs and identify the specifications for the construction of a personalized profile based on the occupational status of the mentoree. A "Framework for Promoting Mobility for Blue Sector Courses" has also been developed in an effort to identify the necessary critical career skills for professionals in the blue economy and to provide accessible pathways for facilitating career advancement and job mobility in the selected MEAs.

OPTIMIZATION OF PHAGOCYTOSIS ASSAY IN RAINBOW TROUT *Oncorhynchus mykiss*

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Requirements for disease diagnosis increase along with the global aquaculture growth. An effective way of examining the fish immune system and its ability to actively react to stimulants is the usage of functional immunological assays. During phagocytosis assay, phagocytes are incubated with fluorescently labeled particles (e.g. zymosan, prepared from yeast cell walls). Peripheral blood is an optimal sample for this assay. Hemolysis in a hypotonic environment is a quick and cheap way of leukocyte isolation. The phagocyte representation and the intensity of phagocytic activity can then be measured by flow cytometry, which offers many advantages, in particular rapid examination of large numbers of cells and easy differentiation of individual cell populations. In fish, however, large variability needs to be taken into account. Phagocytic activity was also recorded in B-lymphocytes. The methodology varies between different authors – incubation time, incubation temperature and concentration of zymosan particles need to be optimized.

Leukocytes were obtained from rainbow trout (*Oncorhynchus mykiss*) blood taken from the tail vein into a heparinized syringe. Incubation with zymosan particles (AF 488, Texas Red) was carried out in a CO_2-free incubator. Leukocytes were isolated by hemolysis in a hypotonic environment (distilled water). Isolated cells were washed by centrifugation in PBS and fixed (Cell Wash, EDTA). As a second option, the cells were hemolysed first and then incubated with opsonized zymosan, medium (HBSS) and serum. Using a flow cytometer (BD LSRFortessa), the numbers of phagocyte cells and the intensity of phagocytic activity was evaluated. Propidium iodide was used to visualize dead cells. Incubation time, incubation temperature, appropriate zymosan particle concentration and volume of blood were optimized.

Presently, best results were obtained with 1-hour incubation at 15°C. Incubation of 10 μl of blood and 1μl of zymosan AF 488 particles followed by hemolysis was proven to be most effective. More experiments are being performed to confirm these results.

AQUACULTURE VS MARINE PROTECTED AREAS: IDENTIFICATION OF POSSIBLE COEXISTENCE AREAS IN MACARONESIA

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In order to supply food for the growing world's human population, sustainable management of natural resources and biodiversity have to focus on how and where the protein source is provided from. Aquaculture production, which is the fastest-growing source of food globally, is promised to achieve this mission. According to the Fish and Food Organization (FAO), this figure will reach around 62 percent of the global seafood by 2030. At the same time that space for food production must be increased, the Convention on Biological Diversity, on its Target number 11, proposes to protect 10% of the entire ocean by 2020. In this case, marine aquaculture brings an alternative option to be performed within Marine Protected Areas (MPAs), where can enhance coastal communities by diversifying local markets and livelihoods acting as a key role for food security, poverty alleviation and economic resilience. Likewise, based on sustainable principles, this activity can promote synergies that are aligned to MPAs conservation aims.

The IUCN (International Union for Conservation of Nature) classifies protected areas into categories where some of them are able to have activities being performed. For that, a more coherent governance and effective planning between marine protected spaces and all several respective activities must be well-studied at local levels, enlightening the most adequate decisions to be undertaken.

As a step in the process of planning, aquaculture allocation is crucial to the maintenance of the MPA's integrity. With this, the present study aims to identify the best spatial scenario for allocation of sustainable aquaculture activities within Macaronesian MPAs. Thus, the methodology consists of implementing state of art, identifying protected areas and analyzing open data basis as EUNIS (European Nature Information System), CDDA (Common Database on Designated Areas) and Natura2000. These repositories include details, such as on the level of protection, biodiversity, preservation status, surface area, percentage of coverage and other data that are used to identify compatibilities with sustainable aquaculture. This presentation will show processed GIS results, percentage of area appropriate for sustainable aquaculture within the areas designated for protection classified by IUCN for Macaronesian region. This study is delivered within project PLASMAR, financed by the European Regional Development Fund.

CRACKING THE CODE OF PACIFIC OYSTER MORTALITY SYNDROME

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For decades, methodological limitations have restricted the study of infectious diseases to simplified experimental pathosystems in which the influences of host and pathogen diversity and the biotic and abiotic environments have been minimized. Such reductionist approaches have made diseases with complex etiologies difficult to characterize. This is the case for some diseases triggering recurrent mass mortalities in non-model species of ecological and/or economic interest such as pollinators, corals and marine mollusks.

The objective of the present work was to examine a disease of complex etiology affecting one of the main invertebrate species exploited in the world, the Pacific oyster *Crassostrea gigas*. Introduced to France in the 1970s, *C. gigas* suffers mass mortalities associated with complex interactions between host, environment and pathogens. The severity of these mortality outbreaks has dramatically increased since 2008. They mainly affect juvenile stages decimating up to 100 % of young oysters in French farms. Over the past years, this mortality syndrome has become panzootic, being observed in numerous other countries worldwide.

By developing a holistic approach to tackle the complexity of interactions, we deciphered the complex intra-host interactions underlying the Pacific oyster mortality syndrome. Using ecologically realistic experimental infections (Figure 1) combined with thorough molecular analyses on oyster families with contrasted susceptibilities, we demonstrated that the disease is caused by a multiple infection whose initial and necessary step is the infection of oyster hemocytes by a herpesvirus. Viral replication leads to an immune-compromised state of the host, evolving toward subsequent bacteremia by opportunistic bacteria.

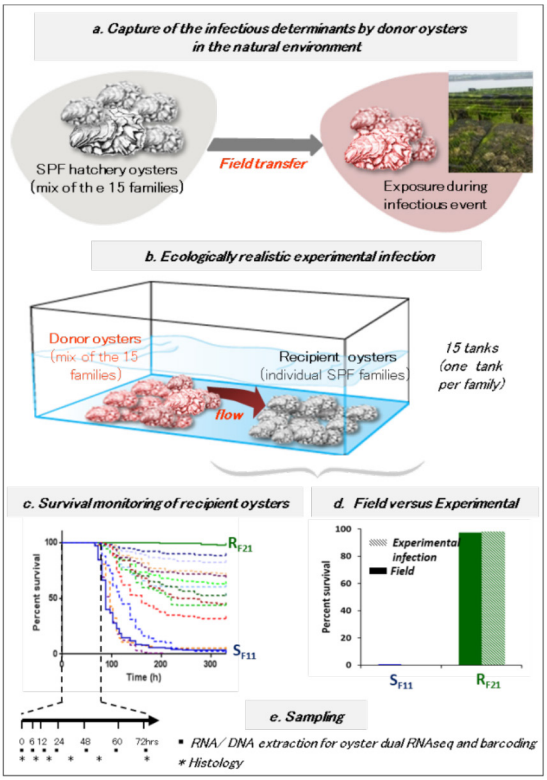

Figure 1: Ecologically realistic infection protocol used in this study.

HEMP SEED PROTEIN AND OIL: ALTERNATIVES FOR FISH MEAL PROTEIN AND FISH OIL IN AQUAFEEDS

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Substitution of fish meal and oil in fish diet with plant sources has high importance in aquafeed production and oilseeds are key alternatives for this purpose. *Cannabis Sativa* L., an annual herbaceous plant commonly referred to as hemp, is a widely cultivated plant in the world. The nutritional characteristics of hempseed are reviewed in this study as an alternative for fish meal and oil in fish diet.

Hempseed meal has high crude protein levels (>50%) and a good amino acid profile (Table 1). Conducted studies showed that using hempseed meal in the diet of farmed fish was more effective than soybean meal as a substitution for fish meal. Hempseed protein has a balanced amino acid profile like fish meal and is rich in glutamic acid, aspartic acid, serine, arginine and leucine, and sulfur-containing amino acids (Met and Cys).

Hempseed contains approximately 25 to 35% oil, with over 80% in polyunsaturated fatty acids (PUFAs), is a good oil source with high nutritional value (Table 2). Hempseed oil is perfectly balanced in regards to the ration (3:1) of linoleic and linolenic essential fatty acids.

Altogether, Hempseed with appropriate protein and lipid composition and quantity and also no considerable limitative effect of growth and health of fish, is a good alternative for fish meal and oil in diet.

Table 1. amino acid composition of hempseed, fish meal and soybean meal (mg amino acid / g protein).

Amino acid	Hemp seed	Fish meal	Soybean meal
Ile	3/99±0/08	4/21±0/22	1/94±0/172
Leu	6/63±0/23	6/69±0/07	3/26±0/274
Lys	4/16±0/87	8/83±0/14	2/69±0/200
Met	1/39±0/06	1/39±0/04	0/61±0/048
Cys	0/17±0/01	ND	0/7±0/063
Tyr	3/67±0/23	2/20±0/14	1/53±0/129
Phe	4/57±0/11	4/79±0/15	2/16±0/207
Thr	4/57±0/35	8/45±0/16	1/62±0/117
Val	4/98±0/13	5/24±0/11	2/06±0/186
His	2/81±0/47	1/84±0/14	1/15±0/096
Trp	Nd	ND	0/5±0/064
Asp	9/41±0/39	2/90±0/19	4/79±0/470
Glu	16/14±0/26	5/27±0/16	7/66±0/868
Ser	5/18±0/02	0/44±0/03	1/92±0/209
Gly	3/99±0/06	5/82±0/09	1/77±0/143
Arg	9/91±0/91	4/56±0/29	3/17±0/397
Ala	4/50±0/36	0/30±0/02	1/79±0/128
Pro	4/53±0/39	0/29±0/02	2/04±0/225

Table 2. Fatty acid composition of hemp seed oil and fish oil (weight percentage of total fatty acids).

Fatty acids	Hemp	Fish Oil
Palmitic acid (C16:0)	5/37±0/13	16/31±4/19
Stearic acid (C18:0)	1/56±0/05	3/62±1/21
Oleic acid (C18:1)	11/51±1/05	27/35±5/1
Linoleic acid (C18:2w6)	59/16±0/85	6/49±3/19
γ- Linolenic acid (C18:3w6)	3/48±0/15	0/26±0/08
α-Linolenic acid (C18;3w6)	17/96±0/23	2/12±1/32
Eicosenoic acid (C20:1)	0/80±0/01	3/92±2/41

IN VITRO STORAGE OF THE EGGS IN PIKEPERCH *Sander lucioperca* AND EURASIAN PERCH *Perca fluviatilis*

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Fertilization success, embryo quality and later performance of the offspring are highly dependent on oocyte integrity. Oocyte ageing has been identified as the most important factor affecting egg quality in several fish species. Delayed fertilization after egg stripping leads to the excessive oocyte ageing and finally egg over-ripening. During ova ageing, major changes occur inside the eggs. These changes deteriorate the quality of ovulated eggs and lead to a limited fertilization rate, increased larval malformation and increased ploidy anomalies. The time period during which unfertilized eggs retain their fertilizing ability after stripping varies from a few minutes to a few days and highly depend on the fish species and the storage temperature. The successful *in vitro* egg storage in percid hatcheries may permit synchronous artificial fertilization for different females and thereby facilitating the hatchery management. This approach can also be helpful when completely mature male brood fish are unavailable or shipment of the eggs is needed. This study was conducted to identify the successful *in vitro* egg storage duration in pikeperch *Sander lucioperca* and Eurasian perch *Perca fluviatilis* as well as the effect of ova ageing on the occurrence of malformed larvae.

Stripped ova of 4 female pikeperch and 4 female Eurasian perch were collected separately and stored in sterile cell culture plates inside the laboratory incubator. All plates were individually covered by their own lids and kept in the dark at 4 and 15°C in case of pikeperch and at 4, 8 and 12°C in case of Eurasian perch. The batches of eggs from a given female were fertilized with constant time intervals until the occurrence of the over-ripening, at different hours post stripping (HPS): 0, 12, 24 and 48 HPS in pikeperch and 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66 and 72 HPS in Eurasian perch.

The results indicated that in pikeperch the highest fertilization rates could be obtained when the eggs are fertilized immediately after stripping. Thereafter, the fertilization rates decreased significantly and dropped to 5% at 48 HPS at the storage temperature of 4°C and to 10% at 24 HPS at 15°C. Eurasian perch eggs however, could be longer successfully stored *in vitro*: 36, 18 and 12 hours at 4, 8 and 12°C respectively. The eyed-egg mortality and larval malformation rates were not significantly affected by post-stripping ova ageing for at least up to 36 h. Thereafter, both values increased significantly and were measured as the highest in the most aged ova. The present study demonstrated that stripped Eurasian perch eggs can be stored for at least 12 h at 4°C to 12°C without a significant reduction in their quality.

Acknowledgements
This study is supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects "CENAKVA" (No. CZ.1.05/2.1.00/01.0024), "CENAKVA II" (No. LO1205 under the NPU I programme), project n. CZ.02.1.01./0.0/0.0/16-025/0007370 and NAZV projects (No. QK1710310 and QK1820354).

DISTAL INTESTINE RESPONSES TO *Aeromonas salmonicida* PATHOGEN CHALLENGE IN ATLANTIC SALMON (*Salmo salar*)

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Development of new diets is important for the continued expansion of the salmon aquaculture industry, some of these diets have been shown to impact genes and morphology of the distal intestine, especially in relation to intestinal cell wall integrity resulting in changes in gut permeability, thus exposing the local immune system to antigens. To extend this line of research we have challenged salmon with different plant rich diets with a bacterial pathogen to examine intestinal responses. Fish were fed an eight-week feeding trial, with fish fed either 10% or 20% bean protein concentrate (BPC) replacing soy protein concentrate. At the end of the trial, the salmon were challenged by intra peritoneal injection with the bacterial pathogen *Aeromonas salmonicida* or PBS as control. Total RNA was collected from distal intestines (n=6) for gene expression analysis following 4 days post challenge to examine the early immune response. We confirmed antibacterial transcriptional response in the liver with significant upregulation in immune genes expression in fish that were injected with *A. salmonicida* compared to control (PBS). In the distal intestine immune genes were also examined indicating transcriptional changes in the intestine. Additionally, genes involved with barrier integrity and mucus production were examined. The mucous producing gene (MUC2) and desmocollins 2 (DCS2) which is important for the cell-cell adhesion showed significant downregulation in fish challenged with *A. salmonicida* compared to PBS. Results demonstrate that the intestinal wall integrity might be impacted by the antibacterial inflammatory bacterial response. There were few differences between the two levels of BPC in the diet. This study was financially supported by Innovate UK (Beans for Feeds) and also Ministry of Higher Education Malaysia for the PhD studentship.

THE POSSIBILITY OF RAISING *Rutilus rutilus caspicus* AND *Rutilus frisii kutum* TOGETHER

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Rutilus rutilus caspicus and *Rutilus frisii kutum* in the Caspian Sea are valuable bony fishes. Due to the decline in fishing and *Rutilus rutilus caspicus* and *Rutilus frisii kutum* stocks, fisheries Organization of Iran each year to reproduce and release the fish fry into the Caspian Sea. Due to count the better the problems of separation of fry from each other that the two species are very similar in terms of appearance at an early age and also obtain accurate statistics released annually, Iran Fisheries Organization take action to monoculture in the pond to since its release in the sea. But what is important for better productivity growth under culture conditions that can be considered apart from the tasks Fisheries Organization. The objective of this study was to evaluate the feasibility necessary in order to foster growth and juveniles together to achieve better growth took place.

This study was operated as long as 6 weeks and in 9 tanks with 20 numbers fish in each tank, with 3 groups and 3 replicate as: Group A: *Rutilus rutilus caspicus*, Group B: *Rutilus frisii kutum* and Group C: *Rutilus rutilus caspicus* and *Rutilus frisii kutum* .Initial body weight and length average were 1.07± 0.007 gr and 3.26± 0.23 cm. At the end of the rearing period (six weeks), the length, weight and growth indices of fish in each group were evaluated.

According to the results of study showed the largest increase in the weight of fish in Group C ($p < 0.05$) (Table 1). The results indicate that breeding *Rutilus rutilus caspicus* and *Rutilus frisii kutum* combined increased weight and length compared to any alone (monoculture) is culturing. The results showed that there isn't any meaningful difference in SGR, % BWI and CF in different groups ($p > 0.05$). But, there is meaningful difference in FCR, GR and Survival ($p < 0.05$). Also, the minimum amount of FCR in combined method (*Rutilus rutilus caspicus* and *Rutilus frisii kutum)*, and the highest survival rate is in this group. The results of this study show that a combination of *Rutilus rutilus caspicus* and *Rutilus frisii kutum* farming improves weight and growth indices in both the fish. Since the two fish canibalism or not carnivorous diet and despite similar diet (omnivorous) and based on the results of this study may be the combination of these two species breeding programs carried out in the future. Since the two fish breeding these fish in brackish water and the environment in the Caspian Sea now the commercial breeding of this species does not occur in Iran. But with the advancement of culture and further studies the adaptation of these fish can be reared in fresh water to mix the two species in the pond did.

Table 1: The weight and length of *Rutilus rutilus caspicus* and *Rutilus frisii kutum*

	Group	Initial weight (gr)	Initial length (cm)	Final weight (gr)	Final length (cm)
A	(*Rutilus rutilus caspicus*)	0.007 ± 1.07^{a}	0.23 ± 3.26^{a}	2.14 ± 0.03^{b}	6.02 ± 0.39^{b}
B	*(Rutilus frisii kutum)*	0.007 ± 1.07^{a}	0.23 ± 3.26^{a}	1.84 ± 0.14^{a}	5.68 ± 0.3^{a}
C	**(***Rutilus rutilus caspicus* and *Rutilus frisii kutum)*	0.007 ± 1.07^{a}	0.23 ± 3.26^{a}	2.4 ± 0.48^{c}	6.3 ± 0.42^{c}

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IMPLEMENTATION OF AN INTEGRATED ZEBRAFISH HEALTH MANAGEMENT PROGRAM AT THE UCL FISH FACILITY

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The importance of sharing health monitoring programme information, currently used by different zebrafish research facilities can contribute to reducing the outbreak of diseases between facilities and lead to greater understanding of the potential impact of pathogens on zebrafish welfare and research studies. Here, we present the UCL integrated health management programme, as a detailed description and the results of the implemented health surveillance measures built over the last 6 years. This includes a body condition scoring program (BCS), database analysis of recurrent phenotypes, biosecurity measures, sentinel program, and health screening tests. The programme includes the use of DNA-based diagnostic tests represented by the use of polymerase chain reaction (PCR), which due to its specificity and sensitivity, result in a good technique for identifying specific pathogens. Database searches represent a good way of identifying patterns of disease across strains of zebrafish. The advantages of this program are to help build more standardized, robust husbandry techniques within the facility. This would also include identifying problematic strains, which may require alternative husbandry. It also helps to secure better health information exchange of zebrafish strains between facilities throughout the world.

HOW TO TRAIN YOUR OYSTER? A STORY OF IMMUNE SHAPING AND PRIMING IN THE PACIFIC OYSTER, *Crassostrea gigas*

Caroline Montagnani[1*], Maxime Lafont[1], Bruno Petton[2], Julien deLorgeril[1], Manon Fallet[1], Agnes Vergnes[1], Jeremie Vidal-Dupiol[1], Yannick Gueguen[1], Cristian Chaparro[1], Eve Toulza[1], Christoph Grunau[1], Benjamin Gourbal[1], Guillaume Mitta[1] and Celine Cosseau[1]

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Over the past decade, a paradigm shift has emerged in the field of immunology bringing new concepts beyond the commonly accepted cleavage between innate and acquired immunity. Numerous findings have shown that a wide range of invertebrates can develop innate immune memory (also called immune priming) leading to improved survival during a second encounter with a pathogen. Moreover, the notion of the organism as a holobiont, characterized by multiple species living together as a metaorganism, has further deconstructed older versions of immune relationships and challenged our vision of immune interactions between hosts and microorganisms. Accumulating studies has brought new highlights on how the host immune system has been co-opted to establish and shape beneficial host-microbiota relationships contributing to the host health status and fitness notably through early interactions with the immune system.

In that context, the present study has undertaken to investigate how we can educate the innate immune system to induce enhanced survival capacities through immune shaping and priming in the Pacific oyster *Crassostrea gigas*. This species is currently the victim of massive recurring mortalities, called the Pacific oyster mortality syndrome (POMS) without existing therapeutic treatment. This syndrome, of complex aetiology, involves different types of pathogen agents including bacteria and a virus, the herpesvirus OsHV-1 µVar. Our results show evidence for within generation innate immune memory leading to enhanced survival capacities following OsHV-1 infection or during a mortality episode in the field. Analyses of the molecular bases by dual RNA sequencing revealed that this priming was based on the triggering of a strong sustained antiviral response limiting replication of the virus, thus allowing the protection of oysters. Moreover, we demonstrated that an early exposure to a non-infectious environmental microbiota could also lead to enhanced survival capacities towards the POMS. Originally, we could show that the impact of early microbial exposure on survival capacities extends to the next generation suggesting a multi-generational effect on offspring performances. Microbiota influence as well as molecular and epigenetic determinants orchestrating these phenomena are currently investigated using integrative, high throughput approaches (16S barcoding, RNAseq, BSseq). Altogether these studies will bring new insights into the oyster capacities to build an innate immune memory, its adaptive capacities and provide a platform to further explore novel strategies to help mitigate disease threats upon marine bivalves.

ISOLATION OF A NEW MICROALGAE STRAINS TO AQUACULTURE AND BIOTECHNOLOGY - IMP3 (*Tetraselmis sp.)* CASE STUDY

Ivo Monteiro[1*], Carlos Cardoso[1], Florbela Soares[1], Maria E. Cunha[1], João Varela[2]; Narcisa Bandarra[1], Pedro Pousão-Ferreira[1]

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Microalgae biomass are considered a potential source of wide spectrum of products to feed, food, pharmacy and services like residual water treatment. Presently, it is estimated that there are more than 1 million of microalgae species, of which only about 30,000 are described and a very small number are used in aquaculture, biotechnology and other uses. Bioprospecting for novel microalgal strains has gained considerable importance in recent years to improving the feasibility of microalgae applications. For this propose, IPMA and CCMAR has developed a protocol for high throughout screening of microalgae, through which we isolated some species, such as *Tetraselmis* sp. IMP3. IMP3 (Chlorophyta, Chlorodendrophyceae) was isolated from EPPO earth pounds, using fluorescence activated cell sorting. After culture, samples were taken for genetic identification. IMP3 demonstrated capacity to grow in seawater solid growth medium and was stored in this way. Liquid culture was growth with a photoperiod of 16h light – 8 h dark (150 photons. s^{-1}. m^{-2}) (Fig. 1). Samples were collected at different stages of growth for biochemical analysis, with special attention to fatty acids (Table 1), concerning the use on live feeds for marine fish larvae. This strain is able to produce biofilm, maybe due to its large size (15 to 20μm), which may be useful for larvae production that attach to the substrate, such as the sea urchin (*Pacentrotus lividus*). Having already been introduced to feed fish larvae (*Sardina pilchardus*), and other marine organisms (*P. lividus*, Artemia, and rotifers).

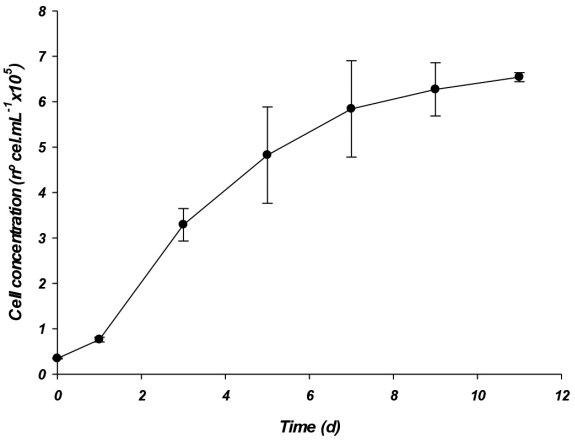

Figure 1. *Tetraselmis sp.* IMP3 growth rate at Aquaculture Research Station (EPPO) culture room.

Table 1 - Fatty acid profile (in % of total fatty acids and in mg/100 g dry weight) of the studied microalgae *Tetraselmis rubens.* Values are presented as average±standard deviation. Different lowercase letters within a row correspond to statistical differences (p<0.05) between the relative (%) FA profiles of microalgae. Different uppercase letters within a row correspond to statistical differences (p<0.05) between the absolute (mg/100 g dw) FA profiles of microalgae.

Fatty acid		Σ SFA	Σ MUFA	Σ PUFA	Σ ω3	Σ ω6	Σ ω3/Σ ω6
***Tetraselmis sp.* IMP3**	**(% total fatty acids)**	29.8 ± 0.1^{a}	25.9 ± 0.1^{a}	38.9 ± 0.1^{a}	31.4 ± 0.1^{b}	6.5 ± 0.0^{a}	4.8 ± 0.1^{a}
	(mg/100 g dw)	3310 ± 52^{A}	2880 ± 36^{A}	4310 ± 71^{A}	3490 ± 41^{A}	730 ± 10^{A}	4.8 ± 0.1^{A}

ASSESSING THE POTENCIAL OF A MEDICINAL HALOPHYTE SPECIES TO BE PRODUCED IN INTEGRATED MULTITROPHIC AQUACULTURE (IMTA) SYSTEMS: GROWTH PERFORMANCE AND FUNCTIONAL PROPERTIES

Ivo Monteiro[1*], Maria João Rodrigues[2], Chloé Placines[2], Pedro Pousão[1] and Luísa Custódio[2]

[1]IPMA – Portuguese Institute for the Ocean and Atmosphere, EPPO - Aquaculture Research Station, Av. 5 de Outubro, 8700-305 Olhão, Portugal
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Aquaculture is the fastest-growing food-producing sector in the world, both in terms of new areas and species produced. However, aquaculture can have alleged negative environmental impacts such as pollution of ground and surface waters by discharges of waste effluents. The improvement in aquaculture waste management is thus a priority, aiming to reduce potential environmental and economic impacts. The purposes of the national fisheries policy regarding aquaculture are to increase production and product diversity, but also to increase product quality to improve the competitive position of the sector and promote environmental, economic and social sustainability. This can be attained through the implementation of integrated multi-trophic aquaculture (IMTA) systems combining the production of fish and marine halophyte plants for commercial purposes. In this work one selected halophytes species, *Polygonum maritimum* L., was produced in greenhouse conditions, and irrigated with water with different salinities (Fig. 1), including that from an outdoor dish tank producing sea bream, sea bass and meagre. Growth performance was evaluated in terms of plant survival, biomass production and yields (kg/m^2 of wet and dry biomass). Plants were cut approximately 7 cm above ground level, for the first time when their shoot sizes were at least 20 cm long. After shoot re-growth, plants were submitted to 2 repeated harvests. Samples from identical conditions were pooled in a single sample, dried and evaluated for biochemical properties. Organic extracts were also prepared and evaluated for functional properties, including *in vitro* antioxidant, anti-inflammatory, anti-melanogenic, and for chemical composition. Biochemical and functional properties were compared with those of plants collected from wild populations. Preliminary results indicate that increased salinity in the irrigation water significantly reduces plant growth and yields (Fig. 1). However, extracts obtained from the produced biomass displayed relevant *in vitro* functional properties, suggesting that this species maintain their functional properties under irrigation with saline water, thus being candidates for production via IMTA systems.

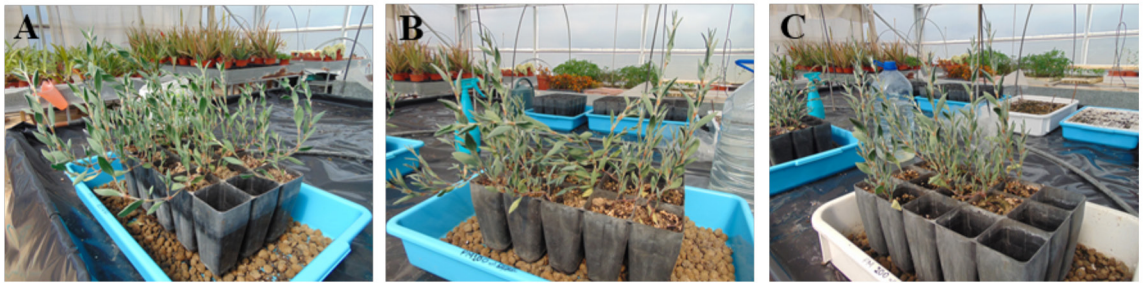

Figure 1. General aspects of *P. maritimum* irrigated with water with 0 (A), 100 (B) and 200 (C) mM of sodium chloride (NaCl).

PARASITE PATHOGENS OF THE ATLANTIC BLUEFIN TUNA *Thunnus thynnus* IN THE MEDITERRANEAN SEA

Francisco E. Montero*, Javier Rodríguez-Llanos, José Francisco Palacios-Abella

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Many parasite species have been recorded in the Atlantic bluefin tuna (*Thunnus thynnus*) (see Munday et al., 2003. *J. Fish Dis*. 26(4):187-206; Culurgioni et al., 2014. *Folia Parasitol*. 61(2):148-156). Parasite records are generally from wild fish, but several species survive in culture conditions, often becoming pathological issues which can affect the production of this highly appreciated fish. Direct life cycle parasites (monoxenous) have usually higher pathogenic potential, as they are dispersed fish to fish; e.g. monogeneans (*Hexostoma thynni* or *Capsala* spp.) or copepods (*Caligus* spp. or *Euryphorus brachypterus*). However, those parasites reported as more pathogenic in cultures of bluefin tunas (*Thunnus* spp.) are aporocotylid trematodes (*Cardicola* spp.); blood flukes whose infectious stages emerge from invertebrate intermediate hosts (i.e. heteroxenous parasites).

In Pacific cultures of bluefin tuna species (*T. maccoyii* and *T. orientalis*), some mortalities and production decrease have been related to parasitoses involving blood flukes and, to a lesser extent, to monogeneans and copepods, often related to secondary bacterial infections. In the case of the fattening industry of the Atlantic bluefin tuna in the Mediterranean, reports of pathological episodes have been scarce and main mortalities have been traditionally related to environmental conditions or bad practices. However, more than fifteen parasite species are often found in cultured tunas, including pathogenic species as *H. thynni* or *E. brachypterus*, and four species of *Cardicola* (the highest diversity of *Cardicola* spp. worldwide). The real impact of these parasites is unknown, despite they are often related to severe local damages and occasionally numerous, and they can seriously compromise tuna health.

Monoxenous parasites mostly reach the cages from the wild, in recently captured tunas. Therefore, preventive monitoring and isolation of just-reared batches and prophylactic treatments are highly recommendable. Heteroxoneus parasites are uncommon, as intermediate hosts are not normally close to the farming facilities. However, remarkable exceptions exist; e.g. blood flukes, transmitted from invertebrates of the fouling. Fouling treatment can be helpful to control these parasites, once the intermediate host is identifies and located.

Finally, parasites infecting trophically could be controlled supplying uninfected food to farmed tunas. In this way, parasites present in feed fish must be inactivated; species harmfully pathogenic for fish, as microsporidians, and more importantly, zoonotic/allergenic species as anisakid and raphidascarid nematodes, can hamper the production of bluefin tunas.

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SUSTAINABILITY INDICATORS APPLIED IN TILAPIA'S (*Oreochromis niloticus*) CAGE FISHFARM IN A BRAZILIAN TROPICAL RESERVOIR

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The culture of tilapia in net-cages set up in reservoirs has been increased in many tropical regions. However, the activity still needs to demonstrate to be sustainable in many countries. Thus, the aim of this study was assess the environmental and economic sustainability of a Nile tilapia (*Oreochromis niloticus*), located in a tropical reservoir in Santa Fe do Sul São Paulo, Brazil (20°15'S and 50°57´W).

The farm had 120 net-cages with 6 m^3, stocked with 1 g juveniles at 660/m^3. Density was reduced to 440/m^3, when the fish attained 50 kg and to 65/m^3 when they attained 100 g. Harvest was performed 24 weeks after first stocking when fish attained 800 g. Environmental variables, samples of diet and fish were obtained in March, May and August 2012. Production costs, harvested biomass and selling price were obtained. The economic indicators suggested that the farm was not economically feasible (Table 1). Farmers sold all fish to a processing plant in the region, which pay low values for small producers. The work showed that the lack of diversified markets that can compensate more the producer and the need of purchase of industrialized diets made the studied model unfeasible. Environmental indicators showed that the culture caused small changes in the water quality surrounding net-cages and produced a large amount of organic sediment. The recovery of nutrients was low (Table 2), suggesting that the system showed low efficiency. Therefore, changes in the system should be introduced to improve economic and environmental sustainability.

Table 1. Indicators of economic sustainability

Internal rate of return	1.7 %
Net present value	$ - 132,505.00
Cost/benefit ratio	$ 1.49 every $ 1 invested
Net income / inicial investment	0.06 %
Product diversity	1
Market diversity	1
Profit	$ - 5,950,00
Annual Income	$ 6,029.00

Table 2. Phosphorus and nitrogen supplied to produce 1 t of biomass percent recovered in the fish body.

Phosphorus applied (kg)	2.03
Phosphorus recovered (%)	0.55
Nitrogen applied (kg)	7.91
Nitrogen recovered (%)	2.11

(CNPq/CAPES/FAPESP)

MUTATIONS INVOLVED IN THE EMERGENCE OF *Yersinia ruckeri* BIOTYPE 2 IN FRANCE

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Yersinia ruckeri is a fish pathogen causing Enteric RedMouth Disease (ERM or yersiniosis). *Y. ruckeri* belongs to the Enterobacteriacea family and is divided into two biotypes: biotype 1 (BT1) which is motile and has a phospholipase activity and biotype 2 (BT2) which is negative for these 2 characteristics. The aim of this study was to identify mutation in BT2 strains responsible for *Y. ruckeri* BT2 emergence in France.

Fourteen BT2 strains isolated from rainbow trout mainly in Brittany and Adour Garonne regions in France between 2005 and 2009 have been selected based on their origins, their API20E profiles, their serotypes, their pulsotypes and their sequence types, (Calvez et al, 2014, 2015). Two reference strains were also included: ATCC 29473 and EX 5, respectively belonging to BT1 and BT2.

Microscopic studies were conducted in order to observe flagellar structures using the Flagella Stain kit (K-13, Presque Ile Culture). The tests revealed presence of flagella in BT1 strains but not in BT2 strains (figure 1).

Genes involved in the motility and/or the phospholipase activity have been selected based on the published genome of *Y. ruckeri* strain ATCC 29473 (Daligault et al. 2014) and on the Evenhuis et al. (2009) study. Genes have been sequenced using the Sanger technology by GATC Biotech (France) and gene sequences have been compared using BioEdit editor. Among French BT2 strains, mutations previously described within *fliR*, *flhA* and *flhB* genes by Welch et al. (2011) have not been found. However, at least 2 new mutations have been revealed within *fli*G and *flh*C genes (figure 2). A 4-bp insertion at nucleotide 853 within *fli*G gene results in a predicted frameshift. Sequencing analysis of *flh*C reveals a single-residue deletion at nucleotide 474 within *flh*C sequence. These mutations concern genes which code for protein involved in flagellar biosynthesis.

Discovery of two new mutations within these genes could explain, in part, the loss of flagella and motility in BT2 strains and *Y. ruckeri* BT2 emergence in France. Several independant mutations seem to lead to. *ruckeri* BT2 phenotype in the world.

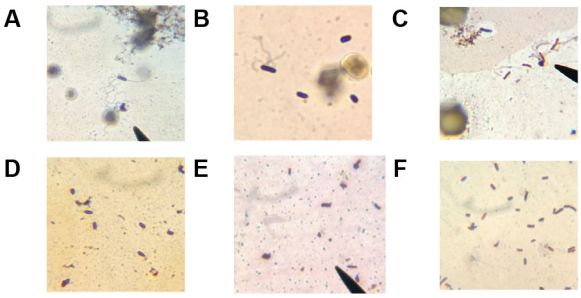

Figure 1 : Flagella Stain of *Y. ruckeri* BT1 ATCC 29473 (A) and CAE 1266 (B), *B. subtilis* (C) and *Y. ruckeri* BT2 : EX 5 (D), CAE 700 (E) and CAE 642 (F). *Bacillus subtilis* is a motile bacteria used as positive control. Light microscopy, oel objective x 100.

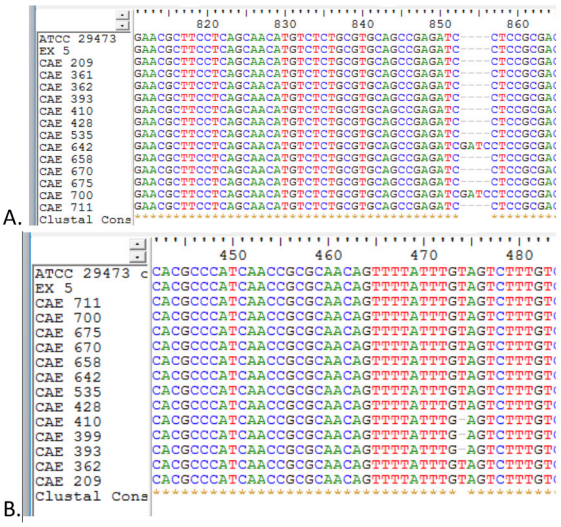

Figure 2 : Mutations revealed within the *fliG* (A) and the *flhC* genes.

REVEALING *Mytilus galloprovincialis* TRANSCRIPTOMIC PROFILES DURING ONTOGENY

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Mediterranean mussels are a worldwide spread bivalve species with extraordinary biological success. One of the reasons of this success could be the reproduction strategy of bivalves, characterized by the presence of trochophore larvae. The principal objective of this work was to study the transcriptomic profile of the ontogeny of *Mytilus galloprovincialis*. For this purpose, a new DNA microarray was designed and developed.

The studied developmental stages: unfertilized oocytes, veliger, pediveliger, settled larvae and juveniles, showed very different transcriptomic profiles and clustered in groups according to their characteristic gene expression along ontogeny (Fig. 1). Our results show that oocytes present a distinct and characteristic transcriptome. After metamorphosis, both settled larvae and juveniles showed a very similar transcriptome. This suggests: 1.- the progressive loss of RNA of maternal origin through larval development and 2.- the stabilization of the gene expression after settlement. On the other hand, during metamorphosis a specific profile of differentially expressed genes was found. These genes were related to differentiation and biosynthesis, and the processes related to the immune response were strongly down regulated. These suggest a development commitment at the expense of other non-essential functions, which are temporary set aside. Immune genes such as antimicrobial peptides suffer a decreased expression during metamorphosis. In fact, we found that the oocytes which express a higher quantity of genes such as myticins are more likely to reach success of the offspring, compared to oocytes poor in such mRNAs, whose progeny died before reaching metamorphosis (Fig. 2).

Fig. 1: Clustering of the analyzed samples.

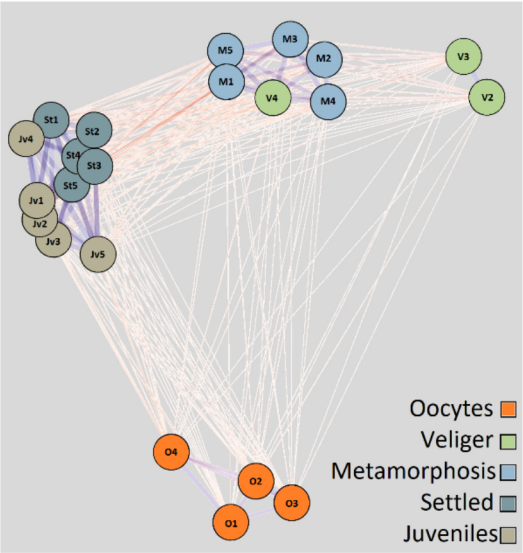
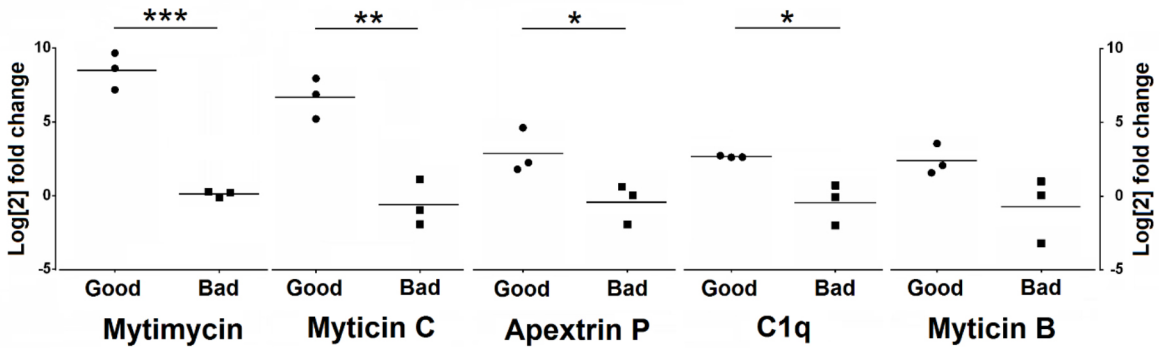


Fig. 2: qPCR analysis of immune-related genes of oocytes which derived in good or bad families.



IS THE INVASSIVE MUSSEL *L. securis* A RISK FOR THE PRODUCTION OF *M. galloprovincialis*?

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Biological invasions started when humans moved species beyond their normal geographic limits, facilitating the spread of alien species over the world. In marine environments bivalves are the most notorious invasive species in intertidal rocky habitats. NGSs are useful to understand the molecular mechanisms involved in the invasion process and to manage it. Eco-immunology studies the magnitude and strength of the immune response of alien species and how it could help to predict the invasiveness.

A remarkable case of invasion has been reported in the ecosystem of the Ria of Vigo (Galicia, NW Spain) by the black pygmy mussel *Limnoperna securis*. This species, endemic of Australia, has spread throughout Asia and the Mediterranean Sea, occupying most of the estuarine ecosystems. The Mediterranean mussel *M. galloprovincialis* is the autochthonous mussel in Galicia, the second worldwide producer of this species. *M. galloprovincialis* is also a successful invader that colonized many areas in Africa, America, Australia and Japan. The true potential of the alien *L. securis* to colonize areas where *M. galloprovincialis* is established and highly produced is an important issue that must be analyzed in detail.

Can we predict the invasiveness of *L. securis* by the magnitude of its immune response? Can the invader *L. securis* represent a serious risk for *M. galloprovincialis*? To evaluate the suitability of several immune-related hypotheses in our invasion model transcriptomic and functional approaches were used.

Our analyses suggest that the the new environment could impose high energetic costs to *L. securis*: 1.- mobilization of energy reserves for the activation of osmorregulatory processes and 2.- regulation of the cell cycle to cope with the salinity stress. The relocation of energetic resources to vital physiological processes could be a reasonable explanation for the low immune capabilities observed in *L. securis*. Although it is difficult to predict the potential replacement of *M. galloprovincialis* by *L. securis*, we could suggest that *M. galloprovincialis* will continue to be the dominant mussel species in the outer part of the Ria of Vigo. The application of this integrated transcriptomic and functional immunological approach to understanding the biology of invasive species is an extremely promising area of research whose knowledge could be used to guide and inform management decisions.

A STUDY OF ANTAGONISTIC ACTIVITY OF PROBIOTIC BACTERIA OF THE GENUS BACILLUS

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To date, in Russia, about 25 types of drugs are produced on the basis of spore-forming bacteria of Bacillus genus, these preparations include biologically active additives and ointments. Of these, we have counted five drugs that are used in aquaculture. In the practice of fish farming, the use of monocomponent probiotics is represented based on *Bacillus subtilis*. Polycomponent probiotics are comprised of the association of two species *B. subtilis* and *B. licheniformis*. There is information about the effectiveness of feed additive that include *Bacillus subtilis, Lactobacillus acidophilus, Ruminococcus albus* to has increase the weight and survival of fish. It is known that probiotic bacteria strains can have a pronounced antagonistic activity to a wide range of pathogenic and conditionally pathogenic microorganisms.

The aim of our research was to study the antagonistic activity of the probiotic bacteria *Bacillus* against the conditionally pathogenic bacteria isolated from carp fishes cultured at farms of the South of Russia. Material was selected from the parenchymal organs (kidneys) of silver carp, carp and golden carp. The standard microbiological methods and the method using the MALDI-TOF mass spectrometer were used. The study covered 37 test cultures: *Acinetobacter lwoffii, Acinetobacter radioresistens, Acinetobacter johnsonii*, *Aeromonas veronii* (10 strains), *Aeromonas hydrophila* (7), *Aeromonas jandaei* (3), *Aeromonas caviae* (2), *Aeromonas ichthiosmia* (2), *Aeromonas media* (2), *Aeromonas salmonicida*, *Aeromonas sobria, Corynebacterium pilosum, Morganella morganii, Pseudomonas putida, Pseudomonas pseudoalcaligenes, Staphylococcus carnosus, Streptococcus uberis*. The antagonistic activity was evaluated by two in vitro methods, namely, by the diffusion method (block modification) and the deferred antagonism method. We tested such strains as *Bacillus subtilis* VKPM-7092 (Vetom 1.1 preparation), *Bacillus subtilis* 945 B-5225 (Bacell-M feed additive), association of *Bacillus subtilis* strains VKM B-2250, *Bacillus licheniformis* VKM B-2252 (Subtilis preparation), *Bacillus amyloliquefaciens* B-1895 (a probiotic additive developed at Southern Federal University, Rostov-on-Don).

According to the level of decrease of their antagonistic activity, the probiotic bacteria were arranged in the following order: *Bacillus amyloliquefaciens* B-1895, association of *B. subtilis* and *B. licheniformis* (Subtilis), *B. subtilis* (Vetom 1.1), *B. subtilis* (Bacel-M). Of the probiotic strains tested, only *Bacillus amyloliquefaciens* B-1895 suppressed the growth of *Pseudomonas putida*. The *Bacillus amyloliquefaciens* B-1895 and the association of *B. subtilis* and *B. licheniformis* also inhibited the growth of all tested cultures of Aeromonas. Only in 20% of cases the strains of *Aeromonas veronii* were insensitive. The strains of *Bacillus subtilis* had a weak antagonistic effect on cultures of Aeromonas, but they inhibited the growth of *Acinetobacter radioresistens* and *A. lwoffii*. The *Bacillus subtilis* strain (Vetom 1.1) revealed antagonistic activity against *P. pseudoalcaligenes, St. uberis*, while *B. subtilis* (Bacel-M) had antagonistic ability against *S. carnosus* and *C. pilosum*.

The obtained data are of interest in connection with the fact that in the South of Russia there is a problem with bacterial diseases of fish. The antagonistic activity of probiotic bacteria is an important aspect in the effectiveness of probiotics.

RESULTS FROM THE NATIONAL COD BREEDING PROGRAM BRINGS NEW HOPE FOR COMMERCIAL FARMING OF ATLANTIC COD *Gadus morhua* IN NORWAY

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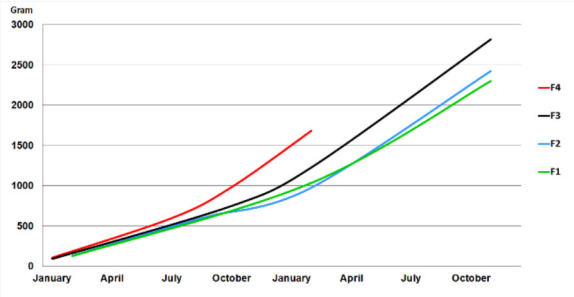
Low quotas for cod and correspondingly high market price, together with a general optimism for marine aquaculture, released a boom in cod farming in Norway from year 2000 onwards. However, the cod farming soon met challenges due to poor quality of intensively produced fish with high mortality at larval stage, high prevalence of skeletal deformities, low and variable growth in sea cages and frequent outbreaks of vibriosis, atypical furunculosis and francisellosis. Coinciding with the economic crises of 2008, it was more than the emerging industry could take. The production of juveniles peaked in 2008 at 22 million, and the slaughter volume in 2010 at 20000 tons. In 2014 there were no commercial cod farms left.

In 2002 the Ministry of Trade, Industry and Fisheries established The National Cod Breeding Program, with the intent to improve important traits of farmed cod through selective breeding and to remove production bottlenecks through targeted research. The activity in the breeding program has continued unaffected by the turmoil in the commercial cod farming industry, and the fourth generation of selected cod (F4) is now under production.

The first priority for the selective breeding has been to increase the slaughter weight. The figure shows that there has been a substantial increase in growth rates the last few years, especially in the F3 and F4 generations. Fast growth gives the opportunity to sell at low availability and high price during autumn. Additionally, faster growth is normally associated with lower FCR, which is attributed to selection and improved production methods. Selective breeding has also increased survival during start feeding, reduced losses in sea cages a reduced prevalence of severe skeletal deformities.

The improvements achieved by the cod breeding program have encouraged a small number of companies to try cod farming again. The results from their tests are quite positive when it comes to growth, survival and market prices, and indicate that the conditions for cod farming is much better now than it was 10 years ago. It brings hope that commercial cod farming will come back as a valuable supplement to the aquaculture industry in Norway.



IMPROVEMENT OF RICE-FISH CULTURE FROM TROPICAL AGROECOSYSTEMS FERTILIZATION, ANTANANARIVO, MADAGASCAR

Jean-Michel Mortillaro*, Ezra A. Raminoharisoa, Diana E. AndriaMananjara, Tiana H. Randriamihanta, Harilala Andriamaniraka, Rija Andriamarolaza, Modestine Raliniaina, Olivier Mikolasek, Lionel Dabbadie, Patrick Andrianetsy and Philippe Martel

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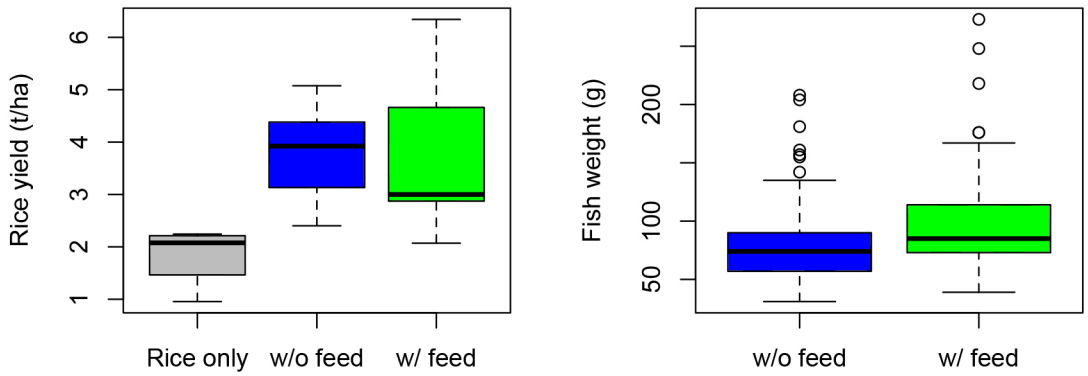
Flooded rice fields are ecosystems favorable to the growth and production of many aquatic organisms. They can even play a major role in the feeding and nutrition of local communities, as a source of self-recruiting species or by supporting the production of farmed fish in association with rice. Integrated rice-fish culture, implies the use of larger and higher side dykes and digging a channel within the field, which serves as a refuge area for fish at all stage of rice culture and when the field is drained. In Madagascar, integrated rice-fish culture has proven a yield increase, without fertilizers or feed inputs, of 19% compared to traditional rice culture. Therefore, after deducing 8.6% loss space for refuge channel, a sustainable net increase of 10.4% in rice production may permit to secure such a basic food commodity for local populations. However, in order to increase productivity and reach food self-sufficiency, fish need to be fed and rice to be fertilized. While agriculture and livestock systems in Madagascar are rather extensive with low if any inputs, agroecosystems are diversified and produce several crop and livestock residues that can be valued as alternatives to mineral fertilizers.

Aside from confirming the yield increase in rice production from integrated rice-fish culture systems, the aim of this farm experiment was therefore to characterize the effects of the qualitative and quantitative use of agroecosystem crop residues on rice and fish yield. Thirty farmers were therefore included in the study and practices investigated, as amount and quality of foods or organic fertilizers provided to integrated rice-fish systems. For instance, qualitative use of termitary had only a significant impact on fish weight (Mann-Whitney-Wilcoxon, $p = 2.7\ 10^{9}$). Further use of fertilizers will be evaluated with an expected effect on fish and/or rice.



Experimental results from rice (left) and fish (right) production with qualitative use of termitary

COMPARISON OF PRE-GROWTH PERFORMANCES OF TWO TILAPIA STRAINS (*Oreochromis niloticus*; GIFT AND HIGHLANDS) IN MADAGASCAR, UNDER FEED ONLY OR GREEN WATER CONDITIONS

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Previous experiments on various tilapia strains, in order to compare their pre-growth performances under low temperature in the highlands of Madagascar, highlighted the potential of the GIFT strain. However, even if growth of the latest can surpasses other strains in intensive aquaculture, no information is available under semi-intensive systems. Also, GIFT displayed high early stages mortality, supposedly due to a bowel obstruction when digestion was weakened by temperature, while the so called "Highland" strain is apparently more adapted and resistant to local environment.

The GIFT strain is up to now not well spread in the highlands of Madagascar where the corresponding strain is widespread. Also, intensive test in hapas are not well representative of aquaculture production systems in Madagascar, which are mainly based on a low input basis. Inputs are indeed limited by low availability of good feeds or fertilization as well as low farmers income for investment. Fertilizers are either often neglected due to leaky earthen ponds or feed not justified as the increasing proteins content of formulated feeds for tilapia pre-growth (El-Sayed 2018).

Given these findings, the aim of this study was to compare the pre-growth performances (from 3 to 100 g) of these two tilapia strains under feed only or green water conditions. To reach this aim, 6 ponds (100 m^2 each) were stocked with either GIFT (n = 3) or Highland (n = 3) strains (2 ind./m^2) under daily extruded feeding, while the same number of ponds and strains were stocked under green water condition in the research experimental station of Andasibe, Périnet, Madagascar. One supplementary pond replicate of 500 m^2 for each of the four treatments was realized in a rural exploitation from the Ankazobe district. Green water was obtained from a weekly distribution basis of 20 kg of Nitrogen and 5 kg of Phosphorus from DAP and Urea per 10 000 m^2. Proteins provided through plankton from the fertilized green water were supplemented by daily inputs of rice bran flour for carbohydrates. Results on Feed – Fertilization comparison is expected to ease aquaculture systems design to local environment and constrains.

Strain	Treatment	Ponds (Station + Farm)	Density
GIFT	Feed	3 x 100 m^2 + 1 x 500 m^2	2 Ind./m^2
	Fertilization	3 x 100 m^2 + 1 x 500 m^2	2 Ind./m^2
Highlands	Feed	3 x 100 m^2 + 1 x 500 m^2	2 Ind./m^2
	Fertilization	3 x 100 m^2 + 1 x 500 m^2	2 Ind./m^2

Table 1: Experimental design for the four treatments

COULD CRYOPRESERVED SPERM BE USED FOR WILD/TANK SPAWNING? ARTIFICIAL INSEMINATION OF AN OVIPAROUS MODEL FISH, THE AFRICAN CATFISH *Clarias gariepinus*, USING THAWED, CRYOPRESERVED SPERM

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In this study, the aim was to develop a practical protocol for using cryopreserved sperm for induced/wild/tank spawning of oviparous fish species. Experiments were carried out on African catfish (*Clarias gariepinus*) as a model fish. Sperm cryopreservation: collected sperm was diluted at a ratio of 1:1 with an extender composed of 266 mM fructose, 4.94 M methanol (20%) and pH was adjusted to 7.73 by the addition of $NaHCO_3$ from a stock solution of 1 M. The final concentration of methanol following dilution was 2.47 M (10%). Sperm was loaded into 0.5-ml straws and cryopreserved. 24 hours later, in preparation for artificial insemination, samples were thawed at 40 °C for 13 s in a water bath, and centrifuged at 500 g for 10 min at 20 °C. The seminal plasma, extender and external cryoprotectant were removed from the concentrated spermatozoa. Centrifuged carp (*Cyprinus carpio*) seminal plasma (10 000 g for 10 min at 20 °C) was added and mixed with spermatozoa pellet, dilution ratio was the same with removed fluid. The sperm samples were then injected by catheter into the ovarian cavity of the experimental subjects, through the oviduct (ovarian lavage/artificial insemination method) in parallel with hormonal administration (5 mg carp pituitary / body weight kg intramuscular injection). The inseminated females (n=9) were monitored for 10 hours and ovulated eggs and ovarian storage spermatozoa were stripped. Stripped gamete samples were divided into two batches. The first batch had only the previously injected spermatozoa and was then activated by aerated tap water (WA). The second batch had additional freshly stripped, sperm added to the stripped eggs before water activation, to act as a positive control (PC). Five females were propagated by using the traditional method as a negative control (NC). All sperm and hormone injected females produced fertilised eggs (hatching rate of the group WA was 17.7±13.2 and for the control groups: PC = 12.5±9.3%, NC = 61±11.5%,). A potential practical protocol was described for induced/wild/tank spawning of oviparous fish species (economical important farm or endangered marine or fresh fish species) by using cryopreserved sperm with ovarian lavage.

Acknowledgements
The work was supported by the EUROSTARS project (E¡9390 TRANSANDER and NEMZ_15-1-2016-0016), the European Fisheries Fund Fisheries Operative Programme III. axis, European Fisheries Fund for Renewable Fisheries provided by the EU and Hungary as well as the Department for Angling and Fisheries, Ministry of Agriculture and the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

THE POTENTIAL OF EARTHWORM (*Eisenia foetida*) IN THE REPLACEMENT OF CONVENTIONAL PROTEINS SOURCES IN THE DIETS OF NILE TILAPIA (*Oreochromis niloticus* l.) PRODUCTION IN KENYA. A REVIEW

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Fishmeal is the main conventional protein sources of fish feeds globally. In Kenya, *Rastrineobola argentea* which is locally known as *Omena* and the freshwater shrimp *Caridina nilotica* are the commonly used protein sources in Nile tilapia (*Oreochromis niloticus* L.) production. This is due to their nutritional quality and palatability properties. However, the high demand and scarcity for fishmeal has escalated its price to levels below break-even point. Various non-conventional animal and plant protein sources have been tested on fish feeds with varying success. The limited success is mainly attributed to the cost factor and in most cases to incomplete essential amino acids for animal and plant ingredients, respectively. Among the non-conventional protein sources which have been tested with relatively promising results is earthworm (*Eisenia foetida*) thanks to its high protein levels, proper amino acid profile, high reproduction and growth rate and ease to culture. However, there is limited information of the utilization of the worm as protein source in fish feeds in Kenya. It is against this backdrop this paper reviews the potential of using; earthworm (*Eisenia foetida*) to replace the conventional protein sources in fish diets. This was achieved by reviewing the main conventional protein sources for fish in Kenya *i.e Rastrineobola argentea* and *Caridina nilotica* then describing the commonly tested non-conventional protein ingredients in Kenya with their associated limitations. Finally the paper evaluates the nutritional properties and culture potential of earthworm (*Eisenia foetida*) for its suitability in fish feed production in Kenya.

FATTY ACIDS AND STABLE ISOTOPES AS TRACERS OF FOOD ASSIMILATION IN FISH-OYSTER-MACROALGAE INTEGRATED MULTITROPHIC AQUACULTURE PONDS

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Integrated MultiTrophic Aquaculture (IMTA) is based on the production of fed species (*e.g.*, finfish) along with extractive species which re-use the inorganic (*e.g.*, seaweeds) and organic (*e.g.*, suspension-feeders) nutrients excess. To increase the development of IMTA practices, it is important to understand the trophic relationship within the system. In Mediterranean earthen ponds, meagre (*Argyrosomus regius*), white seabream (*Diplodus sargus*) and mullet (*Mugil chepalus*) are three fish species of different trophic levels that are good candidates to be cultured with oysters (*Crassostrea gigas*) and sea lettuce (*Ulva flexuosa*) but their trophic behavior are not well known under polyculture. The aim of this study was to identify the food sources used by fish and oysters using fatty acids (FA) as well as carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes.

Within the Era Net COFASP project IMTA-Effect, experiments were conducted in 6 earthen ponds at IPMA´s Estação de Piscicultura de Olhão, south of Portugal. Similar biomass and proportions of meagre, white seabream and mullet were introduced in each pond and three different combinations of organisms were tested: fish-oysters, fish-macroalgae, fish-oysters-macroalgae. According to the combination, oyster spats were introduced while autochthonous macroalgae were let grown or removed from the ponds. Fish were fed daily with a commercial diet. At the end of the experiment, fish and oyster tissues were collected as well as all potential food sources present in ponds: commercial pellet, macroalgae, suspended particulate organic matter (SPOM) and polychetes. FA composition as well as $\delta^{13}C$ and $\delta^{15}N$ values of samples were determined.

The combinations of organisms had no effect on FA composition of fish and oysters. FA composition of fish were significantly different between species. FA composition of meagre and white seabream was close to those of feed. FA composition of mullet was farther than those of feed with lower proportion of C18:1□9 and C18:2□6 and higher proportion of C22:6□3. Compared to feed, polychetes also have lower proportion of C18:1□9 and C18:2□6 and higher proportion of C22:6□3. FA markers of macroalgae were not found in fish. FA composition of oysters was similar to those of SPOM and FA markers of commercial feed were not detected. The combinations of species had no effect on $\delta^{13}C$ and $\delta^{15}N$ values of fish. Meagre, white seabream and mullet were slightly enriched in ^{13}C and ^{15}N in respect to feed. However, mullet were more ^{13}C-enriched than the other fish species and closer to polychetes. Oysters cultured in the fish-oysters-macroalgae combination were ^{13}C and ^{15}N depleted compared to those from the fish-oysters combination. Similar results were found for SPOM.

Our results highlighted that meagre and white seabream only consumed commercial feed while mullets consumed feed and probably, some polychetes whatever the combination of organisms. Macroalgae were not consumed by fish but influenced inorganic C and N recycling in ponds. Oysters fed on SPOM but not on particulate feed. FA and stable isotope analysis are two complementary trophic tracers powerful to study trophic transfers between species co-cultivated in IMTA ponds.

STRATEGIES FOR THE MANAGEMENT OF TILAPIA ALEVINS *Oreochromis niloticus* OF BRAILLOUS ORIGIN FOR SUSTAINABLE DEVELOPMENT OF CULTURE OF FISH IN IVORY COAST

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Fish farming in Côte d'Ivoire faces multiple problems including the lack and poor quality of fry involved in production. To remedy this, in 2014 the country requested Brazil as part of a bilateral agreement to access high-quality, high-profit species. In July of the same year, he received 5,000 suckling stock from *Tilapia Oreochromis Niloticus* from the latter, with the aim of increasing his level of production in the field of fish farming and reducing his dependence on fish. foreign fisheries. These species received by the Ministry of Animal Production and Fisheries of the country should be redistributed thereafter to different production farms nationwide. For an efficient use of these new biological products, the Ivorian State adopts a management method specific to the national context.

The study questions fry management strategies from Brazil for sustainable development of fish farming in Côte d'Ivoire. Its objective is therefore to analyze the methods implemented by the Ivorian government since the reception of the species in question until their diffusion to the final producers installed in the various zones of production of the country.

The working method used is based on a literature search carried out in libraries and documentation centers related to aquaculture production and a field survey especially in the Kongodekro experimental site in Bouaké and the farms chosen as workshop areas.

The management of improved *Tilapia Oreochromis Niloticus* fry enrolled in a program called: sustainable development project of fisheries and aquaculture in Côte d'Ivoire, is taking place in 03 stages. Quarantine phase of said species for testing and acclimation; the stage of their pre-distribution, reserved for 10 pilot farms chosen on the basis of relatively objective criterion and the phase of diffusion towards the final producers distributed throughout the Ivorian territory.

UTILIZATION OF PLANT EXTRACTS AS GROWTH PROMOTERS AND IMMUNOSTIMULANTS IN NILE TILAPIA (*Oreochromis niloticus)* FINGERLINGS IN UGANDA

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Intensification of aquaculture systems in Uganda is increasingly faced with disease outbreaks causing economic losses. Producers commonly use chemical drugs but this approach has negative impacts on environment and human health. The objective of this study was to develop low-cost and eco-friendly prophylactic agents ideal for fish farmers. Plant extracts *Ocimum sp,* N, Garlic and M were screened against eight fish pathogens isolated from commercial fish farms. Ethanol pulverized leaf sample extracts were analyzed and profiled for total phenolic and flavonoid contents, and anti-oxidant properties. Water based and alcoholic extracts were subjected to antibacterial assays to determine sensitivity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration levels. Growth performance and immunostimulant effects on Nile tilapia fingerlings (4.97±0.31g) were examined when fed on a feed infused with 0% (control), 0.1%, 0.5% and 1% of plant N. From the study, all alcohol and water based extracts had relatively high levels of Total phenolics, flavonoids and antioxidants; thus they are useful candidates for preventing fish pathogens in fish farms. It is evident that fish pathogens *Aeromonas sp* (001C), *Staphylococcus aureus* and *Plesiomonas* sp were more sensitive to G, M and N extracts. Preliminary results show gradual increase in weight and showed good health before the challenge test. However, no significant difference was observed in cortisol levels, Erythrocyte count, Hemoglobin, Hematocrit, mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and Platelet count amongst all groups. However, mean values of mean corpuscular hemoglobin concentration (MCHC) and Leucocyte count were lowest in the control group and highest in the 1% group. Differential leucocytes counts including lymphocytes, neutrophils, Monocytes, Eosinophils and basophils were predominated by lymphocytes and all these were not significantly different in the four experimental groups. This study shows that these candidate plant extracts can be used as prophylactics if further studies can explore their potential in aquaculture industry.

DEVELOPMENT AND TESTING OF MOLECULAR-BASED METHODOLOGIES FOR REAL-TIME PROFILING OF BIOLOGICAL COMMUNITIES IN FRESHWATER AQUACULTURE

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The lack of expansion in the freshwater aquaculture sector as a whole has become an alarming concern over the past 20 years in Ireland. This is marked contrast with FoodWise2025 that seeks to increase food exports by €19bn by 2025. Impediments to traditional flow-through production process encompassing fatal disease outbreaks, issues with uncertainty over discharge licensing and a lack of understanding of the overall culture water dynamic have contributed to this stagnation. In order to address the intensive sustainability of Irelands' freshwater aquaculture sector and to inform these bottleneck concerns, it is important to develop an in-depth understanding of the dynamic mix of biological and physico-chemical parameters governing desirable rearing water as a baseline for successful operation.

This project focuses on development and testing of novel molecular diagnostic methods facilitating DNA profiling of predominant bacterial and algal communities in rearing water, with real-time detection of important fish pathogens. Gaining an understanding of target species that contribute to finfish disease outbreaks and poor water quality is essential for prevention and control of problematic species. Molecular-based techniques, such as real-time PCR, have many advantages over traditional plating methods in the identification of species present, as less than 1 % of microorganisms will grow *via* the latter method. The use of species-specific probes enables detection of problematic pathogens and therefore aids future characterisation of harmful bacteria and algae. Linking the biological profile with water quality parameters such as nitrates, nitrites and ammonia in a pill-pond farm when production is thriving will allow for the amendment of the process when production regresses or fails. It will also identify the potential to up-scale and replicate this type of fish farm in the culturing of a variety of freshwater species such as trout, therefore providing an opportunity to expand the industry. Findings from this project will inform innovation (including sensors) and policy.

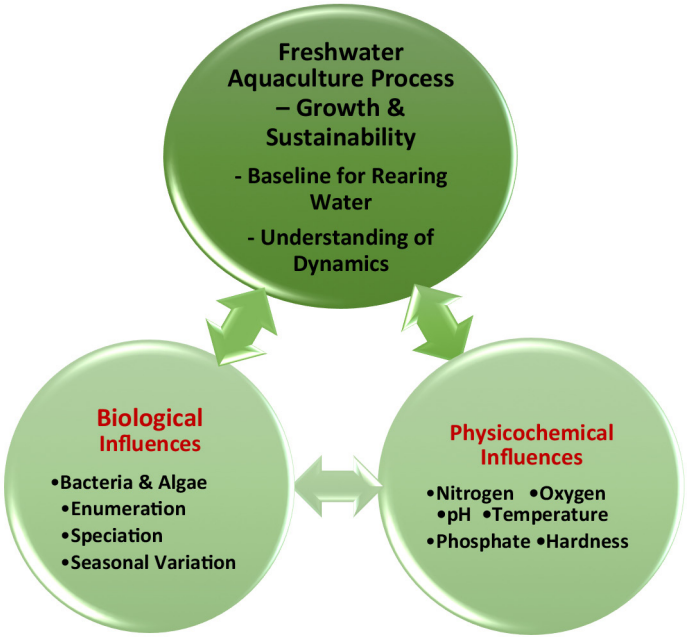

Figure 1. Diagrammatic representation of the dynamic relationships that influence the aquaculture process.

BIOFILTER SALINITY CHANGE STRATEGIES FOR ATLANTIC SALMON *Salmo salar L.* POST-SMOLT PRODUCTION IN RECIRCULATING AQUACULTURE SYSTEMS

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The use of Recirculating Aquaculture Systems (RAS) for the production of Atlantic salmon (*Salmo salar* L.) on land has dramatically increased during the last ten years. The past decade has also seen an extension in the land production phase from smolt in freshwater RAS to post-smolt in brackish or seawater RAS. This has prompted the need to study the performance of the RAS water treatment system under varying salinities. RAS biofilter efficiency is known to be impacted by changes in salinity, but there is a lack of knowledge on how to vary biofilter salinity in accordance with post-smolt production methods. Studies have shown that biofilter nitrification rate is significantly reduced by a sudden change in salinity from fresh (0‰) to seawater (32‰). This experiment studied the nitrification in moving bed biofilm reactors (MBBR) under varying rates of salinity change – gradual to acute, from fresh to seawater.

This study consisted of five treatments (in duplicate) in continuously operated MBBRs of 40L each at 12.2 ± 0.3°C. The MBBRs were shifted from freshwater to seawater at different rates of salinity change: 0, 1, 2, 6 and 15‰/day (Figure 1) over a period of 40 days. The reactors were started in freshwater with mature AnoxKaldnes™ biomedia from a freshwater smolt RAS. Ammonium sulfate and nutrients were supplied to the MBBRs through a synthetic stock solution. Total ammonia nitrogen (TAN) removal rate was calculated daily using feed flow, TAN in feed, makeup flow and TAN in each MBBR. Spike tests were conducted at regular intervals to measure the TAN removal capacity in each MBBR.

Results showed that MBBR nitrification depended on the rate of salinity change, but no matter how gradual the change, nitrification rate was significantly reduced during the period of salinity change. This implies that increasing salinity in RAS during fish production may lead to a reduction in the MBBR performance, causing a potential increase in TAN and nitrite, which may be detrimental to the fish. Daily TAN removal rate and TAN capacity test results will be presented.

The research activities were conducted within CtrlAQUA SFI, Centre for Closed-Containment Aquaculture funded by the Research Council of Norway (Project no. 237856/O30) and collaborating partners.

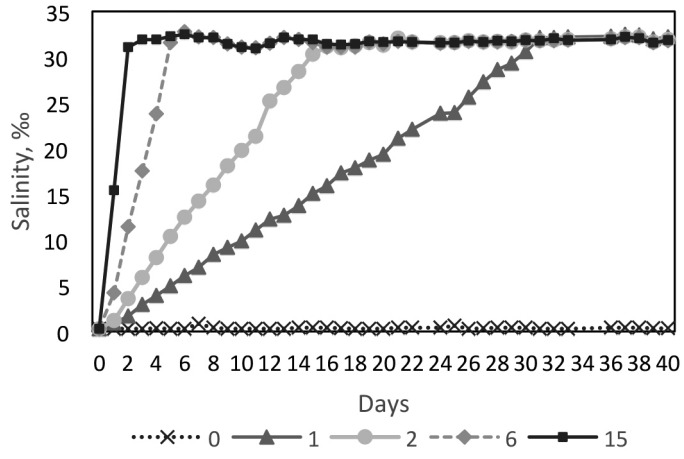

Figure 1 Salinity as a function of time in the MBBRs for different treatments: 0 (Control), 1, 2, 6 and 15‰/day

A POTENTIAL CONTRIBUTION OF AQUACULTURE TO THE EMERGENCE OF *mcr-1* MEDIATED COLISTIN RESISTANCE? NEW EVIDENCE COMING FROM FRANCE

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The old antibiotic colistin is used since recently in human medicine as a last resort to treat infections caused by antibiotic-resistant Gram negative bacteria. In 2015, a Chinese team reported a disturbing discovery: horizontally transferable colistin resistance mediated by the gene *mcr-1* localized in a plasmid (a mobile genetic element). The testing of archived isolates of Enterobacteriaceae has evidenced the worldwide unnoticed dissemination of *mcr-1* in humans and animals for years. Cabello and Godfrey (2017a) suggest that aquaculture may be involved in the origin and selection of *mcr-1*.

In this retrospective study, we aimed at detecting resistance to colistin in a historic collection of *Yersinia ruckeri* causing disease in farmed fish in NW France (Table 1). We tested 104 *Y. ruckeri* clinical isolates for susceptibility to colistin by the broth microdilution method following the guidelines by the Clinical and Laboratory Standards Institute (2014). Because of the lack of a cut-off value for *Y. ruckeri*, we used the one reported by the European Committee on Antibiotic Susceptibility Testing for another Enterobacteriaceae, *Escherichia coli* (Minimum Inhibitory Concentration >2μg/ml). One isolate (0.09%) showed phenotypic resistance to colistin (MIC=128 μg/ml). Its plasmidic DNA was investigated for the presence of *mcr-1* by the Polymerase Chain Reaction, being positive. This isolate corresponded to a case of yersiniosis in an Atlantic salmon from 1985. Pending sequencing results will shed light on the genetic relatedness between this *mcr-1* and those carried by other isolates.

To our knowledge this is the first report of *mcr-1* in *Y. ruckeri* and the oldest bacterial isolate discovered in Europe that is carrying *mcr-1*, with the previous oldest isolate dating from 2004. Our findings are in agreement with the hypothesis of an origin and mobilization of *mcr-1* in the aquatic or aquaculture environment (Cabello *et al*., 2017b), and also suggest that *Y. ruckeri* does not act as a reservoir of *mcr-1*. The lack of use of colistin in French fish farming may have hindered the selection and dissemination of *mcr-1* in *Y. ruckeri* in NW France. In Asia, the heavy use of this antibiotic in aquaculture and the extent of this farming activity are considered responsible for the wide distribution of *mcr-1*.

Cabello and Godfrey 2017a. Comment on: Transferable resistance to colistin: a new but old threat. J. Antimicrob. Chemother. 72:636–637

Cabello *et al*. 2017b. Aquaculture and mcr Colistin Resistance Determinants. MBio 8:1–2

Clinical and Laboratory Standards Institute (CLSI). 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA

Table 1. Colistin Minimum Inhibitory Concentration (MIC) of the 104 *Yersinia ruckeri* isolates tested.

Host species	Years	Colistin MIC (N° of isolates)
Rainbow trout (*Oncorhynchus mykiss*)	1985 to 2014	<0.5 µg/ml (94) 1µg/ml (2)
Atlantic salmon (*Salmo salar*)	**1985**, 2013	**128 µg/ml (1)** <0.5 µg/ml (1)
Brown trout (*Salmo trutta*)	1997	1µg/ml (3)
Turbot (*Scophthalmus maximus*)	1998	<0.5 µg/ml (1)
Unknown species	1985	<0.5 µg/ml (1)

THE EFFECT OF WEIGHT CHANGES IN DRY MATTER, PROTEIN, FAT AND ASH AT SILVER CARP (*Hypophthalmichthys molitrix,* Valenciennes, 1844)

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Body composition in different species of fish may vary in different conditions. This difference is due to different nutritional conditions, water quality, sexual orientation, age, and so on. Also, the amount of protein, fat and ash in the muscle of aquatic animals varies in different species. Knowing the chemical composition of fish meats helps to select the right species for human nutrition and food industry. Silver carp is one of the most valuable fish in the world. This fish is one of the main types of human nutrition in northern Iran.Many people in Iran are keen on feeding large-sized fish. While 500 g of silver carp is available for human consumption.But the basic question is, what size of fish has a higher nutritional value for consumption. In this study, the amount of protein, fat, ash and dry matter of silver carp meat was investigated and compared in different sizes.For this porpose, the study was performed in 5 groups of weight (500 g, 700 g, 1000 g, 1300 g and 1600 g). The results of the examination of the quality of fish meat in various sizes are given in Table 1.

Based on the results, dry matter, protein, fat and ash content in different weight groups were not significantly different ($p > 0.05$). Therefore, weight gain does not affect meat quality in silver carp. The protein content, which is the most important compound, tends to be modest in fish. Although the amount of protein in silver carp (14.18 %) was higher than some fish, such as common carp (10.2 %) and *Liza dussmieri* (10.13 %) , according to the results of this study and its comparison with other fish such as *Tilapia zilli* (18.80 %), *Hemisynodontis membranacea* (20.26 %), *Clupea harengus* (18.45 %), *Scomber scombrus* (20.20 %), *Otolithes ruber* (19.64 %), *Scomberomorus guttatus* (19.9 %), *Scomberomorus commerson* (19.5 %) and *Euthynnus affinis* (24 %) it is determined that the amount of meat protein silver carp is less than other fish. The amount of fat in fish species varies depending on the age, sex, and season of the hunting season. The amount of fat in silver carp (7.55 %) was higher than some fish, such as *Upeneus moluccensis* (4.35 %), *Carcharhinus macloti* (6.69 %), *Otolithes ruber* (1.23 %), *Scomberomorus guttatus* (2.1 %) and *Scomberomorus commerson* (3.4 %), but it is higher than common carp (9.42 %), *Orcynopsis unicolor* (16 %), *Euthynnus affinis* (14 %), *Liza dussmieri* (20.26 %), *Clupea harengus* (11.14 %), *Scomber scombrus* (12.33 %) and *Dicentrarchus labrax* (21 %). Also, the amount of ash in silver carp (1.49 %) lower than common carp (1.4 %), *Otolithes ruber* (1.32 %), *Scomberomorus guttatus* (1.13 %) and *Scomberomorus commerson* (1.63 %), but it is higher than *Orcynopsis unicolor* (2 %), *Euthynnus affinis* (3.27 %), *Liza dussmieri* (1.36 %), *Clupea harengus* (1.51 %), *Scomber scombrus* (1.79 %) and *Dicentrarchus labrax* (11.5 %).

Table 1: Changes in dry matter, protein, fat and ash at silver carp in different sizes

Factor (%)	500 (g)	700 (g)	1000 (g)	1300 (g)	1600 (g)	Average
Dry matter	22.80 ± 0.34 [a]	22.53 ± 0.61 [a]	22.72 ± 1.47 [a]	23.62 ± 0.28 [a]	24.71 ± 0.41 [a]	23.14 ± 1.05
Protein	15.22 ± 2.13 [a]	12.94 ± 1.86 [a]	13.75 ± 2.04 [a]	14.75 ± 2.19 [a]	14.52 ± 0.67 [a]	14.18 ± 1.86
Fat	5.81 ± 1.68 [a]	8.04 ± 1.59 [a]	8.12 ± 0.61 [a]	7.45 ± 2.26 [a]	8.41 ± 0.15 [a]	7.55 ± 1.57
Ash	1.43 ± 0.01 [a]	1.55 ± 0.3 [a]	1.42 ± 0.06 [a]	1.41 ± 0.09 [a]	1.77 ± 0.41 [a]	1.49 ± 0.2

❋The small Latin letters show that there are significant differences among different groups

FISH ARE WHAT THEY EAT: KRILL EFFECTS ON COMPOSITION, HISTOPATHOLOGICAL CHANGES AND HEALTH MARKERS OF ATLANTIC SALMON (*Salmo salar* L.) GILLS

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Gill health issues are an increasing concern in areas where salmon are farmed worldwide. This organ is in intimate contact with the environment and as such is potentially exposed to multifactorial harmful influences, which can lead to serious animal welfare implications and even mortalities. Gills take part in various biological processes, and any deficiency in these functions will reduce feed utilisation and ultimately diminish growth and performance. In addition, impairment of gill health can translate into downstream impacts on health management, including commercially used parasitic treatment strategies.

BioMar has commercially developed functional feeds containing krill, which have been shown to increase appetite and feed intake in fish. Krill meal is also rich in n-3 fatty acids and phospholipids, previously shown in other fish species to affect gill inflammatory and immune processes.

A series of trials using Atlantic salmon as a model have been conducted under different experimental conditions to evaluate the effects of incorporation of different levels of krill in health-associated gill parameters. Gills from salmon of different sizes and grown under semi-controlled (experimental sea cages) and controlled conditions (experimental tanks) were sampled and evaluated for changes in phospholipid composition, variations in histopathological lesions using a gill scoring method developed in cooperation with Pharmaq Analytiq and modulation of gene expression using Nofima's Atlantic salmon microarray SIQ6.

Supplementation of dietary krill produced relatively large dose-dependent changes in all parameters measured. Quantities of n-3 fatty acids EPA and DHA were significantly increased in gill tissue of fish fed the highest krill dose, and the phospholipid composition was modified in favour of a higher proportion of phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylserine (PS). Histopathological gill scores were also significantly improved in two different trials with the highest krill supplementation, in particular regarding lesions associated with inflammatory and haemostatic changes. In the gill transcriptome of salmon from the same trials, the strongest up-regulatory effects were observed in immune genes, especially genes associated with innate antiviral responses. Together these results show that the supplementation of dietary krill has the potential to become a strong tool to improve gill health status in Atlantic salmon.

GNANAGONFLA : ABOUT INVENTIVENESS OF FORGOTTEN LAND, AN EXAMPLE OF UNEXPECTED DEVELOPMENT OF MERCHANT FAMILY FISH FARMING

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In the field of agricultural development, the goal of many interventions is to spread the promoted innovation and a perenial replacement of the production methods for the largest number of farmers. It is such a necessity to study how innovations spread themselves within agriculture and change farming systems in order to assess the effective validity of these innovations for the producers. For West Africa, in the case of merchant family fish farming, the activity was initially promoted by schemes and the development dynamics offer a great variety. Despite an apparent match to the agricultural challenges and a relative agro-economic profitability, family fish farming spreads irregularly inside and outside scheme intercession. This paper describes a spontaneous substantial fish farming development in a border area, with poor water resources. It questions the impact of a change in the initial technical model and tries to assess the social condition of its accessibility and of the relationship network mobilisation.

Gnanangonfla is a village in the Bediala subprefecture in the central western part of the Cote d'Ivoire. This locality is at the border of the rainy forest area, so savannah covers large parts of its territory. Rainy season is quite short, inducing temporary surface water availability.

However, before 2000, family fish farming appeared during the cacao crisis context affecting cacao smallholder incomes. Today this innovation seems part of the Gnanagonfla agrarian system and now has a social dimension. New labour organisation modalities can be observed inside the fish farmers' production units or among groups of farming systems sharing ponds along the same low-land. Nevertheless this probable self-development has never been recorded as a village of beneficiaries of the various schemes that locally take place unlike Luenoufla, another village 10 kilometres away, where the impact of scheme intervention has been described. At the very beginning, they benefited from Luenoufla fish farming group. However they freed themselves from this model by setting up their own dam chains with the flow. This design allows more water to be retained on condition that farmers submit themselves to common rules for preventing the resource: the first farmers who drain are the owners of downstream ponds, then gradually the upper ones drain, enabling the downstream one to save the water. This is opposite to the standard quality where each pond can be separately drained. Other common organisations give some protection against lack of water, over-flow, and wild fish propagation. The duration of a pond cycle is one year (instead of the usual 6 months). Farmers set up an official cooperative whose major benefit is the sale of day licences allowing farmers to freely sell their production to women fishmongers.

This development has a surprising extension: 136 fish farmers, 318 ponds of 128 ha, 22 women, fishmongers (partial collected data evaluates at 76 t the yearly production of 67 fish farmers). This example questions the basis of fish farming propagation methods and shows the benefit to take into account spontaneous development.

THE USE OF THE PHYTASE IN AQUACULTURE, ITS ZOOTECHNICAL INTERESTS AND THE POSSIBILITIES OF INCORPORATION IN THE AQUAFEED

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The study turns on the use of the phytase in aquaculture, its zootechnical interests and the possibilities of incorporation in the feed. The goal is to reduce the waste in phosphorus linked to the feeding of fishes, without any loss of zootechnical performances and with a decrease of feed costs. We have studied the bibliography data, in order to enhance the value of the raw materials (total phosphorus, phytate and available phosphorus) used Gouessant for the manufacturing of rainbow trout of feed ; to determine the needs of phosphorus for aquaculture species; to determine the needs of phosphorus for aquaculture species, to determine the sings of lack of phosphorus for fishes; to study the antagonism between the phosphorus and the calcium and to study also the different forms of waste for the rainbow trout. The results found in the bibliography enable us test several Hypothesis of feed formulation for rainbow trout with different raw materials. This simulation and the calculation for wastes allowed to validate two formulation of feed: a control feed (0.5% of monocalcique phosphate) and a trial feed (supplementation with 0.002% of phytase Ronozyme PL and without inorganic phosphate). The feeds have been produced and sent to a experimental structure (agricultural college of Brehoulou).The result of the formulation give a decrease of the phosphorus waste of 28% for the trial feed compared to the feed. The supplementation enables a gain of 2.3 euro per ton. The partial results of the current test show no significant difference yet for the zootechnical parameters (growth rate, mortality, weight gain and obvious conversion rate) between control feed and the trial one. The waste measures do not show either significant difference between the control feed and the trial one, but however, the average difference would to decrease the wastes of 35.6% thanks to the use of phytase.

BACTERIOPHAGE IN THE CONTROL OF PATHOGENIC *E. tarda* IN CULTURED FISH

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In many cultured fish, *Edwardsiella tarda*, a Gram-negative bacterium causes variety of diseases including edwardsiellosis, and encounters severe economic losses. Though the vaccination is an ideal method for prevention of infectious diseases in the aquaculture field, experimental *E. tarda* vaccines are still difficult to develop as an effective vaccines due to the resistance of *E. tarda* to phagocyte-mediated killing. Licensed antimicrobial agent also has not been reported against *E. tarda* infections of fish. Phage therapy is an attractive approach to prevent and control bacterial infections, and potentially applicable for antibiotic resistant bacterial infection in aquaculture.

We isolated and characterized the *E. tarda* phage (ETP-1) from marine fish farm water against *E. tarda*, which is Streptomycin (10 μg), Erythromycin (15 μg), Vancomycin (30 μg), and Ampicilin (10 μg) resistant. ETP-1 was characterized based on diameter of head and tail length by Transmission electron microscopy (TEM). The head size was 55.27 nm, and tail length x base width were 23.05 x 26.3 nm. The TEM observations of ETP-1 suggested that the phage belongs to a member of the family *Podoviridae*. Bacterium growth inhibition analysis was performed for the ETP-1, and it showed MOI dependent *E. tarda* growth inhibition by the ETP-1. The isolated ETP-1 phage was investigated for their host range specificity, and 5 out of 8 *E. tarda* strains showed efficiency of plating (EOP) as 1 or <1.3. ETP-1 was stable at broad range of pH 3-11) and optimal temperature and pH were 4 °C and 7.5, respectively. Moreover, ETP-1 exhibited complete or greater loss of activity in ethanol, diethyleather and acetone, and optimal stability showed in PBS. Genome sizes of the isolated phage was estimated by restriction enzyme digestion of genomic DNA followed by agarose gel electrophoresis, and the size of the entire genome was predicted as 25-30 kb approximately. In future, protective effects of phage therapy against *E. tarda* will be investigated *in vivo*.

EVALUATION OF THE BIOENERGETIC AND PROTEIN FLUX MODEL FOR GROWTH SIMULATION OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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The application of the extended bioenergetic model to simulate shrimp growth is presented. This generic modelling approach is based on the bioenergetic factorial approach and explicitly includes the energy and protein flows. As previously illustrated for the seabream following this approach it is possible to define a model that is species specific based on experimental datasets. The objective of this work is to present the preliminary evaluation work for simulation of the Pacific White Shrimp, *Litopenaeus vannamei*. The model was parameterized based on a set of growth and metabolic trials that can be considered as a standard for the setup/calibration of individual growth models. The experiments were carried out at constant temperature (28°C). Evaluation was carried out with published data using similar temperature (28.4±0.8°C). The comparison of the experimental final body weight (BW) with model outputs (Table 1) indicate the model potential to simulate the shrimp growth. Nevertheless, a proper model validation still must be carried out.

One of the advantages of this modelling approach is its suitability to simulate planned or on-going production in commercial farms with limited field data (initial BW, temperature, feeding rate and feed composition) when compared to data available from scientific experimental settings. At this stage the model for the Pacific white shrimp can only be applied for temperatures around 28 °C.

Acknowledgements
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Table 1. Preliminary model evaluation.

		Calibration		Evaluation	
	Datasets	**1***	**2***	**3**	**4**
Published data	**Initial BW**	1.5 g	7.7 g	0.3 g	
	Final BW	5.7 g	18.4 g	5.0 g	5.1 g
Simulated final BW		5.9 g	20.4 g	5.4 g	5.2 g

* Lupatsch I., Cuthbertson L., Davies S., Shields R.J., 2008. Studies on Energy and Protein Requirements to Improve Feed Management of the Pacific White Shrimp, Litopenaeus vannamei. 281- 295. Avances en Nutrición Acuícola IX.

A TOOL FOR SEED STOCK MANAGEMENT IN COMMERCIAL OYSTER NURSERIES

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Models designed to provide practical guidance about food limitation in oyster nurseries can support seed stock management. However, most of the available shellfish models are developed for adult oysters and seldom the target end-users of these models are farmers. The goal of this work is to present a nursery model application for seed stock management, which is available online for widespread use at http://seaplusplus4.com/oysterspatbud.html. This approach applies to nurseries, including floating upwelling systems (FLUPSY) or land-based tanks, and is implemented for the Pacific oyster (*Crassostrea gigas* Thunberg). The model was evaluated with published experimental dataset and was further tested to simulate the application of a general rule of thumb regarding spat holding capacity for a given nursery. The model can be used by Pacific oyster farmers to improve the application of general rules of thumb and estimate stock biomass to hold in their nursery as a function of typical external food concentrations. The model estimates fit well within a general rule of thumb for spat of around 0.38 g. However, considering the same conditions but targeting to stock smaller spat of around 0.04 g the biomass stock sustained is lower (Table 1).

The application of the model allows to improve the rule of thumb for a given set of conditions, and thus to lower the set of trials and errors required to determine the biomass of spat to hold in a pond system. This presentation illustrates the application of this model to a nursery located in a Southwestern European coastal lagoon.

Acknowledgements
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Table 1. Simulation of a general rule of thumb regarding spat holding capacity.

	Spat	**Holding stock**	
General rule for 1 hectare surface area of shallow pond	(Not specified)	1 to 3 ton	
Model outputs *	0.38 g	1 to 3 ton	3 to 8 million
	0.04 g	0.7 to 2 ton	17 to 47 million

* Model assumption: 10% water renovation, 1 ha for the bloom pond + 1 000 m2 for the stock pond, 1 m water depth, water temperature 19ºC, 0.5 - 2 µg Chl-*a*.L^{-1} at inflow, 0.5 d^{-1} to 1.2 d^{-1} phytoplankton growth rate

DEMONSTRATION OF A NITROGEN BUDGET MODEL TO ASSESS WATER RENEWAL RATES AND NITROGEN LIMITATION IN COMMERCIAL SEAWEED FARMS

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This work presents a simple nutrient mass balance model, developed for land-based seaweed farmers. The model provides the farmer, via a simple interface, the value of the water renewal rate that is necessary for a desired seaweed production (Figure 1), given a farm configuration and N input concentration in the inflow water.

The model was calibrated and evaluated for one of the main seaweed species in pond cultivation, *Ulva spp*.

The model application with published data illustrates the calculation of the water renewal required in a commercial seaweed farm with 2-ha area of *Ulva* production, a water depth of 0.5 m and a N input concentration averaging 50 µmol L^{-1}. For that hypothetical case, the model advises the farmer to renew the water volume of the seaweed pond from 2 to 4 times per day at initial and harvesting seaweed densities, respectively. Those water renewal rates could be decreased to the range of 1.5 to 3 times per day during winter. The model is available online with the *Ulva* spp. calibration as a default, for widespread use, at http://seaplusplus4.com/algaeplanning.html. It can also be applied to other species by replacing the growth and N uptake kinetics.

Acknowledgements
Financial support was provided by the Portuguese Foundation for Science and Technology (FCT) as a Postdoc scholarship to Ana Nobre (SFRH/BPD/109442/2015) and by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF) in the context of Project INNOVMAR/INSEAFOOD (ref. NORTE-01-0145-FEDER-000035).

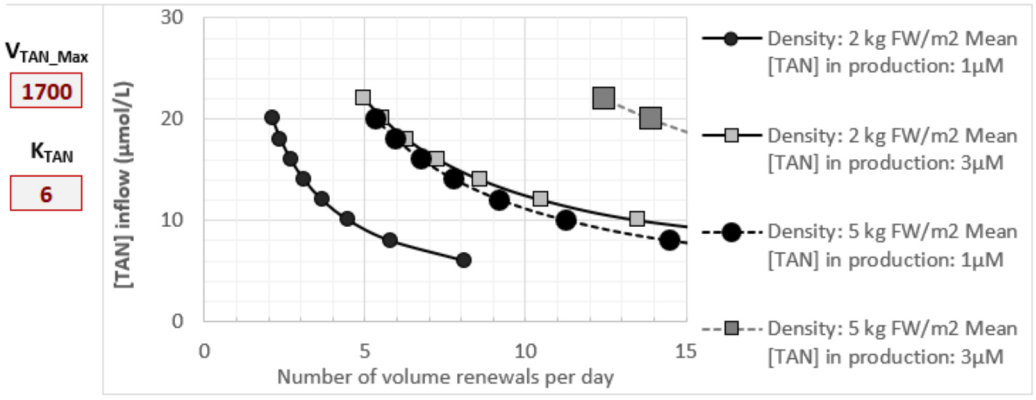

Figure 1. An illustration of one of the model outputs: rate of water renewal vs total ammonia-N (TAN) concentration in the inflow. The legend on the right delineates different given scenarios of seaweed density and a desired mean concentration of TAN in the pond water.

FULL-FAT INSECT MEAL IMPROVE RAINBOW TROUT (*Oncorhynchus mykiss*) GROWTH

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In the last decade it has been promoted the use of insect meals in fish nutrition as an alternative to fishmeal. It has been tested different levels of inclusion although the use of different insect species at the same level has been not tested yet, for that reason the aim of this trial was to analyze the effect of the inclusion of four insect meals in rainbow trout (*Oncorhynchus mykiss*) nutrition, evaluating the growth performance and feed efficiency.

The 1950 rainbow trout juveniles of 53.39g were fed with five extruded diets, the control diet (CT) without insect meal, the 2nd diet with a 20% of *Hermetia illucens* meal (HI) inclusion; the 3rd diet with a 20% of inclusion of *Tenebrio molitor* meal (TM); the 4th diet with 20% of *Gryllodes sigillatus* meal (GS) inclusion and the 5th diet with 20% of *Blatta lateralis* meal (BL) inclusion. Every ten days the animals were weighed to determinate growth performance and feed efficiency and after 60 d., the experiment finished. The fish were randomly allocated into 15 tanks of 600 dm^3 capacity and they are part of an open-flow system. The water temperature ranged from 7.1 to 20.6°C and the dissolved oxygen ranged from 4.3 to 11.2 mg O_2 dm^{-1}.

At the end of the trial, there were no significant differences in mortality among treatments ($p>0.05$). The final body weight of individual fish significantly increased ($p<0.05$) in the BL treatment in comparison to the CT, HI, and GS groups. In the GS group, it was lower than that of the TM and BL diet groups. The specific growth rate was negatively affected by the GS diet. The feed conversion ratio increased ($p<0.05$) only in the GS treatment, while no differences were recorded for all other treatments. The protein efficiency ratio did not differ ($p>0.5$) between treatments. The effects of insect meal inclusion in fish diets vary depending on the fish and insect species used in the experiment. Furthermore, insect feeding and processing seem to be important issues due to their effects on the chemical composition and nutritional quality of the meal. However, it may be concluded that at the present level of knowledge, a 20–25% share of insect meal may be included in fish diets, and further studies are needed to increase this proportion.

TABLE 1. Effect of selected insect meals on growth performance, nutrient retention and survival of rainbow trout.

	Experimental diets				
	CT	**HI**	**TM**	**GS**	**BL**
Body weight gain	165.92^{bc}	167.67^{bc}	171.49^{ab}	161.46^{c}	178.14^{a}
Specific Growth Rate	1.67^{a}	1.62^{a}	1.63^{a}	1.56^{b}	1.65^{a}
Feed Conversion Ratio	0.95^{b}	0.97^{b}	0.96^{b}	0.99^{a}	0.95^{b}
Protein Efficiency Ratio	2.27	2.28	2.26	2.25	2.27
Survival (%)	97.29	99.22	98.21	100.0	95.83

Values in the same row having different superscript letters are significantly different at $P < .05$.

FULL-FAT INSECT MEALS IMPROVE RAINBOW TROUT (*Oncorhynchus mykiss*) MICROBIOTA

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Insect meals are not only a source of protein meal but also a significant source of antimicrobial peptides (AMPs), which are also considered growth and health promoters with beneficial effects on intestinal microbiota. The aim of this research was to observe how the different insect meals affect the gastrointestinal tract microbiota of rainbow trout juveniles.

The rainbow trout juveniles fed with five experimental diets, the control diet (CON) without insect meal, the 2nd diet with a 20% of *Hermetia illucens* meal (HI) inclusion; the 3rd diet with a 20% of inclusion of *Tenebrio molitor* meal (TM); the 4th diet with 20% of *Gryllodes sigillatus* meal (GS) inclusion and the 5th diet with 20% of *Blatta lateralis* meal (BL) inclusion. After 60 d., the fish were sacrificed and the samples of the gastrointestinal content were collected and immediately stored at –80ºC for FISH analysis. The analysis was performed according to Jozéfiak et al., (2011) method. The numbers of detected bacteria are expressed in colony-forming units/g of digesta (CFU/ml).

The total number of bacteria increased (P<0.05) in all experimental treatment groups compared with the CT group. The concentration of Enterobacteriaceae increased (p<0.05) in the TM treatment in comparison to the CT, the HI, and the GS treatment groups, but it did not differ from the BL diet. *Clostridium leptum* subgroup increased (P<0.05) in the TM, GS and BL treatment groups in comparison to the CT and HI treatments. Among the TM, the GS and the BL groups, the highest value was observed in the GS group, and the lowest value was observed in the BL group. All experimental treatments (HI, TM, GS, and BL) significantly increased (P<0.05) the number of *C. coccoides* in comparison to the CT diet in trout. Among them, the highest values were observed in the TM and BL treatment groups. Populations of *Lactobacillus* sp./*Enterococcus* sp. increased in the insect–containing diet groups. The results of our study indicate a simultaneous increase in population of *C. coccoides* in the BL treatment group and confirm that the presence of Clostridia in fish may have a balancing effect on gut homeostasis and contain potentially pathogenic species for fish.

Table 1. Intestinal microbiota of rainbow trout gastrointestinal tract (Log CFU/g of digesta).

	Diet				
Item	**CT**	**HI**	**TM**	**GS**	**BL**
Total number of bacteria	9.51^{d}	9.55^{c}	9.80^{a}	9.56^{c}	9.61^{b}
Enterobacteriaceae	8.46^{b}	8.45^{b}	8.52^{a}	8.44^{b}	8.50^{ab}
Clostridium leptum subgroup	8.30^{d}	8.26^{d}	8.53^{b}	8.69^{a}	8.38^{c}
Clostridium coccoides *Eubacterium rectale* cluster	8.13^{d}	8.23^{c}	8.44^{a}	8.28^{b}	8.47^{a}
Lactobacillus sp. */Enterococcus* sp.	8.10^{c}	8.69^{b}	8.73^{a}	8.68^{b}	8.67^{b}

Values in the same row having different superscript letters are significantly different at $P < 0.05$

THE USE OF FULL-FAT INSECT MEALS IN SIBERIAN STURGEON (*Acipenser baerii*) NUTRITION

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The Siberian sturgeon (*Acipenser baerii*), is one of the most valuable fish farming due to the caviar production, as well as their meat quality. Nevertheless, the diets used on its nutrition as well as in other fish species are based on fishmeal and soybean meal, which have been constantly increasing their prices more than 300 % in the last decades. Considering these aspects the aim of this trial was to compare the growth performance and feed efficiency of Siberian sturgeon juveniles fed with two insect meals. One hundred eighty Siberian sturgeon (Initial body weight (IBW) 640 ±3.9 g) were fed with three diets, the control diet based on fishmeal (FM), the 2nd diet with 15% of *Hermetia illucens* larvae meal (HI) and 3rd diet with 15% *Tenebrio molitor* larvae meal (TM) in triplicate tanks. The animals were randomly allocated into nine tanks (600 dm^3 capacity) under an open-flow water system. The water temperature 19.8 ± 1.4°C; the dissolved oxygen (Fig 1) was 3.8 ± 0.9 mg O_2 dm^{-3}. The trial lasted 60 d., it was performed in summer time. The fish were fed with automatic band-feeders and the daily feed ratios were varying from 1.8 to 1.4%, according to the biomass weight and the water temperature. Every ten days the animals were weighed to determinate growth performance and feed efficiency

At the end of the experimental period, the growth parameters (Body weight gain and Specific Growth Rate), as well as the feed efficiency values (Feed conversion Ratio and Protein Efficiency Ratio) were similar in all treatments; at the same time at the end of the experiment (Table 1) there were no significant differences in all the parameters analyzed ($P > 0.05$). The survival rate at the end of the trial was a 100 % in all the treatments. The 15% of inclusion of *H. illucens* and *T. molitor* meals are suitable meals to be used in Siberian sturgeon nutrition.

TABLE 1. The growth performance and feed efficiency parameters of Siberian sturgeon fed with the experimental diets.

	Diets		
	CT	**TM**	**HI**
Final Body weight (g)	1196.81	1195.04	1194.81
Body weight gain (g)	546.67	553.33	555.00
Feed Conversion Ratio[a]	1.47	1.47	1.48
Specific Growth Rate[b]	1.03	1.03	1.03
Protein Efficiency Ratio[c]	1.52	1.50	1.52

All values are means of triplicate cases (n = 3). There were no significant differences among treatments ($P>0.05$).

DO FULL-FAT INSECT MEALS IMPROVE SIBERIAN STURGEON (*Acipenser baerii*) MICROBIOTA?

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Fish are continuously exposed to the microorganisms present in water and in sediment including the contaminants in sewage/feces. In the gastrointestinal tract colonization commences when fish start feeding. The bacterial populations become dense and these densities are likely to be influenced by the feeding regime. Considering these facts the objective of this trial was to determinate how the 15% of insect meals inclusion in extruded diets affecting the microbiota population in the gastrointestinal tract of the fish. The animals were fed with three experimental diets, the control diet (CON) that only contain fishmeal, the 2nd diet with a 15% of *Hermetia illucens* meal inclusion, the 3rd diet with a 15% of inclusion of *Tenebrio molitor* meal. After 60 d., the fish were sacrificed and the samples of the gastrointestinal content were collected and immediately stored at –80ºC for FISH analysis. The analysis was performed according to Józéfiak et al., (2011) method. The numbers of detected bacteria are expressed in colony-forming units/g of digesta (CFU/ml).

The analysis of the microbiota of Siberian sturgeons fed with the three experimental diets (Table 1) showed that fish fed with HI diet obtained the highest total number of bacteria (Enterobacteriaceae family, and *Clostridium leptum* subgroup) in the case of *Clostridium coccoides – Eubacterium rectal* cluster HI and TM diets achieved similar values and higher than CT diet ($P < 0.05$). In the content of *Lactobacillus* spp./*Enterococcus* spp., there were not observed significant differences among treatments ($P > 0.05$).

Perhaps this acquired microbial richness may potentially provide further metabolic capabilities to the host, that could be the case of Siberian sturgeon fed with HI diet. In the case of TM diet, this insect meal seems to behave similar to FM diet.

TABLE 1. Selected intestinal microbiota populations of Siberian sturgeon digesta at the end of the experimental period.

	Diets		
Target	**M**	**HI**	**TM**
LOG CFU/g of digesta			
Total number of bacteria	8.27^{b}	8.87^{a}	8.14^{c}
Clostridium leptum subgroup	7.66^{b}	8.12^{a}	7.73^{b}
Enterobacteriaceae	7.69^{b}	7.94^{a}	7.73^{b}
Clostridium coccoides – Eubacterium rectale cluster	7.40^{b}	7.66^{a}	7.55^{a}
Lactobacillus spp./ *Enterococcus* spp.	7.63	7.67	7.65

All values are means of twelfth cases (n = 12). Different letters indicate significant differences between treatments ($P < 0.05$).

ENVIRONMENTAL IMPACT ASSESSMENT UNDER MSFD FOR AQUACULTURE IN THE MACARONESIA

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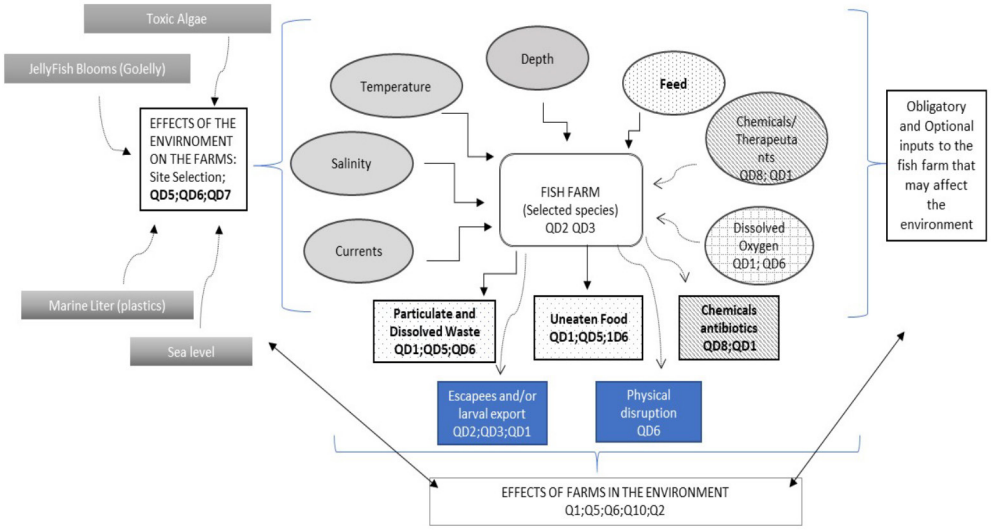
Macaronesia is composed by four archipelagos in the North Atlantic Ocean, that have to deal with a number of difficulties related with their geographical characteristics: remoteness, insularity, size and consequently economic constraints. Maritime-based economic activities contribute significantly to the overall economy of the Macaronesia sea basin. Aquaculture in these Regions, particularly Madeira and Canary Islands is currently characterized by offshore finfish cage production. As this is an emerging industry to the supply of seafood constituting an opportunity for Blue Growth economic development, it is fundamental to ensure sustainable management. Following EU environmental legislation framework (MSFD/GES) and within the PLASMAR project goals, we aimed to identify relevant environmental issues, as well as development of a framework for Environmental Impact Assessment for aquaculture in the Macaronesian Region.

Following the structure given by Gentry et al. (2016), we identified possible inputs and outputs of aquaculture activity in the Macaronesia, related with MSFD on GES (Commission Decision 2017/848). Due to scarce literature specific for the Macaronesia, we took into consideration other areas with similar site and species specifications, as well as local and international experts' consultancy. This methodology allowed us to identify the most relevant descriptors and applicable criteria within offshore finfish production, considering the sensitiveness and specificities of the Macaronesia archipelagos. Moreover, the identification of potential recent effects of the environment (e.g. sea level or Jellyfish blooms) to the fish farms, emphasize the importance of connectivity between the industry, scientists and population.

The outputs of this study will help Public Institutions to develop monitoring plans for aquaculture taking into consideration MSFD. Moreover, performing the exercise for all the Macaronesia Archipelagos we aim at contribute for an effective development of the industry, pointing out the advantages and constraints of aquaculture in the Macaronesia within the Blue Growth Development.

Gentry R.R. et al. 2016. DOI:10.1002/ec3.2637.



LIPID AND FATTY ACID COMPOSITION OF *Chlorella autotrophica* (CHLOROPHYTA) GROWN OUTDOORS IN TWO DIFFERENT PHOTOBIOREACTORS

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In recent years, microalgae have been given considerable attention for their potential as oil-producing organisms and thus a potential feedstock for biofuel production. Still, regardless of the product's final ending, taking the leap into large scale industrial production requires pilot-scale research, adjustment to environmental conditions, cultivation technology and selection of the strain. *Chlorella autotrophica* is a euryhaline marine algae that was used for this study.

C. *autotrophica* (UTEX580) was cultured outdoors in Madeira (33º03'47.7''N 16º18'54.3W) for 3 weeks. Two types of photobioreactors (PBR´s) designs were used with the same optical path (5cm): 20L flat panel PBR´s and 100L annular PBR´s. All PBR´s were inoculated with the same starter cultures ($73*10^6$ cels.mL^{-1}) and nutrient sufficient medium (Fábregas et al. 1996). Cultures were bottom aerated with 250 mL.min^{-1} air mixed with CO_2 to keep pH adjusted. Daily temperature was 20.2± 3ºC and mean solar radiation was 160 W.m^2. Cultures were subjected to periodical renewals (10-30%) and samples were collected for lipid and fatty acid determination. Daily count and dry weight were performed in all PBR´s.

Results show that C. *autotrophica* was able to grow well outdoors. Differences were found between PBR´s: flat plate cultures reached higher densities ($320±10*10^6$ cell.mL); dry weight (1.1 g.L^{-1}) and chlorophyll content (0.11pg.cel^{-1})(*data not shown*). PBR design clearly affected biochemical composition, as mean lipid content was generally higher in flat PBR´s (23±2.7%DW), but PUFA content was higher in annular PBR´s (Fig. 1). EPA, which was almost absent in annular PBR´s (Fig. 1), accounted for more than 80% of PUFA in flat plate PBR´s. The results suggest that PBR design not only affects productivity (areal and volumetric), but it can actually affect gross biochemical composition.

Fabregas J., Patiño M., Morales E.D., Dominguez A. & Otero A. 1996. Distinctive controlo f metabolic pathways by Chlorella autotrophica in semicontinuous culture. Can. J. Microbiol. 42:1087-1090.

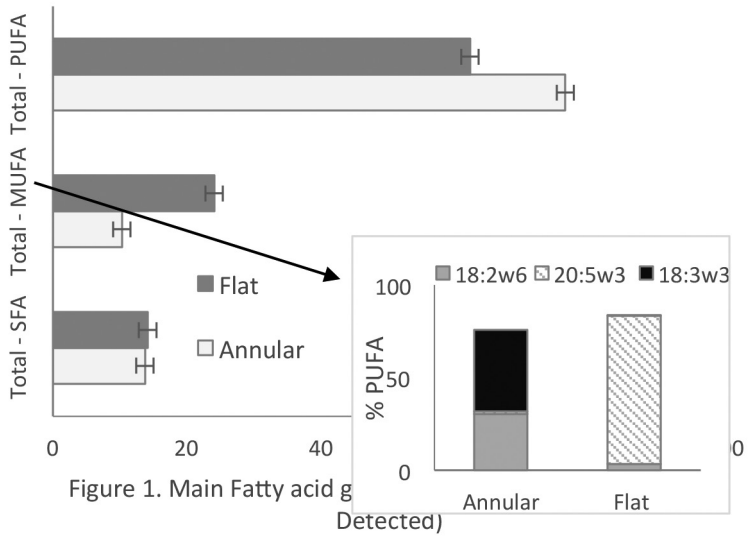

Figure 1. Main Fatty acid g
Detected)

EFFETS OF LYSOLECITHIN ON PROTEOLYTIC ENZYMES ACTIVITY OF STELLATE STURGEON

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In present study, effect of dietary lysolecithin was investigated on proteolytic activity of pancreas (trypsin and chymotrypsin) and stomach (pepsin) in juvenile *Acipenser stellatus*. Four experimental diets including control diet (with 0% lysolecithin) and three diets containing 0.5%, 1% and 2% lysolecithin were used. At the end of the experiment, activities of proteolytic enzymes (trypsin, chymotrypsin and pepsin) were assessed. The trypsin and chymotrypsin activity in the crude enzyme were measured using N-α-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) and Succinyl-$(Ala)_2$- Pro-Phe-*p*-Nitroanilide (SAPNA) as substrate respectively according to Erlanger et al., 1961. The pepsin activity in the crude enzyme extract was estimated according to Rungruangsak and Utne (1981) using casein as substrate. Results showed a decrease in trypsin and pepsin as an increase in lysolecithin, highest trypsin and pepsin activity was observed in fish fed control diet ($p< 0.05$). However no differences in chymotrypsin activity were detected between treatments (Fig. 1). It is concluded that the proteolytic enzymes activity in stellate juveniles are not affected by 1-3% dietary lysolecithin.

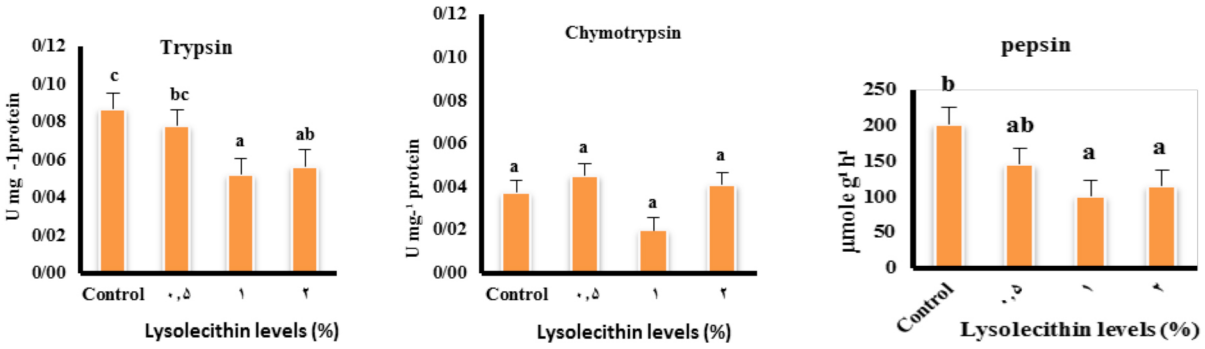

Fig.1. Specific activity of trypsin, chymotrypsin and pepsin content in *Acipenser stellatus* fed four different lysolecithin levels at the end of 56 days culture period. Different superscripts indicate significant differences ($p < 0.05$).

INNATE IMMUNITY OF THE BRINE SHRIMP *Artemia* SHOWS LONG-LASTING ANTI-BACTERIAL FEATURES: PHENOMENON VERIFIED DURING A TRANSGENERATIONAL STUDY

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Disease outbreak (due to vibrios) is a major constraining factor for sustainable development of the crustacean aquaculture sector. Since crustacean's innate immune system is considered to be primitive, lacking adaptive (i.e. long lasting and memory) features, animals were considered to be non-responsive to vaccination. However, more recently, invertebrate's innate immune system was reported to show some form of adaptive responses, termed trained immunity. The memory characteristics of innate immune system and the mechanisms behind such phenomena remain unclear. Using the crustacean model *Artemia*, we verified the possibility or impossibility of trained immunity, examining the presence or absence of enduring memory against homologous and heterologous antigens (*Vibrio* spp.) during a transgenerational study. We also determined the mechanisms behind the possibility of such phenomenon. Our results showed the occurrence of memory and partial discrimination in *Artemia's* immune system, as manifested by increased resistance, for three successive generations, of the progenies of *Vibrio*-exposed ancestors towards a homologous bacterial strain, rather than to a heterologous strain. This increased resistance phenotype was associated with elevated levels of the signaling molecules *hsp70* and *hmgb1*, and alteration in the expression of key innate immunity-related genes. Our results also showed stochastic pattern in the acetylation and methylation levels of H4 and H3K4me3 histones, respectively, in the progenies whose ancestors were challenged. Overall results suggest that the innate immune system of the crustacean can be trained to induce long-lasting anti-bacterial effectcs, and epigenetic reprogramming of (selected) innate immune effectors is likely to have central place in the mechanisms leading to trained immunity.

PARENTAGE ASSIGNMENT USING MICROSATELLITE LOCI IN A PROJECT FOR AQUACULTURE OF *Argyrosomus regius*

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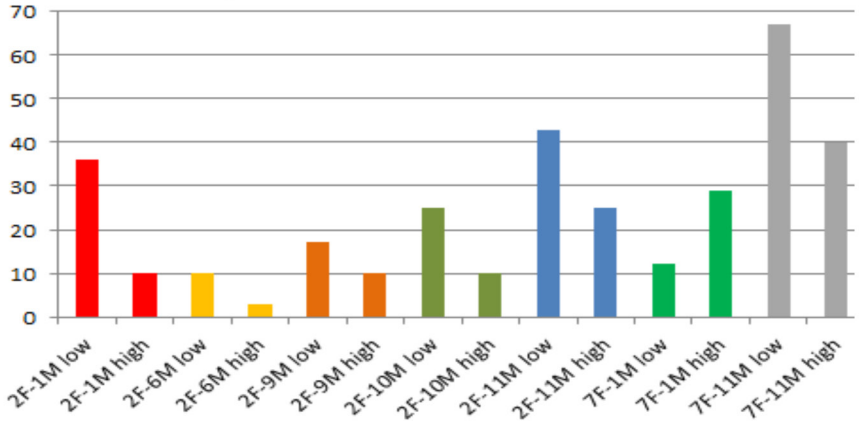
Argyrosomus regius is a fish species that is currently being studied for its potential in large scale aquaculture. Our main aim in this study is to assign the offspring to their parents and to make assumptions that potentially show a pattern of the weight attribute among the families. 19 breeders (8 females and 11 males) were used and 400 of their offspring were selected for low weight. Breeders and progeny were analyzed with two multiplex PCRs using 10 microsatellite loci. Parentage was assigned at a rate of 93.5% with Vitassign. The dominance of some parents is evident. The individuals we assigned were farmed on 4/5/2016 and were selected for low weight. Accordingly there was a population of 400 individuals selected for high weight from the same 19 breeders, which were assigned in another study . Among the two populations, differential composition of some families was encountered.

DIAGRAM1. Differential composition of families. Parents are depicted on X axis and families on Y axis.



REDUCING CRUDE PROTEIN LEVELS IN THE DIETS OF JUVENILE *Litopenaeus vannamei* THROUGH SUPPLEMENTATION OF ESSENTIAL AMINO ACIDS

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Protein is qualitatively the main nutrient associated with tissue synthesis in animals. In aquaculture, high protein diets are often used to meet the minimum amino acid (AA) requirements. Use of supplemental AA has been shown to provide opportunity to minimize excess levels of dietary proteins in other animal species including fish. Commercial shrimp feed often contains 35-40% crude protein (CP) or even higher, depending on feed type or life stage of shrimp. The present study investigated the effect of reducing the amount of CP in diets for the whiteleg shrimp raised in intensive green-water conditions. In addition, the study evaluated response of shrimp to varying levels of total methionine (Met) content at each CP level. Four sets of feeds were prepared containing (% on a fed basis, mean ± sd) 31 ± 0.7, 34 ± 0.4, 37 ± 0.4 and 40 ± 0.1% CP, respectively. Total dietary Met level (% on a fed basis, mean ± sd) evaluated in each set of diet include 0.56 ± 0.02, 0.71 ± 0.01, 0.88 ± 0.02 and 1.04 ± 0.02%. Feeds were formulated applying the ideal protein concept with a fixed amount of 5% fishmeal (as is), and dietary supplementation with DL-Methionyl-Methionine (AQUAVI® Met-Met). Dietary CP were gradually decreased by using more of other supplemental AA. Juvenile shrimp of 1.00 ± 0.08 g were stocked in 84 outdoor tanks of 1 m^3 under 100 shrimp/m^2. Five rearing tanks were randomly assigned for each dietary CP and Met level. Shrimp were fed from 4 to 8 times daily over a continuous 75-day rearing period. At harvest, shrimp were counted, individually weighed, and their growth performance and feed efficiency were determined. Shrimp final survival exceeded 83% and was unaffected by CP and Met content. On the other hand, yield was significantly depressed when shrimp were fed the 31% CP diet, Shrimp grew at a weekly rate between 0.79 and 0.97 g, achieving a final body weight between 10.8 and 11.6 g. There was a significant interaction between CP and Met over body weight. Shrimp fed 0.6% Met achieved the lowest weight at harvest. Increasing the CP content beyond 34% did not result in a further improvement in weight (Fig. 1). With a dietary Met content of 0.7%, the highest mean body weight was achieved with the 34% CP diet compared to other CP levels (Fig. 1). There was a significant improvement in FCR when CP was raised from 31 to 34%. Similarly, dietary Met levels above 0.7% resulted in a significantly better FCR compared to 0.6%. From the present study, it was possible to determine that dietary CP and Met can both have an individual and combined effect over shrimp performance and feed utilization. Juveniles of the whiteleg shrimp can maximize their growth performance and feed efficiency when fed practical diets containing a total CP and Met (Met+Cys) content of 34 and 0.7% (1.22%), respectively. A reduction in the dietary CP from 34 to 31% leads to poor survival, reduced yield and increased FCR. Similarly, reducing dietary Met from 0.7 to 0.6% deteriorates FCR. On the other hand, levels of dietary CP beyond 34% are not required, as long as a minimum of 0.7% Met and other EAA are met through a well-balanced supplementation with crystalline sources.

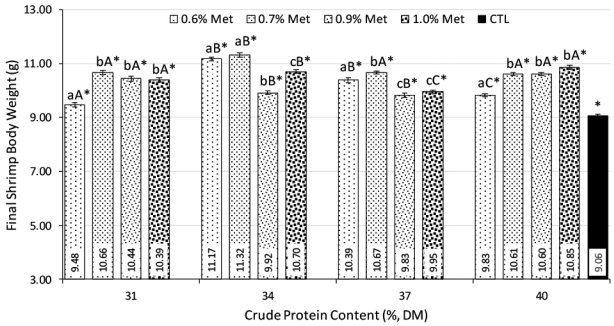

Figure 1. Mean (± se) shrimp body weight (g).

DISRUPTIVE AND FORWARD-LOOKING OPPORTUNITIES FOR COMPETITIVE AND SUSTAINABLE AQUACULTURE

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Aquaculture is expected to supply over 60% of fish by 2030.Every 1% growth in EU aquaculture delivers 3000-4000 jobs in SMEs. Contrary to 6.1% average yearly growth worldwide, EU aquaculture is stagnant. Amidst other challenges, growing cost and volatility of fish feeds are the primary obstacles. Insect protein can replace up to 100% of proteins in salmon diets, and marine invertebrates are an important fraction of the natural diet of farmed fish. Food security, environmental health and sustainable development are global challenges, thus more sustainable aquaculture is crucial. Producing alternative feeds while improving their availability and reducing their footprint and cost, is a must for competitive aquaculture.

The EUSME/EMFF funded EU Project INvertebrateIT aims to help aquaculture operators mitigate their current dependence on costly, volatile, and unsustainable fish feeds, to diversify their business and to contribute to a better management of valuable organic waste and/or new algal substrates for invertebrate production. The proposed integrated scheme builds on available technology in invertebrate production and strategic policy for the aquaculture and waste sectors.

The main results and specific achievements expected from INvertebrateIT are to:

- Develop 3 demonstration projects setting up PPPs
- Build up critical mass and visibility, "occupying" a new industrial niche
- Increase awareness to exploit invertebrate production for Blue Growth and the Circular Economy

To accomplish the first goal, an open contest was organised and three projects selected for further capacity building:

- **EntoGreen** in Portugal is dedicated to insect rearing and processing and will upscale production, contributing to the marine economy and sustainability in rural areas.
- **MUSFLOUR** in Spain seeks to revalorise waste from the mussel industry with the aim of obtaining flour to be used in fish feed.
- **IPATAC** in France produces high-quality protein for aquaculture and will soon commercialise, in collaboration with farms and retail, the first trout in Europe fed with insects, aiming to build up consumer support.
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INvertebrateIT is led by public and private entities in an effort to gather together regional and national maritime clusters to develop sustainable aquaculture, inform marine policy and create innovation and entrepreneurship through knowledge transfer.

AN ASSESSMENT OF CONSTRAINTS TO AQUACULTURE PRODUCTION IN CENTRAL NIGERIA

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High cost of animal protein has forced people to rely on fish. In sum, Nigeria's fish import has been growing owing to the decline of her artisanal fisheries output. It is hoped that aquaculture will alleviate this problem. However, while aquaculture contribution to total fish is high in some developing countries, its contribution to total fish supply in Nigeria is far less than 10 percent, although it is growing at about 20% per annum in recent time. Serious developmental effort at aquaculture is still nascent. Hence, aquaculture is bedeviled by problems. This study assessed constraints to fish production in North Central, Nigeria using Kogi State as a case study. A three staged random sampling technique was used to select 200 cat fish farmers in the state. Data obtained through structured questionnaire were analysed using mean score from a three point Likert type of scale. The findings indicated that the major constraint to cat fish production in the state is high cost of feed (M=2.80). This could be attributed to the fact that feed stuff and most inputs are sourced externally. Other problems include inadequate finance (M=2.61) and lack of encouragement from government (M=2.30). Amongst others, provision of inputs to cat fish farmers at subsidized rate was recommended.

Table 1: Mean Score on Constraints to Aquaculture Production in North Central, Nigeria

Constraint	Mean	Rank
Cost of feed	2.80	1st
Inadequate finance	2.61	2nd
Lack of encouragement from Government	2.30	3rd
Predator	2.20	4th
Inadequate electric power supply	2.02	5th
Inadequate research in aquaculture	1.86	6th
Lack of storage facility	1.89	7th
Poor harvesting tools	1.61	8th
Cost of fingerlings	1.36	9th
Inconvenience source of drug	1.34	10th
Flood	1.11	11th
Water pollution	1.07	12th

COMMERCIAL *Artemia* BIOMASS PRODUCTION SYSTEM USING DISTILLERY AND WINERY WASTES IN AUSTRALIA BASED ON PHILIPPINES PARADIGM

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Successful commercial production trials were conducted in the 1980s using integrated brine shrimp *Artemia* – poultry flow through systems in salt ponds. Optimisation of *Artemia* production was subsequently investigated in Mindoro Occidental, Philippines that can support daily biomass harvests for aquaculture use. Feed experiments were carried out to compare various agricultural wastes, with emphasis on livestock manure and sugar-mill effluent (vinasse). Feeding trials based on dry weight, microbial cell counts and different waste mixtures and processing were implemented to compare the waste input and *Artemia* output.

Aerobically-digested sugar mill vinasse and liquid manure showed significantly superior performance compared to mono-sodium glutamate by-product and unaerated vinasse or undigested manure. Growth rates in treatments where feed rate was based on cell volume calculations were significantly better than those fed according to dry weight basis.

Large-scale production of *Artemia* biomass in ponds are hampered by insufficient supply of suitable feed. With an *Artemia* adult clearing at least 20-ml pond water daily, based on natural algal productivity at best fertilization regime, a consistent supply of large volume agricultural waste as food source is required. This food source must support a daily standing crop of 0.2-1kg cu.m^{-1}., equivalent to 25-100 adult *Artemia*/L, to allow up to 10% daily biomass harvest. Feeding rate to produce this *Artemia* biomass range would be 25-100mg dry weight $L^{-1}day^{-1}$, a feed concentration above 5 g L^{-1} on a dry weight basis or an algal/microbial cell volume above 0.05ml per ml feed.

In New South Wales, Hunter Region in Australia, distilleries, wineries and breweries produce significant amounts of wastes and by-products. For example distilleries produce as high as 100 tons day^{-1} sugar vinasse. This in turn incurs expenses in disposal and poses serious environmental hazards if accumulated over time. Laboratory trials using these wastes were conducted to study the best waste source and processing for *Artemia* feeding, with a view to produce highest quality *Artemia* biomass as food for aquaculture.

Distillery raw sugar and dextrose vinasse, gave promising outcomes as a sustainable food source for *Artemia,* singly or in combination with winery or other wastes. Using parallel Philippine experience with sugar-mill vinasse, the implication of these findings for development of a continuous intensive *Artemia* biomass production system that also help reduce the local industry's ecological footprint are discussed.

OXIDATIVE STRESS, BIOCHEMICAL, LIPID PEROXIDATION AND ANTIOXIDANT RESPONSES IN *Clarias gariepinus* EXPOSED TO IVERMECTIN DRUG

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Objective: To investigate short-term Ivermectin (IVMT) toxicity on *Clarias gariepinus* juvenile with reference to antioxidant enzyme activities, lipid peroxidation (LPO) and serum biochemistry. **Methods**: Fish specimens were exposed to acute concentration of IVTM at 24h, 48h, 72h and 96h exposure durations. The antioxidant enzyme profile assessed included: glutathione reductase (GR), Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). Serum glucose, protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also determined. **Results:** The 96 h LC_{50} of IVMT was 15μgL^{-1}. $LC_{10\text{-}90}$ recorded values which significantly varied ($p<0.05$) in the different durations of exposure. LPO showed biphasic response with initial elevations and significant ($p<0.05$) inhibition with increase in duration of exposure. Significant dose dependent increases ($p<0.05$) were obtained in SOD, CAT and GR when compared to control. However, GPx significantly decreased. Dose and time dependent significant ($p<0.05$) increases in the liver function enzymes was obtained. Protein decreased, while Glucose showed a mixed trend. **Conclusions:** Changes in the antioxidant enzyme activities indicated that IVMT caused oxidative damage in *C.gariepinus*. The formation of oxygen radicals may be responsible for the toxicity of Ivermectin.

THE VENTURA SHELLFISH ENTERPRISE: OPPORTUNITIES FOR MUSSEL FARMING IN SOUTHERN CALIFORNIA

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The Ventura Harbor in southern California is a very active diverse port where the fishery for market squid (*Doryteuthis opalescens*) is the largest commercial fishery. The Port Commissioners, seeking to increase and diversify commercial landings, began exploring the feasibility of long-line mussel farms in the area. One impediment to creating a mussel farming sector in the area is the high cost of permitting, making it out of reach for most entrepreneurs.

To overcome this hurdle, the commissioners and a diverse group of interested parties created the Ventura Shellfish Enterprise (VSE) to pre-permit mussel farming leases off the coast of Ventura and then sublease them to individuals interested in mussel farming, who thereby avoid the complex and expensive permitting process.

The VSE is a multi-party initiative to permit twenty 100-acre plots for growing the Mediterranean mussel (*Mytilus galloprovincialis*) via submerged long lines in coastal waters within the Santa Barbara Channel near Ventura Harbor. VSE partners include Ventura Port District, Coastal Marine Biolabs, The Cultured Abalone Farm, and Ashworth Leininger Group, in coordination with aquaculture scientists and experts including the California and NOAA aquaculture coordinators, and California Sea Grant. Supported by a grant to the Ventura Port District from the National Oceanic and Atmospheric Administration (NOAA) through the National Sea Grant College Program, the Ventura Port District will obtain all the permits and entitlements needed to cultivate mussels on leases that will be farmed by commercial fisherman and other entrepreneurs with mussels landed at existing off-loading facilities in the Ventura Harbor. Coastal Marine Biolabs, a nonprofit research-based science education organization with facilities in the Ventura Harbor organized a comprehensive public outreach campaign and a series of workshops to inform commercial fishermen, consumers, Ventura residents and the public at large of the project's features, benefits and impacts. The VSE represents an opportunity for interested parties to develop mussel farming operations next to one of the largest seafood markets in the world.

NUTRIFISH PROJECT: INSECT PRODUCTION FROM COFFEE WASTE FOR FEEDING VALUABLE FISH SPECIES

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Introduction: Intensive fish farming relies on the use of feeds based on fish meal (FM) and oil (FO) as optimal sources to ensure fish growth, health and nutritional quality. Because of the high demand for these ingredients by the aquaculture industry and the impossibility for further exploitation of the ocean resources, their inclusion levels in commercial aquafeeds has drastically been reduced during the last years. Consequently, the search for alternatives to FM and FO represents a major challenge for the sustainability of intensive aquaculture. Alternatives must ensure fish health and welfare standards by providing proper feeding stimulants, proper levels of indispensable amino acids and PUFAs, high nutrient and energy bioavailability, reduced antinutritional factors. Due to a minimal environmental impact, compared to most conventional feed commodities, insects deserve a growing attention as candidate ingredient for aquafeeds. They are cultured through environmental-friendly, cost-effective farming processes, since they can be produced on by-products/wastes. They are rich in proteins with an essential amino acid composition similar to that of FM, and in lipid content. However, compared to that of FM, the fatty acid profile is poor in PUFAs (particularly in omega-3 and omega-6). Nevertheless, the lipid quality can be manipulated by culture techniques because insects show high plasticity in their nutritional characteristics, dependent on the growth substrate. Certain marine microalgae combine medium-high levels and good quality of proteins with high omega-3-6 contents, making them ideal, ecologically-sound ingredients, for the improvement of the insect growth substrates. This opens the way for enhancing the nutritive value of insect meal in order to use it as an alternative to FO and FM. However, deeper scientific knowledge, beyond the current state of the art, is necessary on crucial aspects of the biological responses of fish to major changes in the nature of protein and oil source of the diet.

Project description: NUTRIFISH is a project which proposes well-performing, cost-effective and eco-sustainable new fish diets based on the circle economy concept. To this aim, the research will be focussed on feeding strategies for fish production by investigating the use of insect meal as a novel ingredient, obtained from *Hermetia illucens* prepupae, cultured on coffee industry wastes enriched with microalgae. A multidisciplinary approach will be applied including five work-packages aimed at (i) improving the strategies for insect production and quality (in terms of nutritional value and safety as a feed in aquaculture) by adding to the growth substrate proper amounts of eco-sustainable microalgae rich in polyunsaturated fatty acids (PUFA); (ii) formulating innovative test diets for fish; (iii) investigating the biological responses of fish to the formulated diets in laboratory feeding trials with experimental model (zebrafish), through an integrated approach assessing fish health, welfare, growth and appetite with conventional and innovative tools (e.g. FT-IR spectroscopy); (iv) evaluating the overall quality of the best diet (identified through the lab-scale experiments) for sturgeon culture in a local aquaponics fish farm, and assessing its potential benefits (as a source of PUFA) on consumers; (v) assessing the overall environmental impact and the cost-effectiveness of the experimental fish diets by Life Cycle Analysis (LCA).The project provides new scientific knowledge and competitive feed formulations to be transferred to feed industries, farmers and other stakeholders to foster the economic growth of the aquaculture industry and its sustainability, and opens new opportunities to young scientists for collaboration and learning.

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NEW INSIGHTS ON THE MACROMOLECULAR BUILDING OF RAINBOW TROUT INTESTINE: FTIR IMAGING AND HISTOLOGICAL CORRELATIVE STUDIES

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Introduction
In fish species, the integrity and control of the intestinal barrier is often attenuated by both nutritional and immunological challenges. At this regard, many traditional analytical techniques and methods are available for gut analysis, including visual inspection and microscopy analysis, as well as biochemical, molecular and proteome assays. Nevertheless, they are normally expensive, time-consuming and laborious. Currently, there is a growing interest in the application of the Fourier Transform Infrared (FTIR) spectroscopy to the biochemical field. This is a fast, label-free analytical technique, which analyses the vibrational transitions induced on matter by the interaction with the electromagnetic radiation in an appropriate continuous range of infrared frequencies. It couples a quick response in terms of biochemical composition of the sample to low cost-effectiveness and short time of sample preparation and data acquisition. The coupling of IR spectrometers with visible microscopes led to the development of FTIR Imaging spectroscopy (FTIRI) which allows performing the infrared imaging analysis of non-homogeneous biological samples, such as tissues and cells. In the present, for the first time, FTIRI spectroscopy was applied to analyze samples of rainbow trout (*O. mykiss*) medium and hind intestinal tracts, with emphasis on the mucosa macromolecular composition. A comparison of the FTIRI results with more traditional histological analysis was also performed.

Material and methods Fish: Trout were reared in different $1m^3$ fiberglass tanks at the University of Udine aquaculture station and the intestines (midgut and hindgut intestine) were immediately sampled and stored for further analysis. **Histological analysis:** Five samples of the intestine (midgut and hindgut intestine, in triplicate) were fixed by immersion in 4% paraformaldehyde and stored at 4°C overnight. Samples were then washed in PBS 1X, dehydrated in an ethanol series and embedded in paraffin. Slides of 4 μm sections (cut with a microtome LEICA) were stained Mayer's hematoxylin and eosin, Masson's trichrome staining with Aniline Blue solution (Bio Optica), and PAS (Periodic Acid Schiff, Hotchkiss-Mc Manus, Bio Optica) and examined under photomicroscope (ZEISS, Axio Imager 2, Germany) . **FTIRI analysis**: FTIRI measurements were carried out at SISSI beamline, Elettra Sincrotrone Trieste, in transmission mode (spectral resolution 4 cm^{-1}) using a Bruker VERTEX 70 interferometer coupled with a Hyperion 3000 Vis-IR microscope and equipped with a liquid nitrogen-cooled bidimensional FPA detector.

Results and Discussion: FTIRI analysis not only confirmed, in both MI and HI samples, that the distribution of the most represented macromolecules followed the well-known arrangement of intestine tissues, but also provided, through a semi-quantitative information obtained from univariate analysis of the spectral data, a proper outline about the macromolecular composition and building blocks of rainbow trout intestinal mucosa. Thus, the spectral outcomes evidenced by FTIRI spectroscopy, together with the analytical semi-quantitative results, can provide a deeper knowledge about the biochemical composition and health status of the intestine mucosa. Therefore, this fast, label-free analytical technique can be considered a complement to traditional histological analysis.

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THE POWER OF PERCEPTIONS: IN SEARCH FOR SOCIAL ACCEPTANCE

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Environmental challenges, especially challenges with sea lice, have been emphasized as the most prominent risk towards sustainability in the salmon aquaculture industry in Norway. Alongside, it has been a rise in public awareness and demands towards the industry. Even though the industry is seen as an important global producer of food, the industry faces critiques and challenges with social acceptance from the public, consumers, markets, and policy makers. Lack of social acceptance and legitimacy can be a constraining factor for further growth in the industry. Important for political decision-making and societies risk perception is the public discourse, as seen in media and in other arenas. Participation in the discourse, and gaining discursive hegemony give actors power of definitions, which can be decisive for how the aquaculture industry is judged by the public. Further, discourses and its structures can also constrain the set of actions feasible for political actors.

We have studied the debate about salmon aquaculture to discover different mechanisms and processes where various actors influence and try to influence how salmon aquaculture is perceived and how the industry should be regulated. The perception of risk, and consequently how one responds to such risk, can be changed by social interactions. Faced with public demands of sustainability, authorities seek good approaches to regulate the industry towards environmental sustainability. From the industry perspective, for being deemed as trustworthy and legitimate, it is important to demonstrate how they act upon public concerns and regulatory demands.

Salmon aquaculture is based on production in the commons, and in order to make natural resources governable, we must choose representations for nature. By translating the ungovernable complexity of nature into a defined governable object we attempt to measure, control and manage these representations. However, the processes of constructing a governable object, and what is chosen to become a governable object, have consequences for the perception of the industry and its social acceptance. In the aquaculture discourse, we find that environmental risks are emphasized, while other risks (and sustainability dimensions) are displaced. Also, there is a simplification of the risks emphasized, e.g. how sea lice are presented as a risk and a governable object. The complexity of creating governable objects and the uncertainties within the knowledge base which political decisions rest upon is not communicated to the public. This means that the underlying uncertainties related to the possible consequences of sea lice and the treatments that are used are seldom part of the public debate. Excluding these uncertainties contributes to black boxing; when the public is not aware of the uncertainties underlying regulatory measures, this could leave an impression of an unwilling industry, and conclusions about appropriate regulations may differ amongst stakeholders and policy-makers. Instead, the public is left with an impression that the knowledge and technological solutions required to improve the industry's sustainability already exist. To improve the industry's social acceptance, it is necessary with a more nuanced debate about aquaculture.

PARASITES of *Oreochromis niloticus* OBSERVED IN THE FISHERIES UNIT OF THE NIGER DELTA UNIVERSITY TEACHING AND RESEARCH FARM

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Abstract
Parasites of Orechromis niloticus was studied at the Teaching and Research Farm of the Niger Delta University. The prevalence, abundance and intensity of infection were determined. The prevalence of Dactylogyrus parasitae in Oreochromis niloticus showed the highest value of 65%, followed by Cestode, ligula 60%, Ergasilus 10% and Protozoa and Nematode 1.66% with the least prevalence. The intensity of Dactylogyrus obtained was 2.79,while Cestode,(ligula) had 1.10,, Ergasilus spp 1.33, Protozoa and Nematode 1.2 respectively..

Introduction
Pond fish farming is faced with several constraints, among which are fish diseases (Hecht and Endemann, 1998; Roberts, 2001). Helminthes have being reported as among the predominant group of parasite that significantly affect fish in most of the fish pond in Africa (Paperna, 1996; Hecht and Endemnann, 1998). Helminthes of importance to fish are in groups of Nematode, *Cestodes and* Trematodes (Paperna, 1995). In addition, other parasites in particular protozoan such as Coccidian are also common (Hecht and Endemann, 1998). Fish parasites are of economic importance because they affect productivity through mortalities, decreased growth rate, reduced efficiency in feed conversion ratio and lower the quality of the fish meat (Aiello and Mays, 1998; Hect and Endemann, 1998).

Oreochromis niloticus, a member of the Cichlids family is one of the largest freshwater tilapia found in most tropical waters (Thistleton, 1986; Lamtane, 2008). This is the most common artificially raised species of fish in Nigeria, because it can be easily managed by farmers, has indiscriminant appetite even in poor water environment (de Graaf, 2004; FAO, 2005). It has been the focus of the major aquaculture efforts worldwide because the fish is easy to cultivate, adapts to wide range of environmental condition, grow fast, tolerant to high stocking density and is relatively resistant to stress and diseases (Robert, 2006).

Information on the parasite of fish become very necessary and important because apart from affecting fish tissues directly, fish are vectors of some parasite of human for example, helminthes. And they also cause huge economic loss in fish farming (Wooten, 1991).

Identification of parasites infecting *Oreochromis niloticus is required* in order to implement an effective therapeutic or prophylactic strategy if necessary in the Niger Delta University Teaching and Research Farm.

MATERIALS AND METHODS

Study Area
The study was conducted at the Teaching and Research Farm of Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State. It was established in 2010. Amassoma is located at about latitude 5^0N and longitude 6.05^0E in the Tropical Rain forest area of Niger Delta Area of Nigeria with an average temperature of 26^0C-30^0C.

Collection of Fish Samples
Sixty fish samples (*Oreochromis niloticus*) were collected from the 2 production ponds in the Fisheries Unit of Teaching and Research Farm in Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State. Seine nets was dragged in the ponds and samples selected on each sampling day. samples were examined in the laboratory for parasites. The sample size collected was according to Paperna (1996), a total of 60 *Oreochromis niloticus* were therefore examined for this study. The sampling was done in months of July and August 2015.

The standard length and total length of *Oreochromis niloticus* were measured with a meter rule. The weight of the fish was taken using a weighing balance. The sex of the fish was determined by observation of the external and internal organs. For male the genital opening is usually two pairs and redish in colours while the female genital opening is also two pairs rounded swollen and whitish.

(Continued on next page)

Examination of fish and identification of parasite

The fish were examined visually for ectoparasites mucous scraping were examined microscopically from the skin. Fish samples were dissected and various organs such as the gills, gills bladder, liver, flesh, stomach and intestine were collected, squashed and placed in a petridish containing normal saline, stained with parasitology iodine and examined under the microscope (Binocular and Stereo microscope) at x40 magnification for parasite.

The macro-parasite found were picked out with a pincher for processing and identification. The number of parasite per fish and organ location were recorded. The parasite were identified using the key according to Paperna (1996).

Results

The parasitic infection of *Oreochromis niloticus* considering the prevalence, intensity, Abundance is as shown in Table 1. The percentage of prevalence of *Dactylogyrus parasitae* in *Oreochromis niloticus* showed the highest value of 65%, followed by *Cestode*, *ligula* 60%, Ergasilus 10% and Protozoa and Nematode 1.66% which is the least prevalence. The intensity of *Dactylogyrus* is a beat 2.79% value which *Cestode*, *ligula* 1.10%, *Ergasilus spp* 1.33%, Protozoa and Nematode 1.2%.

Table 1: Parasite incidence in *Oreochromis niloticus*

PARASITE	PREVALENCE	INTENSITY	ABUNDANCE
Cestode(ligula)	60%	1.10	0.7
Dactylogyrus	65%	2.79	1.81
Ergasilus spp	10%	1.33	0.13
Protozoa	1.66	1	0.03
Nematode	1.66	2	0.03

Results showed that parasitic infection rate on the gill was 119%, on the intestine it was 42%, while rate of infection on the skin was 2%. The *Dactylogyrus* infection on the gills recorded the highest value of 119% followed by the intestine 42%. It was observed that the blood and the body cavity were not infected as shown in Table 2.

Table 2: The rate of parasite infection on different organs of *Oreochromis niloticus.*

PARASITE	BLOOD	GILL	BODY CAVITY	SKIN	INTESTINE
Cestode.ligula	0	0	0	0	42
Dactylogyrus	0	109	0	0	0
Ergasilus sp	0	7	0	1	0
Nematode	0	2	0	0	0
Protozoa	0	1	0	1	0
TOTAL	**0%**	**119%**	**0**	**2%**	**42%**

Parasitic infections of *Oreochromis niloticus* based on the sex, is shown in Table 3; the percentage of total parasitic counts is 40% for the males. Parasite found on male are; *Cestode ligula* 45%, *Dactylogyrus* 40%, *Ergasilus sp* 12.5%, protozoa 0%, Nematode 2.5%. Furthermore, the total percentage count for females *Oreochromis niloticus* is 44% and parasites found are *Cestode ligula* 45.5%, *Dactylogyrus* 42.7%, Ergasilus 2.3%, Protozoa 4.5% and Nematode 0%.

(Continued on next page)

Table 3: Parasitic infection of *Oreochromis niloticus* based on sex

Parasite	**No of male fish infected**	**%**	**No of female fish infected**	**%**	**Total**
Cestode(ligula)	18	45	20	45.5	38
Dactylogyrus	16	40	21	47.7	37
Ergasilus sp	5	12.5	1	2.3	6
Protozoa	0	0	2	4.5	2
Nematode	1	2.5	0	0	1
Total	40	100	44	100	84

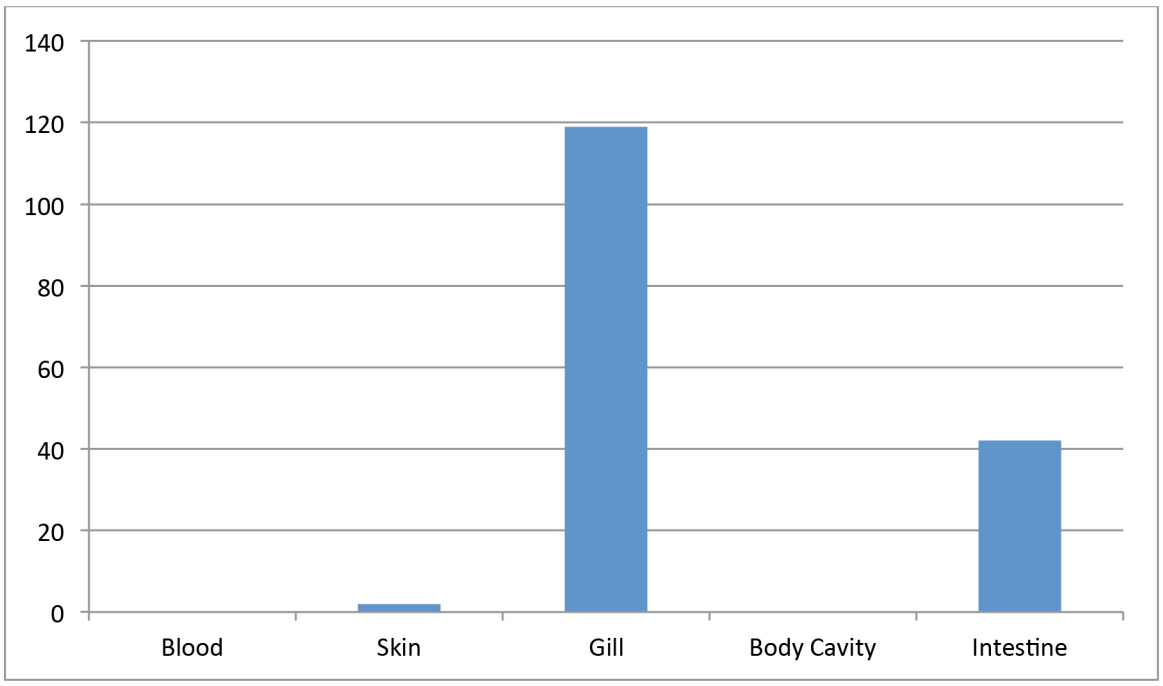
The parasitic infection of *Oreochromis niloticus* based on classes shown in table 3. Classes 8-11cm and 12-14cm show the highest total percentage count of 33% respectively, followed by 12% and the lowest total percentage count of 4% was recorded in the higher classes 20-22cm.

Table 4: Parasitic infection of *Oreochromis niloticus* based on size.

Size class(cm)	***Cestode.ligula***	***Dactylogyrus***	**Ergasilus**	**Protozoa**	**Nematode**	**Total**
8-11	12 36.4%	14 42.2$	4 12.1%	2 6.1%	1 3.0%	33 100
12-14	15 45.5%	14 42.4%	3 9.1%	1 3.0%	0 0	33 100
14.5-17	5 41.7%	3 25%	3 25%	0 0	1 8.3%	12 100
20-22	2 50%	2 50%	0 0	0 0	0 0	4 100

In terms of organ preference of parasites in *Oreochromis niloticus*, in figure (1) histogram was used to illustrate the most preferred organs for parasitic infection and the parasitic load in the host (*Oreochromis niloticus*). It was recorded that *Dactylogyrus* is the dominating parasite found mostly in the gill of the host at 119% followed by *Cestode.ligula* found in the intestine.

(Continued on next page)



Organ preference of parasite infection on *Oreochromis niloticus*

Discussion

Parasites may cause injury on tissues and blood vessels of host by burrowing into them or by blocking their blood vessels entirely with their bodies or their eggs thereby causing anaemia (Sarig, 1985). Fish with blood parasites may develop anaemia, listlessness, emaciation and pop eye, heavy infestation may result in death. The common fish blood parasites include trypanosome and trypanoplasma (Paperna, 1996).Roger and Geines (2000) reported that pathological changes in infected fish include lesion, inflammation of the intestine and severe "catarrh, hemorrhagic enteritis at the parasitic attachment point. (Kearn, 1998). *Microosporidian* infection has been described from many *Holarctic* marine and freshwater fish. There are very few reports of infections in fish in the tropics. *Microsporidian* infections were reported from Lake George, East Africa from the fin and bladder of *Haplochromis sp* (Paperna, 1973), and in the Republic of Benin, in gills and viscera of tilapia S*arotherodon sp*) (Sakiti and Bouix, 1987). *Microspoxidian* spores were also detected in kidneys of *Oreochromis aureus* and its hybrids. There are several factors that influence the occurence of parasitic diseases in fish. These factors are biological factors directly related to the fish and environmental factors related to the water body and culture system (Cedric and Neil, 2007).

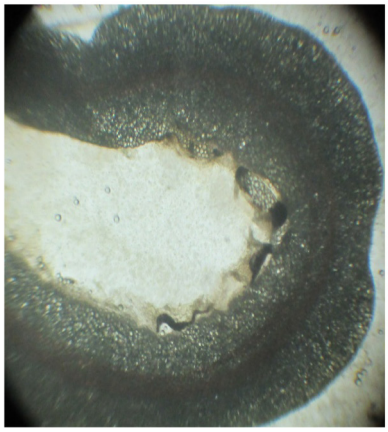


Plate 1: Nematode found from the intestine of male *Oreochromis niloticus*. Magnification x 40

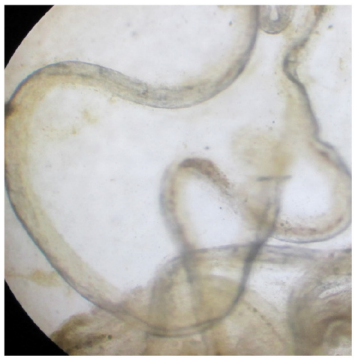


Plate 2: C*estode (Ligula)* found in the intestine of *Oreochromis niloticus* female. Magnification x 40

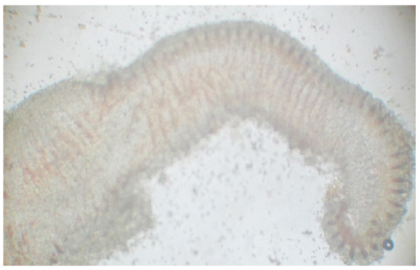


Plate 3: *Dactylogyrus* found in the gill of *Oreochromis niloticus*. Magnification x 40

(Continued on next page)

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EXTENSION OF ROTIFERS (*Brachionus plicatilis*) INCLUSIONS AND ENRICHMENT OF LIVE FOOD WITH PROBIOTIC IN THE LARVAL FEEDING REGIMES OF MUD CRAB (*Scylla paramamosain*): EFFECTS ON SURVIVAL, GROWTH, METAMORPHOSIS AND DEVELOPMENT TIME

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Mud crab (*Scylla paramamosain*) is an important aquaculture species with high commercial value. Although there were research focused on the time of withdrawal of rotifers from the larval feeding regimes, these results were ambiguous and contradicting. In addition, survival of hatchery-produce megalopa is still unreliable and inconsistent, mainly due to mass mortality during metamorphosis from zoea 1 (Z1) to Z2 and Z5 to megalopa caused by poor larval nutrition and selection of improper feeding regimes. The present study was conducted to determine the extending use of rotifer inclusion in the feeding regimes and to evaluate enrichment of live food with probiotics in the selected feeding regimes of mud crab larvae.

Trial 1: The five feeding regimes of mud crab larvae were tested by including rotifers onto them. Mud crab larvae in the first feeding regime were fed exclusively with *Artemia* (control), while those in feeding regimes 2, 3, 4 and 5 were fed rotifers starting from Z1 to various development stages of mud crab larvae whereas feeding with *Artemia* was commenced from the Z2 stage until the end of the trial (megalopa stage). In trial 2: two optimum feeding regimes of trial 1 *viz*. extending rotifer inclusion mixed with *Artemia* until Z4 and Z5 stages were selected. Rotifers and *Artemia* in these feeding regimes were enriched with a commercial probiotic (Lymnozyme FT-2B, Cisbay, USA). A 4 x 2 factorial experimental design with eight various combinations of enriched or un-enriched or a mixture of enriched and un-enriched live food (factor 1) in each of two feeding regimes (factor 2) was tested. Both trials were carried out on 1.5-L plastic beakers filled with 1 L of disinfected seawater (30‰). Each beaker was stocked with 30 healthy one-day-old mud crab Z1.

After 24 day of culture, trial 1 shows that extending inclusion of rotifers feeding until Z5 stage resulted in higher ($P < 0.05$) survival than in the control. Extending inclusion of rotifers feeding until the Z3, Z4 and Z5 stages also resulted in higher ($P < 0.05$) percentages of megalopa metamorphosis than in regime control, but did not significantly ($P > 0.05$) impact development time, and growth of megalopa (Table 1). In trial 2: there were no significant ($P > 0.05$) interactive effects between the two selected factors, so the main effect was independently examined for two factors (Table 2). The survival and metamorphosis rates of larvae fed both enriched rotifers and *Artemia* were higher ($P < 0.05$) than those of the larvae without enriched live food. Similarly, the extension of rotifers mixed with *Artemia* until Z5, irrespective of enrichment, showed higher ($P < 0.05$) survival, metamorphosis and growth of megalopa than when the rotifers were included only until Z4 stage. These results provided valuable information for a commercial crab hatchery.

Table 1: Survival, carapace width, body length, wet weight, metamorphosis and development time of megalopa fed five different feeding regimes.

Parameters	Feeding regimes				
	A	R2-A	R3-A	R4-A	R5-A
Survival (%)	7.80^{a}	10.0^{ab}	23.30^{ab}	25.60^{ab}	28.90^{b}
Carapace width (cm)	1.65^{a}	1.70^{a}	1.71^{a}	1.68^{a}	1.69^{a}
Body length (cm)	5.00^{a}	5.03^{a}	5.02^{a}	5.11^{a}	4.98^{a}
Wet weight (mg)	4.05^{a}	4.02^{a}	3.83^{a}	3.93^{a}	3.94^{a}
Metamorphosis rate (%)	2.20^{a}	6.70^{ab}	17.80^{b}	20.00^{b}	27.80^{b}
Development time (days)	22.50^{a}	19.67^{a}	18.00^{a}	19.33^{a}	17.00^{a}

Significant differences were found among all treatments with different superscript letters ($P < 0.05$) in the same row

Table 2: Survival, carapace width, body length, wet weight, metamorphosis and development time of megalopa fed various feeding regimes

Parameters	Factors					
	Enriched/un-enriched live food				Extension of rotifers	Interaction
	R & A	En-R & A	R & En-A	En-R & En-A	Z4 vs Z5	
Survival (%)	13.90^{a}	20.00^{ab}	21.70^{ab}	38.90^{b}	<	ns
Carapace width (cm)	1.63^{a}	1.64^{a}	1.63^{a}	1.66^{a}	<	ns
Body length (cm)	4.91^{a}	4.97^{a}	4.96^{a}	4.98^{a}	ns	ns
Wet weight (mg)	3.84^{a}	3.92^{a}	3.90^{a}	3.94^{a}	<	ns
Metamorphosis rate (%)	12.80^{a}	17.20^{a}	19.40^{ab}	37.20^{b}	<	ns
Development time (days)	19.00^{a}	18.30^{a}	18.50^{a}	18.0^{a}	ns	ns

Significant differences were found among all treatments with different superscript letters ($P < 0.05$) in the same row for enrichment. < or >: for variables with a significant effect of extension of rotifer inclusion, ns: non-significant ($P > 0.05$)

TECHNICAL EFFICIENCY ESTIMATES OF CATFISH FIRMS IN KOGI STATE, CENTRAL, NIGERIA

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This study was conducted to estimate the technical efficiency and understand the influence of education on technical inefficiency of fish farmers in Ijumu local government area of Kogi state. Multistage randomly sampling was employed in the selection of 100 fish farmers for questionnaire administration. The Cobb-Douglas stochastic frontier production function was applied to the data. Findings show that all Fish farmers in Ijumu local government area are educated and have moderate level of farming experience. The number of fingerling stocked and labour have significant positive effects on the volume of production but the return to scale of 0.88 was estimated. Technical efficiency estimates for fish farmers in the area range from 0.47 to 0.97 and averaged 0.88%. This means that increase in output by about 12% may be achieved through more efficient use of the existing input and technology. Contrary to general notion, formal education was found to increases technical inefficiency. Age and training were however negatively related to technical inefficiency. Therefore in order to achieve production at the isoquant frontier in Ijumu, policy attention needs to focus on variables other than education. Thus we recommend mentoring relationships between younger fish farmers need and older ones; as well Improvement in quality and numbers of training opportunities.

Table 1: Stochastic frontier estimates for catfish production

Variables	Parameter	Coefficient	t-ratio
Constant	β_0	1.36	2.42**
Pond size	β_1	0.11	0.88
Fingerlings	β_2	0.60	4.26***
Feed	β_3	0.19	2.62**
Labour	β_4	0.04	0.89
Drugs	β_5	-0.06	-1.85*
Inefficiency Model			
Constant	δ_0	3.38	2.64**
Age	δ_1	1.16	-2.91**
Education	δ_2	0.99	3.09***
Experience	δ_3	0.07	0.49
Training	δ_4	-0.18	-1.79*
Variance Parameter			
Sigma square	δ^2	0.069	4.9***
Gamma	Γ	0.71	7.1***
Mean technical efficiency	0.88		
Minimum technical efficiency	0.47		
Maximum technical efficiency	0.97		

***, **, * = Significant at 1%, 5%, 10%

FOAM FRACTIONATION AND OZONE: INTERACTION BETWEEN SALINITY, REDOX POTENTIAL, AND TOTAL RESIDUAL OXIDANTS CONCENTRATION

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The use of foam fractionation, as a key water treatment process in aquaculture operations, allow the removal of organic load (fine suspended solids) using gas bubbles as carrier material. This process is not only used in land-based recirculating production systems (RAS), but also for the treatment of intake, backwash recovery, and discharge water systems. Additionally, this technology can be used in fresh water, brackish water, and marine environments.

Foam fractionation, also called protein skimming, removes surface active substances that are produced in the farming process, or are present in the natural water source. The process itself involves a turbulent water and air flow pattern to produce the foam. This takes place in a contact column. The most important ingredients are the dissolved organic compounds and fine suspended solids. Therefore, the performance of the foam formation will depend mainly on the organic load and composition, but also the surface tension, salinity, bubble size, air-water ratio and contact time, among others characteristics of the water. The properties of the final foam in terms of stability and drainability are crucial and will allow a proper transport, flow, overflow, and flushing.

One of the most critical factors affecting the process of foam formation is bubble size, especially in fresh water systems or in marine systems after feeding with e.g. extruded oily feed. Practical observations in fresh water culture systems showed a great amount of foam, related to the extremely high organic load more than a non-ideal bubble size and/or a high coalescence. Furthermore, to enhance the effectiveness of the foaming process, ozone can be mixed into the bubbles. Ozone will enhance the natural electrostatic charge of the active substances, which will contribute to a better foam formation and stability. In fresh and brackish water treatments, the use of ozone will also reduce coalescence.

Results obtained from batch experiments with water containing different salinity (0-9-18-27-36psu) showed the relationship between redox potential (mV) and ozone concentration, expressed as total residual oxidants (TRO). The Michaelis-Menten kinetic explained these relationships with high accuracy, taking into consideration that the Michaelis constant value (Ks) is dependant of the salinity. At zero ozone concentration the redox potential value was 200mV for all salinities.

From the operational point of view, the foam fractionation process in waters below 12psu will need more bubbles in order to form a proper foam. This is in direct relation with the diameter and flow speed of the bubbles.

PUTTING CERTIFICATION SCHEMES INTO WATER - EXPERIENCES FROM CERTIFIED FISH FARMERS

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The salmon aquaculture industry is criticized for not being sustainable because of their use of feed and negative impact on local biotic and abiotic conditions, but is also often praised for its ability to produce protein and omega3 in a highly efficient manner. (Klinger & Naylor, 2012) For the general public it is difficult to deduce whether aquaculture products are sustainable or not, and there is a growing public awareness of how both aquaculture and traditional fisheries carries environmental risks. To relieve the doubt that consumers may have, ensuring that the seafood that is sold or consumed is sustainable has become a rapidly growing industry. Certification labels and schemes are available on a large scale, fashioned to respond to the many different roles that aquaculture has in society (i.e. employer, a user of marine space, a handler of animals, a food producer), and to assure the customer that the fish is produced in adherence to certain standards. But, does certifying production and produce have a positive effect on sustainability, or is it merely a marketing ploy?

Interviews with fish farmers in Chile and Norway confirm that the use of certification schemes is mainly about gaining access to the market. Wholesalers who need to protect themselves from negative publicity and litigations drive the demand for certification, while the costs of certification is placed on the manufacturer. On the other hand, the fish farmers also describe benefits that are both indirectly and directly related to sustainability. Norwegian fish farmers point to improvement in procedures, preparedness and increased disclosure of documentation as important effects. Also, requirements such as limitations on the number of medical lice treatments and copper free pens are considered important. Chilean fish farmers emphasize in particular benefits related to the national environment, and in improving the relations to other stakeholders and activities in the coastal zone. Because the purpose of certification is commensurability that relies on standardized parameters and indicators there is however little room for variations in local environments and species-specific needs. Certification, thus, also leads to requirements fish farmers perceive to be counter to what they consider sustainable practices, for instance related to fish welfare.

One of the implications we can draw from this study is that “sustainability reporting” is one of the most important effects of certification in practice and that there are fewer direct effects on sustainability. The main challenge of using certification to promote sustainable production is intrinsic to what certification schemes are; - a standardized protocol to promote predictable and accountable behavior - undeterred by local circumstances and needs. This study thus sheds light upon one of the paradoxes of using certification schemes to promote sustainability; the gap that is created between the reality where sustainable actions take place and the virtual space where sustainability is envisioned.

TWENTY YEARS AFTER ITS EMERGENCE, WHAT HAS HAPPENED TO FISHFARMING DIVERSIFICATION IN FRENCH WEST-AFRICA (THE EXAMPLE OF COTE D'IVOIRE AND GUINEA)

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In the 1990's in West Africa, various assessments in West Africa converged: no promoted fish farming system responded to the rural population's demand in these areas (Prein et al, 1996, Morissens et al., 1993). Côte d'Ivoire and Guinea continue to benefit from various fish farming schemes of which some deal with the integration of fish farming inside the smallholder farming system. In 2013, following a period of crisis in Côte d'Ivoire, an official statement concluded that this kind of fish farming as a diversification from the cocoa economy was the first contribution to national aquaculture data. At the same time, in Guinea, the Government set up the "Direction Nationale de la Pisciculture" showing a willingness to integrate fish farming into the rural household as the basis of the national aquaculture sectoral policy.

Despite these positive signals, the development has been sometimes difficult and faces numerous challenges: at a macro level, it hasn't affected the increase in imported fish, which from China was over20 000 t/y in 2016 in Côte d'Ivoire. Neither has it met most international standards for promoting entrepreneurship, intensification and funding facilities, even though this type of production has numerous benefits (ecologically friendly, green intensification, ability to producecheap fish for a vulnerable population).At the villages' level, the on-going increase of ponds area (mostly dam-ponds) leads to heterogeneous situations: some places vegetate while others boost.

In order to deepen the diagnosis of theses dynamics, this paper is a summary of three approaches. An agro-economic assessment comparing profitability of the fish farming system against the profitability of cash crops and food crops, furthermore these results are reread at the fish value chain level. An anthropologic approach describes the nature of the relationship between fish farmers themselves, and between farmers and others stakeholders. A geographic approach attempts to show pond development in various places.

The main results are : urban markets drain the higher quality fish produced in most places (mostly big size tilapia) through a value chain managed mostly by women, consequently local distribution is made up of small size fish which face difficult competition from imported fish (frozen or smoked *Ethmalose*). The distributed added value is high but the chain suffers from information and coordination difficulties. Strong dynamics of spontaneous development can be seen in some places with key actors accompanying lots of new fish farmers with the installation, but the reality is that some seem in danger of collapsing, while others show very interesting innovation practices in pond design and in the organisation of fish farming and selling. The quantitative review shows an important under evaluation of these family farming systems and a lot of the dynamics are just ignored. On this basis, the paper questions the modalities of assisting these dynamics and shows that institutional assistance doesn't meet the demand.

TOXICITY OF ZINC OXIDE NANO-PARTICLES ON SOME ENZYMATIC PARAMETERS OF *Heterobranchus longifilis* AND AMELIORATIVE POTENTIAL OF ASCORBIC ACID

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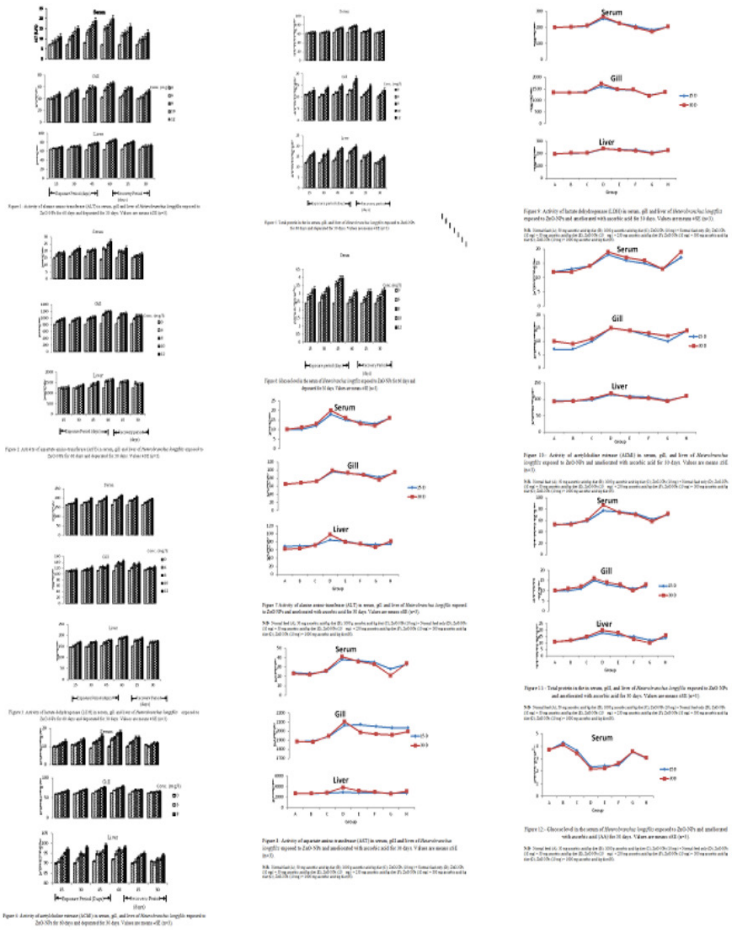
The rapid increase in the use of Nano-particles (NPs) of metal oxides in the production of consumer's products has greatly impacted the environment negatively. The study examined the effects of ZnO-NPs on the enzymes activities in selected tissues of *Heterobranchus longifilis*. Juvenile *H. longifilis* were exposed to sub-lethal concentrations (6, 8, 10, 12 mg/l) of ZnO-NPs for 60 days. The ZnO-NPs-exposed fish were subsequently subjected to depuration phase to evaluate recovery. Fish were later treated with graded levels of ascorbic acids (AA) diet (50, 250, 500, and 1000 mg/kg diet) for 30 days to ameliorate the ZnO-NPs toxicity. After each experimental stage, fish from the control, ZnO-NPs-exposed, depurated and ameliorated groups were sacrificed for enzyme assay from the blood, gill and liver.

There was significant increase ($P < 0.05$) in the activities of enzymes alanine aminotransferase (ALT); aspartate aminotransferase (AST); lactate dehydrogenase (LDH); acetyl cholinesterase (AChE); total protein and glucose (Figures 1, 2, 3, 4, 5, & 6) as the concentration and exposure period increased compared to the control. At depuration phase, there was a reduction in each of these parameters but none was comparable to the control. However, ZnO-NPs-exposed fish ameliorated with 500 mg/kg diet exhibited significant reduction ($P < 0.05$) in all the parameters (Figures 7, 8, 9, 10, 11 & 12) compared to the control. The results indicated that the release of ZnO-NPs into the aquatic environment is dangerous to fish and may also cause health risk in man through food chain, while 500 mg AA/kg diet is capable of ameliorating the toxic effects of ZnO-NPs on fish.



EFFECT OF THE DOMESTICATION PROCESS ON THE INTENSIVE REARING OF PERCH, *Perca fluviatilis*, LARVAE

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In animal husbandry process of domestication offers many advantages, but to date, the number of information on the domestication process and its effects on the fish is still insignificant. The Eurasian perch, *Perca fluviatilis* L., is one of the very promising candidates for diversification of European intensive freshwater aquaculture, but very limited data is still available on the first days of life of perch larvae under controlled conditions. In addition, biological parameters of the development of larvae from wild and domesticated specimens have never been compared before.

The Eurasian perch larvae (both wild and domesticated) were obtained after induced spawning and kept in identical recirculating aquaculture systems (RAS). The rearing protocol was the same for domesticated and wild larvae. In each rearing some features, as for example: total length (TL±0.01mm), wet body weight (WBW±0.1mg) swim bladder inflation effectiveness (SBI, %), hatching effectiveness (HE, %) and the percentage of deformation (%) were determined. Perch larvae were sampled in define development moments, such as: mouth opening, moment when 50% of fish starts to exogenous feeding, moment of weaning onto dry feed and at the END of the experiment (after 10 days on dry feed).

Average WBW of perch larvae is presented on Fig.1 ($p<0.05$), while TL at the END of the experiment was 14.09±0.46mm for wild and the 18.28±1.48mm for domesticated ones ($p<0.05$). The SBI was 41.3±15.6 % for wild and 33.7±4.9%, for domesticated larvae ($p<0.05$). The HE was 62.5±15.8% for wild and 63.7±15.7%, for domesticated ones ($p>0.05$) and the percentage of deformed larvae (counted one day after hatching) was 5.4±2.8% and 4.0±2.9% for wild and domesticated larvae, respectively ($p>0.05$).

Obtained results let us conclude that rearing perch larvae obtained from domesticated females is more promising for the aquaculture sector. However, this are only the first results. To have the full picture of the situation it is need to conduct more detailed analysis.

This research was funded by the Polish National Sciences Centre (UMO-2016/21/D/NZ9/02519).

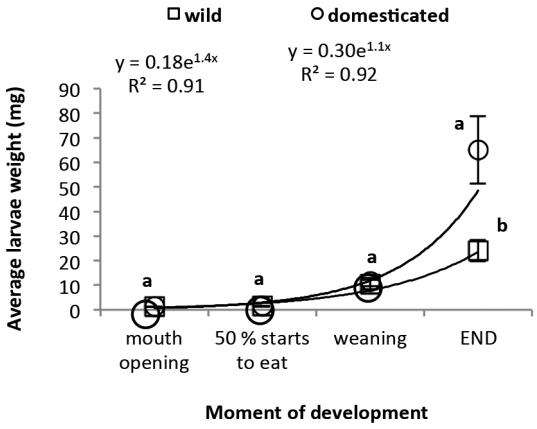

Fig. 1. Average weight (WBW, mg) of wild and domesticated perch larvae, during experimental rearing. Data (mean ±SD) marked with different letters were statistically different ($p<0.05$).

DAILY RHYTHMS OF *IN VITRO* FERTILIZATION IN FISH

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Predictable environmental changes (alternation of day/night, tides, lunar phases and seasons) have fostered organisms to develop a biological clock to keep track of time. Fish reproduction, as well as many physiological functions, exhibit rhythmicity to ensure the survival of the offspring. Furthermore, the timely secretion of *gonadotropin*s and sexual steroids is crucial to synchronize spawning and fertilization. Consequently, it should not be surprising a daily rhythm of *in v*itro fertilization, which could be used to optimize artificial fertilization protocols. The aim of this research is to investigate the existence of daily *in vitro* fertilization rhythms in zebrafish, as an experimental model, and in Senegalese sole (*Solea senegalensis*), as a fish species of great interest in aquaculture. To describe daily rhythms in light-dark cycle (LD) we performed *in vitro* fertilization trials at different times of the day. For zebrafish we performed fertilization at different Zeitgeber Times (ZT0, ZT1, ZT2, ZT3, ZT4, ZT7, ZT15, ZT21, ZT23) with lights on at ZT0 and off at ZT14. For Senegalese sole, we performed similar trials every 4 hours during a 24h LD cycle. The cosinor analysis ($p < 0.05$) revealed the existence of a statistically significant daily rhythm for *in vitro* fertilization with a maximum success around the specie-specific natural spawning time (diurnal or nocturnal for zebrafish and sole, respectively). In addition, one way ANOVA also unveiled statistically significant differences between fertilization time points ($p<0.05$). These results suggest that the success for *in vitro* fertilization highly depends on the time of day, which should be considered when establishing protocols for *in vitro* fertilization in broodstock fish farming.

Acknowledgements
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DAILY RHYTHMS OF EXPRESSION OF GENES INVOLVED IN THE REPRODUCTIVE BRAIN-PITUITARY-GONADAL AXIS OF TILAPIA (*Oreochromis niloticus*)

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In aquaculture, the control of reproduction is one of the main problems so it is necessary to expand the knowledge of this biological process. Fish show rhythms in many of their physiological variables, including reproduction. In most fish, reproduction is a seasonal phenomenon. Besides, the existence of daily rhythms in fish reproduction has been recently reported. Rhythms in neuroendocrine factors are present throughout all the levels of the BPG (Brain-Pituitary-Gonadal) axis, which guarantees the timing of reproduction to the most favourable environmental conditions to ensure the success of the spawn. The objective of the present study was to investigate the existence of daily rhythms in BPG gene expression in tilapia (*Oreochromis niloticus*).

The experiment was performed in the chronobiology laboratory located in the Faculty of Biology, University of Murcia (Spain). 38 males and 38 females of Nile tilapia (*Oreochromis niloticus*) were used. Males and females were kept in separated tanks during 4 weeks of acclimation to the laboratory conditions. Fish were maintained in a closed water circulation system, equipped with biological and mechanical filters. Water temperature was controlled at 29 ± 0.5ºC and the photoperiod was set at 12:12 LD (Light: dark), with lights on at 08:00h (ZT0) and lights off at 20:00h (ZT12). After acclimation, male and female tilapia were mixed in 6 groups in a sex ratio of 3:1 female:male. After 2 weeks, fish were sampled every 4 h during a 24 h cycle at the following time points: ZT2 (2 h after lights on), ZT6, ZT10, ZT 14 (2 h after lights off), ZT18 and ZT22. Samples were obtained from brain, pituitary, gonad, liver and plasma. Then the following genes from the BPG axis were analysed by qPCR: *gnrh1*, *gnrh2*, *gnrh3*, *kiss2*, *gnih* and *gnihr* in the brain; *fshb* and *lhb* in pituitary; *star*, *cyp17*, *cyp19a1a* (only females) and *amh* (only males) in the gonads; and *vtg* and *era* in female livers. Testosterone and estradiol were analysed by ELISA in male and female plasma, respectively.

In this study we demonstrated the existence of rhythms in gene expression of several factors from the BPG axis of tilapia. Maximum values were detected in most of them around the light offset. The results of the present experiment can be used to improve the reproduction protocols established in the aquaculture industry of tilapia as well as to have into account the optimum timing with the best environmental conditions for reproduction in captivity.

Acknowledgements: This research was funded by the project "SOLEMBRYO" (AGL2013-49027-C3-1-R) and "BLUESOLE" (AGL2017-82582-C3-3-R) granted by the Spanish Ministry of Economic Affairs and Competitiveness (MINECO) cofounded with FEDER, and "CHRONOHEALTH" granted by Fundación Seneca (19899/GERM/15) to FJSV.

1-MONOGLYCERIDES OF SHORT- AND MEDIUM-CHAIN FATTY ACIDS: AN INNOVATIVE APPROACH TO CONTROL *Lactococcus garvieae* IN RAINBOW TROUT, *Streptococcus agalactiae* AND *Streptococcus iniae* IN TILAPIA

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A specific composition of 1-Monoglycerides of Short and Medium Chain Fatty Acids (from C3:0 to C12:0), available on the market with the commercial name SILOhealth 128, proved to inhibit *Lactococcus garvieae, Streptococcus agalactiae* and *Streptococcus iniae in vitro* at pH 7, that is at a pH value that we find in the gut of several aquatic species. The trial was conducted by the Public Animal Health Institute Bruno Ubertini (Forlì – Italy). The MIC of SILOhealth 128 against these bacteria was comprised in a range from 0.01% to 0.1%. *Lactobacillus* was not inhibited. The MIC of butyric and formic acid against the pathogenic bacteria was >1%. 1-Monoglycerides resulted to be by 10–100 folds more effective than organic acids at pH 7.

Three field trials were conducted with *Oncorhynchus mykiss* in three farms in North Italy.

In a pond populated with 35,000 individuals with average weight of 300g typical symptoms of *L. garvieae* infection were observed: exophthalmos, dark skin, lethargy and a mortality of 30–50 fish/day. Liquid SILOhealth 128 was administered at a dosage of 1% of the feed, corresponding to 100mg/kg of live weight/day; the feeding rate was 1%. After 10 days the onset of the pathology was stopped: no new individuals showed any signs of the disease; the already infected individuals were not recovered, also because they stopped eating. SILOhealth 128 was administered until the end of the production cycle, to the commercial size of 400-500g. No more out-breaks of disease were observed.

In the second trial the initial typical signs of lactococcosis were observed in two ponds populated with about 40,000 and 45,000 individuals with average weight of 330g and 270g respectively: dark skin, exophthalmos, lethargy and mortality equal to 20–30 fish/day. The feed was supplemented with 1.5% of powder SILOhealth 128 and administered immediately after the first clinical signs occurred. The *L. garviae* infection did not spread and did not affect other individuals; the mortality increase stopped after 4 days of SILOhealth 128 administration and decreased close to zero in 12 days. The feed was supplemented with SILOhealth 128 during the subsequent 20 days and then a feed without supplementation was given. No more lactococcosis outbreaks were observed during the production cycle.

The third trial was conducted after grading procedure of trout on 20t of biomass in total. The fish size was 1kg. After grading the fish was placed into two ponds. The high density of 80 kg/m^3 and the stress facilitated the lactococcosis outbreak. Liquid SILOhealth 128 was administered at a dosage of 1.5% of the feed for 15 days and then at 1% up to the commercial size of 1.5kg. The mortality at the beginning was 30kg/day and it decreased to 10kg/day (less than 10 fish/day) in four days and remained constant up to the end of the production cycle.

In all the trials SILOhealth 128 proved to be effective in stopping lactococcosis spreading and in reducing the mortality, in some cases to zero, and prevented the recurrence of the disease until the end of the production cycle. In case of treatment with antibiotics like Erythromycin, a suspension period of 30 days must be respected before selling the fish for human consumption, with economic losses for the farmer. Furthermore, the usage of Erytromycin can create antibiotic resistance: in the farm practice it is observed that the first treatment with Erytromycin to control *L. garviae* infection usually lasts for 10 days. If a second treatment is required, the period of antibiotic administration may be extended to 15 days, due to the resistance created with the first treatment. SILOhealth 128 can be considered as a promising tool to control *L. garviae* in trout in alternative to antibiotics. Its usage is recommended in tilapia to prevent or to control infections caused by *S. agalactiae* and *S. iniae*.

TABLE I - MIC at buffered pH 7 of SILOhealth 128 or butyric acid or formic acid

	SILOhealth 128	Butyric acid	Formic acid
Lactococcus garvieae	0.1%	>1%	>1%
Streptococcus agalactiae	0.01%	>1%	>1%
Streptococcus iniae	0.01%	>1%	>1%
Lactobacillus plantarum	No inhibition	ND	ND
Lactobacillus acidophilus	No inhibition	ND	ND

1-MONOGLYCERIDES OF SHORT- AND MEDIUM-CHAIN FATTY ACIDS PROVED TO IMPROVE SURVIVAL RATE OF YELLOW TUNA *Thunnus albacares* AT EARLY DEVELOPMENTAL STAGES

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A trial was conducted at the University of Miami-Rosenstiel School of Marine and Atmospheric Science, Marine Biology and Ecology Division. Enriched rotifers (Ori-one®; Skretting, Norway) were supplemented with 1-Monoglycerydes of Short- and medium Chain Fatty acids before feeding the YFT larvae. 1-monoglycerides product contained a mixture of short and medium-chain fatty acids in powder form (SILOhealth108P; SILO, Italy).

Mass cultures of rotifers were reared in two plastic tanks of 1,000L and fed with a mix of yeast (Levapan® 0.3-0.5 g for each 10^6 rotifers day $^{-1}$ split into four rations) and live microalgae Nannochloropsis sp. (30-50% tank volume day $^{-1}$). The rotifers required for feeding the YFT larvae were harvested daily from the mass cultures and rinsed with filtered/UV seawater for 30-40 minutes. After this clean-up, rotifers were transferred into two enrichment tanks of 100L, one assigned to the control group and the other to the supplementation group. Rotifers in both units were enriched overnight for 12 hours (0.3-0.5 g Ori-one®10^6 rotifers^{-1}, mixed with 3L of water and blended for five minutes). In the case of the enrichment tanks receiving supplementation, 1-Monoglycerides product was blended along with Ori-one®. Enrichment solutions (control and supplementation) were fed the rotifers at three rations, 1L every four hours. Rotifers were supplemented with SILOhealth108P at 4% of the total feed (Ori-one®). After 12 hours of enrichment and supplementation with 1-monoglycerides, rotifers were harvested, cleaned-up and transferred into coolers set up with gentle aeration and oxygen supply. The rotifer population was estimated by counting and averaging three samples of 0.1mL, extrapolating to the cooler volume. YFT larvae were fed three times a day (6:30, 11:30 and 3:30 hours). Before each feeding, the residual rotifer density in the larval rearing tanks was estimated by counting and averaging the rotifers present in four 200mL samples, extrapolating to the larval rearing tank volume. The amount of rotifers required for maintaining a particular rotifers density was estimated and scooped out from the rotifer coolers. After delivering the first feeding of the day, ice bottles were used inside the coolers to keep the temperature low (10-12°C) and reduce the catabolism of enriched rotifers.

Fertilized eggs were collected from volitional spawning events occurred in the YFT broodstock of ITTC-Achotines Lab between 00:00 and 02:30 hours. After an estimation of the fertilization ratio and total egg number, eggs were incubated in 160L conical tanks. Larvae hatched approximately after 24 hours and were volumetrically estimated and equally distributed into the experimental tanks at 2 DPH.

The trial with larvae was carried out using three replicates per treatment consisting of 1,000L tanks, a stocking density of 22 YFT larvae L^{-1} and a supplementation level of SILOhealth108 equivalent to the 4% of the enrichment feed.

The mean number of fish produced was higher in the SILOhealth group and nearly double the mean number of fish produced in the control group (1,692 ± 308.5 vs. 824 ± 949.5).

In the control group, survival ranged from 0.9 to 8.5% whereas in the SILOhealth group it ranged from 4.9 to 9.6%. A total of 7,548 9 DPH YFT larvae were produced between both treatments, with the control group representing 33% and the SILOhealth group representing 67% of the total. The supplementation with 1-Monoglycerides yielded a two-fold increase in survival compared to the control group. 1-Monoglycerides might have contributed as additional energy sources, both providing the YFT with a higher nutrient uptake and improved performance. On the other side Short-and medium chain Chain Monoglycerides like Monobutyrin are known to have a positive impact on gut microbiota, shifting it towards beneficial lactic bacteria. Furthermore some 1-monoglycerides like Monobutyrin proved to stimulate tight junction expression and angiogenesis in gut mucosa, which may have contribute to gut development and nutrient utilization.

HEMATOLOGICAL CHARACTERIZATION OF HIGH TEMPERATURE STRESS ON ABALONE*, Haliotis discus hannai*

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Haliotis discus hannai is commercially important aquaculture species in South Korea. In South Korean, the production of *H. discus hannai* in 2017 was 16,027 tons and the value was $ 549 million. However, massive mortality had been occurred due to high water temperature during August-september 2017 and it will be able to produce serious threat of the abalone aquaculture industry in South Korea. Therefore, we will report hematological changes of H. discus hannai for seek to determine the mechanism of mortality on high water temperature.

We investigated hemolymph response of *H. discus hannai* using the flow-cytometry (apoptosis and ROS) and TEM from 18°C to 32°C at intervals of 2°C for 72 hours. Unfortunately, all abalones for test were dead before the 72 hour at 32°C and we could not be analyzed. The apoptotic rate was increased from 26℃ and it was significantly higher at 28°C than at 18°C and especially, it was increased as the exposure time longer in the same temperature. ROS was significantly increased from 28°C. The early apoptotic hemolymph was transformation that the body bodies were rounded and dilated endoplasmic reticulum, numerous vacuoles fibrillary aggregated in cytoplasm. The progressing apoptotic cells observed apoptotic body of nucleus and fibrillary aggregation occupied a large part of in the cytoplasm. The outer nuclear membrane was detached from the inner nuclear membrane, forming wide spaces. The late apoptotic hemolymph showed a condensed nucleus, vesiclulation Golgi complex and endoplasmic reticulum, in its cytoplasm. Finally, nucleus was hypercondensed with membranes missing and a number of fragmented apoptotic bodies were observed. Therefore, the decrease in apoptotic rate at 30°C and 32°C seems to be that increased dead cells and fragmented apoptotic bodies.

MORPHOLOGICAL AND BIOCHEMICAL STUDIES OF MANILA CLAM PARASITE UNKNOWN (MPX) IN THE MANILA CLAM (*Ruditapes philippinarum*) ON THE WEST COAST OF KOREA

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The Manila clam (*Ruditapes philippinarum*) is endemic to the Yellow Sea of Korea and China and is commercially important in this region. Mass mortality of the Manila clam has been occurring since the mid-1990s, with various kinds of pathogens infecting the clams, including *Perkinsus olseni*. In the present study, an unidentified protozoan parasite (MPX) of Manila clams collected from the western coast of Korea was isolated and characterized biochemically and morphologically and compared with *P. olseni*, which co-parasitizes this host. When trophozoites were incubated in Ray's fluid thioglycollate medium, MPX increased in size and was stained with Lugol's iodine, like *P. olseni*, but it was lysed with 2 M NaOH, unlike *P. olseni*. Moreover, MPX was not recognized by the *P. olseni*-specific polyclonal antibody or PCR primer. These findings suggested that the cell-wall composition and DNA sequences of MPX differ from those of *P. olseni*. PCR diagnosis using an MPX-specific primer pair showed that MPX was distributed in Manila clams collected from the south and west coasts of Korea but not in those collected from the east coast. Clams injected with MPX trophozoites showed approximately 50% mortality, indicating that MPX has high pathogenicity in the Manila clam.

EXTERNAL ULTRASTRUCTURE OF *in vitro*-CULTURED *Perkinsus olseni* ISOLATED FROM THE Manila CLAM *Ruditapes philippinarum*

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Perkinsosis is a major disease affecting the commercially important marine mollusk *Ruditapes philippinarum* (Manila clam) worldwide. In this study, we report the external ultrastructure of *Perkinsus olseni* cultured under laboratory conditions during different stages of its life cycle. Prezoosporangia that were formed after induction with Ray's fluid thioglycollate medium developed into zoosporangia, following the development of a discharge apparatus ranging in length from 9.8 to 18.6 μm. The discharge apparatus worked as a stopper and detached before zoospores were released. Biflagellated zoospores were ellipsoidal in shape (3.72 × 2.04 μm), with 2 flagella each; only the anterior flagellum had a unilateral array of mastigonemes. Liberated zoospores gradually transformed into immature trophozoites by losing the anterior flagella first and the posterior flagella later. The transformation of zoospores into trophozoites took approximately 2 weeks at 26°C. Mature trophozoites underwent schizogony by cell cleavage, and numerous merozoites developed into schizonts and were finally released by the rupture of the cellular membrane of the schizont within a few days. Our ultrastructural study will be useful for understanding the life cycle and propagation of *P. olseni*, which will benefit aquaculture systems around the world.

SOFT TUNIC SYNDROM IN THE EDIBLE ASCIDIAN *Halocynthia roretzi* DISTRIBUTED ON THE SOUTHERN COAST OF KOREA

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Halocynthia roretzi is a commercially important edible ascidian species in Korea; however, it has been facing mass mortality for several decades in spring and early summer on the southern coast of Korea. The associated disease is referred to as soft tunic syndrome (STS) owing to the characteristic softening of the ascidian tunics. Our recent studies demonstrated that STS is caused by *Azumiobodo hoyamushi*, a kinetoplastid flagellate exhibiting both flagellate and cyst stages during host infection. The flagellates invade the ascidian through the damaged cuticle layer of the siphon and spread to other parts of the tunic. Upon entering the ascidian, the parasite secretes protease and decomposes the tunic fiber bundles, resulting in STS. The optimal water temperature and salinity for *A. hoyamushi* growth and STS development are 10–15°C and 30–40 psu, respectively. Growth reduction of *A. hoyamushi* and STS disappearance occur under cultivation conditions beyond these values. The life cycle of *A. hoyamushi* consists of a flagellated phase and a cyst phase. Two types of cysts of *A. hoyamushi* were formed when the flagellates were exposed to hypoxia (<5 ppm) and high pH (>8.1): suspended cysts and adherent cysts, respectively. Adherent cysts are excysted in weakly acidic seawater (pH 6.8) within 1 to 3 days. However, excystment conditions for suspended cysts are not known. These findings suggest that adhesive cysts can be formed in the aquatic environment when they are released into sea water (pH 8.1) from hosts (pH 6.8), but most flagellated *A. hoyamushi* might transform into suspended cysts because a majority of the areas for ascidian culture on the southern coast of Korea are hypoxic during summer. Thus, the disappearance of STS in summer might be caused by the death of *A. hoyamushi*, while STS recurrence is boosted by the rapid proliferation of the remaining *A. hoyamushi* in various tunicate species at the bottom of the water column during winter, when the water temperature is low.

PERFORMANCE ANALYSIS OF GLOBAL VALUE CHAINS: A PROPOSAL OF INDICATORS FOR AQUACULTURE IN BRAZIL

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Tilapia is the most farmed fish in Brazil, with several poles of production dispersed in all regions of the country, each presenting significant differences in terms of production volume, processing and input structure, governance and division of value added between the agents. Therefore, the research purpose is to understand what factors influence for such differentiation of economic results between different poles of production and their respective value chains. Based on the Global Value Chain (GVC) approach, this research aims to define a set of indicators and then to construct an index capable of comparing the performance of different tilapia production zones, with reference to the six dimensions of the CGV. In spite of making possible a holistic analysis, the six dimensions of the GVC (i.e. Input-output structure; Governance; Upgranding; Local institutional context; Industry stakeholders) are essentially based on qualitative and subjective data, which makes difficult comparisons between different value chains. Thus, the first challenge of this research is to develop indicators that correspond to these dimensions of the GVC analysis. From these indicators, mostly qualitative, will be elaborated a quantitative index capable of measuring the performance and/or potential performance of the production zones of tilapia and their respective agents. The research methodology predicts the use of fuzzy logic in order to mathematically translate the qualitative /subjective information that will be collected in the field with the value chain agents and consequently to consolidate a quantitative index that is able to compare producers and value chains. As a result, it will be possible to identify the factors that influence the economic performance of producers and their value chains. In addition, this model of performance analysis of global value chains could be used in other aquaculture chains or even in other economic sectors, supporting strategic orientation at the level of public policies or of companies and producers.

EVALUATION OF MUSSELS SEED SURVIVAL *Mytilus edulis* DURING EARLY PERIOD OF COLLECTION, DEVELOPPMENT OF A NEW TOOL AND METHOD

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In France, today, more than 80% of farmed blue mussel seed collection is based on larvae fixation on collector ropes. Mussels are traditionally grown on bouchot, which are rows of wooden poles placed perpendicularly to the shore, stuck in the ground within the gently sloping intertidal strip. Mussels are transferred either as seed attached on collecting ropes or as juveniles placed in net sockings and then wound up around the poles or suspended to long-lines, where they will fatten and grow (Garen *et al.,* 2004).

In order to optimize seed collection and to limit unwanted biofouling on ropes, mussel growers local organizations identify best timing of seed collector ropes deployment by monitoring the number and size of planktonic mussel larvae from water column. Then, number of mussel seed collected on rope per meter is estimated every two weeks and for 4-5 months to establish initial reference data of « quality collection » according origin site (Centre Régional Expérimentation et Application Aquacole –CREAA-, 2017).

In Charente Maritime-Vendée, first shellfish-producing region (Agreste, 2012), monitoring of seed collection with this method over the last ten years showed that more than 50% of mussel seed collected on rope disappeared within May to July. The main deficiency of this method is that these high losses, from seeding to thinning, cannot be clearly attributed to mortalities, stalling, predation, spatial competition,…?

Here, we present the development of an original tool and method to assess precisely level of survival of mussel seed (1-5mm) collected on rope within the first months after spawning period. Our approach is based on little repeated pieces of rope put in semi-closed conditions witch prevent the loss of animals or empty shells.

Our study allowed to assess and compare survival level of seed from three different origins, placed in three stations from Pertuis Charentais (Bay of Biscay). In this region, although adults mussels suffered heavy mortalities (>60%, Normand 2017), average mortality estimated with the present method in seed was around 10% suggesting a less sensitivity of seed and spat to the phenomenon.

This work took place in 2016, in the context of massive mortality events of blue mussels in France since 2014, as a part of a specific project (MORBLEU) dedicated to identify the factors favoring the development of massive mortalities in farmed adult mussels.

SNP MARKERS FOR THE GENETIC CHARACTERIZATION OF MEXICAN SHRIMP BROODSTOCKS

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Mexican shrimp aquaculture is hampered by a high incidence of infectious disease outbreaks. To help mitigate outbreaks, Mexican shrimp breeding companies have introduced broodstock from Ecuador and other countries that have improved disease resistance compared to native lines, as indicated by higher survival rates in disease-affected environments. Selective breeding programs for genetic improvement of shrimp are at a formative stage in Mexico. A key element for these selective breeding programs is the characterization of genetic diversity and composition of stocks, as it will enable decisions on inbreeding restrictions, family structure, and the potential use of genomic selection. Single Nucleotide Polymorphisms (SNPs) are suitable genetic markers for this purpose. In this work, a set of SNPs was developed to identify and characterize current commercial breeding stocks in Mexico.

A total of 95 individuals from 21 putative lines from five commercial hatcheries were selected for DNA extraction and sequencing. The nextRAD genotyping service involved the amplification of selected genomic regions ('sites'), construction of individual Nextera libraries (Illumina) and sequencing in a HiSeq4000 Illumina platform. Reads were demultiplexed, filtered and aligned using de novo reference sequences for each site. Strict SNP calling and filtering parameters resulted in identification of 2,703 SNPs.

Genetic composition and diversity (heterozygosity, number of alleles per locus) were estimated for each breeding line. Genetic distance and clustering analyses was carried out to characterise the variation within, and differences between, broodstocks. Three main genetic clusters were found (2 from Ecuador, one from Mexico; Fig. 1), which can be further subdivided into five genetic groups (Fig. 2). Mixed stocks or mixed-origin individuals were observed in some hatcheries. A subset of 448 SNPs showing medium to high genetic differentiation levels between populations (Fst > 0.1) are candidate markers for stock identification and management. Three SNPs with Fst > 0.4 among 16 sexed individuals are proposed as candidate sex-linked markers. The usefulness of the marker sets for broodstock management and selective breeding programs is discussed.

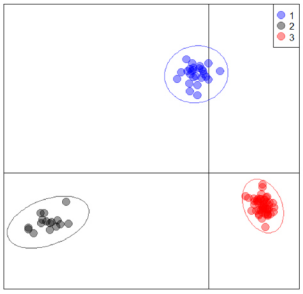

Fig. 1. Discriminant Principal Component Analysis of the genetic composition of the 95 samples from 5 shrimp hatcheries.

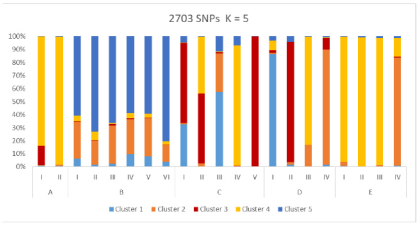

Fig. 2. Bayesian clustering analysis of 21 broodstocks of 5 shrimp hatcheries.

INTENSIVE CULTURE OF NILE TILAPIA *Oreochromis niloticus* IN BIOFLOC WITH REUSE WATER

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The freshwater dispute between different productive sectors limits the availability of good quality water to the aquaculture sector. Thus, it is necessary to prolong the useful life of this resource to guarantee production which can be accomplished using cultivation techniques that take advantage of traditional practices, for example, by using biofloc technology (BFT). The adoption of cropping techniques that both intensify production and increase the use of natural food resources, such as BFT, could reduce water use by up to 40%. Moreover, the reduction in water consumption could be greater if the same water is reused in multiple culture cycles. The objective of the study was to evaluate intensive culture of juvenile of Nile tilapia *Oreochromis niloticus* cultivated in biofloc with reuse water from BFT systems.

The study was evaluated over a period of 14 weeks. A completely randomized design was used with two treatment groups: tilapia culture in biofloc (TB) and tilapia culture in biofloc with reuse water (RWTB), with three replicates. The experiment was conducted using two macrocosm-microcosm systems. The systems consisted of one main culture tank or macrocosm (3 m^3) and three experimental units or microcosm (0.2 m^3). The quality of the water in the culture system was evaluated during the study. The systems were stocked with fish without sexual selection and maintained at a density of 100 fish m^{-3}. The biofloc was maintained with a ratio C:N of 12.5:1, molasses was added as a carbon source. Fish were fed a formulated feed daily (09:30 and 15:30 h) and the biometric data of the fish was obtained every two weeks. During the study the physicochemical parameters of the water were measured, in addition to the following productive variables: survival, final weight, condition factor, specific growth ratio, feed conversion ratio, fillet yield, proximal composition and somatic indices. For the data analysis, bifactorial ANOVA and one-way ANOVA was used.

The RW water parameters such as pH value, total dissolved salts, nitrite, nitrate and carbonate hardness were significantly greater ($p < .05$), when compared to those obtained in the TB treatment. Survival, productive performance, body composition and organo-somatic indices of Nile tilapia, were similar between treatments ($p \geq .05$). The results suggest that the intensive culture of Nile tilapia in biofloc can be developed with reuse water from BFT systems, without adverse effects on their survival, productive performance and body composition.

Table 1. Productive performance of the Nile tilapia *Oreochromis niloticus* cultivated in biofloc with reuse water.

Physicochemical parameters of the water	TB	RWTB
T (°C)	24.73 ± 2.3	24.71 ± 2.3
DO (mg L^{-1})	6.16 ± 1.2^a	5.96 ± 1.3^b
TDS (mg L^{-1})	853.3 ± 283.9^a	1335.6 ± 134.4^b
NH_4-N (mg L^{-1})	0.81 ± 0.3	0.80 ± 0.2
NO_2-N (mg L^{-1})	0.94 ± 0.7^a	1.47 ± 0.7^b
NO_3-N (mg L^{-1})	37.45 ± 39.2^a	51.94 ± 42.9^b
NH_3-N (mg L^{-1})	0.02 ± 0.03	0.02 ± 0.03
pH	6.90 ± 0.6^a	7.28 ± 0.4^b
SS (mL L^{-1})	33.36 ± 8.3	33.81 ± 13.3
GH (mg L^{-1})	214.80 ± 0.0	214.80 ± 0.0
KH (mg L^{-1})	42.96 ± 20.6^a	65.99 ± 31.3^b
Productive variables		
S (%)	100 ± 0.0	98.75 ± 2.5
IW (g)	79.83 ± 12.8	78.74 ± 15.9
FW (g)	163.09 ± 42.3	159.23 ± 38.9
K	3.99 ± 0.3	4.01 ± 0.3
SGR (% d^{-1})	0.72 ± 0.2	0.70 ± 0.2
FCR	2.22 ± 0.7	2.40 ± 1.0
Fillet yield (%)	21.83 ± 2.1	22.39 ± 2.9
Proximal composition (%)		
Moisture	77.31 ± 0.9	77.35 ± 0.5
Protein	19.11 ± 0.5	19.08 ± 0.7
Lipids	2.24 ± 0.6	2.22 ± 0.2
Ash	1.36 ± 0.1	1.34 ± 0.1
Somatic indices (%)		
Gonadosomatic	2.12 ± 1.7	2.48 ± 1.7
Hepatosomatic	1.78 ± 0.6	1.95 ± 0.6

TB= tilapia culture in biofloc, RWTB= tilapia culture in biofloc with reuse water, T = temperature, DO = dissolved oxygen, TDS = total dissolved salts, NH_4-N = ammonium, NO_2-N = nitrite, NO_3-N = nitrate, NH_3-N = non-ionized ammonium, SS = settleable solids, GH = general hardness (as CaCO3), KH = carbonated hardness (as CaCO3), S = survival, IW = initial weight, FW = final weight, K = condition factor, SGR = specific growth ratio, FCR = feed conversion ratio. Rows with different superscripts present significant differences ($p < .05$).

COMPENSATORY GROWTH OF NILE TILAPIA *Oreochromis niloticus* SUBJECTED TO CYCLIC FASTING AND RE-FEEDING PERIODS IN BIOFLOC SYSTEM

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During the commercial culture of fish, the variation in the availability and quality of feed are factors that limit the growth and, therefore, the production. However, after a period of fasting or with limited feed, fish can exhibit a greater growth rate when subsequently provided with a high-quality feed, a condition known as compensatory growth. The application of the compensatory growth technique in various aquaculture fish species can bring benefits associated with a better utilization of the feed and feed logistics. In this way, it is important to evaluate the compensatory growth in Nile tilapia *Oreochromis niloticus*, which is a species that supports more than 90% of the tilapia production worldwide.

The study was evaluated during a 144-day period. The experimental design involved five treatment groups with three replicates, in which the frequency of re-feeding (R) and fasting (F) were varied by day: R3:F1, R9:F3, R18:F6, R24:F8, F36:F12, in repeated cycles during the culture period, the control group received feed daily. The culture was developed in 18 circular geomembrane tanks (3 m^3), at a density of 50 fish m^{-3} (7.81 ± 1.91 g, 5.92 ± 0.73 cm). The biofloc was maintained with a ratio C:N of 12.5:1, molasses was added as a carbon source. Fish were fed a formulated feed daily (32% protein and 5 % lipids; 09:30 and 15:30 h) and the biometric data of the fish was obtained every 12 days. During the study the physicochemical parameters of the water were measured, in addition to the following response variables: survival, final weight, final length, coefficient of variation, condition factor, specific growth ratio, coefficient of compensation, total feed consumed, feed conversion ratio, total biomass, proximal composition and somatic indices. A one-way and two-way analysis of variance and covariance was used, as well as a t test for a sample to evaluate significant differences between treatments.

The parameters of water quality, except temperature, were within the acceptable range for the Nile tilapia culture. Complete compensation in weight was achieved in R18:F6 and R36:F12 (Table 1). The proximal composition of the Nile tilapia was not affected; however, a reduction in hepatosomatic and gonadosomatic indexes was observed. Different cyclic fasting and re-feeding strategies permitted a saving of between 25 – 38 % in feed, where the treatments R3:F1 and R36:F12 reached a similar production as the control. The results indicate that Nile tilapia can achieve complete compensation when cultured in biofloc under regimes of re-feeding and fasting for periods as long as in R36:F12. The exploitation of the compensatory growth in Nile tilapia could potentially reduce labour and production costs during its culture with biofloc technology.

Table 1. Productive performance of the Nile tilapia cultivated in biofloc under a cyclic regime of fasting and re-feeding.

PARAMETER	CONTROL	R3: F1	R9:F3	R18:F6	R24:F8	R36:F12
S (%)	95.09 ± 1.1	96.56 ± 3.7	97.05 ± 0.7	90.68 ± 2.4	97.79 ± 3.8	93.62 ± 7.1
FW (g)	168.71 ± 36.7^{a}	150.49 ± 33.6^{bc}	142.50 ± 26.2^{cd}	159.57 ± 30.7^{ab}	136.08 ± 34.7^{d}	163.94 ± 34.4^{ab}
FL (cm)	15.79 ± 1.2	15.51 ± 1.2	15.24 ± 1.0	15.69 ± 1.0	14.99 ± 1.2	15.66 ± 1.1
CV (%)	22.25 ± 3.9^{ac}	22.91 ± 3.6^{a}	21.03 ± 3.9^{ab}	20.16 ± 4.4^{bc}	21.83 ± 4.9^{ab}	19.88 ± 3.6^{b}
K	4.19 ± 0.8^{a}	4.24 ± 0.7^{b}	4.22 ± 0.6^{b}	4.23 ± 0.5^{b}	4.19 ± 0.7^{ab}	4.33 ± 0.7^{c}
SGR (% día^{-1})	1.97 ± 1.8	1.92 ± 2.0	1.87 ± 1.8	1.91 ± 1.9	1.82 ± 2.0	1.93 ± 1.9
CC	-	1.07 ± 0.3 ^{ab}	1.05 ± 0.2 ^{a}	1.25 ± 0.2 ^{b}	1.13 ± 0.5 ^{ab}	1.13 ± 0.2 ^{ab}
TFC (kg)	169.5 ^{a}	113.1 ^{b}	107.1 ^{b}	113.1 ^{b}	105.1 ^{b}	128.3 ^{b}
FCR	2.64 ± 1.4	2.31 ± 1.5	1.93 ± 0.8	1.67 ± 0.7	2.20 ± 1.3	1.76 ± 0.5
TB (kg m^{-3})	7.08 ± 0.5^{ab}	6.63 ± 0.5^{ac}	6.23 ± 0.1^{bc}	6.14 ± 0.2^{c}	6.35 ± 0.2^{bc}	7.30 ± 0.3^{a}

R = re-feeding days, F = fasting days, S = survival, FW = final weight, FL = final length, CV = coefficient of variation, K = condition factor, SGR = specific growth ratio, CC = coefficient of compensation, TFC = total feed consumed, FCR = feed conversion ratio, TB = total biomass, rows with different superscripts represent significant differences between treatments ($p<0.05$).

COMPOSITION OF BACTERIAL BIOTA LIVING IN THE BIOFLOC AND INTESTINE OF NILE TILAPIA *Oreochromis niloticus* CULTIVATED IN BFT WITH DIFFERENT FEED RATIONS

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Biofloc technology (BFT) is a farming system that allows the proliferation of microorganisms, mainly bacteria. The bacterial consortium is necessary to the function of the biofloc system; however, it is also important to better understand the biofloc system since the culture medium is suitable for the development of both non-pathogenic and pathogenic bacteria. Current research indicates that bacteria in the culture medium in BFT systems affect the shrimp intestine by modifying bacterial biota. In this sense, a possible influence of the bacteria, present in the biofloc, on the bacterial biota of the digestive tract of the Nile tilapia *Oreochromis niloticus*, specifically those of the intestine, is potentially high. The aim of this study was to evaluate the bacterial composition present in the water and in the intestine of Nile tilapia cultivated in biofloc, and to determine its productive performance under different feed rations.

The study was carried out over a four-month period. A completely randomized design with a single factor was employed. The control group was considered as a complete feed ration and the fish were cultured in the culture system containing clear water. The treatment groups were established in the culture system with biofloc: B-100, complete feed ration 100 %; B-90, reduction by 10%; B-80, reduction by 20%; B-70, reduction by 30%, and B-60, reduction by 40% of the feed. For the study, 18 circular tanks (3.14 m^3) were used which held a density of 50 fish m^{-3} each. Fish were fed twice daily with a balanced commercial feed; the fish were also weighed and measured every two weeks to evaluate growth and feeding performance. For the quantitative and qualitative evaluation of bacteria, 15 mL of water was taken from the experimental tanks at the end of the study, and two fish per tank were sacrificed to extract 5 cm of intestine. The CFU count was performed on plaque, and bacterial identification was carried out using PCR, amplifying the 16s rRNA gene. For data analysis, one-way ANOVA was performed ($p < 0.05$).

Twenty species of bacteria were identified, 17 in water and 15 in the intestine. The results showed that bacteria present in both water and intestine were statistical higher in B-70 and B-60 compared with the other treatments. In culture water biofloc treatments, we found *Micrococcus sp* (49.8 – 73.6 CFU mL^{-1}), *Aeromonas hydrophila* (33.0 – 61.4 CFU mL^{-1}), *A. sobria* (50.0 – 81.2 CFU mL^{-1}), *Pseudomonas cepacia* (57.6 – 93.0 CFU mL^{-1}), while we found *Bacillus badius* (119.8 – 160.0 CFU mL^{-1}), *B. subtilis* (83.8 – 136.4 CFU mL^{-1}), *P. cepacia* (40.8 – 146.0 CFU mL^{-1}) in the intestine samples. The productive performance of tilapia in B-100, B-90 and B-80 treatments was similar to the control treatment (Table 1). The results suggest that is possible to reduce 20% of the balanced feed ration without negatively effecting the survival or growth in the Nile tilapia. The contribution of bacteria to the productive performance of tilapia is related to a natural probiotic effect.

Table 1. Production variables (mean ± SD) for different feed relations for Nile tilapia *Oreochromis niloticus* using biofloc technology.

Variable	Control	B-100	B-90	B-80	B-70	B-60
S (%)	84.50±1.33^b	90.87±1.60^a	89.60±0.97^a	91.93±0.37^a	88.96±1.33^a	90.23±1.84^a
Wf (g)	138.51±4.64^a	140.15±4.15^a	141.38±5.45^a	138.27±6.0^a	112.08±6.88^b	75.51±12.93^c
WG (g)	135.3±4.75^a	136.94±4.20^a	138.17±5.44^a	135.06±6.26^a	108.87±6.99^b	72.30±12.91^c
DWG (g d^{-1})	1.12±0.03^a	1.14±0.03^a	1.15±0.04^a	1.12±0.05^a	0.90±0.05^b	0.60±0.10^c
K	2.51±0.41	2.53±0.38	2.59±0.42	2.61±0.45	2.78±0.71	2.37±0.59
FCR	1.36±0.02^a	1.31±0.06^a	1.17±0.02^b	1.03±0.09 ^{c}	0.97±0.03^c	1.04±0.02^c
TB (kg m^{-3})	5.85±0.12^a	6.36±0.10^b	6.33±0.11^b	6.35±0.04^b	4.98±0.01^c	3.40±0.14^d

Control = complete feed ration and system containing clear water, the treatment groups were established in the culture system with biofloc: B-100, complete feed ration 100 %; B-90, reduction by 10%; B-80, reduction by 20%; B-70, reduction by 30%, and B-60, reduction by 40% of the feed, S = survival, Wf = final weight, WG = weight gain, DWG = daily weight gain, K = condition factor, FCR = feed conversion ratio, TB = total biomass. Rows with different superscripts represent significant differences between treatments ($p<0.05$).

COLLAGEN TYPE-1 DIFFERENCES IN FARMED CHINOOK SALMON *Oncorhynchus tshawytscha* IN NEW ZEALAND

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Introduction: The integrity and function of collagen type-1, a fundamental structural molecule, is central to fish movement. Farmed Chinook salmon in New Zealand are reported to develop a late onset curvature syndrome(1) in which Lordosis, Kyphosis and Scoliosis (LKS) is associated with excess collagen and fibrosis(2). LKS affects fish movement and the efficiency of production. Matching husbandry conditions as closely as possible, collagen type-1 abundance and crosslink profile in a population where LKS is prevalent (farm 1) was compared with a population where LKS is rare (farm 2).

Methods: The properties of type-1 collagen were investigated using Liquid Chromatography Electrospray Ionization Mass Spectrometry. Triplicate samples from two regions of production salmon (n = 9), targeting the muscle and myosepta compartment, were analysed. Hydroxyproline concentration [Hpro] and the collagen crosslink profile is reported. A mixed linear model with farm, region and their interaction as fixed effects, and fish nested within farm as a random effect was fitted to the data.

Results: There was a significant (~2 fold) difference between farm 1 and farm 2 for [Hpro]. PYR (mature crosslinks) were readily detectable in fish from farm 2 but below the threshold for reliable detection in fish from farm 1. Mature crosslinks (HHMD) were ~ 3 fold higher in fish from farm 1 than from farm 2. Immature cross links were 3-4 fold higher (DHLNL) and ~ 4 fold higher (HLNL) in fish from farm 1.

Discussion: The reasons why collagen abundance and crosslink profiles of farmed salmon could differ, alongside population and husbandry differences are presented. The location and maturation of type-1 collagens, in relation to the condition factor of the fish, are discussed.

PYR - Pyridinoline; HLNL - Hydroxylysinonorleucine;
DHLNL – Dihydroxylysinonorleucine; HHMD - Histidinehydroxymerodesmosine.

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THE TROPHIC CARRYING CAPACITY OF THE THAU LAGOON: DEALING WITH HUMAN ACTIVITIES AND ECOLOGICAL CONSERVATION

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The decrease of nutrients inputs from the watershed has long dominated Thau lagoon ecosystem management objectives. Phytoplanktonic biomass and abundance have drastically decreased for more than a decade and *Zostera* meadow have gradually recovered, expressing lagoon ecosystem restoration. Do the progressive recovery of the ecosystem health and associated development of carrying capacity possibly threatens the shellfish industry? To provide answers about the right balance to be achieved between conservation and exploitation, numerical tools are developed to help in decision-making. We hereby propose to adapt an existing lagoon ecosystem box-model, by incorporating a Dynamic Energy Budget type shellfish production model.

Model reliability was tested using global sensitivity analysis, to provide an evaluation tool of admissible nutrients fluxes from the watershed in the context of shellfish exploitation and lagoon ecosystem restoration. The carrying capacity of the lagoon was hence determined according to different scenarios of nutrient inputs related to projections of population growth or improvement of wastewater treatment plants. The effect of shellfish farming practices on the lagoon trophic carrying capacity was also investigated with regards to ecological states indices used within EU the water frame directive.

Monitoring OF THE PRESENCE OF CARP EDEMA VIRUS (CEV) IN AQUACULTURE OF THE CZECH REPUBLIC in 2017

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In the last few years, increasing of spring mortality of common carp in European ponds was recorded. It affected mostly larger, almost market-sized fish. In 2011, first detection of a virus from the case of spring mortality of carp was noticed in CEFAS (Way et al., 2013). It was very similar to Carp Edema Virus (CEV; family Poxviridae) which was isolated from koi suffering from Koi Sleepy Disease in Japan in the late 1970s (Oyamatsu et al., 1997; Miyazaki et al., 2005). Since then, CEV-like virus has been considered as one of possible causal agents of SCMS (Lewisch et al., 2015). Clinical as well as pathological symptoms of the „CEV disease" in carp noticeably remind koi herpesvirus disease (KHVD) but in case of CEV, the symptoms and mortality usually evolve in lower temperature (8 – 20 °C). In the Czech Republic, mortality caused by CEV was first confirmed by PCR in 2015 (two-round PCR developed by CEFAS was used) in archived samples from 2013 and 2014 (Vesely et al., 2015). Since that time, the diagnostic of increased mortality of carp in ponds after the winter was focused on CEV, especially when the clinical and pathological signs were as lethargy, asphyxia, gathering of fish at the surface and near the shore or inflow, irregular mucus layer on the skin, sunken eyes, necrotic gill etc. and the temperature of water ranged from 5 to 13 °C. Till the end of 2016, sixteen suspect cases of "spring carp mortality" were investigated by PCR. Seven from them were CEV DNA positive.

In 2017, the national grant project supported by Ministry of Agriculture of the Czech Republic started. Twenty localities were investigated from 30th March to 28th June. Four from them were koi breeds, sixteen carp ponds. In all localities, increasing mortality was reported. Testing for CEV presence employed a two-round PCR method developed at CEFAS, with external primers yielding a product of 528 bp, and internal primers yielding a product of 478 bp (Way et al., 2014). Nine localities were CEV DNA positive (3 koi, 6 carp) but not all fish from these localities were positive although they showed similar signs.

In 2017, the first case of autumn mortality of carp connected with the presence of CEV was registered. This outbreak started some days after the transfer of harvested market-sized fish to a storage pond. In this case, all investigated fish were CEV DNA positive. Mortality reached 100 % during three weeks.

It is necessary to continue in the monitoring of the presence of CEV in carps suffering from typical clinical and pathological signs in spring and autumn to understand better the role of CEV in these epizootics and the pathogenesis of this disease.

Acknowledgement:
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FISH PARISITES WITH AQUACULTURE RELEVANCE IN MECKLENBURG-WESTERN POMMERANIA

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The parasite fauna and feeding ecology of 375 wild freshwater fishes belonging to ten species mainly from northern Germany (Lake Malchin, Lake Hohen Sprenz, Mecklenburg-Western Pomerania (MV)) and Latvia (Lake Baltezers) were studied between 2011 to 2017. *Abramis brama* (bream), *Alburnus alburnus* (bleak), *Anguilla anguilla* (eel), *Blicca bjoerkna* (silver bream), *Carassius gibelio* (prussian carp), *Gymnocephalus cernua* (ruffe), *Perca fluviatilis* (perch), *Rutilus rutilus* (roach), *Scardinius erythrophthalmus* (rudd) and *Tinca tinca* (tench) were investigated. In total **74 parasite species**, 11 protozoan and 63 metazoan, were detected, whereby **new host** and **locality records** for German inland waters could be provided.

The purpose of this detailed investigation was to compensate the underrepresented field of fish parasitology in MV. Beside the development of precise identification methods for ectoparasites of the genera ***Argulus*, *Ergasilus*, *Diplozoon*** and ***Paradiplozoon***, which have a high damage potential to fishes especially in farms, the importance of this research was shown by the discovery of a **human pathogenic** roundworm. The achieved results are **transferable** to other North European ecosystems. Moreover, the results improve the **control of pathogens** in fishery industry and aquaculture facilities in MV. The detected parasite fauna, combined with ecological issues, has a high relevance for future research work in the field of aquatic pathology, ecology and aquaculture.

The knowledge gained from this previous work will now be used for a new project "**Fish health monitoring in MV**". Within this study the potential risk (transfer of pathogens) from wild fishes to aquaculture fishes is going to be examined. For this purpose there will be a comprehensive fish health monitoring in aquaculture facilities where besides the **water quality**, **parasites** but also **bacteria** will be analyzed.

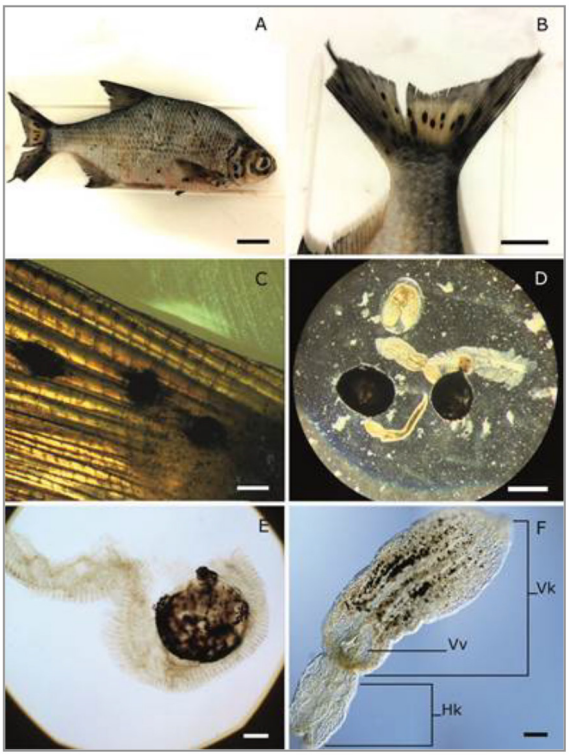

Figure 1: Digenea *Posthodiplostomum cuticula* on silver bream (*B. bjoerkna)*. VK: front part of the body, HK: hind part of the body, Vv: ventral hollow

TAKING A RIDE WITH COD - NORWEGIAN SALMON IN THE BRAZILIAN SEAFOOD MARKET

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Norwegian salmon aquaculture has been a success story in terms of innovations in production technology, marketing and regulations. New markets are important to secure the future value of the industry and the foundation for additional growth. Brazil is Norway's closest trade partner in Latin America and a big emerging economy. Currently, Brazil buys salmon from Chile, but the long history of trade in cod between Norway and Brazil and the reputation of Norwegian fish in Brazil provides a potential entry point for Norwegian salmon in the emerging Brazilian seafood market. The expansion of seafood markets requires the understanding of how different seafood products interact with each other (e. g. substitution effects). The higher the degree of integration in the markets, the easier for aquaculture to expand if it is competitive in existing markets and can influence price. Moreover, given the global market for salmon, understanding the market in Brazil that is mostly supplied by Chile, means also to understand part of the bigger picture. The Chilean salmon production is the second largest in the world, and the salmon supplied by Chile to Brazil means a reduction in supply to other market in direct competition with Norwegian salmon.

This paper uses market integration analysis to investigate Norway's potential market expansion into Brazilian market for salmon and for Brazil´s role as an increasingly important part of the global salmon market. The multivariate Johansen test was applied on monthly prices of seafood categories from the wholesale market CEAGESP (São Paulo wholesale market) for the period between 2014 and 2017 (Fig. 1).

Results indicate a common market for the domestic whitefish group, but not when considering salmon. This suggests that salmon is not competing in the same market with the other domestic whitefish in Brazil. Thus, considering the global market for salmon, Norwegian salmon potential

market expansion would be depending more on its interaction with the Chilean salmon than with the whitefish market in Brazil.

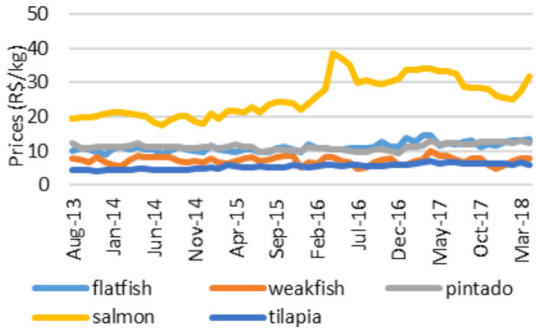

Figure 1. São Paulo wholesale market seafood prices from 2013 to 2018.

STUDY OF THE BACTERIAL COMMUNITIES OF THE SEAWEED (*Ulva* spp.) HOLOBIONT TO BASE MANAGEMENT STRATEGIES FOR THE CONTROL OF HARMFUL BACTERIA IN IMTA-RAS

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Bacterial communities associated to *Ulva* spp. (Ulvales, Chlorophyta) are different from those of the surrounding water both in terms of biodiversity and function and play an essential role for the function of the algae, implying that the macroalgae and all their associated microbiota form a singular entity or holobiont (Egan et al 2012). The specific composition of those bacterial communities may be formed by different members of equivalent functional guilds and a stable core set of functional genes in the bacterial communities associated with different *Ulva* species has been demonstrated (Roth-Schulze et al 2018). *Ulva* spp. provide an important niche for biofilm-forming bacteria, including those belonging to the genus *Phaeobacter* with antagonistic activity towards fish pathogens, such as *Vibrio anguillarum* (Prol-García and Pintado 2013). Based on the flexibility in colonization patterns and on the mentioned antagonistic properties of *Phaeobacter*, the possibility of experimental colonization of *Ulva* spp. with antagonistic *Phaeobacter* strains, previously isolated from *Ulva* species, has been demonstrated (Pintado et al 2017) and small-scale trials showed a probiotic effect of *P. gallaeciensis*-colonised *Ulva*, decreasing the mortality of *V. anguillarum*-infected turbot larvae. However, the environmental conditions for *Ulva* spp. culture (agitation and aeration and high light intensity) would have a determinant influence on the maintenance of the biofilms and the production of TDA.

The aim of the research was to study the bacterial communities of different *Ulva* species and the effect of culture and experimental colonization with different species of *Phaeobacter*, studying the influence of factors as light and agitation on bacterial epiphytic communities in *Ulva* spp. and on the colonization by *P. gallaeciensis*.

Samples were taken at different times for bacterial community analysis by PCR-DGGE. Small-scale experiments were conducted with algae thallus discs of 2 cm diameter of different species (*U. austrails*, *U. rigida* and *U. ohnoi*) in well plates with 10 ml sterile Guillard's F/2 medium adjusted to 20 $mg.L^{-1}$ of N (from nitrate) to mimic the concentration on fish-algae IMTA-RAS systems. Algae cultures were inoculated with *P. gallaeciensis* or *P. inhibens* (10^7 CFU ml^{-1}) and controls were conducted in parallel without addition of bacteria. The plates were cultured at 18ºC and 80 rpm orbital agitation, with a daylight-type LED panel and a 12:12 photoperiod. Scale-up of the selected combination *U. ohnoi – Ph. gallaeciensis* was done up to 40L of non-sterile F/2-N medium.

DGGE profiles showed different bacterial communities between *Ulva* species and locations, in samples collected from the sea. Culturing promoted changes on epiphytic bacterial communities, which were affected by the introduction of the *Phaeobacter* strains. Shifts were also observed in the different steps of the scale-up. The study of the effect of light intensity (300, 170, 100 e 50 μmol m^{-2} s^{-1}) and agitation is currently in progress. The results will permit to define the culturing conditions that would favour the establishment of *Phaeobacter* biofilms in *Ulva* spp. and base management strategies for the control of harmful bacteria in IMTA-RAS systems.

Acknowledgments
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EFFECT OF AUTOCLAVING PROCESS AND ENZYMATIC HYDROLYSIS OF OKARA (BYPRODUCT FROM SOY BEVERAGE) ON GROWTH OF NILE TILAPIA (*O. niloticus*)

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Among plant protein ingredients, soybean meal has high nutritional value and is the most widely used in the aquaculture industry. On the other hand, the consumption of soy products increases worldwide, either whole or as an ingredient for soy beverage. Currently, the food industry generates large amounts of byproducts, such as okara, obtained from production of soy beverage. This byproduct is often discarded, but okara can still be a valuable source of nutrients and natural antioxidants for animal nutrition. In addition, several processing techniques, like enzymatic hydrolysis, can improve the nutrient bioavailability of okara. On the other hand, a thermal treatment, such as autoclaving, can eliminate antinutritional substances that soybean contains and often limit its use. So, the present study was conducted to evaluate the potential use of different processed okara meals, as an alternative protein sources in Nile Tilapia (*O. Niloticus*) juveniles. The okara was used after enzymatic hydrolysis, using *Cynara cardunculus* enzymes- CYOK or autoclaving process- AOK (1 atm, 121ºC and 15 min). Both processed okara were dried at 65 ºC until constant weight and included at 10 and 20% in isonitrogenous diets for Nile tilapia, at the expense of soybean meal. Diets were fed to triplicate groups of fish during 10 weeks. At the end, growth performance and nutrient utilization was evaluated. In general, the diets were well accepted by Nile tilapia, resulting in similar voluntary feed intake and feed conversion ratio among dietary treatments. The results obtained showed that all groups of Nile Tilapia increased 6-fold the initial weight. Furthermore, the final body weight, length and the overall growth performance (SGR) of the fish were similar among the different experimental diets. The dietary inclusion of different okara meal (autoclaved or hydrolysed) did not affect fish final whole body composition nor nutrient gain (Table 1). The present work demonstrated that it is possible to include up to 20% okara meal in diets for Nile Tilapia without major effects on growth, nutrient utilisation or body composition.

Table 1. Final growth performance and whole body composition of Nile tilapia fed the experimental diets for 10 weeks.

	CONTROL	AOK20	CYOK10	CYOK20
Growth				
Final body weight (g)	110.6± 4.5	99.4 ± 6.1	107.3 ± 5.1	102.2 ± 5.0
SGR	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
FCR	1.1 ± 0.1	1.1 ± 0.3	1.1 ± 0.1	1.1 ± 0.0
Whole body composition (% wet weight)				
Dry matter	28.0 ± 0.2	27.4 ± 0.4	26.9 ± 0.8	27.2 ± 0.7
Crude protein	16.3 ± 0.6	15.6 ± 0.5	15.8 ± 0.4	15.7 ± 0.7
Crude fat	8.0 ± 0.7	7.9 ± 0.5	7.3 ± 0.3	7.8 ± 0.4
Gross energy (kJ g^{-1})	6.8 ± 0.1	6.7 ± 0.2	6.4 ± 0.1	6.6 ± 0.2

AOK10 and AOK20 – diets with 10 and 20% autoclaved okara meal, respectively; CYOK10 and CYOK20 - diets with 10 and 20 % of okara hydrolysed by *Cynara cardunculus* enzymes meal, respectively.

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ECONOMIC VIABILITY OF TILAPIA FARMING IN NORTHEAST BRAZIL

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Commercial fish farming is a modern system of agricultural production, with high profitability rates compared to other alternatives of investment. Economic viability of aquaculture is crucial for farmers since it provides key data for the decision-making process and the implementation of managerial actions for the sustainability of the business.

The economic analysis of tilapia fish farming in cages in Northeast region in Brazil was carried out using data collected from six fish farmers employing panel methodology in a technical meeting in order to characterize the typical fish farm of the region and to gather production costs along the previous production year.

Synthetic farms are often based on economic-engineering machinery budgets and livestock production coefficients (FAO, 2016). Technical coefficients of a typical farm informed by farmers set up the starting point for the development of the analysis (Table 1).

The index of economic viability followed the methodology described by Faro (1979), that presents: Net Present Value (NPV), Investment Income Relationship (IIR), Internal rate of Return (IRR), Payback, Benefit Cost Relationship (B/C). Production costs data were based on the concept of Effective Operating Cost (EOC) according to Matsunaga et al (1976). EOC includes all expenses assumed by the farmer over a productive cycle and consumed in the same time interval. It comprehends variable costs (eg feed, labor, fingerlings, energy, fuel, vaccines, fertilizers, maintenance) and part of the fixed costs (eg taxes, labor charges, among others). The investment comprises capital invested in land, facilities, improvements, machinery, equipment, taking into account the apportionment of the use of these assets in fish farming. Gross revenue consists of multiplying the selling price of the kilogram of tilapia received by the farmer by the annual production in kilograms. The reference discount rate considered is 6% per annum, and 10 years for project evaluation.

Financial indicators (Table 2) obtained in the typical farm were very optimistic, compared to other tilapia centers in Brazil. Although feed price in Northeast is 20% higher than other regions in the country due to the cost of transport from south/southeast regions, such good outcomes can be explained in part by the positive influence of climate on zootechnical performance of fish during fattening, which, along with production control measures, results in shorter duration of the crop cycle. This region also benefits from the high price paid to the producer compared to other regions.

Table 1: Zoo technical indicators

Cycle of production (days)	180
Final Feed Convertion Ratio	1.61
Final Density kg/m³	144
Fingerling's initial weight (kg)	0,025
Final weight of fish (kg)	1.1
Production of fish (ton)	361

Table 2: Financial results and indicators

Initial investment	US$ 221,929
EOC	US$ 395,777
Gross Revenue	US$ 613,258
NET	US$ 1,378,747
IRR	97,89%
B/C	1.44
Payback	2.09
IIR	2.76

THE VALUE OF CREATING PLUS PRODUCTS – THE CASE OF THE NORWEGIAN MARINE INGREDIENTS INDUSTRY (NMII)

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Over the last decade, there has been challenges in creating profitability for the Norwegian fillet industry, especially in white fish production, but lately also for the producers of salmon fillets. Falling profitability is partly due to a falling willingness to pay for the fillets, but also increased production costs and lack of value creation from what is left after the production (Iversen, A. et al. 2016). Simultaneously, scientific reports claim substantial value creation potential for marine rest raw material (Richardsen, R. et al. 2015). In our study, we look at the performance of the firms that are creating value of these "leftovers", the marine ingredients industry.

The most referred to definition of rest raw material is "what is not the primary product in the utilization of a raw material" (Fiskeri- og Havbruksnæringens forskningsfond (FHF)). This includes heads, backbones, guts, skins and fins. However, products are also made of dead fish. The norwegian marine ingredient industry also comprise of firms that utilize seaweed and similar, that is not a left over from other industry, but the main utilization of material.

We have done a desk study of (the little?) available information and scientific reports of this industry. Further, we made a short survey, developed with colleagues across disciplines, fit for telephone interviewing. We managed to interview 28 of 61 firms. The industry consists of firms that either have production based on raw material in its original form or on further processed raw material. In this study, we have categorized the products into ensilage, powder/meal and oil. The firms produce either one or combinations of these. The average profitability turns out to be very high in this industry (2014-2016). Results show that 18 of 28 firms have good or very good profitability. We have looked to see if we can find any commonalities within the different performance categories. Salmon is present in all profitability categories while those producing purely on raw material from whitefish has satisfactory to very good profitability.

Oil production is most common, and we see that those including oil in the production have a better profitability, whilst those producing only powder/meal has a lower profitability than the rest. 21 of 28 of the interviewees are targeting the ingredients market. A larger share of the firms producing for the ingredients markets have a good profitability, than those producing consumer products.

The results show that the firms producing products from marine rest raw material have potential to achieve very good profitability. These results illustrates which product categories and where in the value chain there is greatest potential for value creation. However, some of the firms also struggles to survive. In general, it is difficult to see any strong common factors among the firms in the different performance categories, meaning that the profitability probably depends more on less measurable factors like human recourses than raw material and product properties.

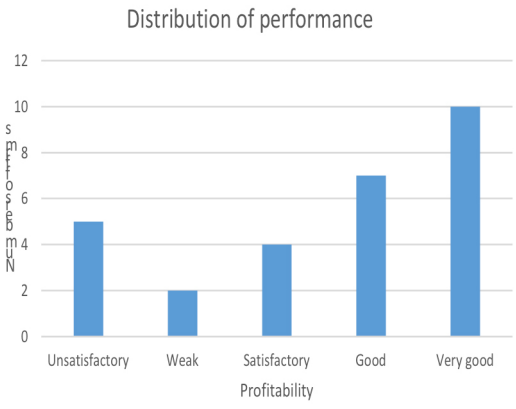

FIGURE 1: Numbers of firms in the NMII distributed in profitability

POST-SPAWNING TREATMENT AND MORTALITY ELIMINATION AFTER TWO DIFFERENT SPAWNING TECHNIQUES IN POND-CULTURED PIKEPERCH (*Sander lucioperca*) BROODSTOCK

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Pond-cultured pikeperch (*Sander lucioperca*) broodstock are widely used for both spawning techniques: egg stripping (ES) with artificial fertilization and tank spawning (TS) with egg fertilization on a nest in pond aquaculture of Central Europe. However, broodstock manipulation, hormonal treatment, spawning procedure and post-spawning fish attacks can often cause stress, skin damage, secondary fungal infection and finally high mortality. Broodstock loss is serious production cost and following problem for next spawning season. The whole pikeperch commercial sector has been trying to improve the broodstock welfare and eliminate fish stress and mortality with the goal to reuse high quality broodstock in the next reproduction season and reduce production costs. The aim of this study was to find the best way for elimination of post-spawning mortality in both sexes and spawning techniques – ES and TS.

After ES (36 pairs) and TS (36 pairs) of broodstock, 6 different post-spawning treatments were applied in this study. Immediately after the spawning, both sexes were divided in to six groups consisted of twelve pairs (six from ES and six from TS). Fish in each group were kept in 3 tanks (each 4 pairs: two from ES and TS) with different post-spawning treatments: 1) water flow tanks supported with river water, 2) RAS – Recirculating Aquaculture System tanks with filtrated and UV treated water, 3); 4) and 5) the same RAS tanks supplemented by salt bath (2.5; 5 and 10 $g.l^{-1}$) lasting six days and 6) the same RAS tanks supplemented by 48-hour formaldehyde bath (38%, at dose of 0.015 $ml.l^{-1}$). Fish were fed with prey fish (daily rate 5 fish TL=60 mm a W = 2 g per one broodstock). Fish survival, conditions (FC – Fulton´s condition) and skin injuries (% of wounded are was evaluated by image analyses) were observed and compared among all the post-spawning treatments, both sexes and spawning technique at 7 and 21 days post spawning (DPS). After that, all the surviving fish were tagged and stocked to a production pond (area 1 ha) with prey fish. The survival of these fish was calculated at 90 DPS.

The lowest survival was found in both sexes after ES and TS held in water flow tanks (16.7 – 50% at 7 DPS and 0% at 21 DPS). Higher survival was found in both sexes and spawning techniques in RAS without any treatment at 7 DPS (50 – 66.7 %). Total mortality was also found here after 21 DPS. Mortality was caused by skin damage and secondary fungal infection covering 30 – 75 % of body surface between 7 and 21 DPS. Salt bath (2.5 and 5 $g.l^{-1}$) in RAS increased survival of all fish at 7 (83.3 – 100%) and 21 DPS (66.7 – 100%). The highest survival (100% at 7 and 83.3 – 100% at 21 DPS) was found in RAS with salt (10$g.l^{-1}$) and formaldehyde bath. Females after 21 DPS increased their FC from 0.78 to 0.88. Final survival rate (75 – 83.3 %) was found in fish of all surviving groups at 90 DPS without any effect of sex, spawning techniques and post spawning treatment.

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EGG QUALITY AND GYNOGENETIC DEVELOPMENT OF RAINBOW TROUT (*Oncorhynchus mykiss)*

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Rainbow trout (*Oncorhynchus mykiss*) is one of the most important species in the aquaculture. In rainbow trout, males usually mature before reaching a market size in contrast to females which achieve a required size before maturation. From the economic point of view, this fact makes females more preferable and profitable for the aquaculture. One of the methods enabling production of "all female" stocks is gynogenesis. However, induced gynogenesis requires use of the very high quality eggs to assure high survivability of gynogenotes during incubation and larval growth. Eggs quality can be defined as the ability to be fertilized and to develop into viable offspring. The egg quality is influenced by many factors including broodstock genetics, environmental conditions, broodstock management. Furthermore, delayed collection, inappropriate striping, extended egg storage before insemination can cause post-ovulatory oocyte aging and further decrease of the egg quality. Egg quality can be assessed by lipid droplet distribution, characteristics of ovarian fluid, fertilization and survival rates. Moreover, pigment content in fish eggs seem to have a positive correlation with their quality. The main goal of the present research was to examine the hypotheses that highly pigmented rainbow trout eggs assure high survival of the gynogenetic Doubled Haploids (DHs).

Gametes originated from the spring spawning rainbow trout broodstock raised in the Department of Salmonid Research, Inland Fisheries Institute in Olsztyn, Rutki, Poland. Gametes were collected from four males and four females. Gynogenetic offspring were obtained by activation of eggs with UV irradiated sperm. Activated eggs were subjected to the high hydrostatic pressure (HHP) shock (9500 psi/3 min), 5 h and 50 min after insemination in order to inhibit the first cell cleavage and to restore diploid state in the gynogenetic zygotes. The coloration of the eggs from each female was analyzed and photographed under binocular using a digital camera.

The color of the eggs of one of the females was significantly different from three others. Its eggs had more intense shade of orange compared to the other three females where the color was significantly brighter. The survivability of the gynogenetic specimens varied between 1% to more than 40% in the swim-up stage. The survival rates of rainbow trout from the control groups equaled about 90% at the same stage. Molecular analysis of gynogenetic DHs confirmed their homozygosity.

The high differences in survivability of offspring can be related to the varied quality of eggs produced by different females. Furthermore, surprisingly high survival rate (over 40%) of gynogenetic offspring of one of the females suggest that eggs originated from this female were at the same stage of maturation and HHP applied 5h 50 min. after activation inhibited 1st cleavage in majority of exposed eggs. In case of other females, asynchronous cleavages in the gynogeneticaly activated eggs result in low efficiency of HHP.

ANDROGENESIS IN RAINBOW TROUT (*Oncorhynchus mykiss*) – IONIZING RADIATION (IR) – INDUCED REDUCTION OF EGG QUALITY?

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Introduction

Eggs quality may be described as the ability of eggs to be fertilized and subsequently develop into normal embryos (Aegerter et al., 2005). External factors such as food quality, environmental conditions and broodstock genetics can decrease quality of eggs. Moreover, it is considered that increase of reactive oxygen species (ROS) accelerates the post ovulatory oocyte aging. ROS cause oxidative damage to DNA, lipids and proteins. ROS are generated by ionizing radiation (IR) which is used i.e. to inactivate maternal nuclear genome during induction of androgenetic development in fish (Komen and Thorgaard 2007; Gagnaire et al., 2015). IR-induced ROS may lead to the premature aging of the irradiated eggs and reduction of their developmental abilities. The major aim of this study was to assess influence of ionizing radiation on quality of the irradiated rainbow trout (*Oncorhynchus mykiss*) eggs. Distribution of lipid droplets in irradiated and untreated eggs and survival rates and ratio of abnormal larvae in androgenetic and control fish were compared to assess whether radiation applied for androgenesis reduces quality of the irradiated rainbow trout eggs.

Material and Methods

Gametes were collected from four males and females rainbow trout from spring spawning, raised in the Department of Salmonid Research, Inland Fisheries Institute in Olsztyn, Rutki, Poland. Eggs were irradiated with 350 Gy of X-rays using TrueBeam linear accelerator (Varian Medical Systems, Palo Alto, CA, USA). Sperm from each male was used to activate irradiated eggs (about 2000 eggs per one male) in the presence of Billard fertilization diluent. About 90 of the irradiated and inseminated eggs from each batch were left to develop as haploid androgenotes. 350 minutes after activation, rest of the irradiated and inseminated eggs were exposed high hydrostatic pressure (HHP) shock (9500 psi/3min.) using TRC-APV hydraulic apparatus (TRC Hydraulics Inc. Dieppe, Canada) in order to duplicate paternal set of chromosomes. To provide control groups spermatozoa from each male were used separately to inseminate non-irradiated (c. 230) eggs. Batches of eggs from all experimental variants were placed in the hatching apparatus and incubated at 10°C under routine conditions. Randomly chosen irradiated (n= 30) and non-irradiated (n= 30-33) eggs were photographed under binocular Nikon SMZ18 with constant magnification (13,5 x) using a digital camera Opta-Tech 5 MPIS and OptaView-IS software (Opta-Tech). Distribution of lipid droplets in the eggs was assessed according to classification proposed by Mansour et al.(2007) with slight modification. Survival rates of the androgenetic and control embryos and larvae were calculated 27 days post fertilization (dpf) (eyed stage) and 57 dpf (swim-up stage). Dead larvae were collected and analyzed under binocular. Microsatellite DNA analysis was applied to confirm homozygosity and only paternal inheritance in the androgenetic individuals. Androgenetic progenies were genotyped using at least three polymorphic microsatellite markers in each male used as a milt donor for androgenesis.

Results

In the present research, four common categories of lipid droplets distribution were distinguished in the non-irradiated and irradiated eggs. Only few non-irradiated and irradiated eggs from each female exhibited distribution of lipid droplets characteristic for category I (most of the lipid droplets were distributed homogenously). Before irradiation, most of the eggs from each female were classified into the category II (lipid droplets started to merge) and category III (many lipid droplets were coalesced in one bigger droplet). Only few eggs had lipid droplets coalesced in one of the egg poles (category IV). After irradiation, most of the eggs were classified into category III or IV. Nearly three times more specimens with improper development were observed in the androgenetic groups when compared to the control groups. Most of the abnormal individuals suffered from the head deformations. Deformations such as abnormal tail, malformation of yolk sac, lordosis, scoliosis, C-shape, spiral and kifosis were also noticed. Analysis of microsatellite markers proved homozygosity in the studied double haploids.

Discussion

Ionizing radiation (IR) generates increased amount of ROS that are considered to be responsible for the post-ovulatory oocyte aging in fish (Samarin et al., 2015). The present experiment showed that IR applied for genetic damage of maternal

DYNAMICS OF BIOCHEMICAL PROCESSES IN THE REPRODUCTIVE CYCLE OF FEMALES OF HYBRID STERLET X GREAT STURGEON *Acipenser ruthenus* Linnaeus, 1758 x *Huso huso* Linnaeus, 1758 WHEN CULTIVATED IN RAS

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Biochemical blood indices have long been successfully used to correct the orientation of reproductive cycle of farm animals. The current study focuses on the investigation of variability of biochemical parameters of blood and urine in hybrids' females of sterlet x great sturgeon (*Acipenser ruthenus* Linnaeus, 1758 x *Huso huso* Linnaeus, 1758) in the process of reproductive cycle.

The trial fish specimens were maintained in a modular water recirculation installation (RAS) with controlled hydrological-hydrochemical conditions. The functional state of female hybrids with different degree of gonads' maturity was assessed by the concentration of osmotically active substances in the urine. The osmolality of urine serum was determined by cryoscopic method using vapor-pressure osmometer OSKR-1 with further transfer into salinity.

The conducted trials have confirmed the previously established fact that the natural maturation of gonads and hormonal regulation of this process causes a profound change in the water and electrolytes metabolism of sturgeon fishes. This leads to water retention in the body of the fish, reducing the osmolality of blood and increasing the osmolality (salinity) of urine. The osmolality of urine in young females of the studied hybrid was significantly lower ($p<0.05$) than in mature fishes at II, III, and IV stages of maturity of the gonads. Reliability of differences between mature females before and after the artificial wintering was revealed, and the osmolality of urine decreased by almost 1.5 times ($p<0.05$).

Thus, the study of orientation of biochemical processes in wild breeders and in hybrids' females cultivated in RAS during the reproductive cycle indicated that the dynamics of the studied biochemical substrates closely correlates with the degree of maturity of sexual products.

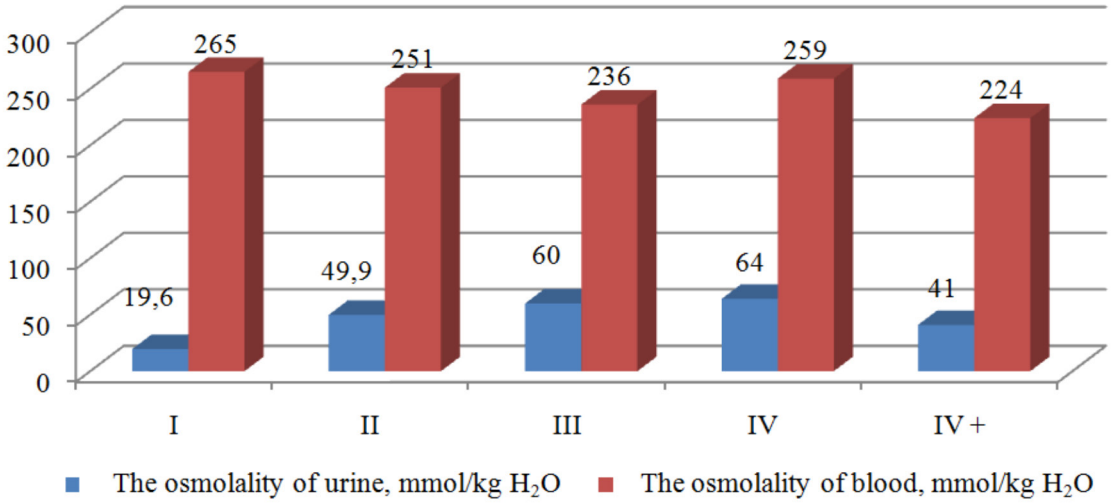

Fig. Variability of osmolality of blood and urine of hybrid's females in the process of reproductive cycle

EFFECT OF A SINGLE-STRAIN YEAST FRACTION ON ATLANTIC SALMON SKIN MUCOSAL BARRIER AND SUSCEPTIBILITY TO SEA LICE

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Sea lice (*Lepeophtheirus salmonis*, Lep, and *Caligus elongatus)* remain a persistent problem for the European salmon industry. The use of in-feed functional compounds and yeast cell wall fractions against these ectoparasites has shown some mixed results to date. We aimed to assess the effect of a single-strain yeast fraction (SsYF) product, rich in mannan-oligosaccharides, on Atlantic salmon post-smolt performance, chalimus settlement and skin mucosal barrier.

A 10-week tank-based trial (MERL, Scotland, UK) was conducted using Atlantic salmon post-smolts (BW_i ~250g; 40 fish/ tank) fed one of two test diets in triplicate: 1] control, 2] SsYF (Lallemand SAS). All tanks were exposed to a standard Lep challenge after 6 weeks of feeding (50 copepodids/fish). Sampling was performed prior to challenge, and at 2 (chalimus stage) and 4 weeks (motile stage) post-challenge, to assess lice count; skin mucus quantity and composition; mucosal immunity (in distal intestine, gill, skin) was evaluated using the Fluidigm BioMark™ platform (64 target genes).

Specific growth rate was numerically improved in the SsYF group (Control: 1.01 ± 0.22 %/day; SsYF: 1.12 ± 0.13 %/day), further showing significantly fewer settled chalimus compared to the control (-16.6%; Fig 1). The SsYF was associated with a significant or trend increase in skin mucus quantity (Fig 2a) and lysozyme activity (Fig 2b). Expression level of selected immune genes across mucosa will be presented and discussed.

The data presented demonstrates the positive contribution of a SsYF product on the growth, skin mucosal barrier and mitigation of Lep settlement in Atlantic salmon. This highlights the potential of this natural dietary product as a practical tool against sea-lice; in particular when considering its suitability for long-term application expected to yield accumulative benefits against lice propagation over the rearing cycle.

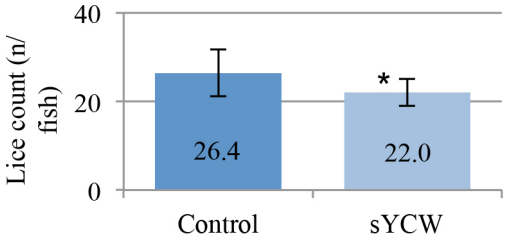

Fig 1. Lice count 2 weeks-post-challenge (Mean ± SD)

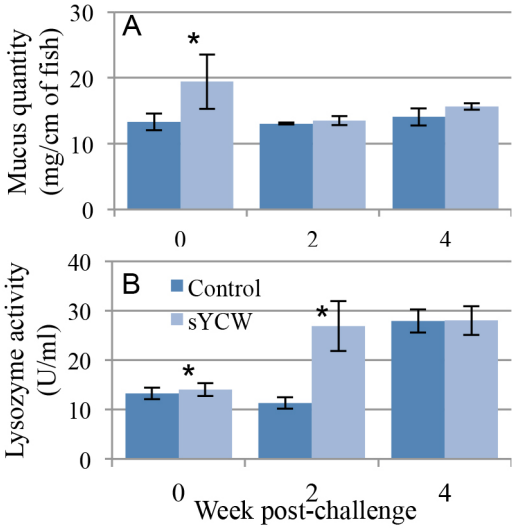

Fig 2. Skin mucus **A**) quantity relative to fish length and **B**) lysozyme activity. Mean ± SD; Asterisk indicate statistical differences ($P<0.05$) between treatments.

DEVELOPMENT OF A HIGH THROUGHPUT REAL-TIME qPCR ASSAY TECHNIQUE FOR THE TARGETED GENE EXPRESSION ANALYSIS OF ATLANTIC SALMON *Salmo salar* MUCOSAL TISSUE RESPONSES

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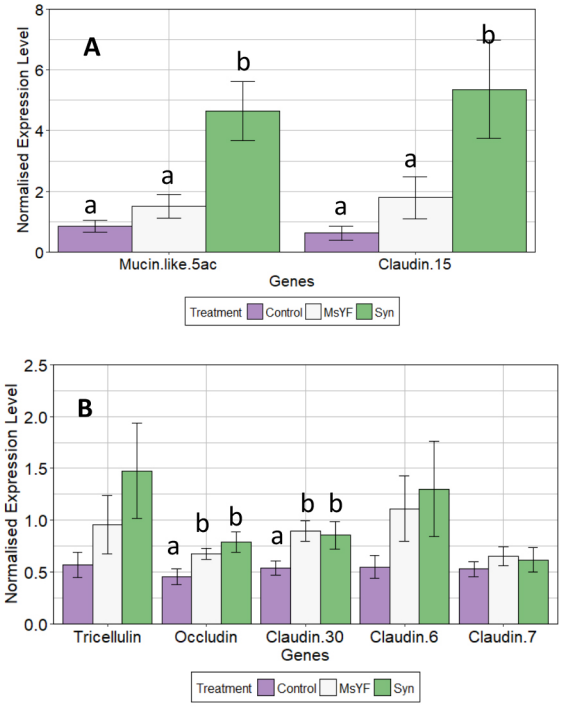
The BioMark™ System developed by Fluidigm provides orders of magnitude higher throughput for real time qPCR compared to conventional platforms. Research exists on the beneficial applications of immunomodulants incorporated into fish diets, yet few studies report a holistic approach to gene expression analysis and the interconnectivity of different immune cascades. We investigate the potential of feeding a novel MsYF product to Atlantic salmon mucosal responses, alone and in combination with a commercial probiotic as a synbiotic (Syn), employing the Fluidigm platform to analyse the expression of 62 selected gene targets.

The trial was conducted under semi-commercial conditions (Ardnish, Scotland, UK) using Atlantic salmon post-smolts (BW_i ~120g; 150 fish/ pen) fed one of three dietary regimes in triplicate: 1] control, 2] MsYF, and 3] synbiotic (MsYF + probiotic (Bactocell)). Standard lateral skin-wipes were performed to collect epidermal mucus for quantity analysis; samples of skin, gill and intestine were collected for gene expression (15 fish/ treatment) at two successive time points (8 weeks and 14 weeks). For the analysis of mucosal responses, cell mediated immunity, stress, and humoral immunity, 62 sets of primers were designed.

A similar positive effect on skin mucus quantity was observed for MsYF and Syn after 8 weeks (+35.7% and +39.3%) and 14 weeks (+24.0% and +11.5% respectively) compared to the control. Upregulation of a number of genes associated with mucin production and tight junction permeability was observed in the gills (Mucin-like 5ac, Claudin 15) (Fig1A) and skin (Tricellulin, Occludin, and several Claudins) (Fig 1B) suggestive of a stimulatory effect upon the mucosal responses, especially in regard to Syn.

Additionally, several genes associated with the Th1 (IL-12, INF-γ and T-bet) and the Th2 (Stat6, GATA3, and GOS2) pathways were affected in the skin after 14 weeks, suggesting a diet-dependent activation of PRR and downstream immune signaling cascades.

Figure 1. Normalised expression level of selected genes in the gill (A) and skin (B) after 8 weeks of feeding (mean ± SE; n=10/ treatment). Different letters indicate statistical differences (P<0.05).



A SPATIAL MULTI-CRITERIA EVALUATION APPROACH TO SITE SELECTION OF ALLOCATED ZONES FOR AQUACULTURE

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According to FAO (2015), the selection of Allocated Zone to Aquaculture (AZA) plays a key role in supporting the sustainable development of this industry within the framework of the Ecosystem Approach to Aquaculture (EAA). The identification of AZA should be framed within the Maritime Spatial Planning (MSP) process which, in the EU, is being carried out in accordance with the EU Directive 2014/89/EU. In this context, the mapping of use conflicts and of suitability indices concerning different uses of the maritime space is of paramount importance for carrying out the planning process in a transparent way. Tools, such as GIS, and remotely sensed data, which can cover large areas at a low production cost, are essential for mapping sea uses connected to different management scenarios and thus adopting science-based planning solutions. In this paper, we present a framework for supporting the AZA identification for finfish farming, based on the Spatial Multi-Criteria Evaluation (SMCE) methodology. The methodology, previously applied to shellfish farming, is here tested for identifying AZA for seabass/seabream farming within the Italian EEZ.

We based our analysis on eco-physiological models, driven by satellite data, and data concerning cumulative impacts. Input criteria included: indicators of biomass yield, *e.g.* the number of days required to reach the market size; indicators of environmental impact and of economic profitability, *e.g.* distance to harbours. Criteria were normalized, weighted and aggregated applying the weighted linear combination method, in order to map the Suitability Index (SI). Three suitability scenarios were considered, in relation to different weights: "BG", Blue Growth; "Eco", Economic; "Env", Environmental. Constraints were subsequently added in order to exclude all areas in which fish farming is not allowed. The analysis was carried out using free open software R 3.4.3, R packages RAC, raster, ncdf4 and maptools.

Results are summarized in Fig. 1, which shows the areas corresponding to 4 suitability classes for the three scenarios. These findings highlight the highly suitable area in the Eco scenario, is lower compared with the other ones, as water temperature limits the biomass production along the Adriatic coast.

The results of this study show that SMCE, supported by satellite data and models, is a flexible methodology for site selection, which can be applied also in data poor areas. Our framework, thanks to the generality and transferability of the applied methodology could be applied to the AZAs identification in other case studies.

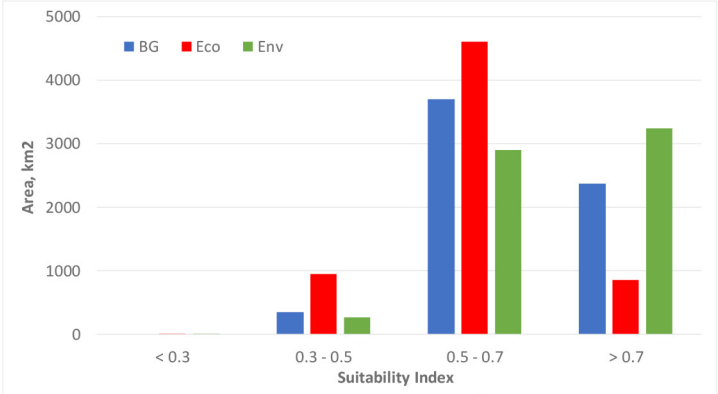

Figure 1. Extension of the available space in km^2 for the three scenarios BG, Eco and Env: results were aggregated in four suitability classes.

TEMPERATURE EFFECTS ON CAPTIVE LUMPFISH (*Cyclopterus lumpus)* BROODSTOCK SPAWNING SEASON AND GAMETE QUALITY

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There is an increasing demand for lumpfish for biological control of sealice with production in the UK and Norway exceeding 15 million in 2016. Hatcheries are heavily reliant on wild caught broodstock, however to alleviate sustainability concerns and to open potential for selective improvement of stocks there is a need for closed lifecycle management. While there has been small scale success there is a lack of information on basic rearing requirements to make captive production commercially viable. As with any "new" species, successful captive spawning requires a clear understanding of their reproductive development as well as environmental and nutritional requirements. This study aimed to describe reproductive development in lumpfish and the effects of holding temperature during the spawning season on productivity and gamete quality of captive lumpfish broodstock.

Just prior to their first spawning season 506 farmed lumpfish (245M: 261F) were separated into 6°C, 9°C and 14°C treatments and maintained on these regimes until spawning was complete. Every 4 weeks sacrificial samples were taken to assess gonadal development (histological) and milt quality (sperm density by spectrophotometry). A large scale assessment of gamete quality was conducted one month into the study where a full factorial cross compared eggs and milt from each treatment (6M: 6F) plus a pool of wild sourced milt (n = 3). Eggs were assessed after 150 degree days and recorded as a percentage eyed rate for each individual cross.

An oocyte developmental scale was validated to create species specific definitions of size ranges of key developmental stages. This scale was used to interpret oocyte population spread and progression in relation to environmental treatment which confirmed that lumpfish are a batch spawning species. Total fecundity was estimated to be 40440 ± 12434 oocytes per Kg body weight, independent of treatment. The spawning window was inversely related to rearing temperature (72 days, 6°C: 28 days, 9°C: 11 days, 14°C) and while the number of natural spawning events was comparable in the 6 °C (25 events) and 9°C (20 events) treatments it was notably lower under the 14 °C treatment (3 events). Furthermore females reared at 14°C showed clear evidence of early and rapid gonadal regression. A significant reduction in sperm density was observed in the 14 °C treatment (5.8 x 10^9sperm/ml) compared with pre-study measures (11.9 x 10^9 sperm/ml) which was not apparent in the other conditions. Eggs from the 14°C treatment were unviable while the 14°C milt remained viable when crossed to oocytes from lower temperatures, furthermore there was evidence of a negative interaction, in terms of quality, between gametes from fish reared at 6°C.

To assure viable gametes captive reared lumpfish broodstock must be held <10 °C during the spawning season. This study is an important first step in understanding environmental optima regulating reproductive development in the species. Going forward, farm rearing practices will be adjusted accordingly to work towards a viable captive rearing program.

FIRST STEPS ON SARDINE (*Sardina pilchardus*) AQUACULTURE PRODUCTION: ADAPTATION TO CAPTIVITY AND SPAWNING

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The southern stock of European sardines (*Sardina pilchardus*) is exploited by Portugal and Spain, where the species has high cultural and gastronomic demand and is also absorbed by the canning industry of both countries. Present historical sardine low biomass levels led to strict catching/landing restrictions to the fishing sector, which raised the question on the viability of the production of the species in captivity. Culturing marine species depends on many factors from adapting wild broodstock to captivity, regular natural or induced spawning, to the domain of larval and fattening culture techniques. The first assays of adapting European sardines in captivity took place in Spain in 1990 and in Portugal at IPMA/EPPO in 2003. Further studies regarding rearing the species from egg to adult phase were also led by both countries (IPMA-Portugal in 2012 and Spain 2014). However, because it is a pelagic species of small dimensions, lives in large schools, has planktonic feeding and is more sensitive to husbandry, this specie constitutes a challenge for aquaculture. In 2016 EPPO adapted to captivity two sardine broodstock with different ages. The broodstock A (n=80) is composed by animals with an initial mean weight of 21,2 ± 2,8 g and the broodstock B (n=300) by individuals with an initial mean weight of 16,4 ± 2,2 g. These breeders have adapted well to captivity, with a low mortality and good growth rate (6,7g/month in the first 6 months of captivity) within an optimum wide thermal range (13-20 ºC). Both stocks have adapted well to an artificial diet composed (sea bream commercial inert feeds 1mm, 2mm). The daily feeding rate was approximately 1,5% of the total biomass. Natural spawning started at the end of January and, until Abril 2018, a total weight of 252 g (stock A) and 367 g (stock B) eggs was obtained with a viability of around 65%, sometimes reaching the 99%. Work in progress includes trials of larval production with the aim of obtaining information on the viability to culture the species at an industrial scale.

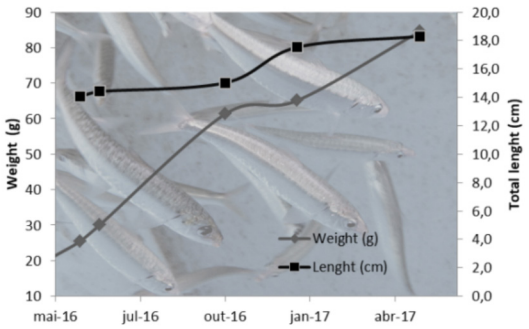

Figure 1- Length and weight variation of sardine (*Sardina pilchardus*) (stock A) during the first year of captivity.

NUTRIENT CHARACTERIZATION AND HARZARAD IDENTIFICATION OF THREE SELECTED INSECTS: SUPERWORM (*Zophobas morio*), MEALWORM (*Tenebrio molitor*) AND CRICKET (*Gryllus. Bimaculatus*), A FUTURE INNOVATIVE PROTEIN FOR SUSTAINABLE AQUACULTURE

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The use of insects as an alternative source of protein in animal feeds is becoming more globally appealing. Among the promising species for industrial scale are superworm (*Z. morio*) and Cricket (*G. bimaculatus*) because they are efficient at converting their food into body mass, making them a candidate for food or feed production. The three selected insects are commonly used to feed carnivorous pets and zoo animals. At present, information about nutrient characterization and hazard identification of insects is scarce.The aims of this study are: (1) to evaluate and compare the nutrient characteristics and (2) to identify the possible hazardous components of three selected insects. Protein of three selected insects was ranged between of 20.1–23.7% and fat was ranged between 3.3–12.8%. Fiber and ash content were observed between 2.2–3.4% and 1.3–1.6%, respectively. Total energy was between 126.8 – 214.8 Kcal/100 g. Factors influencing the nutrient content were species and stage in the life cycle. Cricket, adult stage had highest protein content (23.7%), but juvenile stage had lowest protein (20.7%). High fat content was observed in superworm. Three selected insects showed potential as protein ingredient for aquaculture; however, pesticides (carbaryl and chlopyriphos-methyl) and heavy metals (cadmium, mercury and lead) must also be taken into account as a critical point of using insect as protein ingredient for aquaculture. Moreover, investigation on the effects of dietary inclusion of insect on growth and immune response.

Table 1 Comparative nutrients of three selected insects: superworm, mealworm and cricket

Nutrients (g/100 g, as is basis)	Mealworm (60-day old)	Superworm (60-day old)	Superworm (30-day old)	Cricket (Adult)	Cricket (Juvenile)
Protein	21.4	21.4	20.1	23.7	20.7
Fat	4.7	12.8	8.4	3.3	4.3
Fiber	2.7	2.2	2.3	3.4	2.5
Ash	1.5	1.5	1.6	1.6	1.3
Carbohydrate	3.9	3.4	7.9	2.0	1.3
Moisture	68.5	60.8	62.1	69.4	72.3
Energy (Kcal/100g)	143.5	214.8	187.2	132.4	126.8
Indispensable amino acids					
Arginine	1.58	1.63	1.13	1.77	1.24
Histidine	0.62	0.61	0.60	0.55	0.40
Isoleucine	0.67	0.66	0.66	0.63	0.55
Leucine	1.21	1.18	1.06	1.32	1.05
Lysine	1.61	1.55	1.08	1.65	1.19
Methionine	0.13	0.13	0.12	0.12	0.13
Phenylalanine	0.74	0.77	0.68	0.79	0.57
Threonine	0.70	0.64	0.63	0.55	0.47
Tryptophan	0.16	0.24	0.15	0.16	0.14
Valine	0.83	0.86	0.86	0.85	0.73

Table 2 Comparative heavy metals and pesticide contents of three selected insects: superworm, mealworm and cricket

Hazardous components (mg/kg, as is basis)	Mealworm (60-day old)	Superworm (60-day old)	Superworm (30-day old)	Cricket (Adult)	Cricket (Juvenile)
Heavy metals					
Arsenic	ND	ND	ND	ND	ND
Cadmium	0.03	0.01	0.02	ND	ND
Lead	ND	0.05	0.05	0.05	ND
Mercury	ND	0.02	ND	ND	ND
Pesticides					
Carbamate (Carbaryl)	ND	0.21	0.29	ND	ND
Organochlorine	ND	ND	ND	ND	ND
Organophosphate (Chlopyriphos-methyl)	ND	0.14	0.14	ND	ND
Pyrethroid	ND	ND	ND	ND	ND

POTENTIAL FOR GENETIC IMPROVEMENT OF THE MAIN SLAUGHTER YIELDS IN COMMON CARP WITH *IN VIVO* MORPHOLOGICAL PREDICTORS

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Slaughter traits are of high importance especially for species sold as processed body parts, similarly as in common carp. However, recording of processing yields is impossible on live breeding candidates, and alternatives for genetic improvement are either sib selection based on slaughtered fish, or indirect selection on correlated traits recorded *in vivo*. However, morphological predictors that can be measured on live fish and that correlate with real slaughter yields hence remain a possible alternative. Thus, the aim of this study was to i) determine morphological predictors by external (phenotyping, 2D imaging) and internal measurements (ultrasound imagery) that can be combined by linear regression to predict slaughter yields (headless carcass and fillet yields), ii) estimate genetic parameters of slaughter yields and their predictors, iii) predict and compare the potential genetic gain based on mass selection, sib selection and indirect selection of slaughter yields.

The experimental stock was established by a partial factorial design of 20 dams and 40 sires, and 1553 progenies were assigned to their parents using 12 microsatellites and reared communally until market size under semi-intensive pond conditions. Fish were phenotyped for body weight (BW), standard length (SL), head length (HL), body height (BH), muscle fat (% Fat) and slaughter yields – headless carcass (hl-Carss) and fillet yields (Fill). External measures were performed using 2D imaging (Canon EOS 1000D) and twenty coordinates of morphological points were digitized using ImageJ with the Point Picker plugin. These coordinates were used to calculate lengths, heights and areas. Five internal measurements were collected through ultrasound imagery (Hospimedi LC1000, 7.5 MHz). Slaughter yields were calculated as log-log residuals instead of common percent-expressed slaughter yields, and their predictors were estimated using a multiple linear regression model, including BW, % Fat and external and internal morphometric variables using the reg.best function of the FactoMineR of R software. Genetic parameters were calculated using single trait (heritabilities) and multi trait (genetic correlations) animal model in DMU software. Genetic gains were calculated for theoretical mass selection (MS), full-sib selection (FSS) and indirect selection (IS) using 10% and 30% selection intensities.

Slaughter yields were highly heritable (h^2 = 0.46 for headless carcass yield, 0.50 for fillet yield) and strongly genetically correlated with each other (r_g = 0.96). The accuracy of the phenotypic prediction was high for headless carcass yield (R^2 = 0.63) and intermediate for fillet yield (R^2 = 0.49). Interestingly, heritability of predicted slaughter yields (0.48 – 0.63) was higher than that of the real yields to predict, and had high genetic correlations with the real yields (r_g = 0.84 – 0.88). In addition, both predicted yields were highly phenotypically and genetically correlated with each other (0.95 for both), suggesting that using predicted headless carcass yield in a breeding program would be a good way to also improve fillet yield. Besides, two individual predictors (P_1 and P_2) included in the prediction models and two simple internal measurements (E4 and E23) exhibited intermediate to high heritability estimates (h^2 = 0.34 – 0.72) and significant genetic correlations to the slaughter yields (r_g = |0.39 – 0.83|). The expected genetic progress per generation on fillet yield using indirect selection criteria was even slightly higher than the expected gain obtained by traditional sib selection (IS = 0.40%, 0.61%; FSS = 0.43%, 0.66% for 30% and 10% selection pressure, respectively). The overall results show that there is a solid potential for genetic improvement of slaughter yields by selecting for predictor traits recorded on live breeding candidates of common carp.

PROTEIN*plus*: A BIOTECHNOLOGY PLATFORM TO MEET THE NEEDS OF AQUACULTURE

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As aquaculture production must double in the next 30 years to meet the increased demand in fish consumption, our industry needs to offer innovative technologies that are sustainable, environmentally conscious and appeal to both the feed manufacturer and the farmer. Creating alternative protein sources for aquafeed that produces healthy fish while at the same time decreasing the land use and offering the quality products that consumers are expecting is one of the challenges that KnipBio is tackling.

KnipBio's single cell protein (SCP) technology platform enables a high-quality protein alternative to fish meal (FM) or soybean meal (SBM). Our SCP is produced from cheap renewable resources and provides important molecules like antioxidant carotenoids or molecules that can be missing when replacing FM. The lack of antinutrient factors as well as the presence prebiotics with immune-enhancing properties lead to healthier fish and shrimp. **PROTEIN***plus* products lower the cost of high-value biomolecules and significantly increase the survivability of aquatic animals on the farm, which make operations more profitable.

KnipBio will present an update on its technical progress including the recent progress of its R&D platform and animal trials, its main product characteristics and the status of its production and commercialization phases.

THE DEVELOPMENT OF HIGH THROUGHPUT DIAGNOSTIC TECHNIQUES TO ASSESS FISH HEALTH AND THE IMPACT OF SEA LICE TREATMENT IN AQUACULTURE

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This industry led project uses a translational approach towards the re-purpose and validation of human medical diagnostic technologies to assess the health and impact of sea lice treatment on salmonid fish (rainbow trout and Atlantic salmon) in aquaculture in Scotland. There is currently no rapid, non-lethal method to assess fish health in aquaculture. This novel and innovative approach using automated and semi-automated technologies shall allow the rapid testing of various biomarkers of health (Table 1) from large numbers of fish, allowing for the first time the validation and establishment of background levels of these highly conserved endpoints commonly used in human and veterinary medicine

This approach provides farmers with a means to assess the chronic impact of sea lice treatment, allowing the optimisation of the treatment method. The impact was assessed using clinical chemistry, haematology and histopathological methods.

Rainbow trout treated with azamethiphos (Salmosan) were assessed for 22 clinical chemistry endpoints. Creatinine, a biomarker of kidney damage was significantly elevated post treatment (Fig 1a). ALP, a typical marker of liver condition, was significantly elevated 10 days post treatment (Fig 1b). Increases in total bilirubin and haemoglobin indicate haemolysis occurred. These results were correlated with histopathology and shall be discussed for both salmon and rainbow trout.

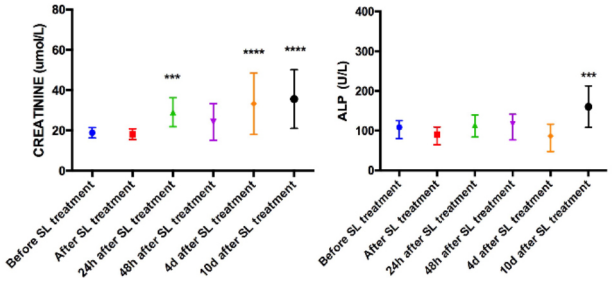

Figure 1. Expression of Creatinine (a) and ALP (b) in rainbow trout following azamethiphos treatment.

Salmo salar	REFERENCE RANGE	LITERATURE
ALP	257 – 643 U/L	380 – 821 U/L
ALT	15 – 43 U/L	4 – 27 U/L
LDH	84 – 3906 U/L	302 – 5579 U/L
CK	839 – 22201 U/L	2258 – 20567 U/L
AMY	298 – 1357 U/L	560 – 1534 U/L
TP	39 – 56 g/L	40 – 52 g/L
ALBUM	17 – 22 g/L	13 – 17 g/L
CREAT	17– 47 µmol/L	9 – 51 µmol/L
AMMONIA	642 – 1408 µmol/L	
SODIUM	154 – 168 mmol/L	158 – 170 mmol/L
CHLORIDE	111 – 128 mmol/L	128 – 136 mmol/L
POTASSIUM	1.19 – 2.45 mmol/L	0.6 – 1 mmol/L
IRON	10.6 – 27.2 mmol/L	
COPPER	7.6 – 15.3 mmol/L	
PHOSPHATE	5 – 12 µmol/L	3.5 – 8.5 µmol/L
MAGNESIUM	1.2 – 2.9 µmol/L	1.2 – 2.0 µmol/L
CALCIUM	2.9 – 3.8 mmol/L	3 – 3.4 mmol/L
ZINC	141 – 402 mmol/L	

Table 1. 12 month background clinical chemistry references range for *Salmo salar*.

CARP EDEMA VIRUS (CEV) AND *Cyprind herpesvirus 2* (CyHV-2) AFFECTING SERBIAN CYPRINID AQUACULTURE AND ICHTHYOFAUNA

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Fish farming industry in Serbia is mainly based on cyprinid species common carp (*Cyprinus carpio*) and salmonid species rainbow trout *(Oncorhynchus mykiss)*. In addition, silver carp (*Hypophthalmichthys molitrix),* bighead carp (*Hypophthalmichthys nobilis*), grass carp (*Ctenopharyngodon idellus*), wels (*Silurus glanis*), pike-perch (*Stizostedion lucioperca*), northern pike (*Esox lucius*) and sterlet (*Acipenser ruthenus*) are cultured.Total fish production in Serbia varied from 12 to 14 thousand tonnes per year. Due to the increasement of disease problems in the fish farming industry and the impact disease may have on both feral and farmed fish, monitoring and surveillance on diseases have for many years been considered to be of great importance.

In mid-2017 two epizootic incidents were recorded in common carp and Prussian carp in Serbia with the suspicion of an involvement of cyprinid herpesviruses. During April and May 2017, mortalities caused by CEV occurred in common carp at water temperatures between 9 and 15°C, in two carp farms located 50 kilometres apart in two north-western districts of Serbia, close to the Hungarian and Croatian borders. At the turn of July and August 2017, a wild population of Prussian carp in Grlisko Lake in eastern Serbia was affected by a massive mortality of about 500 specimens caused by CyHV-2.

The confirmation of CEV and CyHV-2 outbreaks in Serbia is in line with the actual epidemiological situation in Europe, in which both viruses are found to be more widespread than supposed earlier.

STUDYING HOST – PATHOGEN INTERACTIONS BY A MUTAGENESIS APPROACH: THE CASE OF *Vibrio tapetis* TYPE IV SECRETION SYSTEM IN THE BROWN RING DISEASE (BRD) AFFECTING THE MANILA CLAM *Ruditapes philippinarum*

Alexandra RAHMANI*, F. Delavat, V. Pichereau and C. Paillard, 2018

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Type IV secretion systems (T4SS) were first described in the plant pathogen *Agrobacterium tumefasciens (Stachel et Nester, 1986)* and are well known to be involved in pathogenicity in multiple infections such as Brucellosis (*Brucella spp.)* or Legionnaires' disease (*Legionella pneumophila*) *(Vorth et al., 2012)*. T4SS can deliver proteins directly into host cells to cause infection. BRD (*Paillard et al., 1994)* is a disease affecting the Manila clam *Ruditapes philippinarum* due to the Gram-negative bacterium *Vibrio tapetis*. The effects of infection on clams are well documented (e.g. reduction of hemocytes' adhesion properties due to a disorganization of the actin cytoskeleton, decrease in ROS production or alteration of biomineralization of the shell) *(Paillard, 2017)*. However, very little is known about the mechanisms linked to pathogenicity in *V. tapetis*. Our approach is to delete genes potentially linked to pathogenicity in order to improve knowledge on the mechanisms associated to pathogenicity of *V. tapetis* in the context of BRD.

Madec et al. (2014) characterized the *V. tapetis* secretome upon exposure to Manila clam hemocytes, and identified proteins that may be potentially involved in pathogenicity. In addition, *Bidault et al. (2015)* developed a method for quantifying *V. tapetis* in extrapalleal fluids revealing the presence of a gene, *virB4*, encoding a type IV secretion system protein, which is only present in strains of *V. tapetis* pathogenic to clams. Recently, *Dias et al. (2018)* demonstrated the high level of conservation of the gene cluster coding for the type IV secretion system in strains of *V. tapetis* pathogenic to clams.

We chose to focus on VirB4 protein within the T4SS by an approach of gene deletion to further analyze the role of this protein in pathogenicity of *Vibrio tapetis*. Our approach is based on a mutagenesis technique by two homologous recombinations enabling to delete only the targeted gene. We succeeded to construct a *Vibrio tapetis* strain lacking the *virB4* gene. Δ*VirB4* mutant exhibits growth similar to the wild strain.

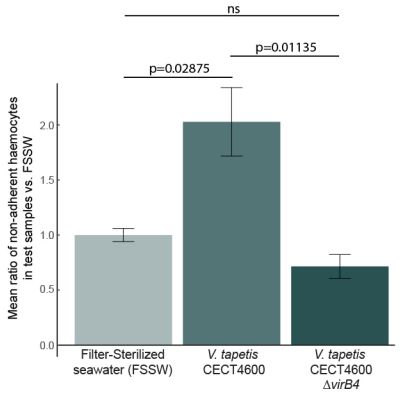

figure 1 : Virulence test based on ratio of non-adherent hemocytes in exposed samples (to V. tapetis CECT4600 WT or V. tapetis CECT4600 VirB4) / control samples (FSSW).

In Figure 1, we performed a virulence test *(Choquet et al., 2003)* that shows a loss of adherence properties of clam hemocytes, in the presence of the wild type strain, but not in the presence of the ΔVirB4 strain of *V. tapetis*. This shows an essential role for the *virB4* gene in the *in vitro* cytotoxic activity of *V. tapetis*.

These results strongly suggest that the T4SS plays an essential role in the pathogenic capacity of the bacterium. With this technique we have developed, we will be able to better understand the genes and thus the mechanisms related to the pathogenicity of V. tapetis in the context of BRD.

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OXIDATIVE STRESS PARAMETERS AFTER PROBIOTICS ADMINISTRATION TO RAINBOW TROUT (*Oncorhynchus mykiss*)

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In a demanding era as ours, were *sustainability* and *one health* approaches are mandatory to any animal production system, we must use all the possible strategies to promote the efficiency and health of animals. The administration of probiotics may be one of such strategies, specially at intensive fish farming, challenging for the animals. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Through distinct anti-microbial properties and/or immune system stimulation, probiotics can increase protection towards opportunistic pathogens, as several works attested (Merrifield, Ringø, 2014). The antioxidant defence system of the organism and immune system are closely linked. Enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are part of the antioxidant defence system, aiming to maintain low cellular levels of reactive oxygen species avoiding oxidative damage to macromolecules and influencing the response to pathogens. Nevertheless, the knowledge of how the antioxidant responses are affected during supplementation with probiotics is limited. This work aimed to study how the probiotic supplementation influences some oxidative stress indicators of rainbow trout juveniles (*Oncorhynchus mykiss*), since we have observed some immune modulation effect previously (Ramos, et al., 2015).

A basal diet (44 % CP, 15 % CL, 19.8 MJ.kg^{-1} DM) was supplemented with a commercial multi-species probiotic (*Bacillus* sp., *Pediococcus* sp., *Enterococcus* sp., *Lactobacillus* sp.) at two concentrations: A_1 (1,5 g.kg^{-1}; 8,6 × 10^5 CFU.g^{-1}) and A_2 (3 g.kg^{-1}; 1,6 × 10^6 CFU.g^{-1}) or a mono-species probiotic (*Pediococcus acidilactici*) at two concentrations: B_1 (0,1 g.kg^{-1}; 2,6 × 10^4 CFU.g^{-1}) and B_2 (0,2 g.kg^{-1}; 7,2 × 10^4 CFU.g^{-1}) and tested against the non-supplemented diet (Control). Juvenile fish (16.4 ± 0.5 g) were fed the diets until satiation (n=6) for 8 weeks at 17 °C.

After 8 weeks, while no significative influence of probiotics was observed on liver CAT, GST, GR or GPx, the hepatic lipid peroxidation (LPO) was influenced by treatments (table1). Comparatively to controls, the probiotic administration increased LPO levels, significantly in the animals fed the B_2 diet.

First results indicate the necessity to evaluate the antioxidant responses during probiotic supplementation, since the immune stimulation could result in a disbalance in oxidative system, prejudicial at a long term, and/or to a possible need to raise antioxidant levels in probiotic diets.

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	Control	A_1	A_2	B_1	B_2	*p-value*
LPO	*31,86 ± 10,60* ^{a}	*34,30 ± 7,84* ^{ab}	*32,40 ± 2,44* ^{a}	*33,35 ± 6,18* ^{a}	*46,38 ± 8,71* ^{b}	*0,012*

Values are mean ± standard deviation (n=6). Different superscripts letters stand for statistical differences between treatments (Tukey test).

EVALUATION OF GROWTH PERFORMANCE OF PIKEPERCH *Sander lucioperca* FED WITH FLOATING AND SINKING PELLETS UNDER PRODUCTION CONDITIONS IN A RECIRCULATING AQUACULTURE SYSTEM

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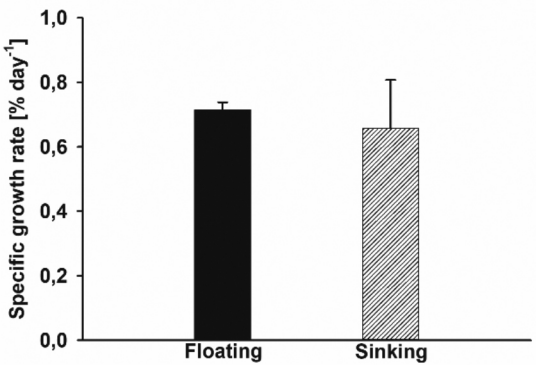
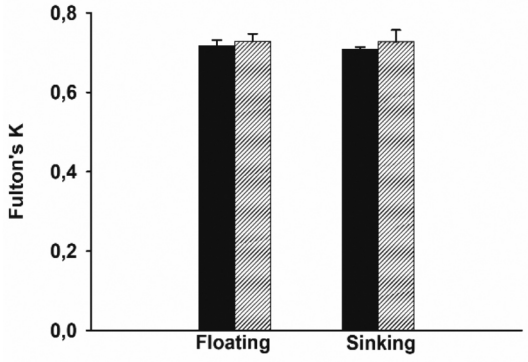
Although floating pellets offer some advantages under production conditions, such as visual monitoring of fish and cleaning efficiency in recirculating aquaculture systems, sinking pellets are the standard in pikeperch feeding regimes under controlled conditions. To evaluate the growth performance and survival of juvenile pikeperch fed with floating pellets we conducted a study at the pikeperch aquaculture facility of the Mecklenburg-Vorpommern Research Centre.

The study was carried out in 5 m^3 tanks with circular flow at a exchange rate of 10 m^3 per hour. Each tank was stocked with pikeperch of a mean weight of 100 g at intial densities of 20 kg/m^3. Fish were fed with either floating pellets or sinking pellets six times per hour at a rate of 0.8 % of the biomass per day. Light intensity was kept at 40 lux and a day:night light cycle of 8:16 hours. Each treatment was replicated three times. After eight weeks we compared specific growth rate (% day^{-1}), Fulton's condition factor (K), coefficient of variance (%) and surivival between the two treatments. Specific growth rate ($p = 0.700$), Fulton's condition factor ($p = 0.681$), coefficient of variance ($p = 0.992$) and survival ($p = 0,299$) didn't differ between treatments. Our results suggest that a floating diet produce similar results as a sinking diet in terms of growth and survival in pikeperch aquaculte under production conditions



Specific growth rate (% day^{-1}) of juvenile pikeperch fed with floating and sinking pellets. Differences were not significant.



Fulton's condition facor (K) of juvenile pikeperch at the beginning (black bars) and at the end (hatched bars) of a feeding trial with floating and sinking pellets. Differences were not significant.

AQUA ACCEPT: BUILDING KNOWLEDGE FOR A SUSTAINABLE AQUACULTURE INDUSTRY

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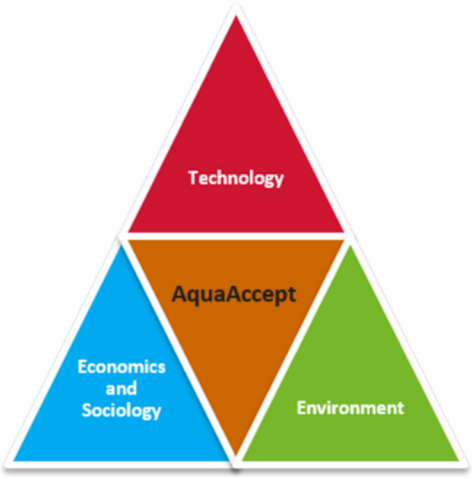
The main objective of the AquaAccept project is to explore what are considered to be acceptable impacts from aquaculture on coastal areas. This is done from a multidisciplinary perspective, drawing on expertise in biology, ecology, economics, sociology and computer science. The ultimate goal of the project is to develop a decision-making support tool to aid stakeholders and decision-makers address conflicts and overlap between aquaculture and other activities in the same area.

The project has evaluated the indicators currently used to measure the environmental impacts of the aquaculture industry and has tested new biological indicators. The new biological indicators will provide a more accurate assessment of the environmental impacts and can be integrated into fixed or mobile sensor platforms.

The project has also carried out a representative survey in Norway at a national level and in a study area of Finnøy Municipality, Rogaland, characterised by numerous marine related activities. Interviews with various interest groups were also conducted to explore and understand attitudes towards the aquaculture industry and its interaction with other marine activities. The project held public meetings in Finnøy to encourage a constructive discussion among the local participants and stakeholders. A similar survey was also conducted in Mullon Bay, Ireland. The results from Norway and Ireland will be compared and will inform a comparison of decision-making support tools in the EU and Norway.

The decision-making GIS-based support tool (SEAGRID) is under development and tested in the Finnøy area and in Mulroy Bay (Ireland). SEAGRID can assess the most suitable places for aquaculture. Furthermore, when potential sites are identified, users can simulate a number of scenarios and possible spatial overlap with other marine activities in the area.



SUREAQUA NORDIC CENTRE OF EXCELLENCE ON BIOECONOMY

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SUREAQUA is a multidisciplinary center that will create knowledge, innovation and technology to develop sustainable and robust aquaculture production.

SUREAQUA (Sustainable and Resilient Aquatic Production) is a “Nordic Centre of Excellence” in bioeconomy. NordForsk’s research program on bioeconomy will give us new knowledge about how we can stimulate and develop the transition to a society based on the bioeconomy in the Nordic region. The program has a budget of just over 9 million Euros, divided amongst three Nordic Centres of Excellence, and began in 2017.

The Nordic region’s abundance of raw materials from both oceanic and land-based environments provides us with a unique opportunity to produce and process biomass. Aquaculture can provide us with extensive quantities of nutritious food, but requires careful management to ensure that the production and management of valuable resources is sustainable.

SUREAQUA consists of more than 40 Nordic partners from research and development, industry, public entities and environmental organisations. Joint projects are being developed to find innovative solutions to ensure safe and stable feed raw materials, increase aquaculture production and profitability, and maintain fish welfare and water quality, increase value-added from by-products and reduce energy consumption in production. These will be developed and implemented in collaboration with industry actors and public administration. Aquaculture is thus central to the Centre, which operates under a value-conscious perspective. The Centre has a special focus on ensuring that the products and technologies being developed will be economically, socially and environmentally sustainable.

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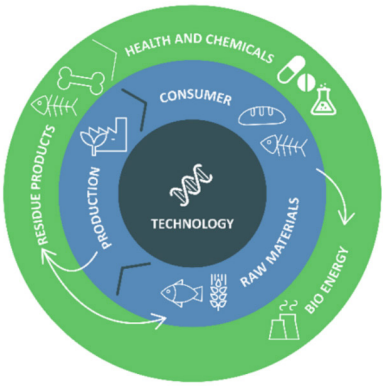

THE EFFECTS OF FEEDING A NOVEL MULTI-STRAINS YEAST FRACTION ON THE MUCOSAL IMMUNE RESPONSES OF RAINBOW TROUT *Oncorhynchus mykiss*

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The use of single-strain yeast fractions (SsYF) such as mannan-oligosaccharides based products has gained momentum in aquatic feeds in the last decade. We investigated the combination of several yeast fractions (MsYF) from different yeast species/strains with the hypothesis that contrasted yeast cell structures would interact with an enlarged set of pattern recognition receptors and in turn induce specific downstream signalling pathways. The product was incorporated into experimental diets and its effect on performance and various mucosal parameters in the skin and mid intestine was investigated.

An 8-week trial was carried out at the Aquaculture and Fish Nutrition Research Aquarium, University of Plymouth, UK. Rainbow trout (BW_i = 23.1 ± 0.2 g) were randomly distributed into 16 x 150-l fibreglass tanks (30 fish / tank; 14.5 ± 0.5 ºC) and fed at a fixed rate of 3% biomass daily. The trial tested three dietary regimes in quadruplicate: 1] Control, 2] MsYF-continuous at 1.5 kg/t (MsYF_C), and 3] MsYF-pulsed at 1.5 kg/t and switched with control diet every 2 weeks (MsYF_P). Mucosal responses was assessed at week 4 and 8 measuring skin mucus quantity (15 fish / group), dorsal skin gene expression (10 fish / group) and mid intestinal analysis (6 fish /group).

Skin mucus level was ~30% higher in fish fed MsYF compared control fed fish. At week 8, skin expression *Calreticulin (Cal)* was significantly elevated in both MsYF groups. In humans, *Cal* is a chaperone protein for MHC1 conformational status suggesting that feeding the MsYF could be activating cytotoxic T-cell responses in the epidermal mucosa. Further, both MsYF groups exhibited strengthened innate and adaptive responses in the intestine by augmenting pro-inflammatory cytokine expression and regulation of inflammatory signals (up- regulation of *Tollip2*, *TGF-* β and *IL-10*). After 8 weeks, gene expression profile in the MsYF_C group was suggestive of a Th2-driven humoral immunity. There was no augmentation of Th1 cell mediated immunity although up-regulation of both IL-12 and IFNg may be indicative of strengthening innate macrophage and natural killer cell responses to both extracellular and intracellular-resident pathogens (Fig 1). Together, these preliminary findings indicate that feeding MsYF revealed a positive effect at maintaining mucosal barrier protection of rainbow trout at intestinal mucosal level with a positive effect also measured in the skin.

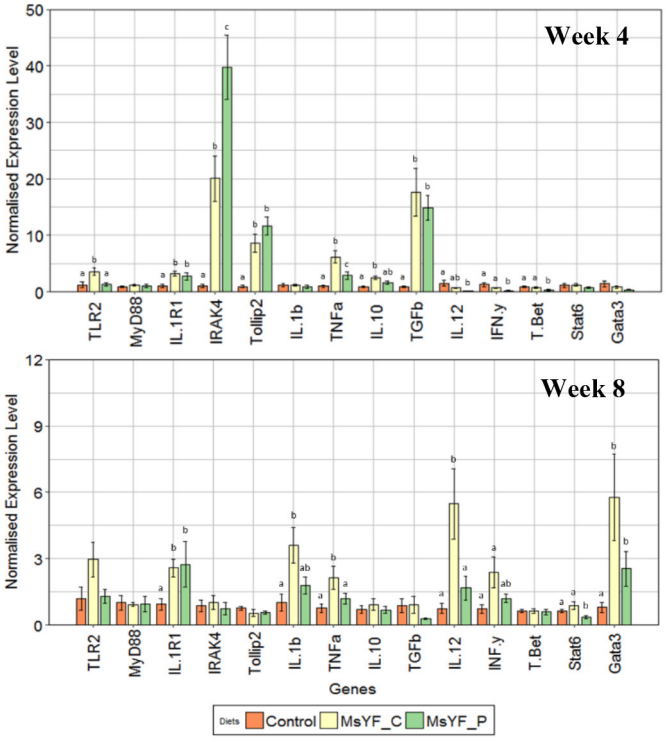

Fig 1 Normalised expression level of selected genes in the distal intestine (n = 6/ treatment). [abc] denotes significant differences between groups P <0.01

THE EFFECTS OF FEEDING A NOVEL MULTI-STRAIN YEAST FRACTION ON THE MUCOSAL RESPONSES OF JUVENILE GILTHEAD SEABREAM (*Sparus aurata*)

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The mucosal surfaces of fish are constantly exposed to a vast number of potential pathogens and non-infectious disrupting factors, such as physical transfer or chemical exposure during production. Lallemand Animal Nutrition have developed a novel multi-strains yeast fraction (MsYF) formulation with reported positive effects on pathogen binding, immune modulation and skin mucous secretion in a number of aquatic species (WO2017/005936). A trial was undertaken to ascertain the effect of the novel MsYF product at two commercially relevant doses on the performance and mucosal responses (skin, gill, intestine) in the gilthead seabream.

The 10-week trial tested three diets in triplicate tanks: A control diet and the same diet supplemented with MsYF at 600 or 800 g/T feed (Lallemand SAS, Blagnac, France). Gilthead sea bream fingerlings (20 fish / tank; body-weight = 24.0 ± 0.1 g) were stocked within 9 x 140 L circular flow-through indoor tanks (T = 22 ± 1°C, salinity = 30 ppt) at the Nireus Research Station (Chalkida, Greece). At the end of the trial, skin mucus quantity (45 fish / group) and quality (24 fish/ group was measured as well as the normalised expression level (NEL) of selected genes in dorsal skin, gill and distal intestinal (12 fish / group) by RT-qPCR (*Mucin 18, Mucin 13, I-muc, Mucin 2* relative to reference genes *Elf1-α* and β-actin).

Feeding the MsYF to seabream significantly increased skin mucus quantity (+60 %; Fig 1A) and protein level (+102%: Fig 1B) compared the control diet (Fig 1B). Gene expression analysis for gill, skin and intestine will be presented. The current trial and previously reported data indicates that feeding the novel MsYF at 600 or 800 g/tonne can positively modulate the physical and mucosal barrier of gilthead seabream, which could help mitigate pathogen and, or physical insults during challenging rearing or transfer periods.

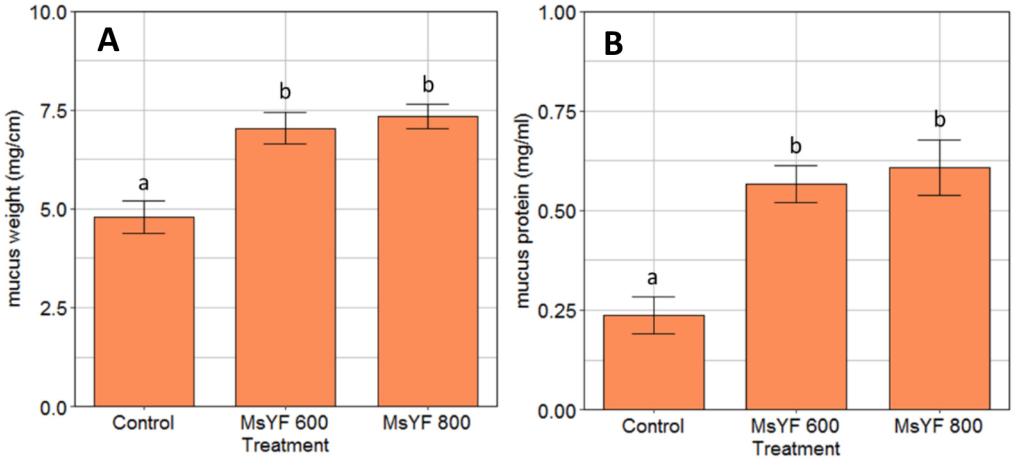

Figure 1: Skin mucus A) quantity (mg/cm of fish) and B) protein level (mg/ml of mucus) at the end of the 10-week trial. Mean ± SEM; different letters denote significant differences between treatment ($P < 0.001$).

THE USE OF *Bacillus subtilis* PB6 IN SPINY SOFT-SHELL TURTLE (*Apalone spinifera*) AS FEED AND WATER SUPLEMENT

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Nowadays due to increasing market demands and decrease of wild turtle populations new species are introduced to aquaculture. The most common among them are soft shell turtles *Apalone* spp. which are used due to their bigger size and higher fertility in comparison to *Pelodiscus sinensis*. The objective of the present study was to evaluate the effect of dietary and water addition of *Bacillus subtilis* PB6 on growth performance, shell composition, gastrointestinal tract development and intestinal microbiota in *Apalone spinifera*.

A 14-week-long growth experiment were carried out on 48 spiny soft-shell turtles (*Apalone spinifera*).. The animals (mean body weight = 77.8 g; mean straight carapace length = 92.4 mm) were randomly assigned to 4 dietary treatments using 12 animals per treatment: control (CON) with no additives; probiotic in water (PIW); probiotic in feed (PIF) and probiotic in water and feed (PWF). The probiotic preparation used in the experiment contained *Bacillus subitlis* PB6: $2 \cdot 10^9$CFU/ g of the product (Kemin Industries, USA). It was added to the diets of PIF and PWF treatments '*on top*' in the amount of 500 ppm, in PIW and PWF treatments 500 ppm of *Bacillus subtilis* PB6 preparation was added to the water after every its change. Turtles were kept in individual plastic tanks (25x20x20 cm), filled with 5 l of water, treated as individual repetitions. The intestinal microbiota was analysed with Fluorescent In Situ Hybridization (FISH). For the assessment bone mineralization Ca, P and Mg concentrations were measured. All obtained data were tested Duncan's multiple range test at the significance level of $P \leq 0.05$.

The use of *Bacillus subtilis* PB6 significantly improved body weight gain and straight carapace length gain. There were no main significant effects of probiotic use in water. However, in the case of its use in feed improved in all above mentioned parameters, and in the case of condition index was observed. The Ca level was lowered by probiotic use in feed, P was significantly increased by the use of probiotic in water, while Mg decreased by probiotic in feed. Intestinal concentrations of Bacteroidetes, Enterobacteriaceae, *Clostridium perfringens*, *Akkermansia muciniphila* and *Campylobacter jejuni* were decreased by the use of probiotic in feed. It increased numbers of Bifidobacteria and *Lactobacillus/Enterococcus sp*. populations. The use of probiotic in water decreased number of *Lactobacillus/Enterococcus sp*. and *Campylobacter jejuni*. Thus we can confirm our previous results which show probiotics as effective tools in turtles (Rawski et al. 2016; Rawski et al 2018). However, the oral way of their applying seems to be the most effective.

VEGETABLE AND FRUIT POMACES USE IN COMMON CARP NUTRITION – THE EFFECTS ON GROWTH PERFORMANCE AND MEAT QUALITY

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The modern animal production must deal with feeding an increasing human population in environmentally sustainable fashion. Thus our efforts should be focused on using omnivorous species as well as cheap by-product based feed resources to make it environmentally friendly and economically efficient (Wadhwa a Bakshi, 2013). The aim of the study was to evaluate the influence of carrot and apple pomace content in fish diets on growth performance and meat quality.

The growth trial lasted 60 days. A 240 of two-year old common carps were used (mean body weight 1 kg ± 0.007). The animals were randomly divided to four dietary treatments including three replications per treatment and 20 fish per replication. The fish were placed in 12 concrete ponds (area 40 m^2, max. depth 1.5 m). Control treatment (CON) received feed with wheat bran as main components. CPL treatment received diet with 15.5% of carrot pomace dried in low temperature (40 ºC). The CPH treatment received 23.6% of dried in high temperature (120 ºC). CAC group was feed with addition of 8% of carrot and apple concentrate.

In comparison to the control diet growth performance parameters (BWG and SGR) were improved by CPL treatment, however, CPH diet impaired BWG, SGR and FCR. The protein efficiency ratio and survival rate were unaffected by diets used in the experiment. There were no effects of the diet on $pH_{15'}$ and pH_{24h} as well as basic chemical composition and free water content. A similar level of meat lightness was observed in control (CON) and carrot pomace (CPL) treatments, however, it was significantly decreased in the treatments with addition of carrot pomace (CPH) and apple and carrot concentrate (CAC) use. The highest value of red colour (a*) and the highest chroma (C) were observed in the (CPH) treatment. All experimental diets caused darker meat hue in the comparison to the control treatment.

Basing on the results of the study we can conclude that vegetable and fruit pomaces may be used as feed components in common carp nutrition. However, there are factors which are limiting their usability including the temperature of drying which may affect their nutritional value and bioactive compounds content or crude fiber content which must be considered during feeds formulation.

ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL ISOLATES FROM COMMON MUSK TURTLE (*Sternotherus odoratus*)

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Reptiles are frequently considered as pathogen carriers and as a zoonotic threat to people. Salmonellosis caused by pet reptiles kept in 1–2% of households are supposed to be responsible for 6–11% of this disease in the USA. In European countries, such as Germany and Poland, the reptiles are kept in similar numbers and are found in about 1% of households. The most important way of the pathogen spreading is direct contact with reptiles or their environment contaminated by feces, which is particularly hazardous for children which often play with pet turtles. The aim of the study was to asses the frequency of selected Enterobacteriaceae excreted by common musk turtles (*Sternotherus odoratus*) and their antibiotic susceptibility.

Samples of the water of 30 turtles of 1 year-old kept under laboratory conditions were taken. They were spread plated using 10-fold dilutions, using modified Hungate method in anaerobic pre reduced medium (Miller and Wolin 1972). The obtained material was incubated for 24h in anaerobic conditions using McConkey no.3 agar plates. Colonies which were isolated from them were preincubated overnight and tested for antibiotic sensitivity according to Kirby-Bauer method with use of Mueller-Hinton agar and diffusion discs. For further identification of bacterial isolates Remel RapID system was used.

The most frequently isolated Enterobacteriaceae were *Escherichia coli* (50%) and *Citrobacter freundii* (26%). There were also isolated *Citrobacter amalonaticus* (9.5%), *Salmonella* sp. (7%) *Acinetobacter amalonaticus* (5%) and *Edwardsiella tarda*. General antibiotic resistance pattern was similar for all above mentioned bacteria. They were sensitive for chloramphenicol, gentamycin, norfloxacin and doxycyclin. They showed high resistance to amoxicillin, erythromycin, and lincomycin.

In opposition to most of studies on microbiota excreted by turtles which focus on *Salmonella* sp. we analyzed wider spectrum of Enterobacteriace. It should be underlined that bacteria like *E. coli, C. freundii, A.amalonaticus* or *E. tarda* are potentially pathogenic for turtle keepers. Similar patterns of antibiotic resistance among Enterobacteriaceae show that antibiotic treatment used in *Salmonella* prevention on farms may be effective against other pathogens. However, we should keep in mind that frequent antibiotic use may result in multi-drug resistance which is highlighted as increasing problem in reptiles since early 1990s.

THE EFFECTS OF DIETARY PROBIOTICS ON MICROBIOTA AND GROWTH PERFORMANCE OF FLORIDA SOFTSHELL TURTLE (*Apalone ferox*)

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Global soft-shell turtle production is increasing rapidly, turning into a multi-billion dollar industry. However, the microbiology of the reptilian gastrointestinal tract (GIT), its composition and effects on the host still remain almost unknown and only a few studies have been published. The aim of the study was to investigate the effects of selected probiotic preparations on growth performance and microbiota of *Apalone ferox*.

A 52-week growth experiment was carried out on 36 young (mean body weight 13.69 g, mean straight carapace length 47.17 mm) Florida soft-shell turtles (*Apalone ferox*). The turtles were randomly allocated to plastic tanks (20x15x15 cm) filled with 2 l of water. The animals were kept individually - 1 turtle per tank. They were feed with gelatine-based diet (35.5% of protein, 7% of fat, 1.1% of fibre and 9.7% of ash). The animals were assigned to 3 dietary treatments: control (CON) – with no additives; single species probiotic (SSP) - with the *Bacillus subtilis* PB6 addition (Kemin Industries, USA); multiple species probiotic treatment (MSP) containing: *Lactobacillus plantarum*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *Bifidobacterium bifidum*, *Streptococcus salivarius* subsp. *thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae*, *Candida* (Probiotics International Ltd, UK). Probiotic preparations were added to the diets '*on top*' according to the producers' recommendations for poultry: SSP - 500 ppm, MSP - 500 ppm. The intestinal microbiota was analysed with Fluorescent In Situ Hybridization (FISH). All obtained data were tested Duncan's multiple range test at the significance level of $P \leq 0.05$.

The bodyweight, straight carapace length and carapace width gains, as well as condition index of turtles were increased by SSP preparation in comparison to control treatment. The microbial populations of small intestine including total number of bacteria, *Clostridium perfringens* and *Salmonella* sp. were lowered by SSP treatment. However, the MSP treatment increased microbiota concentrations including number of *Bifidobacterium* sp., Enterobacteriaceae, *Lactobacillus* sp./*Enterococcus* sp., *Akkermansia muciniphila* and *Bacteroides–Prevotella cluster*. The results of the experiment show that probiotics may be effective nutritional tool in the case of *Apalone ferox*. However, they are highly specific in terms of host – probiotic strain relations. However, they are highly specific in terms of host – probiotic strain relations. The first case of *Akkermansia muciniphila* detection may be also interesting results due to its mucin degrading and anti-inflamatory features.

BLEACHING OF MELANOMACROPHAGES FROM TISSUES OF ECTOTHERMIC VERTEBRATES FOR LATER USE OF IMMUNOHISTOCHEMICAL AND IN SITU HYBRIDIZATION TECHNIQUE

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Due to the large quantity of melanomacrophages in the organs of the ectothermic vertebrates, with special interest in the ranids and fish, with their brownish melanin granules, we decided to test the MELANIN removal technique, in order to facilitate the observation of the organ fragments in the slides, under the direct light optical microscope, when using the antibodies and biotinylated probes. Thus, the melanin bleaching study favored the visualization of the diaminobenzidine chromogen (DAB) without interfering with the antigen-antibody affinity of immunohistochemistry and without interfering with the technique by which specific nucleotide sequences are identified in histological sections. (of DNA or RNA, endogenous, bacterial or viral). This bleaching of melanin from tissues avoided false positive results, without interfering with the IHQ and ISH techniques for *Mycobacterium* spp and *Francisella* spp in fish.

Material and methods: Fragments of fish organs collected between apparently healthy animals or diseased animals were fixed in 10% formalin, dehydrated in increasing sequence of alcohols and diaphanized in xylol. After embedded in paraffin, histological sections of 4.5 microns in thickness were glued on silanized slides. The slides used for the bleaching were immersed in 10% hydrogen peroxide (H_2O_2) in 0.2 mol / L Tris-HCl buffer pH 7.4 for 24 hours at room temperature. During this process the material was kept in the dark. After this procedure the normal staining protocol for IHC and ISH was followed.

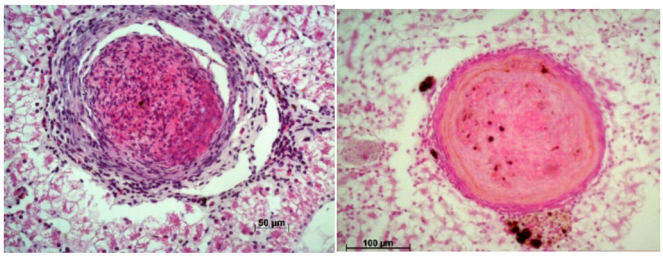

figure on the left: No melanin after treatment

figure on the right: with melanin in the granuloma and in melanomacrophages

CLIMATE CHANGE AND AQUACULTURE: CONSIDERING BIOLOGICAL RESPONSE AND RESOURCES

Gregor K. Reid[1,2,*], Helen Gurney-Smith, Amber F. Garber, Duncan Knowler, David J. Marcogliese, Thierry Chopin, Kathy Brewer-Dalton, Richard D. Moccia, Mark Flaherty, Ian Forster, Caitlin Smith, Sena De Silva

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The heavy reliance of most global aquaculture on the ambient environment and ecosystem services, suggests inherent vulnerability to climate change effects. Climate change mediated physiochemical outcomes important to aquaculture include: extreme weather, precipitation and surge-based flooding, water stress, ocean acidification, sea-level rise, salt water intrusion, and changes to temperature, salinity, and dissolved oxygen. Culture practices, environment and region will influence exposure to stressors. Biological response to climate change stressors between related culture species, or even between populations of the same species, is not universal. Positive, negative or negligible response of a species or species population, to a climate change stressor will be a function of: where changes occur relative to optimal ranges and tolerance limits of an organism's life stage and physiological processes; the average magnitude of the stressor over the production cycle; stressor rate of change; variation, frequency, duration, and magnitude of extremes; epigenetic expression, genetic strain and variation in a population; health and nutrition; and simultaneous stressor occurrence. The effects of simultaneous stressors will frequently interact, but may not be fully additive or synergistic. One dominant driver may cause the greatest effect. Disease is a major aquaculture limiter and climate change is expected to further affect plant and animal health through the host and/or infectious agents with uncertain but potentially profound outcomes. Climate change may introduce further complexity to the capture fishery relationship; over two thirds of global animal aquaculture production is dependent upon external feed inputs, primarily from climate sensitive reduction fisheries. Higher production costs could be an economic outcome of climate change for many aquaculture sectors. Some aquaculture practices may inadvertently reduce resiliency to climate change, such as a reduction of coastal vegetation, coastal ground-water pumping, and reduction of population variability in pursuit of consistent production traits. While aquaculture specific climate change literature is evolving, information from some of the largest aquaculture producers is sparse in the international literature, potentially limiting global comprehensive understanding of climate change effects on some aquaculture sectors

CLIMATE CHANGE AND AQUACULTURE: CONSIDERING ADAPTATION POTENTIAL

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Increases in global population and seafood demand are occurring simultaneously with ongoing fisheries decline, in an era of rapid climate change. Aquaculture is well positioned to help meet the world's future seafood needs, but heavy reliance of most global aquaculture on the ambient environment and ecosystem services, suggests inherent vulnerability to climate change effects. There are, however, opportunities for adaptation. Engineering and management solutions can reduce exposure to stressors or mitigate stressors through environmental control. Epigenetic adaptation may have the potential to improve stressor tolerance through parental or early life stage exposure. Stressor-resistant traits can be selected for and maintaining adequate population variability can improve resilience and overall fitness. Information at appropriate time scales is crucial for adaptive response, such as real-time data on stressor levels and/or species response, early warning of deleterious events, or prediction of longer term change. Diet quality and quantity have the potential to meet increasing energetic and nutritional demands associated with mitigating the effects of abiotic and biotic climate change stressors. Effective research advancements in understanding how climate change affects aquaculture will benefit most from a combination of empirical studies, modelling approaches, and observations at the farm level. Ultimately, in order for aquaculture sectors to move beyond short-term coping responses, governance initiatives are required to facilitate planned climate change adaptation and mitigation.

THE USE OF MARENNINE IN AQUACULTURE– MUCH MORE THAN JUST GREENING

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Mareninne is a blue-green pigment produced by the cosmopolitan marine diatom *Haslea ostrearia*. This diatom often blooms in the oyster ponds thus causing a greening of the oyster's gills and palps[1]. The green-blue pigment called marennine that can be found intracellular and extracellularly[2] when released into the culture medium causes the greening.

Apart from the widely known colouring capabilities, recent research has shown that marennine also has antibacterial, antiviral, antiproliferative[3] and antioxidant[4] properties. In the project CAMAFAN (Characterization and industrial Application of Marennine as Aquaculture Feed Additive and Nutraceuticals) we aim to characterize this so-far unknown pigment and delve into its possible uses in nutraceuticals but more importantly in aquaculture. Given its properties and potential, marennine can be used to green oyster just before harvesting thus increasing their value; be valuable in hatcheries for the production of fish and shellfish larvae by reducing bacterial load; have health benefits for fish, when supplied in specially formulated diets.

After establishing a culture of *H. ostrearia* we are currently undertaking experiments to apply customized modification to optimize/increase the production of extracellular marennine and biomass by defined cultivation modes, such as manipulation of culture conditions (nutrients, light intensity, light spectrum) and testing different culturing systems (suspension cultures and immobilized cultures). We will present these results at the conference.

The extracellular marennine produced during the culture optimization experiment will be used to determine the efficacy of this product to improve oyster (*Crassostrea gigas* and *Ostrea edulis*) larvae rearing and greening of adult individuals. Different concentrations of external marennine will be added to the larval rearing tanks. The larvae will be monitored through to settlement and survival, growth rate, settlement rate and larval abnormalities will be determined. In adults, we will look at the colouring of gills and palps before and after feeding Haslea biomass and exposing the animals to extracellular marennine to quantify greening, as well as monitor clearance and ingestion rates and feeding behaviour. We aim to present the preliminary data of this set of experiments at the conference.

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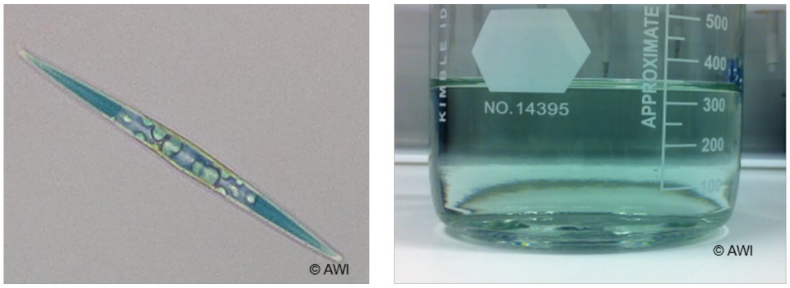

Figure 1: Haslea ostrearia (left) and extracellular marennine in culture medium.

ADAPTATION OF Serrasalmidae FISH, PUREBRED AND HYBRID, TO WINTER FARMING CONDITIONS

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Tambaqui (*Colossoma macropomum*) is one of the most important species of South America aquaculture. But tambaqui is a species from Amazon basin and its cultivation is limited to regions that are hot all year round. On the other hand, pacu (*Piaractus mesopotamicus*) shows tolerance to low temperatures, but has lower growth than tambaqui. Thus, hybrids produced from pacu and tambaqui could be an option for fish farmers in the subtropical and temperate regions of South America. We evaluate the survival of pacu, tambaqui and reciprocal hybrids fed with diets containing different levels of protein, during periods of low water temperatures.

The experiment was carried out from June to August 2016 (51 days) in aquaculture station of São Paulo Agribusiness Technology Agency, Pariquera-Açu, Brazil (latitude 24° 43' 14" S; longitude 47° 52' 43" W). A diallel crossbreeding between pacu and tambaqui was performed by artificial reproduction, generating four genetic groups: pacu (♂ pacu x ♀ pacu), tambaqui (♂ tambaqui x ♀ tambaqui), paqui (♂ tambaqui x ♀ pacu), and tambacu (♂ pacu x ♀ tambaqui). Fish were distributed in 24 experimental plots installed in 12 ponds (110 fingerlings of one of the groups per plot) and were fed twice daily with commercial diets containing 24%, 28% and 32% of crude protein (CP). During the experimental period, plots were monitored daily to record the mortality. During the evaluation period, the water temperature ranged from 14.0 ºC to 22.9 ºC. Lifetime (days) of animals was submitted to Cox regression to test the genetic group and CP effects and the interaction between the two factors. Kaplan-Meier survival curves were adjusted for factors that had a significant effect. The lifetime estimates generated from the survival curves were compared using the log-rank.

Pacu group presented higher survival than other groups for all CP level in the diet (Table 1). Tambaqui and paqui groups were more susceptible to environmental conditions, especially when fed a diet containing a higher level of CP (Table 1). Purebred pacu presented greater adaptation to the experimental conditions, but if the aim is the production of hybrids, crosses between pacu males and tambaqui females (Tambacu) generate fish that are resistant to low temperatures.

Table 1: Estimation of expected lifetime (days) fish juveniles (pacu, tambaqui, tambacu and paqui) fed on diets with different protein levels.

	Pacu	**Tambaqui**	**Tambacu**	**Paqui**
32% CP	48.5Aa	33.7Ab	33.5Bb	27.5Bc
28% CP	43.9Ba	36.6Ab	43.7Aa	29.8Bc
24% CP	43.9Ba	36.6Ab	43.7Aa	29.8Bc

Estimates followed by uppercase letters in the same column are significantly different according to the log-rank test ($p < 0.008$). Estimates followed by lowercase letters in the same line are significantly different according to the log-rank test ($p < 0.0016$).

REPROGRAMMING OF *M. galloprovincialis* HEMOCYTES TRANSCRIPTOME AFTER *V. splendidus* INFECTION

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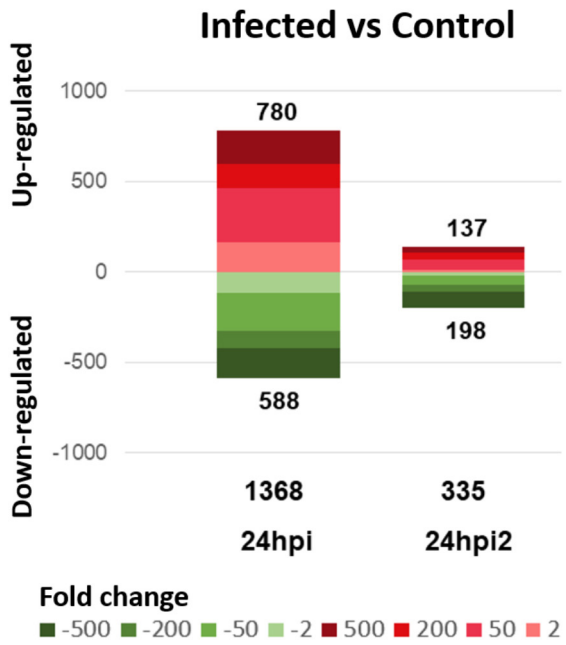
Mediterranean mussels (*Mytilus galloprovincialis*) are sessile filter feeders that live in a close contact with numerous marine microorganisms. As all invertebrates, they lack an adaptive immune response but it has been reported that invertebrates may respond to an infection involving a high degree of pathogen specificity and innate immune memory or "priming".

In this work we conducted Illumina sequencing of the mussel hemocytes transcriptome before and after *Vibrio splendidus* infection: triplicates of samplings at time 0, 24 h after a first infection and 24 h after a second infection. About 90 million raw reads were obtained from each sample, which were assembled into more than 200,000 contigs averaging 527 bp. The putative identities of about 25% contigs were obtained by Blast.

The differential expression analysis show that the priming with *V. splendidus* reprograms the gene expression in the second infection (Fig. 1). After the first infection the immune response (inflammation, interferon and cytokines production, migration and response to bacterium) was significantly regulated. After the second infection it seems important the NF-kappaB signaling pathway regulation, as well as phagocytosis and the glucose homeostasis.

We also analyzed the response of individual mussels, and could confirm an important individual variability. Each animal showed an exclusive repertoire of genes not shared with others. The common group of regulated genes to both control and infected mussels was also analyzed and one interesting fact of this common response is that the sampling before the injection was considered a stress stimulus strong enough to trigger a specific response in hemocytes, promoting cell migration and proliferation.

Fig. 1: Differentially expressed genes in each sampling point.



ASSOCIATION BETWEEN INNATE IMMUNITY AND CORTISOL RELEASE IN GNOTOBIOTIC FULL-SIBLING EUROPEAN SEA BASS (*Dicentrarchus labrax*) LARVAE CHALLENGED WITH *Vibrio anguillarum*

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The neuro-immuno-endocrine network is the main responsible to orchestrate the response to allostatic load in fish. These tightly connected mechanisms are able to mount an immune response but can also induce a subsequent global neuroendocrine response when the alarm messengers will reach and activate the HPI axis. Currently, there are no studies focused on host-pathogen interaction in which the association between glucocorticoid synthesis and immune response has been evaluated in larvae after a challenge with a highly pathogenic bacterium. Therefore, the aim of this study was to evaluate the modulation of innate immune-related genes and glucocorticoid synthesis in gnotobiotic full-siblings in specific time points related to mortality of European sea bass larvae challenged with *V. anguillarum*. For this purpose, full-sibling gnotobiotic sea bass larvae were challenged by bath with 10^7 CFU mL^{-1} of *V. anguillarum* strain HI 610 on day 5 post hatching (dph). The mortality was monitored up to the end of the experiment (120 hours post-challenge, hpc). While no variations were registered in non-challenged larvae maintained under gnotobiotic conditions (93.20% survival at 120 hpc), in the challenged group a constant and sustained mortality was observed from 36 hpc onwards, dropping to 18.31% survival at 120 hpc. Expression analysis of innate immunity-related genes and glucocorticoid quantification were carried out in single larvae. The expression of *lysozyme*, *transferrin*, and *il-10* differentially increased at 120 hpc together with a marked upregulation of the pro-inflammatory cytokines (*il-1β* and *il-8*) and *hepcidin*, suggesting a late activation of defense mechanisms against *V. anguillarum*. Importantly, this response coincided with the lowest survival observed in challenged groups. On the other hand, an increase of cortisol, cortisone and 20β-dihydrocortisone was observed at 120 hpc as well. Therefore, the increase in markers associated with glucocorticoid synthesis together with the upregulation of genes associated with the anti-inflammatory response suggests that in larvae infected with *V. anguillarum* a pro-inflammatory response at systemic level takes place, which then leads to the participation of other physiological mechanisms at systemic level in order to counteract the effect and the consequences of such response. However, this late systemic response could be related to the previous high mortality observed in sea bass larvae challenged with *V. anguillarum*.

This study was financially supported by the Aquaxcel EU project 0133/09/14/31 (FERL), and MINECO-Spain AGL2016-76069-C2-2-R project (LT). EVV is recipient of a grant from CONICYT-Chile Postdoctoral fellowship.

ICHTHYOTOXIC DINOFLAGELLATES INDUCE DNA DAMAGE, LIPID PEROXIDATION, AND ANTIOXIDANT RESPONSE IN THE GILL TISSUE OF RED SEABREAM *Pagrus major*

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Ichthyotoxic dinoflagellates pose a significant threat to aquaculture and fisheries. In this study, we employed several molecular and biochemical response systems of the gill tissue of red seabream *Pagrus major* to understand potential mode of actions of two dinoflagellates, *Cochlodinium polykrikoides* and *Karenia* sp. after exposure to different cell concentrations for 24 h. Overall, both dinoflagellates dose-dependently increased DNA damage, lipid peroxidation (intracellular malondialdehyde; MDA), and glutathione (GSH) depletion/synthesis during both exposure (24 h) and depuration (3 h) phases. We also analyzed enzymatic activities of antioxidant defense systems such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). Both dinoflagellates tested in this study significantly modulated enzymatic activity of antioxidant defense system with strong inductions of SOD and CAT, and the levels were maintained during depuration period. Principle component analysis (PCA) showed potential correlations between molecular markers (i.e. DNA damage, MDA, and GSH) and enzymatic responses by cell concentrations and time-courses. Taken together, our results indicate that representative dinoflagellates have potential hazardous effects on the gill of red seabream within relatively short time period, as the gill is the first organ exposed to water and diverse environmental factors including dinoflagellates. Our results also suggest that analyzing a series of molecular and biochemical parameters can be a way of understanding and uncovering the mode of action of ichthyotoxic dinoflagellates.

MICROBIAL FOOD WEB STRUCTURE CHANGES IN INTERACTION WITH JUVENILE OYSTERS DURING MORTALITY EPISODE IN THE THAU LAGOON (FRANCE)

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Oysters can modify the microbial food web (MFW) by their filtration and excretion activities. While many studies have been conducted on this subject with adult oysters, none had been carried out *in situ* with juveniles. Significant mortality of Pacific oyster juveniles (*Magallana gigas*) has been systematically observed all over the world since 2008. During mortality episodes, sick and dead oysters are not separated from healthy live ones, but left to decay in the surrounding environment, with unknown consequences for the nutrient cycle and MFW components. The present study aimed to elucidate for the first time the interactions between oyster juveniles and MFW components during a mortality episode. Innovative 425-L pelagic chambers (Fig. 1) were deployed weekly *in situ* around oyster lanterns along a stocking-density gradient in the Thau Mediterranean lagoon during an oyster mortality episode in 2015.

This study reveals (i) significant changes of MFW structure during mortality episodes, with a proliferation of picoplankton (<3 μm) and ciliates (*Balanion* sp., *Uronema* sp.) within two weeks when mortality rates and numbers of moribund juvenile oysters were highest. These changes were probably induced by oyster tissue leaching, decomposition and mineralization, which probably began during the moribund period, as suggested by an increase of PO_4 concentration and N:P ratio decrease (Fig. 2), (ii) oyster juveniles mainly consumed 3–20 μm plankton with a preference for autotrophic microorganisms. Oyster juvenile filtration and mortality shifted the MFW structure toward a heterotrophic microbial system, where (iii) ciliates and flagellates acted as a trophic link between picoplankton and oysters (Fig. 3). Based on these results, we hypothesize that decomposed oyster tissue, picoplankton and protists are involved in juvenile oyster infection, OsHV-1 mortality and transmission processes.

NOVEL YEAST PROTEIN SOLUTIONS (PROSAF®) IN REDUCED FISH MEAL DIETS FOR WHITELEG SHRIMP (*L. vannamei*)

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Fishmeal reduction in shrimp feeds has reached a critical point in practical diets. Nutritionists and formulators are working in the strait line between optimum performance and reduced growth and health, due to interactions of nutritional limitations, stress factors and pathogens in the field. In this context, functional ingredients can be a cost-effective solution to support shrimp nutrition and health.

In a series of experiments, the benefits of a novel high protein yeast extract (Prosaf®, Lesaffre Animal Care; 63% crude protein) in reduced fishmeal diets on feed preference, growth performance and immune status of white-leg shrimp (*L. vannamei*) were assessed.

Apparent digestibility coefficients of nutrients (protein, lipid, energy, essential amino acids and phosphorus) of Prosaf® were first evaluated *in vivo* in juvenile shrimp. In a second trial, the effect of the high protein yeast extract inclusion in a low fishmeal diet (3% fishmeal) on shrimp feed preference and intake was assessed over a 15-day trial (shrimp body weight: 10g). Finally, a third trial was conducted to assess the effect of Prosaf® on growth performance, digestibility and immune parameters when included at 0%, 0.5%, 1.5% or 2.5% in a low fishmeal formula (3% fishmeal). Shrimp (initial body weight: 1g) were fed in quadruplicates (20 shrimp per tank) for 9 weeks. Apparent digestibility (dry matter, protein, energy) of the different dietary formulas was assessed *in vivo*. Growth performance were monitored all along the trial. At the end of the trial, samples of hemolymph were collected for immune parameters evaluation (total haemocyte count, phenoloxidase activity, phagocytic activity).

Increasing levels of Prosaf® promoted increased shrimp weight gain and daily growth compared to the low fishmeal formula. Shrimp fed the 2.5% Prosaf®-containing formula had significantly higher performance compared to the control low fishmeal-fed group. Prosaf® supplementation at 2.5% also resulted in significantly higher apparent protein digestibility compared to control low fishmeal. Plasma immune-related parameters from shrimp fed the 2.5%-Prosaf® diet had a significantly higher total haemocyte counts and phenoloxidase activity in hemolymph compared to control low fishmeal diet.

This study demonstrated that the supplementation of a high protein yeast extract can improve the growth performance and health parameters of shrimp fed on low fishmeal diets.

NEW FUNCTIONAL FEED INGREDIENT: EFFECTS OF DIETARY YEAST AUTOLYSATE ON INTESTINAL MICROBIAL COMMUNITIES OF SEA BREAM *Sparus aurata*

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Fish protein hydrolysate is rich in free amino acids, bioactive compounds and water-soluble proteins that improve feed digestibility and palatability, thus promoting fish growth and feed utilization. Protein hydrolysate from yeast (*Saccharomyces cerevisiae*) is assumed to have similar beneficial effects of fish protein hydrolysate but, in addition, it contains several immune-stimulating compounds, such as β-glucans and mannan-oligosaccharides, which have positive influence on immune responses and stress tolerance of fish. However, the introduction of any new ingredient in the diet needs to be carefully evaluated since diet is one of the main factors shaping the intestinal microbiota. The gut microbial communities of fish is indicated being correlated with host metabolism, nutrition, growth, immunity, and disease resistance, in a similar way as reported for mammals.

The present study aimed to investigate the effects of a diet rich in plant proteins (A), supplemented with 5% of either fish protein hydrolysate (B) or autolysed dry yeast (HiCell®, Biorigin) (C) on intestinal microbiota of gilthead sea bream. Totally 102 operational taxonomic units (OTUs) of bacteria were identified from the 2,327,049 sequences obtained by Illumina MiSeq 16S rRNA gene sequencing. Interestingly, our data revealed that inclusion of autolysed dry yeast was associated with an increased bacterial diversity compared to fish protein hydrolysed supplemented diet. Indeed, although most of the bacterial taxa were common between dietary groups, fish receiving autolysed yeast showed higher abundance of *Bacillus* and *Shewanella* genera, besides to be characterized by specific bacterial genera, such as *Megasphera*, potentially beneficial for host (Table 1). In conclusion, autolysed dry yeast could be a valid alternative protein source for aquafeed production, since it positively affects the intestinal microbial communities of sea bream by increasing the number of indigestible carbohydrate degrading and short chain fatty acid producing bacteria.

Table 1. Percentage of most abundant genera (mean ± SEM) found in different dietary groups. "n.d." means not detected. Statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

Genus	A	B	C	
Prevotella	n.d.	n.d.	0.8 ± 0.3^{a}	***
Bacillus	0.9 ± 0.2^{b}	1.5 ± 0.4^{b}	2.3 ± 0.6^{a}	**
Staphylococcus	< 0.5	0.8 ± 0.1	0.8 ± 0.2	
Lactobacillus	70.9 ± 3.9	63.6 ± 6.1	67.8 ± 3.7	
Clostridium	< 0.5	1.3 ± 0.6	0.8 ± 0.5	
Megasphaera	n.d.	n.d.	1.2 ± 0.3^{a}	***
Comamonas	< 0.5	< 0.5	0.6 ± 0.2	
Shewanella	2.0 ± 0.5^{a}	$< 0.5^{b}$	1.1 ± 0.3^{a}	***
Erwinia	$< 0.5^{ab}$	$< 0.5^{b}$	0.6 ± 0.2^{a}	**
Pseudomonas	0.7 ± 0.1	0.9 ± 0.3	1.0 ± 0.3	
Pseudoalteromonas	0.9 ± 0.2^{a}	$< 0.5^{b}$	$< 0.5^{b}$	***
Photobacterium	12.4 ± 4.2	20.2 ± 5.8	10.9 ± 4.4	
Vibrio	4.7 ± 1.3^{a}	0.5 ± 0.2^{b}	2.5 ± 0.7^{a}	***

CHARACTERISATION OF PAMP-PRR INTERACTIONS IN NILE TILAPIA (*Oreochromis nilitocus*) USING A MACROPHAGE PRIMARY CELL CULTURE

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Nile tilapia (*Oreochromis niloticus*) is considered as one of the most important commercial fish in the world and surprisingly there is limited knowledge available related to the molecular and cellular immune response. It is necessary to understand the immune system of fish, particularly on the interaction between pathogen recognition receptors (PRR) and pathogen associated molecular patterns (PAMPs) that operates the immune response, for the both basic research and fish health management. The macrophage is the key orchestrator of the immune response as it has phagocytic activity in order to detect and eliminate invading pathogens. Moreover, macrophages are a major source of cytokines that are the critical factor involved in the communication between innate immunity and adaptive immunity of host. The cytokines play a regulation role in the inflammatory process and the activation of T cells and B cells. Therefore, the development of the macrophage primary cell culture will provide a knowledge platform to understand the molecular regulation of the immune response and reduce dependence upon in vivo disease studies in Nile tilapia.

This study aimed to characterise a pattern of innate immune response to PAMPS (pathogen-associated molecular patterns) in a Nile tilapia macrophage primary cell culture. In parallel we developed a suite of molecular tools to examine immune system performance. For primary cell culture development; macrophages were obtained from head kidney and were cultured in DMEM medium containing difference types of serum (Chicken serum and Fetal Bovine serum) and antibiotics (Primocin, penicillin/streptomycin, and Kanamycin) in various concentrations. The cells were incubated at 28 ˚C with ~3% CO_2. After 3 days of culture, the macrophages were stimulated with 10 μg/ml of ultra-pure peptidoglycan (PGN) and 10 μg/ml of poly I:C for 12 hours before harvesting. The pattern of innate immune response was examined with the expression of TNF-α, IL-1β, IL-6, IL-10, TGF-β mRNA responses representing the antibacterial response and TLR-3, IRF-3 and Viperin for the antiviral response. The phagocytic activity was performed with E.coli and yeast, and analysed by flow cytometer. We identified the optimal culture medium for the macrophage primary cell culture to be DMEM with 10% CKS and 1X Primocin. After cell stimulation with PGN, TNF-α, IL-6 and IL-10 mRNAs were significantly upregulated and TGF-β mRNA abundance down-regulated. After poly I:C activation all antiviral mRNAs increased significantly. Moreover, a similar trend of gene expression was found in selected tissues (head kidney, spleen and liver) of *Oreochromis niloticus* and *Oreochromis mossambicus*.

R+D+i TOWARDS AQUAPONIC DEVELOPMENT IN THE UP ISLANDS AND THE CIRCULARECONOMY(ISLANDAP-INTERREGMAC/1.1a/207).FIRSTCOLLABORATIVE RESULTS

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ISLANDAP aims are to promote the bioeconomy and the circular economy through research, technological development and innovation towards the sustainable aquaponic production, accordingly to the specificities of the UP Region, and to create a Multidisciplinary UP Islands Network for the Circular Economy Development and the education in all related areas.

First project results

1) *Aquaponic prototypes* for fresh and marines species production have been designed and being implemented in the 3 Regions in June 2018 (6 by Region with specific sites and the specific fish species and plants already defined).
2) *Organic by-products:* 2.1. List of the principal organic by-products per Region; 2.2. Three initial key by-product per Region already selected (grape wine discard, aloe, carrots peel, date, acacia pods); 2.3. Definition of protocols for samples compilation, laboratory management and processing and the subsequent biochemical and key components valorisation analysis. 2.4. Some of the samples under analysis or testing.
3) *First aquaponic production* trial (tilapia and lettuce), with a local formulated and produced diet, have being finished and the results under analysis.
4) *Definition of the initial teams and roles by Regions*, and the need for new profiles to be incorporated in the project. New researchers and institutions have being incorporated.
5) *First International Training Course on Aquaponic and the Circular Economy* was held at the Ecoaqua Institute in Gran Canaria, with 25 participants from the 3 regions.
6) *A Sort Term Scientific Mission from Madeira to Gran Canaria was completed*. One moth specific training on aquaponic trials and diets production.
7) *Activities for the local and international project objectives and results dissemination* have been developed for the different partners, with great social impact locally.
8) *Creation of the Network for the UP Islands Bioeconomy and the Circular Economy Development*.

ARTIFICIAL INTELLIGENCE TO GAIN VALUABLE INSIGHT ON AQUATIC ORGANISMS

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Data quantity and reliability has been the main driver for yield optimization in agriculture and most livestock industries. In aquaculture however, accurate and reliable data is hard to obtain since counting and sizing small aquatic organisms mostly still relies on manual methods. These manual methods are time consuming, inaccurate and non-repeatable. Inconsistency in inventory assessments of aquatic organisms leads to mismanagement of feed and poor production performances for aquaculture producers.

In recent years, technologies such as computer vision have been explored with moderate success to provide information about aquatic organisms. However, recent development in artificial intelligence are proving to deliver viable options for efficient development of computer vision based solutions in aquaculture. In this project, an artificial intelligence approach using machine learning and computer vision was used to accurately predict the number of Giant tiger prawn (*Penaeus monodon*) post-larvae in a production setting. Data was gathered using an electronic device that image samples in optimal conditions. A training framework was then used to train and validate a classifying algorithm based on annotated data.

Once trained, the algorithm could count Giant tiger prawn (*Penaeus monodon*) post-larvae with more than 97.6% accuracy and 2.1% standard deviation. Other algorithms were also developed combining different technologies for different species and prediction of the size distribution was also implemented in a similar but more complex way.

Fig. 1. Example of a sample image being processed.

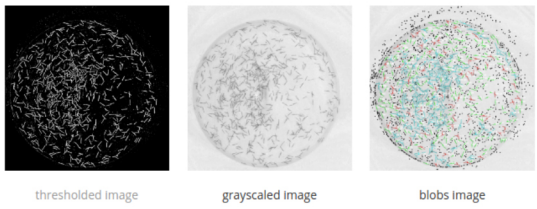

Table 1. Counting accuracy of Giant tiger prawn post-larvae using artificial intelligence.

Mean (%)	Standard deviation	N
97.6	2.1	723

"DIVERSIFY": EXPLORING THE BIOLOGICAL AND SOCIO-ECONOMIC POTENTIAL OF NEW/EMERGING CANDIDATE SPECIES FOR THE EXPANSION OF THE EUROPEAN AQUACULTURE INDUSTRY

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Introduction

The project DIVERSIFY (FP7-KBBE-2013, GA 603121) is already in its final period (2013-2018). This EU funded project has the objective of acquiring the necessary knowledge for the diversification of the European Aquaculture production based on new/emerging finfish species. Six new/emerging finfish species, with great potential for the expansion of the EU aquaculture industry were identified by the project (**www.diversifyfish.eu**). These included **meagre** (*Argyrosomus regius*) and **greater amberjack** (*Seriola dumerili*) for warm-water marine cage culture, **wreckfish** (*Polyprion americanus*) for warm- and cool-water marine cage culture, **Atlantic halibut** (*Hippoglossus hippoglossus*) for marine cold-water culture, **grey mullet** (*Mugil cephalus*) a euryhaline herbivore for pond/extensive culture, and **pikeperch** (*Sander lucioperca*) for freshwater intensive culture using recirculating systems. Research has been carried out in six scientific disciplines, namely Reproduction & Genetics, Nutrition, Larval and Grow out husbandry, Fish health, and Socioeconomics including final product quality.

The **Socio-economic** research in DIVERSIFY has three main objectives: (a) find out the consumer market opportunities for the six new species (*i.e.* greater amberjack, pike perch, meagre, wreckfish, Atlantic halibut and grey mullet), (b) examine the business-to business market opportunities for the species above and (c) develop business models for the new species on the basis of an online market test. These insights are being generated for the five largest European fish markets: France, Germany, Italy, Spain and the United Kingdom. The outcomes of this work will help the EU aquaculture sector and the supply industry in targeted marketing and improvement of its international competitive position.

Acknowledgements

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GENOMIC APPROACHES TO UNDERSTANDING AND IMPROVING RESISTANCE TO SEA LICE IN ATLANTIC SALMON

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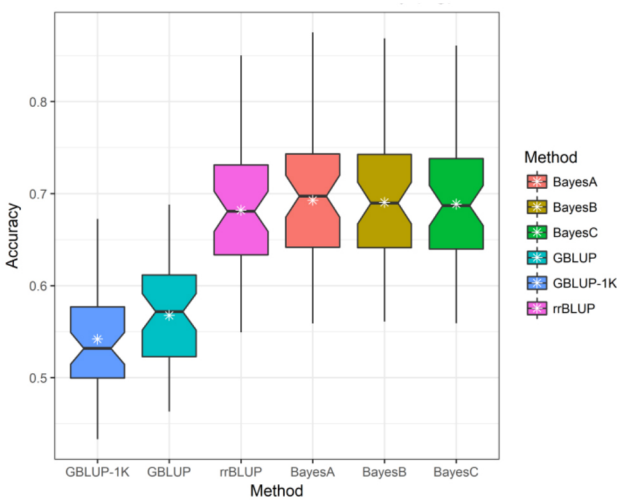
Sea lice are parasitic copepods that cause large economic losses to salmon aquaculture worldwide. Alternative methods to control this parasite, such as selection for host resistance, are increasingly important. Further, insight into the host-parasite interaction and mechanisms of host resistance can lead to improvements in selective breeding for resistance, and potentially novel treatment targets. The aims of this study were to evaluate the potential of genomic selection for resistance to sea lice (*Caligus rogercresseyi*), to characterise the functional genomic basis of host resistance, and to identify potential functional polymorphisms underlying resistance. To achieve this, lice challenge experiments were performed on a population of salmon from a Chilean breeding program. 2,500 salmon were genotyped using a 1K SNP panel and imputed to high density (~40K) from genotypes of 1,000 full-siblings. In addition, salmon from resistant and susceptible families were compared using RNA-Seq of attachment sites and healthy skin. Finally, pooled whole genome sequencing was carried out of identify all potential polymorphisms in the regions of interest.

Heritability of sea lice resistance was estimated ~0.25 and genomic selection using imputed genotypes showed increased selection accuracy, especially with Bayesian methods (Figure 1). Genome-wide association analyses revealed a significant QTL in chromosome 3, and regional heritability methods confirmed that QTL in chromosome 3 and suggested two others in chromosomes 18 and 21, each of them explaining 8-10% of the genetic variance. Analyses of the gene expression signature of host resistance revealed several genes and pathways significantly associated with resistance to lice, and these results were cross referenced with the GWAS and WGS data to identify candidate functional resistance genes and polymorphisms. These results improve our understanding of host response to lice in salmon, highlight the potential of genomic selection to improve host resistance to lice in salmon and identifies possible functional genomic variants that could be used to enhance genomic selection.

Figure 1. Genomic selection accuracy for sea lice density using different methods



GONAD QUALITY AND SAFETY OF TWO WILD POPULATIONS OF SEA URCHIN, *Paracentrotus lividus*, HARVESTED IN THE NORTH ATLANTIC COAST (PORTUGAL)

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Paracentrotus lividus gonads are prized seafood items mostly consumed raw in Europe. The exploitation of wild populations in Portugal has been increasing over the years, but the nutritional quality and risks associated to the consumption of raw gonads have never been evaluated. The aim of this study was to characterize important market-related traits, including the nutritional value and chemical/organic safety of sea urchin gonads from two wild populations seasonality harvested in the north coast of Portugal (Praia Norte and Carreço, Viana do Castelo). Gonad index (GI) was highest during winter and similar between populations (14% in Praia Norte and 11% in Carreço). Both populations had a single spawning event occurring during Spring-Summer, based on histological examination of gonads and the low GI (7%). Sea urchin is a rich protein and low fat seafood item. Biochemical composition of gonads showed a clear seasonal variation in both populations: the highest protein (17% WW) and lipid (6% WW) content were obtained during fall, when the gonadal tissue was growing and filling with nutritive cells. In turn, moisture reached minimum levels during fall (68%). A premium gonad is characterized by its size combined with specific sensory traits sought by the consumers (taste, color and texture). Both populations showed similar color, total carotenoid content and profile, but these traits suffered a seasonal variation. During fall, the concentration of total carotenoids and the main pigments (echinenone and β-carotene) peaked, gonads color began to become brighter and yellowish and tissue firmness achieved its highest level. Most examined chemical and organic contaminants (HNS, BTs, phenols, and PAHs) were found below or near the limit of detention in the sea water and gonadal tissue and, in general, were not affected neither by season nor sampling site. In conclusion, in terms of food safety, gonads harvested in the north coast of Portugal can be consumed all year round, but their nutritional value is higher during fall when market-related traits, such as color and texture are enhanced.

Acknowledgements: This work was supported by the Project INNOVMAR (ref. NORTE-01-0145-FEDER-000035) within the research line INSEAFOOD, founded by the NORTE2020 through the ERDF.

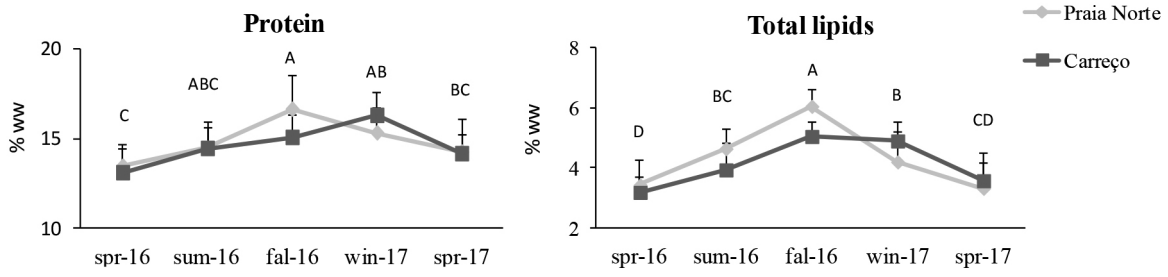

Figure 1: Seasonal changes in protein and total lipids (% WW) in gonads of *P. lividus* harvested in Praia Norte and Carreço; Superscripts indicate significant differences between seasons (two-way ANOVA, $P< 0.05$).

BIOCHEMICAL CHARACTERIZATION OF OYSTERS *Crassostrea gigas* CULTIVATED IN THE NORTHWEST COAST OF PORTUGAL (AVEIRO)

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Quality and safety of seafood products are important for consumers' buying choices and must be assured. This study aimed to assess trace metal contents (Cd, Cu, Cr, Hg, Ni, Pb and Zn) and nutritional composition (moisture, ash, protein, lipid, glycogen and energy) of commercial sized oysters collected seasonally over one-year production cycle in an aquaculture farm located in the North-western Portuguese coast (Canal de Mira, Aveiro). Sampling was carried in four seasons (July 2016, summer; November 2016, fall; January 2017, winter; and May 2017, spring). Oysters (ca. 35 individuals) were collected from production trays, placed in Canal de Mira, i.e. a channel of Ria de Aveiro with an open connection to the sea. For trace metals analysis, a pool of oysters (ca. 20 individuals per season) was opened, soft tissues removed, mixed with the intervalvar liquid and freeze dried until constant weight. For the determination of the nutritional composition, 15 oysters were sampled individually at each season to record biometric information. Body samples were then pooled and freeze dried for biochemical analysis.

Oysters showed a premium quality all year round by presenting an AFNOR quality index higher than 17 (classified as "special quality" when index is ≥ 9). Trace metal values were, in general, low. The concentration of Cd, Hg and Pb were always below the maximum levels (MLs) set for contaminants in foodstuffs by the Commission Regulation No. 1881/2006, amended by 420/ 2011, thus representing a minimal health risk to consumers. The nutritional composition of oysters varied seasonally: during summer, the protein, fat and energy levels were highest, whereas moisture and glycogen showed lowest levels. These results indicate that oysters produced in Aveiro can be consumed all year as a safe and high nutritional quality seafood product.

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Table 1: Nutritional composition of the edible part of *Crassostrea gigas* produced in Aveiro, Portugal. Superscripts indicate significant differences (ANOVA, $P< 0.05$).

	summer-16	fall-16	winter-17	spring-17
Moisture (%)	76,27 ± 0,78 ^{b}	79,05 ± 1,28 ^{a}	80,59 ± 0,81 ^{a}	79,11 ± 0,32 ^{a}
Ash (%ww)	2,40 ± 0,17 ^{b}	2,36 ± 0,14 ^{b}	2,40 ± 0,17 ^{b}	2,70 ± 0,17 ^{a}
Protein (%ww)	15,10 ± 0,40 ^{a}	11,08 ± 0,49 ^{c}	10,36 ± 0,41 ^{c}	12,68 ± 0,49 ^{b}
Total fat(%ww)	3,49 ± 0,83 ^{a}	2,58 ± 0,30 ^{ab}	2,43 ± 0,26 ^{b}	2,60 ± 0,26 ^{ab}
Total glycogen (%ww)	1,74 ± 0,31 ^{b}	5,30 ± 0,89 ^{a}	5,15 ± 0,51 ^{a}	2,42 ± 0,32 ^{b}
Gross energy (kJ/g)	5,17 ± 0,38 ^{a}	4,37 ± 0,30 ^{b}	3,98 ± 0,19 ^{b}	4,35 ± 0,10 ^{b}

CONTRIBUTING TO THE BMP GUIDELINES FOR THE FARMED SHRIMP PRODUCTION CHAIN: A CASE STUDY FROM BRAZIL

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The application of the concept of BMPs and Biosecurity Measures in marine shrimp farming involves steps similar to those in the conventional cycle of managing the activities of a Quality System, known as PDCA - Plan, Do, Check and Act. The Plan phase must be initiated with the previous study of the disease or diseases that you want to control, in order to determine the forms of transmission and proper strategies to reach the objective within the limits of the technical parameters; The Do phase encompasses the implementation of operational strategies necessary for the control of diseases, such as sanitary facilities, personnel training and adoption of recommended procedures; The Check of the samples and analysis of parameters allows for the measurement of the efficiency of the procedures of control of the diseases and, to Act is to adopt the corrective actions necessary for the adjustment of the established limits.

The Biosecurity, in turn, is the term applied to the prevention, containment and elimination of diseases that affect farmed shrimp. Procedures for Best Management Practices and Biosecurity Measures prepared by ABCC (Brazilian Shrimp Farmers Association) in 2.000 bring together and systematize a set of standards and principles that need to be adopted throughout the entire shrimp production chain, involving comprehensive and safe guidelines on how to prevent, control and, if at all possible, eradicate the diseases that affect the sector, ensuring the regularity and sustainability of its production.

The essence of the document, lies in the conscious commitment of all sector stakeholders to follow and apply the recommended measures and procedures as the most suitable and reliable way of confronting and living with the diseases that have caused huge financial losses to global shrimp farming. The final document, updated in 2015, is structured in four Modules that contain the Procedures of Best Management Practices and Biosecurity Measures for each segment of the sector: Module I – Maturation / Hatcheries; Module II – Nursery / Growout Units; Module III – Feed Mills / Feeding and, Module IV – Processing Industries. .

In this way, the Procedures of Best Management Practices and Biosecurity Measures were conceived and structured as a guideline instrumentto maintain under relative control the health of shrimp farming in Brazil, ensuring the sustainability of its development. Parallel to this action with producers, the dissemination of its guidelines is being widespread to technicians, workers, representatives of the related industries and other stakeholders involved in the production chain of the national farmed shrimp industry, so that the sector as a whole can embrace the Best Management Practices and Biosecurity Measures aimed at prevention, control, coexistence and fight against diseases that affect farmed shrimp.

In order to carry out the above-mentioned actions, ABCC and its affiliated State Associations are developing a comprehensive and systematic information and training plan through dozens of theoretical and practical courses aimed at raising the awareness of producers, whether small, medium or large, on the importance of adopting such measures.

DIETARY CAROTENOIDS CAN AFFECT GONAD COLOUR OF SEA URCHIN *Paracentrotus lividus*

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Paracentrotus lividus gonads are considered a prized seafood product. The high quality determines market prices, thus product appearance is an important factor, being firstly evaluated through colour. The desirable yellow-orange colour is determined by carotenoids deposited in the gonads, mainly the accumulation of echinenone that is its dominant pigment. Gonad echinenone content depends on the availability, uptake and bioconversion of β-carotene from dietary sources. Four extruded diets containing 30% protein and 6% fat were enriched with two β-carotene sources: paprika (PAP) and synthetic β-carotene (BC) at two incorporation levels (10 and 25 mg β-carotene/kg). A non-supplemented diet was used as control (CTRL) diet. Groups of 20 wild harvested *P. lividus* (44g; 4.7cm diameter) were randomly distributed by plastic mesh cages within 50L tanks in a saltwater recirculation system (35‰, 18ºC) with a 12hL:12hD regime. Sea urchins were fed the experimental diets for 8 weeks.

At the end of the feeding trial, male and female body weight (47.5 g), gonad weight (6 g), and gonadal somatic index (GSI, 12.5%) were not affected by the dietary treatments (P 0.05). Daily growth index (0.1 %), voluntary feed intake (0.88 %) and feed conversion ratio (4.3-5.5) were also similar among dietary treatments. All diets resulted in a significant increase of gonad weight and GSI.

Redness (a*) values of gonads were significantly higher in urchins fed either the BC10 and the BC25 diet than in those fed the CTRL or PAP10 diets, whereas yellowness (b*) values were highest in BC10, irrespectively of the gender; values were always higher in females than in males (P<0.05). Gonad lightness did not vary among dietary treatments. Sea urchin fed BC10, BC25 and PAP25 diets resulted in similar gonads' redness and yellowness to those observed in wild animals (initial sample). Gonad lightness significantly increased with the experimental diets, and males had lighter gonads than females, irrespectively of the dietary treatment (P<0.05). Gonad texture (firmness and resilience) was not affected by the dietary treatments.

All diets were effective in increasing gonads size, but the inclusion of 10 mg synthetic β-carotene/kg in the experimental diets resulted in a significant increase in gonads redness and yellowness values that were similar to values reported in wild sea urchins. Moreover, colour varied significantly between genders, suggesting that males and females have different metabolic uses of dietary carotenoids and possibly differ in their dietary request for pigments. Further studies are required to fully understand the mechanisms involved in the modulation of gonad colour through diets in order to potentiate the production of desirable gonads of both gender.

Work supported by Project INNOVMAR (NORTE-01-0145-FEDER-000035) within the line "INSEAFOOD - Innovation and valorization of seafood products", founded by NORTE2020 through the ERDF.

DETERMINANTS OF TECHNICAL CHANGE IN THE SALMON AQUACULTURE SECTOR IN NORWAY 2001-2014: A GENERAL INDEX APPROACH

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The salmon aquaculture sector is one of the fastest growing industries in Norway. For more than 30 years the sector experienced high productivity and industry concentration. Many factors influenced productivity growth, including new technologies, improved inputs, and better management practices (Asche, Guttormsen, & Nielsen, 2013). Specially, technical change showed to be the main driver of productivity growth that implies a reduction in production costs over time. However, productivity growth started a reversing tendency since 2005 with a slowdown in technical change and - as a consequence - production costs have increased during the last decade (Nilsen, 2010). This paper examines the determinants of technical change for the salmon aquaculture sector during the period 2001-2014. We estimate a purely general index of technical change from a flexible cost function that allow us to decompose the index in the following 4 effects: Scale augmentation, capital augmentation, non-neutral, and pure effect.

We found in average negative technical change during the period under analysis. This result imply that firms have become less efficient overtime. The decomposition of the technical change index let us see to what extent each effect had contributed to such results. Our estimations show that the pure effect component is the main driver of the reversing tendency as it explains more than 80% of the technical change index. The scale augmentation effect showed little contribution to the index while the capital and non-neutral components were empirically unimportant when evaluated at the representative firm. These results point out that efficiency in salmon aquaculture sector is being affected negatively by external factors that are not under control of the firms. Our results are a starting point for future studies regarding the welfare effects of policy measures in the industry.

Asche, F., Guttormsen, A. G., & Nielsen, R. (2013). Future challenges for the maturing Norwegian salmon aquaculture industry: An analysis of total factor productivity change from 1996 to 2008. *Aquaculture, 396*, 43-50.

Nilsen, O. B. (2010). Learning-by-doing or technological leapfrogging: Production frontiers and efficiency measurement in Norwegian salmon aquaculture. *Aquaculture Economics & Management, 14*(2), 97-119.

ENZYMATIC HYDROLYSIS OF PROTEINS IN THE FISH FROM BY-CATCH OF SHRIMP TRAWL FISHERIES

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By-catch of the shrimp trawling fishing has been an environment issue during the past decades. For each kilogram of shrimp catch shrimp 10 to 20 kg of other organisms are captured and discarded died in the ocean. Nevertheless, this reject can be an abundant source for the extraction of bio-active molecules, which may add value to these wastes. In this study, protein hydrolysates were obtained from the most abundant fish species obtained in the by-catch of shrimp fisheries in Brazilian, *Paralonchurus brasiliensis* and *Micropogonias furnieri,* by enzymatic hydrolysis using the enzymes Alcalase (A) and Protamex (P). The fishes were collected in September 2017 in the region of Ubatuba, São Paulo, Brazil, by double-rig a commercial shrimp trawling. The muscle and skin of the animals were blended and packaged in sealed 100 g plastic bags and transported to the Aquaculture Center. The samples was hydrolyzed using A and P. The hydrolysis were conducted at pH 7 and 50°C, with enzyme-substrate ratio of 2% (w/w) for both enzymes. The degree hydrolyses (DH) was monitored by pH-stat method.

The results showed that both the species hydrolyzed with A possessed a higher DH than did samples treated with P (Table 1). However, did not showed a significant difference ($P < 0.05$) between *P. brasiliensis* and *M. furnieri* using enzyme A. On the other hand, the enzyme P was more efficient in *P. brasiliensis* than for *M. furnieri*. In both the species, the rate of hydrolysis was during the first 100 min. Thereafter, a slower rate of hydrolysis was found after of 150 min. until a steady-state phase.

This characteristic was verified in hydrolysis from different protein sources as reported by some authors. The high DH, observed with enzyme A, is related to a higher proportion of low molecular weight soluble proteins in the muscles. A lower reaction rate observed with the enzyme P could be occurred due to affinity of the enzyme with substrate, thus it could be possibly inferred that A and P have different proteolytic affinities for substrate protein and, therefore, A is more efficient than P. Our results suggesting that the enzymatic hydrolysis proved to be an efficient process to obtain hydrolyzed proteins. Tests about the biological activity of such hydrolyzed are under research.

Table 1. Descriptive analysis of the DH at 300 minutes of the species under the action of the enzymes Alcalase and Protamex.

Species	Enzyme	
	Protamex	Alacalase
	Average (SE)	Average (SE)
Paralonchurus brasiliensis	**24,60 (1,32)^{b}**	**40,87 (2,42)^{a}**
Micropogonias furnieri	**19,48 (0,69)^{c}**	**42,82 (1,29)^{a}**

SE: standard error; Different letters indicate significant differences determined by Tukey tests (p<0,05).

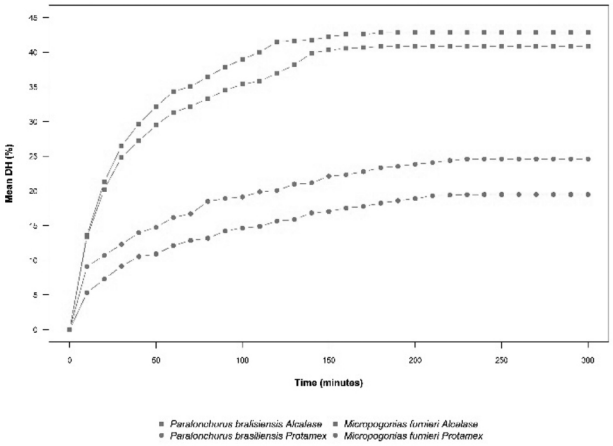

Figure 1. Action of the hydrolysis in the muscle of the two species and the maximum DH for each enzyme.

EFFECT OF SUPPLEMENTAL ADDITIVE EMULSIFIER ON THE GROWTH PERFORMANCE AND NUTRIENT UTILIZATION IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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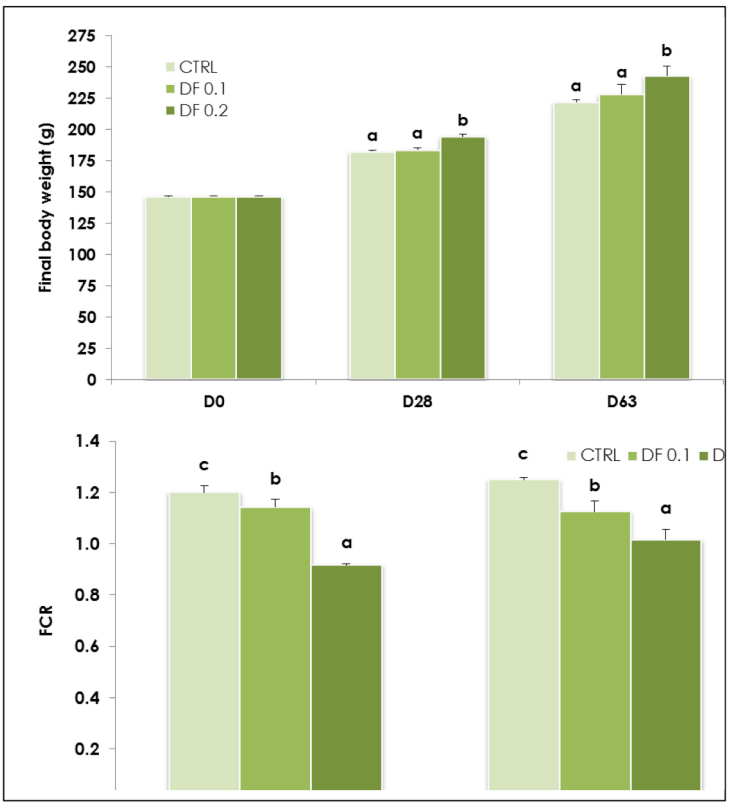
A study was undertaken to evaluate the efficacy of a complementary feed with emulsifier properties plus botanicals on the growth performance, whole body composition, nutrient digestibility and retention of European seabass fed a practical diet. The trial comprised three dietary treatments: control diet (**CTRL**) mimicking a commercial formulation for European seabass; a test diet based on this same formulation but with a complementary feed emulsifier at 0.1% (diet **DF0.1**) and with a concomitant reduction of 1% soybean oil; and a second test based on the same formulation with a complementary feed emulsifier at 0.2% (diet **DF0.2**) with a 2% reduction of soybean oil.

At the end of the trial, fish fed diet DF0.2 showed a significantly higher final body weight (FBW) and specific growth rate (SGR) than those fed diets CTRL and DF0.1 ($P<0.05$). Also, fish fed diets DF0.1 and DF0.2 showed a significantly lower feed conversion ratio (FCR) than those fed the CTRL diet ($P<0.05$). Moreover, fish fed the DF0.2 showed also a significantly lower FCR than those fed the DF0.1 diet ($P<0.05$). Feed intake (FI) was not significantly affected by the various dietary treatments ($P>0.05$).

Fish feed diets DF0.1 and DF0.2 showed a significantly higher protein efficiency ratio(PER) than those fed the CTRL diet. Moreover, fish feed DF0.2 showed also a significantly higher PER then those fed the F0.1 diet ($P<0.05$).

Fish feed diets DF0.1 and DF0.2 showed significantly higher dressing yield than those fed the CTRL diet ($P<0.05$). Fish fed diets DF0.1 and BF0.2 showed a significantly higher fat retention than those fed the CTRL diet ($P<0.05$), while fish fed the DF02 diet showed also a significantly higher energy retention than those fed the CTRL diet ($P<0.05$).

Complementary feed with botanicals and emulsifier properties at 0.2% with a concomitant reduction of 2% soybean oil, resulted in significant beneficial effects on the overall growth performance criteria in sea bass (*Dicentrarchus labrax*).



CHARACTERIZATION OF THE TLR FAMILY IN *Branchiostoma lanceolatum* AND DISCOVERY OF A NOVEL TLR22 INVOLVED IN dsRNA RECOGNITION IN AMPHIOXUS

Jie Ji, David Ramos-Vicente, Carlos Herrera, Enric Navas, José Miguel Lizcano, Jordi García-Fernández, Hector Escrivá, Àlex Bayés and Nerea Roher

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Toll-like receptors (TLRs) are important for raising innate immune responses in both invertebrates and vertebrates. Here we have identified 30 new putative TLR genes of *Branchiostoma lanceolatum* from transcriptome data and we have demonstrated by RT-PCR that all of them are expressed in adult amphioxus. Phylogenetic analysis showed that the repertoire of *B. lanceolatum* TLRs consists in both non-vertebrate- and vertebrate-like TLRs. The phylogeny of these genes also indicates lineage specific expansions in orthologous clusters of the vertebrate TLR11 family. To gain insight in this TLR11 family we studied in depth a particular TLR highly similar to a *B. belcheri* gene. The phylogenetic analysis of this novel BlTLR showed that it clusters with the vertebrate TLR11 family and it might be more related to TLR13 subfamily according to similar domain architecture. Transient and stable expression in HEK293 cells showed that this new receptor localized on the plasma membrane, but it did not recognize most of the common ligands for TLRs. However, when we fused the ectodomain of BlTLR to the TIR domain of human TLR2, it could indeed induce NF-κB transactivation in response to Poly I:C indicating that in amphioxus, specific accessory proteins are needed for downstream activation. Based both on phylogenetic and functional analysis, we propose that the novel BlTLR might be classified as an antiviral receptor TLR22 (BlTLR22). TLR22 is thought to be viral fish specific TLR but here we demonstrate that teleosts and amphioxus TLR22 probably share a common ancestor. Additional functional studies with other lancelet TLR genes will enrich our understanding of the immune response in amphioxus and will provide a unique perspective on the evolution of the immune system.

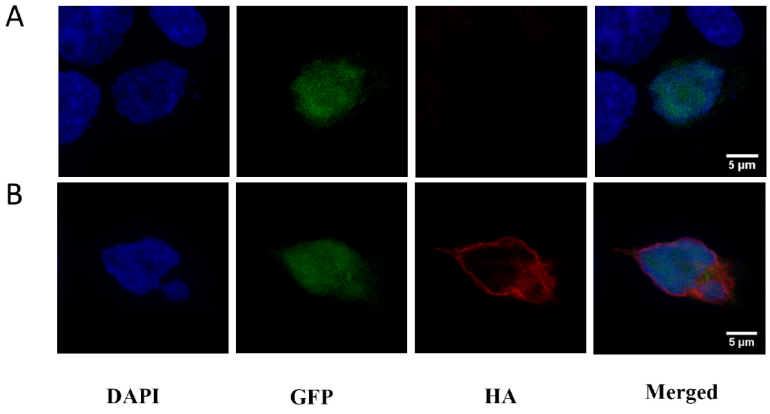

Figure 1. Subcellular localization of BlTLR. (A) Empty vector (B) HEK293 cells stably expressing BlTLR-HA.

MONITORING AND PURPOSE OF MANAGEMENT OF FLAT OYSTER (*Ostrea edulis*) WILD STOCKS IN THE MIDDLE ADRIATIC SEA

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In the past, the recruitment of wild of flat oyster (*Ostrea edulis*) represented a good economic income for local fishermen of the artisanal fishery in the middle Adriatic sea, along the Italian coastline. In recent years, a sharp decline in the collection of spat and juveniles of this species of bivalve has been recorded from this area. The decrease is causing strong concerns among operators and public administrators. Studies showed that among the causes are mainly to consider over-exploitation of natural areas, scarcity of richness of benthic ecosystems and lack of effective management of marine environment.

The present work refers about a study which has been performing in different times in order to investigate the state of natural stocks of flat oysters and purpose management interventions. In a first step, a characterization of energy values was investigated through two years evaluating the nutrients and glycogen content in wild oysters collected using a hand dredge dropped from a boat. In a second step, types of different collectors are going to study and optimize in order to increase the success of collection of flat oysters, to be located on the bottom or suspended in an area of longline plant. Efficiency is evaluated on the basis of used supports, depth and immersion period.

Acknowledgements – Research supported by FAR-UNICAM 2014 and FLAG “Marche Centro” 2018.

TRANSCRIPTOME ANALYSIS OF TURBOT (*Scophthalmus maximus* L.) THYMUS IN RESPONSE TO INFECTION BY *Enteromyxum scophthalmi* (MYXOZOA)

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Enteromyxosis caused by the myxozoan parasite *Enteromyxum scophthalmi* poses a serious threat for turbot (*Scophthalmus maximus* L.) aquaculture. The parasite invades and colonizes the digestive tract, causing severe catarrhal enteritis associated to a cachectic syndrome. The disease, directly transmitted from fish to fish, presents elevated morbidity and mortality rates, and can affect up to 100 % of fish at farm facilities. Currently, no effective therapeutic treatments are available to control this parasitosis. Transcriptome analysis by RNA-Seq has emerged as a very useful tool to better understand host-pathogen interaction, critical to devise successful disease prevention strategies and for achieving more robust broodstock. RNA-Seq analysis was applied to get insights into the pathogenesis of the disease by studying pyloric caeca, spleen and kidney. Nevertheless, the role of the thymus, a primary lymphoid organ, was never evaluated in enteromyxosis, and, in general, it has been poorly studied in teleost pathology despite its relevance for the functionality of the immune system.

In this study, the thymus transcriptomic profile was analyzed in turbot suffering advanced enteromyxosis and their respective controls, using the Illumina HiSeq 2500 platform for cDNA sequencing (100 bp paired-end reads). The recently assembled turbot genome was employed as reference for read mapping and the expression levels were compared using DeSeq2. A total of 4,113 genes resulted differentially expressed (DE) between infected and control turbot, considering DE genes those showing a False Discovery Rate (FDR) corrected P value < 0.05. Among these genes, 1,888 were up- and 2,225 down-regulated in infected fish compared to controls (Figure 1). Genes related to innate and adaptive immune response were mainly found among those up-regulated, as well as apoptosis-related genes, while functional analysis showed that genes involved in cell cycle and cell proliferation were prominent among those down-regulated. The transcriptome analysis was complemented by the *in-situ* evaluation of especially relevant gene products by immunohistochemistry, as a functional read-out of gene expression. This is the first description of the thymus transcriptome in turbot, providing novel insights into the role of this organ during infection in teleosts.

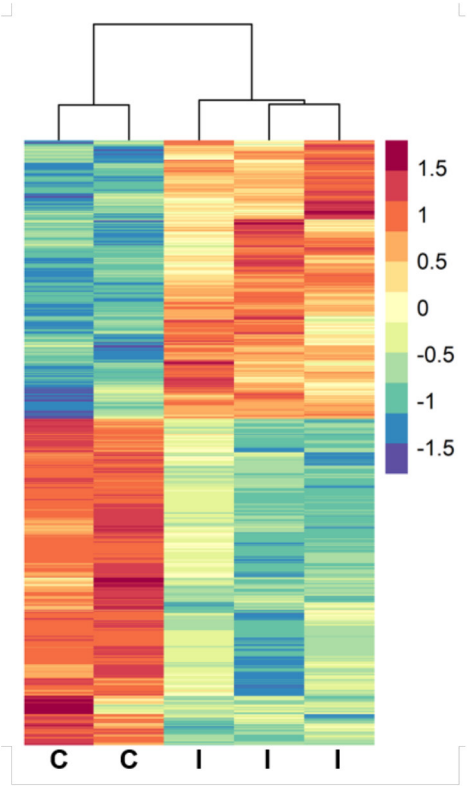

Figure 1. Heatmap of DE genes in turbot thymus. Expression values for each gene have been scaled from −1.5 to 1.5 by subtracting the mean and dividing by the standard deviation. C=control; I=infected.

This work was funded by the Spanish Ministry of Economy and Competitiveness under the Projects AGL2015-67039-C3-1-R and AGL2015-67039-C3-3-R and the European Regional Development Fund (ERDF).

ROLE OF MICROALGAE IN A LANDBASED MULTI-TROPHIC AQUACULTURE SYSTEM: BIOREMEDIATION, PRODUCTION, BIOCHEMICAL COMPOSITION

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The incorporation of microalgae culture in a land-based multitrophic aquaculture system was investigated during the "IMTA-effect project" (2016-2019). Two experiments were conducted in 2017 and 2018 using a landbased IMTA system combining the production of European sea bass (*Dicentrarchus labrax*) in a recirculating aquaculture system (RAS), natural marine microalgal assemblages in high rate algal ponds (HRAP) and oysters growing in separate open tanks. Our results demonstrated: 1) The efficiency of the microalgae for fish effluent bioremediation with an overall removal efficiency of 335.8 ± 0.8 for NO3-N and 22.3 ± 0.2 for PO4-P mg m^{-2} d^{-1}; 2) The rapid shift from green algae to diatoms 15 days after silicate addition (Si:N:P molar ratio of 10:5:1) leading to a dominance of *Phaeodactylum* sp. (Fig 1); 3) A microalgal productivity sufficient to meet oyster food requirement (production peak of 0.42 ± 0.03 mg chl*a* L^{-1}, Fig. 2); 4) The incorporation of produced diatoms by oysters using stable isotope signatures.

The results will be discussed together with the changes in environmental conditions, the nature of algal inoculum, the biochemical composition of algae and oysters and the system characteristics. Perspectives for research opportunities and aquaculture producers will be discussed.

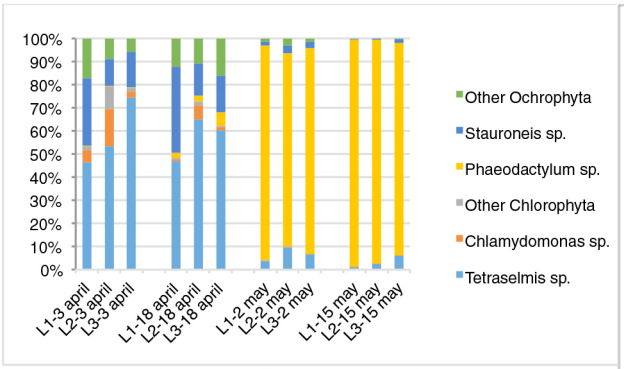

Fig 1. Microalgal community structure composition at genus level. The abundance is defined as the number of sequences affiliated with a genus divided by the total number of sequences obtained per sample. "Lx-Dy" indicates samples taken in Laggon x on day y

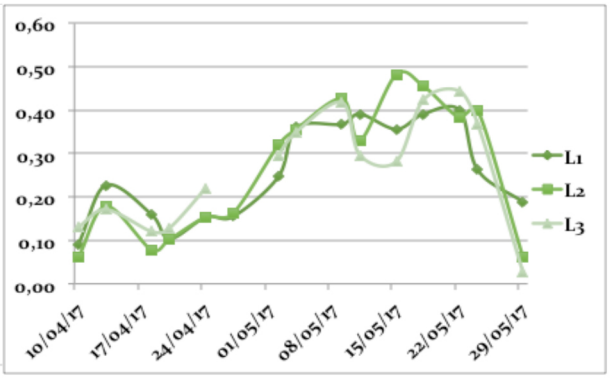

Fig 2. Microalgal biomass concentration expressed in chlorophyll a concentrations over the experimental period, for the 3 raceway lagoons (L1, L2, L3)

1H-NMR METABOLOMIC INVESTIGATION OF THE EFFECT OF ALTERNATIVE DIETS ON *Rainbow trout* PLASMA PROFILES

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Fish farming needs high quality feeds to support the growing global demand for fish. Over the last two decades, the development of sustainable feeds based on plant feedstuffs has strongly reduced the use of marine resources. However, full plant-based feeds still reduce growth performances in carnivorous species such as salmonids. Thus, active research programmes are conducted on alternative feedstuffs in order to provide sustainable feeds for aquaculture especially trout farming.

Our project aims at establishing a link between feed composition and plasma metabolome of rainbow trout (*Oncorhynchus mykiss*) through *i*) the characterization of plant-based diets devoid of marine resources complemented with insect, micro-algae or yeast products, and *ii*) the analysis of trout plasma metabolome fed these alternative diets.

Fish were fed *ad libitum* for three months with nine, iso-proteic and iso-energetic experimental diets: a control plant-based diet (PB) and eight diets containing 5%, 10% and 15% of either insects (INS), micro-algae (SPI) or yeast (YST). Ethanolic extracts of diets were prepared and analysed. Fish blood was collected 48 h after feeding and centrifuged to obtain plasma. The 1D ^{1}H-NMR profiles of both diet extracts and plasmas were acquired on a 500 MHz NMR spectrometer. Spectra were processed to select and integrate spectral regions with NMRProcFlow (nmrprocflow.org) and statistical analyses were realised with BioStatFlow webtool (biostatflow.org).

Diet extract ^{1}H-NMR spectra showed specific compounds for INS and SPI diets compared to PB. These signals were related to triglyceride residues and organic acids and account for the origin of feedstuff and the effect of process. Multivariate analysis of plasma spectral data showed a classification of individuals based on diets according to highly discriminant signals variables. Several specific signals showed an increasing trend with graduate incorporation of alternative feedstuffs.

Integrative metabolomics combining diet and trout plasma characterization highlights the significance of the soluble fraction of alternative diets and their extended impact on plasma metabolome.

This research was funded by FUI 2014 (NINAQUA with Le Gouessant, COPALIS, Algae Natural Food and Phileo Lesaffre Animal Care), ANRT (CIFRE 2016/0775) and MetaboHUB (ANR-11-INBS-0010).

OUTCOME OF THE 8TH INTERNATIONAL SYMPOSIUM ON STURGEONS (ISS 8) AND THE *"Vienna 2018 Declaration on Global Sturgeon Conservation"*

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World Sturgeon Conservation Society (WSCS), Germany

The "international Symposia on Sturgeons" (ISS) are organized by WSCS every four years to re-assess the conservation status of the highly endangered species. These Symposia deal also with issues on aquaculture, trade of sturgeon products and the progress made in rehabilitation programmes, including ex-situ culture for re-introduction into areas where these species are at the brink of extinction. Recognizing the complexity of threats imposed upon sturgeons, WSCS, in 2005, published the RAMSAR DECLARATION ON GLOBAL STURGEON CONSERVATION, outlining the guiding principles for measures urgently needed to ensure that sturgeons have a future. To meet the persiting and emerging challenges in sturgeon conservation, the ISS 8 was held in September 2017 in Vienna with a participation of about 300 sturgeon specialists from 32 countries. The conference dealt with major topics such as (a) general biology, (b) habitat restoration, (c) river connectivity, (d) genetics and ex-situ culture as well as (d) with aquaculture and trade of sturgeon products. At the end it was decided to update and expand the Ramsar Declaration of 2005 by formulating the "VIENNA DECLARATION ON GLOBAL STURGEON CONSERVATION 2017". This declaration includes many aspects relevant to aquaculture and trade control of sturgeon products derived from aquaculture, but also elements on requirements for ex-situ conservation and specific culture requirements for release. The key issues of interest to the aquaculture community will be presented in this contribution, providing an overall picture on the outcome of the ISS 8 elaborations while also presenting the background information on which several of the session papers today are building their concepts and arguments.

EXPANDING THE KNOWLEDGE BASE ON "*Culture requirements to produce strong juveniles for release*": THE NEED TO TRAIN FISH FOR FITNESS OF SURVIVAL IN A HARSH ENVIRONMENT

Harald Rosenthal

World Sturgeon Conservation Society (WSCS),Germany

Aquaculture for production and culture for release of juvenile into natural waters are two VERY different objectives requiring **totally** different methodologies to meet specific goals. In the past, most of the technical approaches for both purposes have been handled in a similar manner. This is no longer acceptable and we need to re-assess our principle handling strategies.

While **aquaculture production systems select for** (a) best survival, (b) high food conversion via feeds designed for best biomass gain, (c) fast growth rates to shorten grow-out time (d) disease resistance, (e) cost-effective production and finally (f) for high quality of the end product, the **culturing for restocking** needs to provide fish with the ability to (a) rapidly respond to changing environmental cues in nature, (b) adjust to behavioural traits (diurnal/nocturnal rhythms), (c) recognize predators by natural cues to avoid them successfully, (d) display robustness to abruptly changes in water quality (e.g. thermoclines; oxygen depletion; high turbidity and changing micro-light climate and wind/wave action), (e) resist challenges by pathogens/parasites (e.g.develop a well-functioning immune system before release), and (f) finally to effectively interact with behavioural traits of con-specifics (perfect intermingling). In short, culture for release must be designed to allow progeny at the time of release to match the "**fitness**" required for survival in the receiving habitat while also "**matching**" the characteristics in all traits of the con-specifics in natural populations. Although this is logical and the concept has been presented already over several years now, most stocking and rehabilitation programmes still lack a cohesive concept and systematic approach to meet these requirements. We present here a framework based on recently developed strategies for sturgeons (species of high longevity, low residual population size and specific habitat-dependent traits). During the past few years several studies have contributed elements in their culture approach that prove the benefits of adjusted rearing techniques. The conventional hatchery systems offer largely monotonous life conditions of little use to prepare the progeny for the harsh and challenging environmental conditions which they are facing in nature after release. Step by step adaptation to these challenges in a standardized format can help improving performance, including the transition to natural foods, exposure to predators, as well as acclimation to diurnal rhythmic cues (lunar cycles, tidal currents, wind and wave action, etc).

IN VITRO STUDY OF A DOSE- AND TIME- EFFECT RESPONSE OF A COMBINATION OF ORGANIC ACIDS AND NATURE-IDENTICAL COMPOUNDS ON GILTHEAD SEABREAM HEAD-KIDNEY LEUCOCYTES

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Organic acids (OA) and botanicals have a long history of use as feed additives in animal production with the aim of improving intestinal health and performance. To the best of our knowledge, the available studies in aquaculture are very limited and little is known about their impact on the cells of the immune system. Aim of this study was to assess the biological properties of OA and nature-identical compounds (NIC) on head-kidney leucocytes (HKLs) viability, functionality and oxidative response in an *in vitro* model. HKLs were isolated from gilthead seabream (*Sparus aurata)* and incubated with a combination of citric and sorbic acid, thymol and vanillin (MIX) at 0, 50, 100, 250, 500, and 1000 mg/L for 30 min, 2 h and 4 h. At any assayed time, the MIX did not affect leucocyte viability (which was determined by flow cytometry using propidium iodide) nor phagocytic ability. Regarding respiratory burst, starting at 30 min the MIX at 50 and 100 mg/L induced a decrease in activity compared to control.

The relative gene expression of HKLs after being incubated for 30 min, 2 hour and 4 hours without (control) or with different concentrations of the MIX was also studied by real time PCR. The selected antioxidant genes were nuclear factor erythroid 2 (*nrf*-2), superoxide dismutase (*sod*), catalase (*cat*) and glutathione reductase (gr). The expression of interleukin 1-beta (*Il-1* beta) and interleukins 6, 7, 8, 15 and 18 (*Il-6, 7, 8, 15, 18)* as pro-inflammatory genes and interleukin 10 (*Il*-10) and transforming growth factor β (*tgf-β*) as anti-inflammatory genes was also determined. Different significant variations were detected in the expression of all the studied genes on HKLs after being incubated with the different concentrations of the MIX. The increases or decreases recorded were related to the MIX concentration and to the incubation time.

In conclusion, the present results showed that the MIX of OA and NIC did not have a negative impact on HKLs viability and the decrease in the respiratory burst activity in a dose-dependent manner underlines the potential antioxidant power of the MIX. The variation in the expression of antioxidant genes and interleukins suggests a possible modulation of the immune-response mediated by the MIX that needs to be further evaluated *in vivo*.

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VIGITHAU: A MODELING TOOL FOR PUBLIC MANAGEMENT OF SHELLFISH FARMING AREAS

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In December 1989, salmonella destroyed much of the shellfish production in the Thau lagoon, causing a serious social crisis. In order to preserve the quality of the lagoon's water and to reduce the number of crises, significant investments have been made in waste water, but these have not been sufficient to stop the crises. Then, the public authority responsible for managing the lagoon catchment area (the Syndicat mixte du bassin de Thau, SMBT) has progressively engaged in the search for environmental excellence by adapting local regulations and implementing an original mode of governance and technical solutions for the prevention of pollution.

A scientific partnership has been established between local authorities and several research organizations (Ifremer, BRLi, EGIS Water). The ambition was to determine the maximum limit in bacteriological flow compatible with shellfish standards. For this purpose, a hydrodynamic model of the Thau lagoon allowed to calculate Admissible Microbiological Flows (FAM) in Ecoli number per day for each stream (29 in total). FAM values are listed in the water policy planning document (SAGE Thau Basin) and are applicable for all catchment area activities. Release authorizations are now issued taking FAMs into account and incorporating the cumulative effects of all pre-existing emissions to water.

The regulation makes it possible to instruct the new requests but is not always sufficient to solve the current problems of overcoming the FAMs in rainy weather. Thanks to the creation of an innovative lagoon catchment area model, which integrates both the river system (stream, river), storm drainage system and sewage networks, solutions are proposed to network managers and decision makers. A contractual tool, built on the model of integrated coastal zone management, has been set up to program actions to reduce pollution. It helps to overcome the partitioning of public policies and then to meet the common objectives of protecting maritime activities and defining cost efficient public works program.

The Vigithau system is an innovative environmental management tool that allows both real-time detection of microbiological pollution sources in the lagoon and health risk warming. The risk prediction system is based on a complex calculation process that transforms a rain forecast into a health risk index for the lagoon's activities (shellfish farming, fishing, swimming). In addition, the warning platform makes it possible to know in real time the state of contamination of the lagoon and its catchment area thanks to the continuous monitoring of water quality. In the end, this system makes it possible to anticipate pollution peaks several days in advance and thus to better manage crises. In case of a high risk of pollution, shellfish farmers are warned by newsletters and SMS messages; the time for them to build up shellfish stocks.

COMPARATIVE ANALYSIS OF DISTINCTIVE TRANSCRIPTOME PROFILES WITH BIOCHEMICAL EVIDENCE IN BISPHENOL S- AND BENZO[*a*]PYRENE-EXPOSED LIVER TISSUES OF THE OLIVE FLOUNDER *Paralichthys olivaceus*

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Flounder is a promising model species for environmental monitoring of coastal regions. To assess the usefulness of liver transcriptome profiling, juvenile olive flounder *Paralichthys olivaceus* were exposed to two pollutants, bisphenol S (BPS) and benzo[*a*]pyrene (BaP), which have different chemical characteristics and have distinct modes of metabolic action in teleost. Six hours after intraperitoneal injection with BPS (50 mg/kg bw) or BaP (20 mg/kg bw), liver transcriptomes were analyzed using the Illumina Hiseq 3000 platform. Interestingly, the transcriptome was highly sensitive and was distinctively expressed in response to each chemical. The primary effect of BPS was significantly increased transcription of egg process and vitellogenesis related genes, including vitellogenins (*vtg1*, *vtg2*), zona pellucida sperm-binding proteins (*zp3*, *zp4*), and estrogen receptors (*erα*, *erβ*), with increases in plasma 17β-estradiol (E2) and vitellogenin (VTG) concentrations. Following BaP treatment, detoxification- and biotransformation-related genes such as *cyp1a1* and UDP-glucuronosyltransferase (*ugt1a1*) were significantly increased, with an increase in EROD activity. In both transcriptomes, mRNA expression of genes involved in antioxidant defense systems was increased, while genes involved in innate immunity were decreased upon BPS or BaP exposure with a decrease in complement activity. This study provides useful insight into the chemical-specific hepatic transcriptional response of *P. olivaceus* and suggests a basis for further studies examining biomarker application of liver transcriptomes for environmental pollution.

SCIENCE TO INFORM THE DEVELOPMENT AND REGULATION OF OFFSHORE AQUACULTURE

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With increased demand for the production of seafood through aquaculture, technologies are rapidly being developed and deployed to grow fish, mussels, and seaweed at sites further offshore or in open ocean conditions. What is science's role in determining where these new facilities are located? How will potential environmental, social, and economic effects be properly addressed, regulated, and monitored? Producers and government agencies need science information to answer questions about resource efficiency (use of space and feeds); environmental effects (e.g., impacts on water quality, benthic habitat, and endangered species); aquatic health management; trophic relationships; social and economic effects; siting analysis and spatial planning. Science-based tools are needed for decision support for permitting, efficient monitoring, and analysis of reporting data. Although much can be learned from nearshore aquaculture, moving offshore requires new scientific information and refinement of models used for near shore marine aquaculture.

This presentation will provide an overview of the science products to address and manage offshore aquaculture now development in the United States and through collaborative international efforts by the International Council for the Exploration of the Seas (ICES) and other transatlantic research collaboration.

NUTRIENT FLOW IN THE INTEGRATED RABBIT–FISH–RICE AQUACULTURE SYSTEM IN RWANDA

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An analysis of nutrient flow, based on nitrogen (N) and phosphorus (P), was conducted on an integrated rabbit–fish–rice system (*IRFR system*) at Rwasave Fish Farming Station (University of Rwanda). Rabbits, stocked at 12 per are (1200 rabbits.ha^{-1}) of pond were reared over fishponds stocked with one and three male Nile tilapia (*Oreochromis niloticus* L.*)* per m^2 for pond treatments PT1 and PT2. Effluent fertilised by the rabbits was drawn away from the ponds by pipes installed at the bottom of the ponds to irrigate rice (*Oryza sativa* L., variety *Yuni yin4*) fields. There were six 400 m^2 ponds and nine 90 m^2 rice fields; three of the latter were irrigated by canal water and fertilised by NPK (200 kg.ha^{-1}.crop^{-1}, 2 applications; 100 kg.ha^{-1}.crop^{-1}, one application).

The results showed that rabbit droppings supplied about 27% N and 79% P of the total N and P inputs, fertilising the ponds at a rate of 3.98 kg N and 1.94 kg P.ha^{-1}.day^{-1}. The Nile tilapia fish recovered 18.57.6% N and 16.9–34.3% P of the total nitrogen (TN) and total phosphorus (TP) inputs. All water quality variables remained within good limits for tilapia aquaculture and nutrient distribution was not dependent on fish density. Large amounts of N and P accumulated in the water, sediment, and effluent fertilised rice fields at a higher rate (118.5 kg N and 27.2 kg P.ha^{-1}.day^{-1}) than that of inorganic fertilisers, resulting in a slightly higher rice yield than that induced by NPK and urea.

Tilapia effluent was thus able to substitute inorganic fertilisers completely, allowing savings to the farmers, and showing its potential as a fertiliser for fish and crop production rather than waste to be discharged, polluting the environment by its solids and organic matter component. Further studies involving a thorough analysis of nutrients lost and diversified uses of the nutrient-rich effluent are needed.

RISK MANAGEMENT TO MINIMIZE DISEASE-ASSOCIATED ECONOMIC LOSSES IN AQUACULTURE OF NEW FISH SPECIES – IDENTIFICATION OF INFLUENCING PATHOGENS

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Introduction
Due to low water consumption and the possibility to grow fish under warm water conditions, recirculating aquaculture systems (RAS) are getting more and more popular. One important problem in RAS are limited possibilities for treatments and disinfection measures, when fish are suffering from any kind of disease. In order to keep the bio filter working, a RAS normally will always be stocked with fish. That means there is no time point where you can empty and disinfect the whole system without a production stop. Additionally, several different fish species and age classes are usually produced in one system. Because of these complicating factors, a good health management and high import control standard including a proper quarantine are pivotal before new fish enter the RAS.

Project and study aims
The business partner of the project is a warm water RAS facility in the Swiss Alps. This fish farm cultivates European perch (*Perca fluviatilis*), pike perch (*Sander lucioperca*) and sturgeon (*Acipenser baerii, Acipenser gueldenstaedti*) for meat production and sturgeon caviar. The University of Bern (Centre for Fish and Wildlife Health, CFWH) supports the project as research partner. The fish health management of the facility is covered by a veterinarian specialised in fish medicine.

One of the main project goals is the identification of relevant pathogens in European perch and pike perch production. We strive to find and establish diagnostic methods and criteria to recognize infectious diseases as early as possible and to establish a prophylaxis and treatment concept to prevent financial losses due to increased mortality.

Methods
Since the start of the project in February 2017, we performed weekly controls to detect external parasites and changes in gill health by taking skin and gill smears.

After arrival of new animals in the quarantine, in addition to routine samples of skin and gills as described above we performed a bacteriology of the liver, kidney and spleen and took samples for histology. In order to detect viral infections, we inoculated organ samples on BF-2 (bluegill fry) and EPC (epithelioma papulosum cyprini) cells. At the same time, we performed a PCR to detect perch rhabdovirus DNA (Talbi et al. 2011, Bigarré et al. 2017).

Results
External parasites, mostly *Ichtyobodo sp.* and *Gyrodactylus sp.*, were identified at several occasions. *Aeromonas salmonicida* was isolated in a group of European perch, suffering from severe skin ulceration and muscle necrosis. In this bacterial strain, a Typ 3 secretion system (T3SS) was identified. The activity of the T3SS was confirmed on cell culture.
During the quarantine phase two cases of rhabdovirus infections were detected.

Funding
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DIFFUSIVE METHANE EMISSIONS FROM CARP PONDS (SOUTH BOHEMIA, CZECH REPUBLIC)

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Atmospheric methane (CH_4) concentrations increased from 715 ppb to 1775 ppb during the post industrialisation era mainly due to human economic activities. Aquaculture is one of the fasted growing economic activities nowadays. However, organic matter inputs that are poorly incorporated into reared animals may be fuelling methanogenesis processes in fishponds and other water bodies. Thus, there is an urgent need to estimate methane emissions from aquaculture in local methane emissions inventory. The aim of this study was to estimate methane emissions from carp ponds and to identify factors driving its missions from two types of carp ponds.

Sampling took place over growing season (from April to September) 2017 in six fishponds (South Bohemia, Czech Republic) of two types (main ponds and nursery ponds) in which common carp is the main reared fish. Dissolved CH_4 was extracted monthly from pond surface water using the headspace technique. Henry's law was used to quantify CH_4 in the headspace after its concentration measurement with gas chromatography. CH_4 emissions from ponds were calculated indirectly using the transboundary layer model (TBL model). Physico-chemical water parameters were measured as well.

All ponds were supersaturated with CH_4 during the study period. Nursery ponds had higher CH_4 concentrations than main ponds. However, CH_4 emission flux rates did not differ substantially between the two types of ponds (Table 1). Chlorophyll a concentrations, water temperature and fish size were the factors influencing CH_4 concentrations. TN, NH_4-N, NH_3-N, TP, SRP, Secchi depth, Chl-a, differed significantly between fishpond types whereas TOC, TIC, and DOC did not differ significantly.

COMBINED EFFECT OF TEMPERATURE AND DIETARY PROTEIN ON MEAGRE, *Argyrosomus regius*, JUVENILES GROWTH AND MUSCLE CELLULARITY

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Fish growth can be affected by temperature and by dietary protein. These two factors have been proven to influence fish muscle cellularity. Muscle cellularity refers to the area and density of muscle fibre and is the result of two different processes: fibre hyperplasia and hypertrophy.

The current study aimed to test the combine effects of water temperature and dietary protein level in the growth and muscle cellularity of meagre juveniles. To achieve this, two isolipidic diets with different protein content (45 and 57 %) were formulated and tested, in triplicates, at 20 and 24 ºC. Therefore, four treatments were used: 45 % protein at 20 ºC (LPLT); and diet with 45% protein (LP; LTLP); 45 % protein at 24 ºC (LPHT), 57 % protein at 20 ºC (HTLP) and 57 % protein at 24 ºC (HPHT). A total of 1056 fish (initial live weight 56 g ± 1.3) were used in each 1500 L tank and the experimental trial lasted 90 days.

Survival was between 94 and 97 %. Fish weight (wet weight) was significantly higher in fish from the HTHP group (156,8 ± 34,77 g) and significantly lower for both LT treatments (100,4 ± 18,68 g for LTLP and 106,3 ± 16,21 g for LTHP). The same was observed for fish length and specific growth rate (SGR), all higher in the HPHT group and lower in the LTLP e HTLP. No differences were found for muscle fibre area or density. This study shows that temperature has more impact on meagre growth compared to dietary protein.

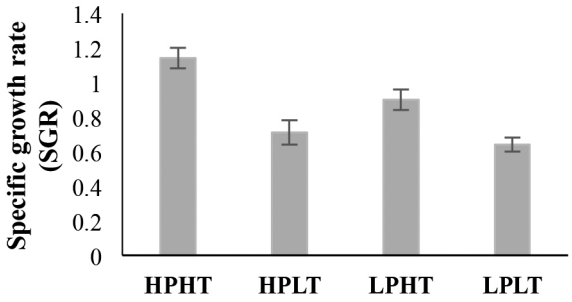

Fig.1 Specific growth rate of meagre juveniles fed diets containing different dietary protein levels (45 and 57 %) fed at 20 and 24 ºC.

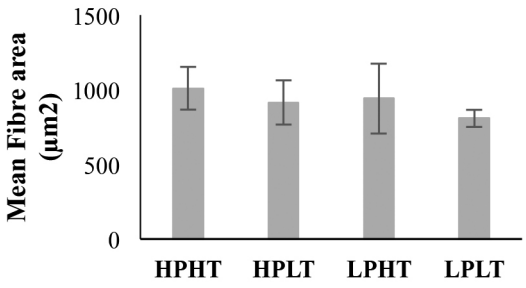

Fig. 2. Mean muscle fibre area of meagre juveniles fed diets containing different dietary protein levels (45 and 57 %) fed at 20 and 24 ºC.

EFFICIENT USE OF RESOURCES FOR THE SUCCESSFUL DEVELOPMENT OF AQUACULTURE IN THE DESERT AND ARID LANDS

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The increasing competition for water and land use for a wide range of economic activities is driving the expansion of aquaculture operations towards unfavorable regions such as desert and arid lands which can now be better exploited through the use of modern aquaculture technology and practices. The integration of aquaculture with agriculture is becoming progressively more attractive in areas where water is a limited resource. In fact such systems can reduce water requirement for the production of high quality protein source (fish) and fresh vegetable avoiding competition in water consumption between aquaculture and agriculture. Integrated desert aqua-agriculture can increase social and economic wellbeing of rural population living in remote areas through the production of food, contribution to livelihoods and generation of income. Current and future developments of fresh and brackish water aquaculture in desert and arid lands will rely greatly on the appropriate use of underground water using farming practices which ensure the smart use of this limited resource. This paper presents the most suitable aquaculture technology (flow-through, RAS, biofloc, aquaponics, etc.) used for rearing fish and shrimp species tolerant to arid land conditions for an efficient use of water and energy (solar panel) and the integration with other agriculture crops (fish/fodder/sheep), with the addition of biogas production. In particular, more detailed information is provided on the use of non-conventional brackish and effluent brackishwater desalination water plants in agriculture in support to sustainable production of halophyte plant crops (*Salicornia, Atriplex* and *Suaeda)* and farming marine fish species such as European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) in arid lands. For freshwater species the use of the insect larvae i.e. black soldier fly, worms (African Night Crawler, Red Wiggler and Mealworm) and algae (azolla and duckweed) to feed the fish using the aquaponic plant remnants in Egypt. Also aquaculture in desert can meets biofuel as food security in UAE by developing a system for raising fish and shrimp hardy salt tolerant species for food, and reclaiming fish farm waste effluent waste to raise biofuel crops mainly the dwarf saltwort (*Salicornia* spp.) in agricultural fields. Similarly, the wastewater would be bioremediated in constructed wetlands, reducing treatment costs down to a fraction. In Israel commercial project is capable to produce intensively the microalgae (*Dunaliela* spp.) using high saline brackish ground water to produce astaxantine. The main obstacles to the development of the sector, are the lack of technical competences and availability of inputs (underground water quality, the smart use of water to the plant crop production, possibility of causing disturbance to the ecosystem if the aquaculture is not sustainable, aquatic feed, seeds and marketing of the aquatic products for the urban desert communities whom are conservative to consume freshwater fish products). Different success stories from Algeria, Australia, Egypt, Israel, Mexico, Saudi-Arabia, United Arab Emirates and USA are presented and described to demonstrate that the integrated agri-aquaculture in the desert is a fact and is rentable. Several recommendations are provided to develop a sustainable and feasible sector (perform business plan before starting, encouraging the establishment of farmer associations that can better assist fish farmers in organizing themselves to produce and/or sharing inputs such as fingerlings, feed and equipment making them available at a more accessible costs, perform fish marketing studies including promotional strategies for aquaculture products acceptance in urban desert communities).

CONSISTENCY IN RISK TAKING TESTS THROUGHOUT LIFE AND CHALLENGING SITUATIONS

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Growth, disease susceptibility and, more generally, energy allocation were all demonstrated to be partly driven by the coping style. Being able to describe individual differences in this coherent set of behavioural and physiological responses to stress, has therefore major implications for aquaculture. Notably, the use of mass-screening tests can help to routinely and effectively select the best dams and sires in a population for selection programs. The group risk-taking test, which has previously been described as a promising tool, assigns to thousands of fish a boldness score (Ferrari et al. 2016). Here, we tested whether sea bass (*Dicentrarchus labrax*) behaviour assessed using the group risk-taking test was consistent throughout long periods of time and across challenging situations.

After being individually PIT tagged and divided in three tanks, fish were challenged in a first group risk-taking test. We repeated the tests on the same fish 3 times over the next 6 months. Tests were however performed with different tank sizes, after a chronic stressor or after mixing fish from different tanks in a common garden. At the end of the experiment, fish were sexed, blood was sampled for cortisol measurements and organs were taken for gene expression analyses.

Our results highlight a strong consistency of the coping style across time and environments (figure 1), and this was independent of the sex and the life history. The strong repeatability of our results also suggests that the group risk-taking test is a reliable and robust test. Finally, our results are related to physiological measures in order to further explain differences in coping styles.

ERANET, COFASP and French National Research Agency funded the project SUSHIFISH.

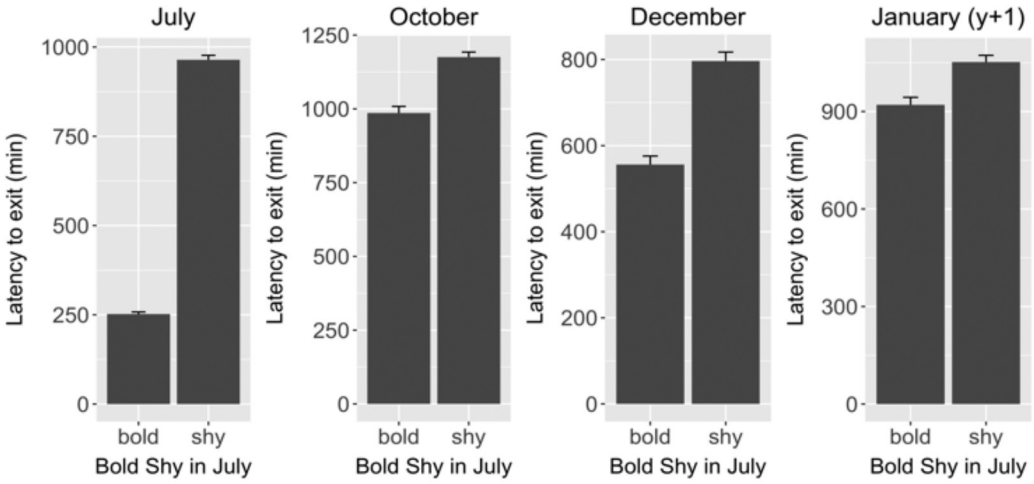

Figure 1. Consistency over time of the coping style in sea bass.

NUTRIENT SENSING IN THE INTESTINE – IMPACT ON PERISTALSIS AND THE TRANSCRIPTOME

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Ballan wrasse is an efficient cleaner fish used in salmon farming to control the ecto-parasite, salmon lice. One of the bottlenecks in the production of Ballan wrasse is low growth, which seems to be associated with poor appetite. The Ballan wrasse has a very different digestive tract and consequently different feeding regime than the established farmed fish species in northern parts of Europe. It lacks a functional stomach and pyloric-ceca and the whole intestine is about 2/3 of the fish total length. The Ballan wrasse also does not have ghrelin, a hormone that is secreted by the empty stomach of mammals and is a strong hunger signal that promotes feed intake. We have used an *in vitro* organ model to investigate how the wrasse intestine responds to nutrients on a molecular level and the effect on motility.

Ex vivo intestines from 15-20 g wrasses were administered a bolus (lipid, protein, inert matter or empty control) before being mounted in oxygenated physiological buffer inside a glass vial. The intestinal motility patterns were documented with time lapse imaging for 14 hours. The images were used to construct spatio-temporal maps for gut motility analysis; i.e. to determine the speed, amplitude, frequency, travelled distance, direction, and initiation site of waves of muscle contractions. The experiments were repeated for RNA sampling and deep seq analysis.

All the administered boli induced change in motility compared to activity in the empty intestine. Comparing hydrolyzed vs. intact lipids, we found that intact lipid had longer residence time in the intestine than lipid hydrolysate. Intact lipid also induced a higher frequency of contractions, despite prolonged residence time. It is likely that the increased frequency of contractions leads to increased mixing of gut content, and also better mixing of bile and digestive enzymes. There was no difference in passage time between hydrolyzed and intact protein (casein), but also here we saw increased frequency of contractions in the group that received intact protein. To investigate the effect of stretch on the intestine, without the impact of potential biochemical signal from nutrients, we analyzed the intestines given inert diets. These induced more longitudinal contractions (peristalsis) than lipid and protein, which led to rapid transport of the inert diet in the distal direction.

Gene expression analysis showed that intestines administered intact lipids clustered closer to the empty intestines compared to those given hydrolyzed lipids. These and more results will be presented and discussed in greater detail.

Based on these studies we conclude that the Ballan wrasse intestine has chemoreceptors that contribute to a nutrient specific control of motility and passage rate.

SEX DETERMINATION, SEX CONTROL AND MONOSEX CULTURE IN DECAPODS

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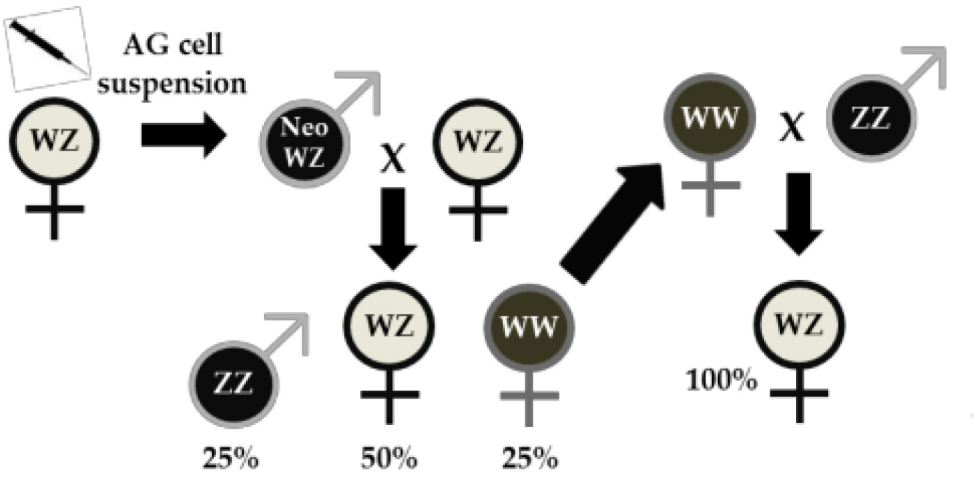
With respect to sex heritability, most cultured decapod crustaceans (shrimp, prawns and crayfish) exhibit homogametic (ZZ) males and heterogametic (WZ) females. The insulin-like androgenic gland hormone (IAG) is functioning as a hormonal switch in decapods in which its presence enables male development while its absence gives rise to female development. IAG-switch manipulations have enabled gender shifts beyond any boundaries in which individuals with any combination of sex chromosomes could be manipulated towards both maleness or femaleness.

Monosex is a desirable practice in crustacean aquaculture. Differences between males and females of the same species, in growth rate, alimentary needs and behavioral patterns, highlight the need to establish novel technologies and management procedures specifically tailored to culturing only one sex or the other. This was achieved by temporal manipulations of the IAG-switch to cause sex reversal. Obtaining functional ZZ females through RNAi to produce all-male prawn populations, and WZ males through parental AG cell transplantation to produce all-female prawn population (e.g. all-female biotechnology, see figure below). Based on these abilities, all-male and all-female populations are now available to be used for efficient, sustainable crustacean aquaculture, increased yields and biocontrol tasks.



Prawn all-female biotechnology through manipulations of the IAG-switch: Crossing of neo-males (WZ) with normal females(ZZ) and normal males (ZZ) with super female (WW)

POPULATION STRUCTURE OF DIGESTIVE PROTEASE PHENOTYPES IN HATCHERIES FOR PACIFIC WHITE SHRIMP *Litopennaeus vannamei*

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Trypsin from Pacific white shrimp *Litopenaeus vannamei* is a polymorphic protein composed of three alleles (A, B, and C). The C allele is monomorphic for the first region, while A and B are allelic variations for the second region that are segregated according to Mendelian rules. These three alleles give rise to three individual trypsin phenotypes displayed in sodium dodecyl sulfate polyacrylamide gel electrophoresis: CBA, CB, and CA. The expected frequencies from mating CBA males and females resulted in offspring that consisted of 25 % CB, 25 % CA, and 50 % CBA trypsin phenotypes. To define a management protocol for studying shrimp performance associated with the trypsin phenotype, the goal of this study was to determine if the expected proportions of the three trypsin phenotypes are represented in the population structure at breeding age in 11 hatcheries. Here, we hypothesized that the population structure of the trypsin phenotypes is represented by frequencies that are subject to selection, and it is possible to establish a management protocol to study shrimp performance. A total of 2,828 breeding shrimp from 11 hatcheries were analyzed to describe their population structure. The results showed a high frequency of CBA breeders and a near absence of the CA phenotype in the population structure. Two possibilities were related with these results: the man-made selection during the breeder selection is the cause of the trypsin phenotype frequency or shrimp with phenotype CA die during the early stages of culture.

Fig. 1. Trypsin phenotype frequencies in 11 *Litopenaeus vannamei* hatcheries.

THE ROLE OF DIETARY ASTAXANTHIN IN EUROPEAN SEA BASS (*Dicentrarchus labrax*) GROWTH, IMMUNITY, ANTIOXIDANT COMPETENCE AND STRESS TOLERANCE

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Sea bass (*Dicentrarchus labrax*) larvae (about 0.4g initial weight) were fed different levels of astaxanthin for 60 days. Diets contained 0, 60, 80 and 100 mg axtaxanthin /kg diet. Results showed marked enhancement in fish growth, feed utilization, survival rate and fish protein content when 100mg astaxanthin /kg diet was supplemented in fish diet.

The activities of hepatic antioxidation enzymes; superoxide dismutase (SOD) and glutathione Peroxidase (GPx) have reduced as the level of dietary astaxanthin increased.

Results showed simultaneous gradual increase in intestinal mucosal phagocytic and lysozyme activities as astaxanthin inclusion level elevates in diets indicating effective role of astaxantin as an immunostimulant agent in sea bass diet.

By the end of the trial, fish were exposed to a sudden drop in water salinity (37 to 0.3‰) and that continued for 24 hours period. Survival rate was significantly the highest in fish that consumed 100mg/kg diet as compared with control group by an increment of 36.9% suggesting an improvement in fish tolerance against osmotic stress. Results demonstrated that astaxanthin is a qualified nutrient for sea bass.

Table (1): Growth & feed utilization indices of sea bass fed AX for 60 days

Parameter	Diets			
	CTRL	AX1	AX2	AX3
Initial body Weight (g)	0.38±0.04	0.40±0.01	0.39±0.02	0.38±0.03
Final body Weight (g)	2.43±0.24b	2.77±0.15b	3.33±0.20ab	3.80±0.17a
Weight gain (g)	2.06±0.13b	2.36±0.13b	2.94 ±0.21ab	3.42 ±0.18a
Specific growth rate	3.10±0.08b	3.21 ±0.03b	3.57 ±0.13ab	3.82 ±0.09a
Weight gain rate (%)	545.57±31.3b	585.19±13.66b	756.61±64.78ab	892.13±52.55 a
Feed conversion ratio	1.69 ±0.05a	1.37 ±0.06b	1.35 ±0.02b	1.29 ±0.06b
Protein efficiency ratio	1.23±0.04b	1.53±0.06a	1.54±0.02a	1.62±0.08a
Proteinproductive value	16.18±0.76 b	18.83±1.49b	21.01±0.43ab	25.74±1.17a
Feed intake	4.12±0.66a	3.40±0.12b	3.56±0.47b	3.52±0.13b
Survival rate (%)	94.0±1.1b	95.3±2.1ab	96.8±1.4ab	98.7±1.2a

Table(2): Sea bass biochemical composition after fed AX feed additive for 60 days

	CTRL	AX1	AX2	AX3
Moisture	73.27±1.93	74.27±1.89	74.06±2.33	73.28±2.43
Protein	13.93±0.82b	14.24±0.42b	14.18±0.37b	16.33±0.24a
Lipid	6.54±0.18	5.98±0.16	6.56±0.2	6.13±0.13
Ash	6.07±0.1	5.67±0.26	5.18±0.17	5.22±0.97

Table 3. Antioxidation enzymes and some immunological parameters (mean ±SE) of sea bass fed astaxanthin (AX) supplemented diets for 60 days

Parameter	CTRL	AX1	AX2	AX3
SOD	65.2±3.80a	57±3.24a	42.4±3.11ab	32.8±3.77b
GPx	59.20±3.85a	56.60±6.19a	43.80±4.04ab	31.0±3.48b
Protein	209.6±14.08	202.2±11.83	213.0±12.10	219.4±18.02
PA%	36.40±3.09b	48.20±4.24ab	49.4±4.60ab	58.2±3.92a
Lysozyme	281.8±13.11b	293.8±15.43b	366.0±14.93a	396.4±14.81a

EVALUATION OF TAURINE AS A FEED ADDITIVE IN SEA BASS (*Dicentrarchus labrax*) DIET

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Over the course of the 13–weeks growth trial, European sea bass juveniles with initial weight 4.7±0.5 g reared in an outdoor 40 m^3 concrete-raceway and fed the diets supplemented with 0, 0.4, 0.7 and 1% taurine (T) as a feed additive and assigned as CTR, T4, T7 and T10 groups.

T–incorporation had caused a graded increase in all measured growth indices, with the highest values for the highest taurine level (1%).

Feed utilization was also improved by adding T to fish diet. Fish biochemical analyses showed that Protein content of fish was significantly elevated for all T–fed fish, but lipid content was significantly reduced in only T4–fed fish as compared to CTRL fish.

Dietary taurine inclusion at all levels had no major effect on amino acids composition of fish.
Also, taurine-added diets had resulted in a significant effect on fish blood constituents (CBC) and also, serum lysozyme and phagocytic activities indicating better immunity competence.

Table (1). Growth and feed utilization indices (*D.labrax*) fed taurine enriched diets for 13

Parameter	Dietary groups			
	CTRL	T4	T7	T10
Initial body weight	4.79±0.12	4.70±0.04	4.70±0.04	4.71±0.04
Final body weight	22.82b±0.20	22.98b±1.27	25.16ab±0.33	26.68a±0.15
Weight Gain	17.92c ±0.34	18.28b±1.30	20.43ab±0.31	21.97a±0.17
Daily Growth Index	1.28b±0.01	1.29b±0.06	1.39ab±0.01	1.47a±0.03
Specific Growth	1.73b±0.03	1.76b±0.07	1.86ab±0.01	1.93a±0.02
Percentage Weight Gain	374.1c±11.60	388.5b±29.40	431.6ab±4.70	466.1a±6.80
Feed intake (%BW/d)	4.58±0.04	4.65±0.32	4.67±0.35	4.69±0.38
Feed Conversion Ratio	1.59ab ±0.02	1.69a±0.05	1.52b±0.07	1.59ab±0.08
Protein Efficiency Ratio	1.00±0.00	1.00±0.00	1.33±0.58	1.67±0.58
Protein Productive Value	29.88b±0.61	28.39b±0.79	31.89a±0.75	32.70a±0.84
Survival Rate (SR, %)	95.00±5.00	100±0.00	98.33±2.89	100±0.00

Table (2). Major nutrients composition (mean ±SD, % wet weight) of ESB

Parameter	Final			
	CTRL	T4	T7	T10
Crude protein	19.06b±0.35	19.60a±0.61	19.45a±0.40	19.11a±0.31
Lipids	8.38ab±0.15	7.94c±0.79	8.19b±0.15	8.48a±0.23
Ash	4.99±0.31	4.97±0.72	4.77±0.21	4.70±0.40
Moisture	67.10±0.46	67.03±0.57	67.20±0.10	67.53±0.21

Table (3): CBC and serum constituents of sea bass fed taurine for 13 weeks

Parameter	CTRL	T4	T7	T10
Hemoglobin	12.77a±0.25	11.83b±0.62	11.55b±1.11	11.50b±0.53
Hematocrit	41.67a±3.79	34.00b±3.46	33.67b±3.06	32.00b±1.00
RBCC ($X10^6$/L)	4.69a±0.18	3.17b±0.35	3.23b±0.15	3.13b±0.06
WBCC($s\times10^3$/L)	31.33b±6.28	47.00a±7.00	35.67ba±6.03	40.33ba±9.87
Serum analysis				
Total proteins	5.67b±0.15	7.80a±0.35	6.27ba±1.42	6.57ba±6.80
Phagocytic activity	3.00b±1.00	9.00a±1.00	7.67a±4.04	7.63a±1.53
Lysozyme activity	429.00b±44.53	530.33a±12.50	487.67a±56.86	475.67a±44.7

EFFECTS OF SUPPLEMENTED PHYTASE ON GROWTH PERFORMANCE OF SEA BREAM (*Sparus aurata*)

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Fishmeal is constantly increasing in price as a result of their scarcity. Therefore, vegetable protein sources are promising alternatives to fishmeal. However, most plant ingredients contain anti-nutritional materials, such as phytate, which is the main storage form of P. Up to 80% of the total P. Phytic acid also chelates with various cations like Na, K, Ca, Mg, Mn, Zn, Fe and Cu, and is practically not available for fishes. Exogenous phytase is found to hydrolyse phytate and increase nutrient digestibility. Therefore the objective of this study was to assess the effect of dietary phytase in sea bream diets, in the presence or absence of mono-calcium phosphate and mixture of minerals.

Five diets were prepared. The dietary treatments consisted of a control diet (0% phytase) supplemented with mono-calcium phosphate and mixture of minerals. The other four experimental diets were supplemented with 0.2% phytase, together with mineral mixture or mono-calcium phosphate in diet 1 or 2 respectively, without mineral mixture or mono-calcium phosphate in diet 3, with mineral mixture and mono-calcium phosphate in diet 4. Each diet was assigned to triplicate groups of fish in floating hapas, and each cage was stocked with 20 fish. At the end of the experiment, the main growth, feed utilization parameters and proximate body composition were investigated.

The results showed that weight gain (WG%) of fish fed on phytase supplemented diets was significantly higher than fish fed on control diet ($P \leq 0.05$) (table 1). Moreover, Feed conversion ratio (FCR) was also affected by the feed additive, where FCR in fish fed phytase supplemented diets was lower than in fish fed control diet (table 2). It could be concluded that Phytase can efficiently act as a growth promoter for Nile tilapia fingerlings regardless the presence of mineral mixture or mono-calcium phosphate in diet or not.

Table 1. Effect of supplemented phytase on growth performance o *S.aurata*)

	Initial	final	Gain	ADG	SGR
0	0.32± 0.023^{a}	3.26± 0.08^{b}	2.94± 0.06^{b}	0.039± 0.001^{b}	3.09± 0.05^{b}
1	0.34± 0.01^{a}	4.52± 0.10^{a}	4.19± 0.9^{a}	0.056± 0.001^{a}	3.48± 0.046^{a}
2	0.31± 0.15^{a}	4.64± 0.10^{a}	4.33± 0.12^{a}	0.058± 0.001^{a}	3.61± 0.084^{a}
3	0.32± 0.10^{a}	4.66± 0.34^{a}	4.34± 0.35^{a}	0.058± 0.005^{a}	3.57± 0.121^{a}
4	0.32± 0.11^{a}	4.67± 0.17^{a}	4.35± 0.17^{a}	0.058± 0.002^{a}	3.57± 0.086^{a}

Table 2. Effect of supplemented phytase on feed utilization of *S.aurata*)

	Survival	Feed intake	FCR	PER
0	83.33± 2.89^{a}	7.40± 0.10^{b}	2.52± 0.43^{a}	0.83± 0.11^{b}
1	86.67± 2.89^{a}	8.19± 0.32^{a}	1.96± 0.09^{b}	1.09± 0.53^{a}
2	86.67± 2.89^{a}	8.67± 0.21^{a}	2.00± 0.56^{b}	1.08± 0.30^{a}
3	83.33± 2.89^{a}	8.27± 0.32^{a}	1.92± 0.21^{b}	1.10± 0.13^{a}
4	85.00± 5,00^{a}	8.57± 0.42^{a}	1.97± 0.07^{b}	1.04± 0.038^{a}

MOLECULAR CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF GLUTATHIONE REDUCTASE MITOCHONDRIAL GENE FROM SEAHORSE (*Hippocampus abdominalis*)

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Glutathione reductase is an antioxidant enzyme which is able to convert oxidized glutathione (GSSG) into reduced glutathione (GSH) to maintain the GSH/GSSG level of the cells during the oxidative stress. Reactive oxygen and nitrogen species such as super oxide radicals, hydroxyl radicals, hydrogen peroxide and nitrogen oxide molecules cause for the oxidative stress in most aerobic organisms. Glutathione (GSH) is the major intracellular thiol compound can found in organs such as endoplasmic reticulum, nucleus and mitochondria. In this study we characterized structural and functional features of the mitochondrial glutathione reductase gene (*ShGsr*) of seahorse. The cDNA sequence of *ShGsr* was 1467 bp long and consist of 1398 bp long open reading frame (ORF). The ShGsr protein composed with 465 amino acids. The ShGsr protein contains typical Gsr structural features, including NADPH binding site (184-189 aa), FAD binding domain (14-32 aa) and glutathion binding site 45-55 aa). Pairwise sequence alignment showed the 82.9% identity and 91.1% similarity with the glutathione reductase mitochondrial isoform 3 gene of *Fundulus heteroliticus*. According to the phylogenetic analysis ShGsr showed close relationship with teleost fish.

A real time polymerase chain reaction data for fourteen tissues of sea horse showed the highest expression of *ShGsr* in the ovaries. Ovaries require high energy for the reproduction activities, so the ROS level may high in the ovaries because of the high level of mitochondria. The seahorse was subjected to the immune challenge with Lipopolysaccharides (LPS), Poly IC, *Edwardsiella tarda* and *Streptococcus iniae* in order to determine putative immune responses of ShGsr. The *ShGsr* mRNA was significantly upregulated after the immune challenge experiment in the seahorse blood cells. Collectively, these results suggest that the ShGSR might be involved in immune responses of the seahorse.

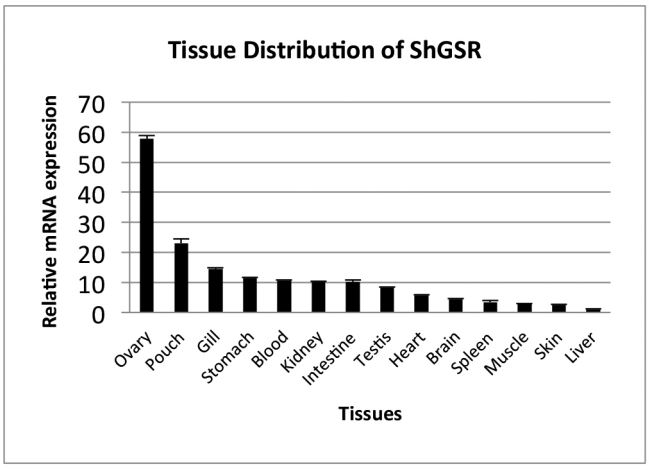

Fig.1 The mean of the relative mRNA level of seahorse ShGSR in each tissue determined by qPCR. Error bars represent the SD (n=3)

EFFICACY OF ICTHIOVAC® VR/PD AGAINST *Listonella anguillarum* IN SEA BASS UNDER UNFAVORABLE CONDITIONS

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Vaccination is one of the key preventive measures against one of the most important diseases in Sea Bass production farms, the Vibriosis caused by *Listonella* (*Vibrio*) *anguillarum*. However, fish rearing conditions vary among farms, making the evaluation of vaccine efficacy at farm level necessary. This is particularly relevant when vaccines face unfavorable conditions for the stimulation of the immune response and there are chances of outbreaks happening. The aim of this study is to evaluate the efficacy of ICTHIOVAC® VR/PD at low water temperatures (<15ºC), and a short time interval between vaccination and challenge (21 days post-vaccination).

A blinded and controlled trial was conducted as shown in Figure 1. Two hundred sea bass (47.1 ± 11.3 g body weight), were appropriately randomized to receive the vaccine (vaccinated group [VG]) or the placebo injection (control group [CG]). The challenge was carried out 21 days after vaccination (dpv) in duplicate using a virulent heterologous strain (serotype O2β). Vaccine efficacy was assessed based on the cumulative survival rate and the relative percentage of survival (RPS). The post-challenge observation period lasted 21 days (dpc).

During the study, fish were kept at 13.8 ± 0.6 ºC during the vaccination period, while the temperature during the challenge period was 20.8 ± 1.1 ºC. Any adverse effect after the vaccination was recorded

The results obtained after challenge show that cumulative mortality rate was significantly lower in VG than in CG (Figure 2). The RPS value obtained was of 97.6%. Deaths occurred between 2 and 5 dpc, reaching a mortality rate of 68.3±7% in CG.

All in all, the results obtained here clearly demonstrate that ICTHIOVAC® VR/PD is effective in conferring immunity to Sea bass against virulent *Listonella* (*Vibrio*) *anguillarum* O2β strain, even under unfavorable conditions like low temperature (<15ºC) and shorter post-vaccination time (21 days).

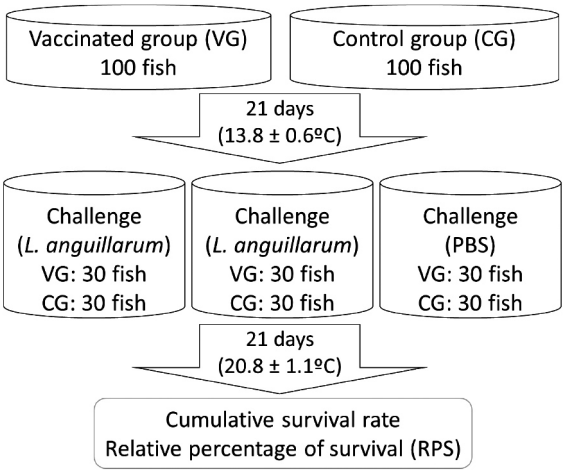

Figure 1. Experimental design of efficacy study of ICTHIOVAC® VR/PD against *L. anguillarum* O2β.

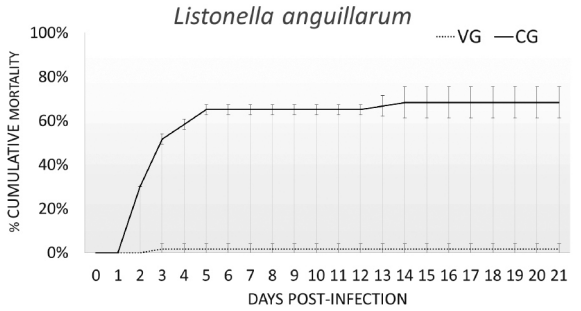

Figure 2. Cumulative mortality after challenge of *L. anguillarum* O2β.

EFFECT OF HIGH TEMPERATURE ON SEX DETERMINISM AND SEX DIFFERENTIATION PROCESS IN AFRICAN CATFISH, *Clarias gariepinus*

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Fish display a large variety of sex determination mechanisms and patterns of sexual differentiation. Two systems of sex determination were reported in gonochoristic fish: genetic sex determination (GSD) and environmental sex determination (ESD). In ESD species, phenotypic sex can be controlled by environmental factors like temperature. The aim of this study was to determine high temperature effect and its mechanism on sex determination process in the African catfish, *Clarias gariepinus*.

Ten high temperature – HT (36°C) treatments groups were constituted, with 500 larvae each (Table 1). Before and after each treatment and in control groups, fish were reared at 28°C. Once the most thermosensitive period was determined, fish were sampled at the beginning and end of HT application, to assess sex steroid hormones (testosterone, 17β-estradiol, and 11-ketotestosterone) concentrations and *foxl2*, *cyp19a1b* and *dmrt1* genes relative expressions in the head.

Our results show that the sex differentiation pathway can be orientated by HT in African catfish with the most thermosensitive period extends from 6 to 8 dph (Table 1). When applied during these 3 days, HT frequently induces 90 to 100% of males in progenies (Fig. 1). Thus, we can conclude that the African catfish display a temperature-induced sex reversal process. Sex-ratio in control groups highly fluctuate according to the progeny (Fig. 1), suggesting that minor genetic factors play an important role in sex determinism process.

Masculinization did not influence sex steroid levels nor *cyp19a1b* and *dmrt1* genes expressions. Nevertheless, after HT application, lower thermosensitive progenies showed high *cyp19a1b* relative expression, while in control groups, higher thermosensitive progenies displayed high *dmrt1* relative expression at 14 dph. Expression of *foxl2* was not detected in the head during the experimental period. However, to our knowledge, this is the first report of *dmrt1* expression in teleost fish head (perhaps in brain).

Table 1: Effect of high temperature (36°C) on the sex-ratio in *C. gariepinus*. χ^2 were calculated between each control and a theoretical expected sex-ratio (50:50). Batches reared at HT (36°C) were compared to their respective control for each progeny. * significantly different ($p<0.05$) from 50 %; ** significantly different ($p<0.05$) from the control. P: Progeny

Moment of HT application (dph)	Progeny 1		Progeny 2	
	% Males	χ^2	% Males	χ^2
Control	36	3.99*	44	0.72
0-2	54	6.55**	58	3.92*
3-5	58	9.71**	70	13.80**
6-8	96	80.21**	96	64.38**
9-11	50	3.99**	66	9.78**
12-14	48	2.96	40	0.33
15-17	52	5.19**	44	0.00
18-20	38	0.09	48	0.32
21-23	50	3.99**	38	0.74
24-26	38	0.09	50	0.72
27-29	48	2.96	57	3.38

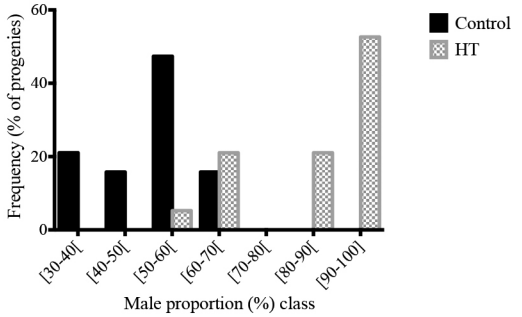

Fig. 1. Sex-ratio distribution in 19 progenies *of C. gariepinus* juveniles exposed to 36°C from 6 to 8 days post-hatching (control maintained at 28°C).

PROBIOTICS INFLUENCES IN GENE EXPRESSION THAT CONTROL MUSCLE GROWTH IN NILE TILAPIA *Oreochromis niloticus*

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The objective of this study is to evaluate the effect of probiotic additive in tilapia growth, assessing muscular gene expression. Tilapia fingerlings of approximately 1.5 g were cultivated in recirculation systems containing 15 water tanks of 0.25 m^3 each at 28 °C, with biological filter and UV system at 80 fish/m^3. The fish are fed three times a day, with the same feed, using the probiotic additive supplied by Biomart Animal Nutrition. They were assessed the inclusion levels of 0.1 and 0.2% of probiotic additive. The probiotic was homogenized in 2% soy oil and sprinkled over the feed. The control treatment consisted only of the addition of vegetable oil in the same proportion. The gene expression in dorsal muscle was evaluated at day 30th in two fish for each tank. The expression of miogenic regulatory factors (MyoD, myogenin), myostatin and IGF-1was performed by real-time polymerase chain reaction after reverse transcription (RT-qPCR), following the guidelines of the MIQE: Minimum Information for Publication of the Quantitative Real-Time PCR Experiment. Relative expression mRNA were analyzed by Kruskal-Wallis Test followed by Dunn's multiple comparisons test at $\alpha = 0.05$.

Final fish weight at day 90th were 165.62 (14.16), 186.73 (12.58) and 183.66 (16.39) to control and 0.1 and 0.2 probiotic level, respectively, and they were different ($P<0.05$). Probiotic did not alter myogenin and IGF expression in white muscle fibers, however, it provided a decrease in MyoD and myostatin expression (Figure 1). In our previews studies the probiotic stimulate the muscle hypertrophy and somatic growth.

The fish growth fed diets containing probiotics occurred more by inhibition of myostatin expression than by stimulation of IGF expression.

Acknowledgments: Financial support was given by Fapesp.

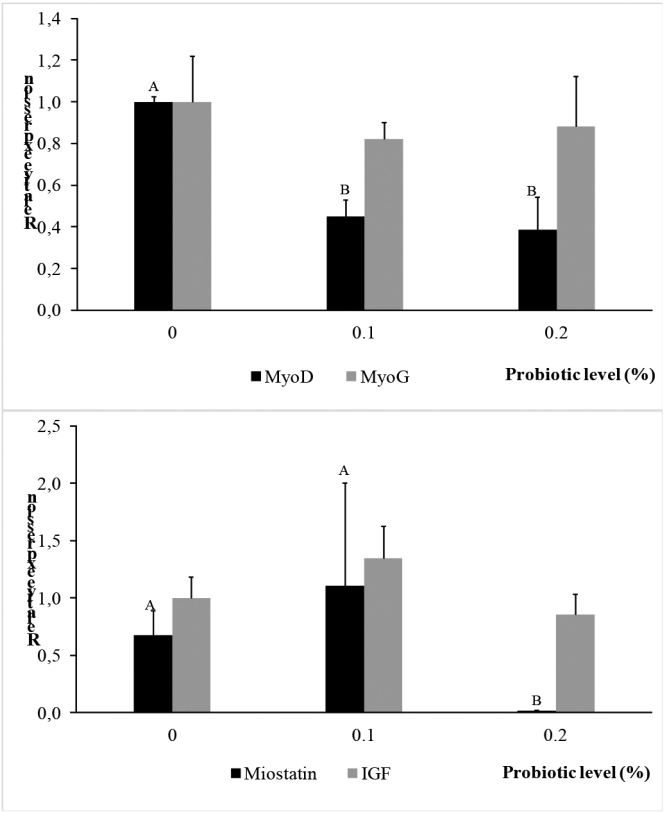

Figure 1. Relative expression of MyoD, myogenin, myostatin and IGF in the dorsal muscle of tilapia fed with feed containing probiotics. Different letters indicate significant differences ($P<0.05$).

MIXED PROBIOTICS STIMULATE THE TILAPIA GROWTH BY INTESTINAL DEVELOPMENT

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The objective of this study is to evaluate the effect of mixed probiotic additive in tilapia growth, assessing the effect in intestine in the morphology of crypts. Tilapia fingerlings of approximately 1.5 g were cultivated in recirculation systems containing 15 water tanks of 0.25 m^3 each at 28 °C, with biological filter and UV system at 80 fish/m^3. The fish are fed three times a day, with the same feed, using the mix probiotic additive supplied by Biomart Animal Nutrition: *Bacillus subtilis, Bifidobacterium bifidum, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Lactobacillus plantarum, Pediococcus acidilactici* ($1.0x10^9$ UFC/g). They were assessed the inclusion levels of 0.1 and 0.2% of probiotic additive. The probiotic was homogenized in 2% soy oil and sprinkled over the feed. The intestinal tissue samples were fixed in formaldehyde and subsequently embedded in historesin. Cross sections of 5 µm were stained in Hematoxylin-Eosin and prepared on microscopy slides for further morphological analysis (Figure 1). The slides of intestinal tissue were analyzed for quantity, height and villi area. It was proceeded ANOVA and Tukey test at 5%.

Final fish weight at day 90^{th} were 165.62 (14.16), 186.73 (12.58) and 183.66 (16.39) to control and 0.1 and 0.2 probiotic level, respectively, and they were different ($P<0.05$). It was observed that fish fed diets containing probiotics at 0.2% had a higher amount of villi (58.11) compared to those that did not receive the probiotic (Table 1). No differences were found in villi height and area ($P>0.05$). The mixed probiotic used in this experiment should be diffused in aquaculture as it stimulated growth by increasing the number of intestinal villi, ensuring greater absorption and utilization of the nutrients provided in the diet.

Acknowledgments: Financial support was given by Fapesp.

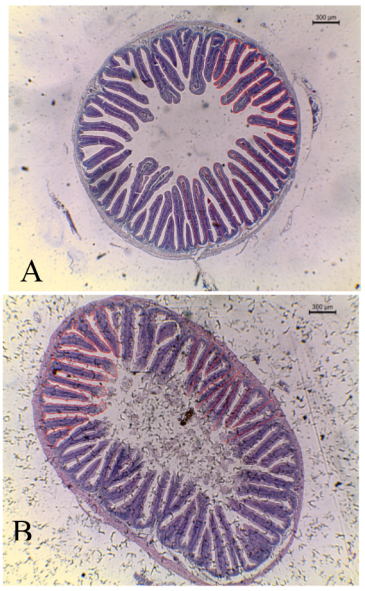

Figure 1. Histological sections of the intestine of Nile tilapia fed with feed containing probiotic. A) control group without probiotic inclusion; B) 0.2% inclusion. Scale bar = 300 µm

Table 1. Means and standard deviation of the amount, height and area of the intestinal villi of tilapia fed with feed containing different levels of probiotic inclusion.

Probiotic level	Amount villi	Height villi (µm)	Area villi (µm²)
0.0 %	48.50 (5.02)b*	389.60 (89.00)a	34.538.39 (8.917.16)a
0.1 %	54.63 (9.52)ab	426.65 (61.06)a	38.113.88 (6.012.52)a
0.2 %	58.11 (5.49)a	387.80 (55.79)a	38.133.73 (5.993.82)a

* Values followed by the same letter in the column are not different by the Tukey test at 5%.

SUPPLEMENTING COMMERCIAL FEED WITH RESTAURANT PLATE-FOOD WASTE IN TILAPIA AQUACULTURE

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More than 40% of human food produced is wasted and much of it in restaurants. An environmentally friendly and economic solution to food waste disposal is to convert wasted human food to animal feed. Two 8-week feeding experiments were performed to evaluate the suitability of using restaurant food waste to supplement commercial feed in the aquaculture of Nile tilapia *Oreochromis niloticus*. In the first experiment, five feeding regimens in which commercial feed was substituted by waste-based feed at 0, 25, 50, 75 and 100 % of daily offering were evaluated. Results show that 25 % of the commercial feed can be replaced with waste based feed without any significant effect on survival, growth, feed conversion, hepatosomatic (HSI), viscerosomatic (VSI) indices, hemoglobin, hematocrit and total plasma protein. In the second experiment, seven feeding treatments were evaluated in which daily offerings of commercial feed was alternated with waste based feed in six-day cycles. Results suggest that replacement between 16 and 33% is feasible without significantly affecting survival or growth. Again, no significant differences were observed in growth, FCR, VSI, hemoglobin, hematocrit, and total plasma protein. Findings suggest that around 25 % replacement of commercial feed with waste based feed can be utilized in the culture of *O. niloticus* thus improving financial returns of farmers while reducing the environmental impact of food waste.

REPLACING SOY BEAN MEAL WITH RESTAURANT FOOD WASTE IN TILAPIA FEEDS IMPROVES PRODUCTION

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A substantial portion of food prepared in restaurants is wasted and often ends up in landfills. This waste can easily be used as animal feed supplement but not as a complete diet. Traditional feed replacement experiments assess the possibility of replacing fishmeal with a more sustainable feed ingredient. We assessed the replacement of SBM with restaurant waste in the feed of Nile tilapia *Oreochromis niloticus*. Five feeds were formulated in which SBM was replaced on a weight for weight basis by restaurant waste. Waste was added instead of SBM at 0, 5, 10, 15 and 20% of the diet before extrusion. The various feeds were offered to tilapia fingerlings for eight weeks. Results suggest that the more the restaurant waste in the feed, the better the growth of the fish. Restaurant waste did not affect proximate analysis or any hematological parameters. Using restaurant waste thus reduces the cost of the feed, improves feed conversion and reduces polluting waste thrown in landfills.

Table 1. Survival (S; %), final body weight (FBW; g), final length (FL; cm), Fulton's condition index at harvest (K), feed conversion ratio (FCR), Hepatosomatic Index (HSI; %) and Viscerosomatic Index (VSI; %) of juvenile *Oreochromis niloticus* offered feeds with increasing inclusion of food waste.

Treatment	S	FBW	FL	K	FCR	HSI	VSI
T1 (0 % food waste)	100^{a}	48.7^{a}	13.9^{a}	1.76^{a}	1.77^{a}	1.10^{a}	8.05^{a}
T2 (5 % food waste)	100^{a}	$52.1^{a,b}$	$14.2^{a,b}$	1.80^{a}	1.77^{a}	1.06^{a}	8.28^{a}
T3 (10% food waste)	100^{a}	$56.3^{a,b}$	$14.5^{a,b}$	1.80^{a}	1.57^{a}	1.34^{a}	7.52^{a}
T4 (15% food waste)	100^{a}	$56.1^{a,b}$	$14.5^{a,b}$	1.80^{a}	1.53^{a}	1.29^{a}	8.33^{a}
T5 (20% food waste)	96.9^{a}	58.1^{b}	14.8^{b}	1.73^{a}	1.60^{a}	1.09^{a}	7.05^{a}
PSE[1]	-	1.81	0.15	0.02	0.10	0.08	0.36

[1]PSE: Pooled standard error

ONE HEALTH APPROACH ON ANTIMICROBIAL RESISTANCE AND THE PRUDENT USE OF ANTIBIOTICS IN FISH FARMING

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Aquaculture provides nowadays half of the world's seafood consumption and it is expected to expand since wild fisheries will remain stable at best. Considering the importance of aquaculture for contributing to food security over the world (fish accounted for 17% of the world population intake of animal proteins), therefore, producers will have to meet challenging goals to make aquaculture more sustainable and productive in future. Indeed, diseases are a severe threat to the sustainability of aquaculture and often jeopardize the efforts of millions of small fish farmers throughout the world. The extensive use of antibiotics to prevent and treat infections over the world, and in some countries for growth promotion, has been associated with the emergence and spread of resistant bacteria to antibiotics through the food chain and the environment, which pose a threat to both human and animal health. Up to 80% of antibiotics, generally administered to fish in feed, are released in water and sediments, where they exert a selective pressure on the microbial communities of the aquatic environment. Knowing that selection of resistant bacteria can occur at extremely low antibiotic concentrations, aquatic environment may provide the ideal setting for antimicrobial resistance (AMR) dissemination. The situation is alarming in food-producing animal, including aquaculture, by the use of antibiotics that are common to human health and by the lack of investment in developing new effective antimicrobials. Thus, the UN General Assembly declared in 2016 that AMR is "the most urgent global risk" and emphasizes the importance of international cooperation in tackling AMR at global level. The European Commission Action Plan specified the most needed measures among which it is worth to mention: preventing microbial infections and their spread; developing alternatives for treatments; cooperating with international partners to contain the risks of AMR; promoting research and innovation and improving communication and training. The new European "Animal Health Law" of March 2016 puts greater emphasis on the prevention and control of animal diseases as an important tool for fighting AMR in human, animals and the environment. At international level, several initiatives are taken jointly by WHO, FAO and OIE for the prudent use of antibiotics toward a One Health approach and for recommendations targeted at reducing AMR in animal production on a global scale (FAO 2016). The concept "prevention is better than cure" remains one of the most important measure for reducing antibiotics and for preserving their efficacy for therapeutic purposes only.

EXPERIENCE WITH ON-BOTTOM MUSSEL CULTURE IN LOW TIDAL AREAS: THE LIMFJORDEN CASE STUDY

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Introduction

On-bottom culture of blue mussels (*Mytilus edulis*) is the dominant aquaculture of mussels in North European countries like the Netherlands, Germany, Ireland and the UK (Capelle 2017). Areas allocated to mussel production in these countries are characterized by high tidal amplitude creating strong horizontal water velocity, thus ensuring advection of food to the production areas, and benthic conditions suited for mussel production. Mussel production areas in Danish coastal waters are characterized by low tidal amplitude, i.e. <0.3 m, but are on the other hand heavily eutrofied with often muddy bottoms. Fisheries on wild stocks or long-line off-bottom farming accordingly dominate mussel production in Danish waters. There is, however, a wish to increase on-bottom mussel production for several reasons: 1) Due to predicted increased productivity of relayed mussels, it is assumed that on-bottom culture will result in less area affected by the mussel dredge and is thus from a management point of view important; 2) By relaying mussels from areas affected by oxygen depletion, resources that would otherwise have been lost are utilized; 3) Relay of mussels for on-bottom culture can secure a stable source of biomass for the fishing fleet and 4) as a potential mitigation tool for removing nutrients back to land from the eutrophied system. It is the purpose of this study to evaluate possibilities and problems for an increased on-bottom mussel aquaculture production of blue mussels in Danish coastal waters.

Materials and methods

The study took place in the Limfjorden, which is the main mussel production area in Denmark. Basis of the investigations are analysis of data sampled in relation to relay carried out by the mussel fishery industry association "Foreningen MuslingeErhvervet (FME)" using their mussel relay vessel "Limfjorden". Ordinary relay activities carried out by FME in the period 2005-15 were analysed for efficiency by comparing relayed biomass with fished biomass from culture plots in the sub-basin Kaas Bredning. In order to explain results of the overall analysis of efficiency of the FME relay activities, a number of experiments were carried out in two other sub-basins. In Løgstør Bredning and Venø Bugt, the relay areas were surveyed before relay, after relay and prior to the fisheries using sidescan sonar mapping and benthic sampling by diver. The mussel biomass variation and size distribution were analysed accordingly. Finally, a dedicated experiment was conducted with the aim to test the effects of relay on survival and growth of mussels transferred from areas potentially affected by oxygen depletion. Mussels from another sub-basin Thisted Bredning were dredged at deeper waters exposed to emerging oxygen depletion and relayed at shallow waters with no signs of oxygen depletion. Samples were taken both before and after relay and analysed for mussel biomass and size distribution.

Results and discussion

The results from the various experiments data analyses indicate that the ratio of gross biomass of relayed to harvested mussels on the relay plots varied from 1:0.1 to 1:4 but was in general close to 1:1 for net biomass, indicating no clear increase in biomass, thus not so promising. In some on-bottom culture beds, density dependence effects could be observed, but the main issue remains the starfish predation. In some plots, while there was a net increase in mussel biomass over time, none was left at harvesting time due to the predation pressure. In other plots where mussels had been transferred from zones of potential mass mortality due to oxygen depletion to shallower areas in the same basin, starfish predation also reduced the final harvested biomass. The mussels survived well the transfer from deep to shallow areas and grew individually, however, frequent monitoring of the relay plots is important in order to exploit mussel biomass prior to starfish predation.

The key challenges to develop an efficient on-bottom culture production in Danish coastal areas is to: a) reduce/avoid starfish predation, b) secure relay before oxygen depletion events during the summer period, c) limit costs associated to relay, d) avoid density dependence effects. Net biomass from on-bottom culture do not seem encouraging, although, with new relaying plots outside zone with high starfish predation pressure, more information regarding environmental conditions and mussel biomass ratio might bring a new representation. More, on-bottom culture might not bring a net biomass increase and thus not be an efficient mitigation tool, but it might be seen as a management strategy to a) reduce the impact of mussel dredge while searching for fishing on commercial size mussel beds, b) save the mussel seeds from zones with predation pressure or with frequent oxygen depletion events and c) keep a safe mussel plot for the fishermen when commercial size mussel beds are scarce.

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MONOSEX PRAWNS AS SUSTAINABLE BIOCONTROL AGENTS OVER DISEASE CARRYING SNAILS IN AQUACULTURE

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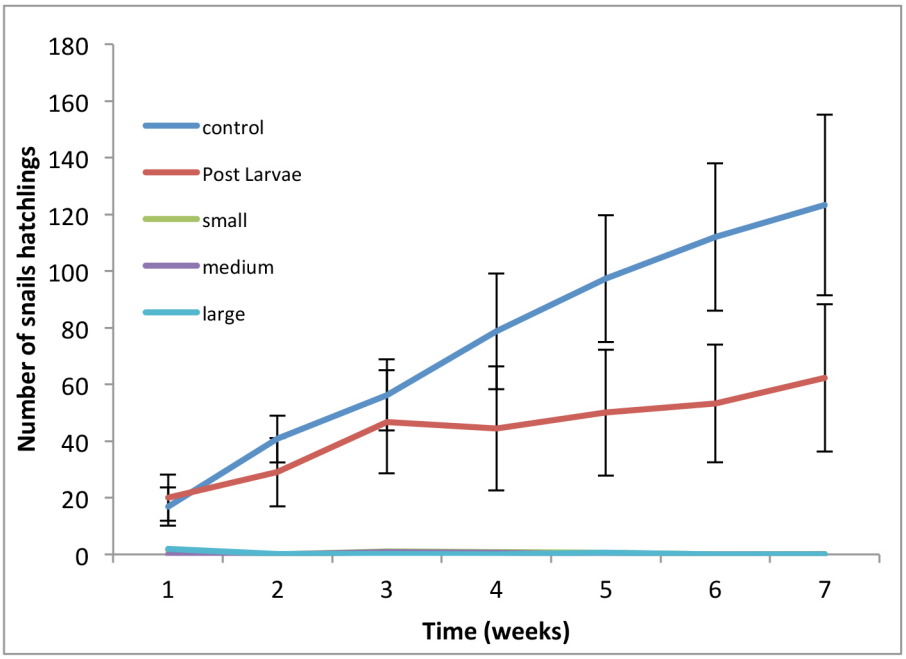
In the past ten years, freshwater fish in aquaculture ponds suffer from increasing levels of parasitic diseases such as *Centrocestus*, that are transmitted by freshwater snails from the *Thiaridae* family. Two species of this family are common in aquaculture ponds in Israel, *Melanoides tuberculate* and the invasive *Thiara scabara*, both are hosts of various disease-causing trematodes. Freshwater prawns of the *Macrobrachium* genus are known to be voracious predators of freshwater snails, therefore following the recent biotechnological advances in monosex freshwater prawn production, monosex prawns are suggested as sustainable biocontrol agents. The prawns are expected to prey upon the disease carrying snails and increase the produced biomass of aquaculture ponds with high-value side-product. Laboratory predation experiment with various sizes of monosex prawns over the above mentioned snail species were carried out. The results show that under laboratory conditions any single prawn above 4 g is capable of exterminating dozens of snail hatchlings, and prevent recruitment of both snail species (see figure). We found significant differences in the predation abilities of the large prawns over the large snails of both species as *Melanoides* snails were more susceptible to predation than *Thiara* snails. A preliminary field observation was conducted with all-female prawns in *Tilapia* growout aquaculture ponds. Under field observation conditions, we found differences between control and treatment ponds with respect to snail sizes, biomass, livability and species abundances. Based on the results we plan to continue and expand the field experiments the coming season to better understand how to maximize the profit and biocontrol power of monosex prawns in polyculture with fish.



Recruitment of Melanoides snails in the presence of female prawns in laboratory predation experiments

REPRODUCTIVE BARRIERS BETWEEN *Macrobrachium* SPECIES PERMIT SUSTAINABLE AQUACULTURE AND BIOCONTROL OF SCHISTOSOMIASIS IN AFRICA

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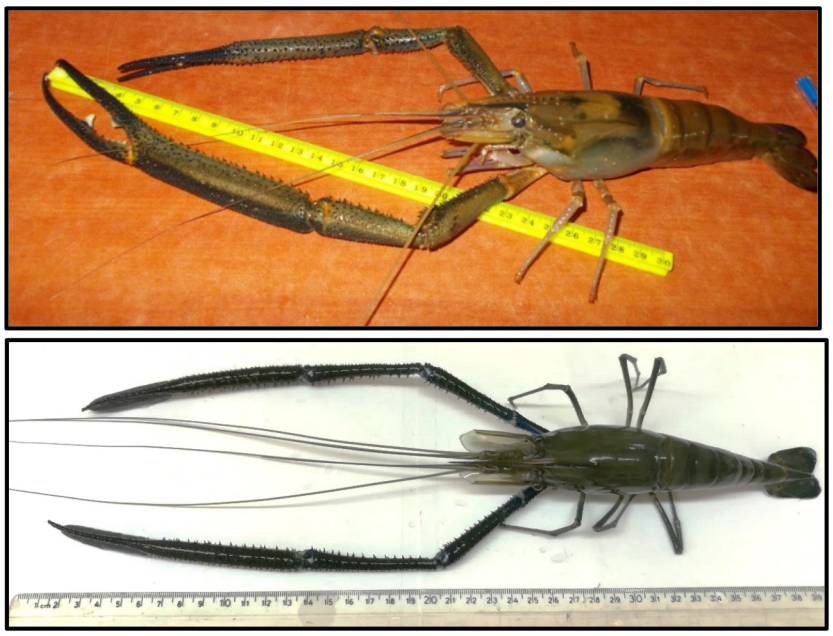
Macrobrachium prawns are voracious predators of freshwater snails that host flatworms responsible for bilharzia (schistosomiasis), a health burden in many African countries. A novel strategy to decrease the disease in Africa involves the use of prawns as biocontrol agents over snails. Although the endemic African river prawn, *Macrobrachium vollenhovenii*, is a natural candidate for aquaculture and biocontrol, efforts to domesticate it have been unsuccessful to date. Thus, it is not available in the large quantities required for aquaculture and biocontrol. The giant freshwater prawn, *M. rosenbergii*, endemic to Asia, has been cultured worldwide for decades. Recently, novel biotechnologies were developed to establish monosex (e.g. all-male) non-breeding populations for aquaculture. such all-male populations are suggested as ideal agents for biocontrol in Africa. Since the above prawn species are of the same genus, exhibit similar sizes (see figure) and require a female pre-mating molt (PMM) prior to egg fertilization, the potential for cross-breeding between the two species must be tested. To assure that all-male populations of *M. rosenbergii* will not pose an ecological threat, we carried out cross-breeding experiments with *M. vollenhovenii*. Both inter-species encounters and artificial insemination attempts, revealed that fertilization does not occur between the two species. Our results indicate that the reproductive barrier between these species is both pre- and post-zygotic. We suggest that all-male *M. rosenbergii* can be safely and sustainably used as an aquaculture species in areas where *M. vollenhovenii* naturally occurs, and as a bio-control agent in Africa, where schistosomiasis is a major health concern.



Large male M. vollenhovenii *(top) and* M. rosenbergii *(bottom)*

WATER PARAMETERSOF KOSI AND SHARDA RIVERS OF UTTRAKHAND,INDIA

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The present study was carried out in Kosi and Sharda Rivers of Kumaun, of Uttarakhand. The various water quality parameters such as nitrate, phosphate, total hardness, TDS and alkalinity were analyzed from the period of November 2015 to April 2016. The nitrate and Phosphate increased in the spring months due to the higher phytoplanktonic production. Total hardness and alkalinity were higher during winter. Studied were indicated that both river water were suitable for aquatic organism, such as fishes. Water parameters levels indicated the moderate quality of water. Thus present study concludes that river water was not polluted; all the results are within permissible limit when compared with safe water quality standards.

Introduction

Water is an important natural resource on earth. It is necessary for all living organisms, biological system, public health, food production and profitable growth **(Shafiq *et al*., 2013). (Okonko *et al*., 2008).** Drinking water is an essential feature in guaranteeing public health, the protection of environment and sustainable growth **(Rajini *et al.,* 2010).** , **EPA 2011**

Estimation of Water quality parameters of rivers

Rivers (Kosi and Sharda) water qualities were assessed by regular sampling throughout the study period for estimation of parameters. **(APHA, 2012)**

Result and Discussion

The physico-chemical parameters of the water normally undergo seasonal and daily fluctuation but not always adhere to a general pattern with regard to different aquatic system.

Nitrate is the highly oxidized form of nitrogen compound usually present in natural waters. The nitrate exhibited the regular trend of variation in both the river water. In Kosi River minimum and maximum value ranged between 0.008 mg/l and 0.023 mg/l. The minimum and maximum value ranged between 0.006 and 0.015 mg/l in Sharda River. Nitrate low values recording in the winter months and high values in spring season. This may be due to the higher phytoplanktonic production, decaying macrophytes and concentration of nutrients owing to the evaporation of reservoir water with subsequent increase in nitrate value. Decrease in nitrate content during winter months was probably due to its utilization as nutrient by the algal community as growth of algae particularly in the winter months. Nitrate occurs naturally and due to its solubility, its mobility in groundwater is very high and hence due its well known adverse effects on health examine the nitrate is very important **(Nas and Ali 2006).** In drinking water the prescribed limit of nitrate concentration suggested by BIS is 45 mg/l

Physico-chemical analysis is very important to evaluate the quality of water for its better utilization. It is also useful in to identify the complex processes, interaction between the environmental and biological processes in the water. All the water quality parameters showed significant seasonal variation. The analyzed results showed that the parameters namely nitrate, phosphate, total hardness, TDS and alkalinity are within permissible limits when compared with safe water quality standards; physicochemical parameters levels indicate the moderate quality of the river water. Present study concluded that the river water was not polluted. Thus Kosi and Sharda River were not polluted in respect to physicochemical assessment.

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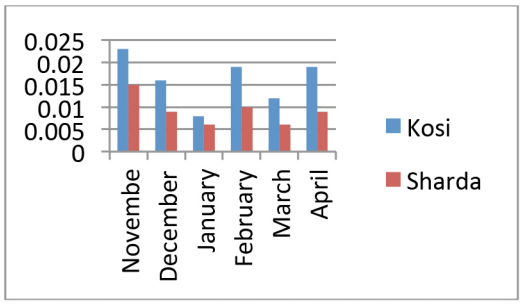
Monthly variation in water quality parameters of Kosi and Sharda River during the study period November(15) to April (16):

Parameter	Nitrate		Phosphate		Total Hardness		TDS		Alkalinity	
	Kosi	Sharda	Kosi	Sharda	Kosi	Sharda	Kosi	Sharda	Kosi	Sharda
November	0.023	0.015	0.042	0.031	75	99	291	146	69	81
December	0.016	0.009	0.0 33	0.026	87	105	257	134	77	88
January	0.008	0.006	0.028	0.021	98	109.5	198	109	89	100
February	0.015	0.010	0.025	0.016	95	113	155	125	87	95
March	0.012	0.006	0.031	0.020	89	99	170	128	71	86
April	0.022	0.009	0.039	0.025	73	95	179	152	60	78



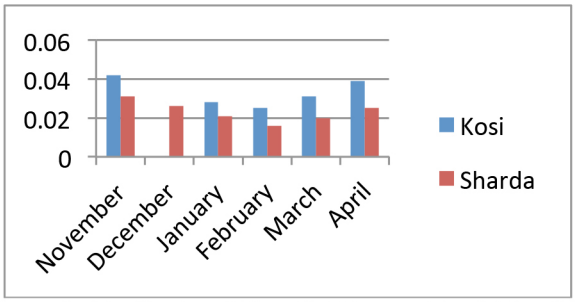
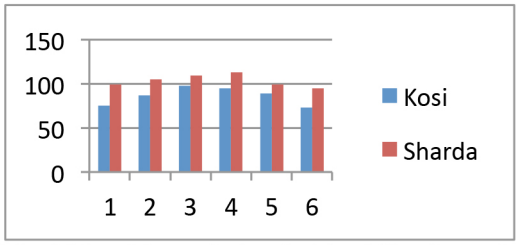


Fig-1 Monthly variation in Nitrates during the study period in Kosi and Sharda River Fig-2 Monthly variation in Phosphate during the study period in Kosi and Sharda River



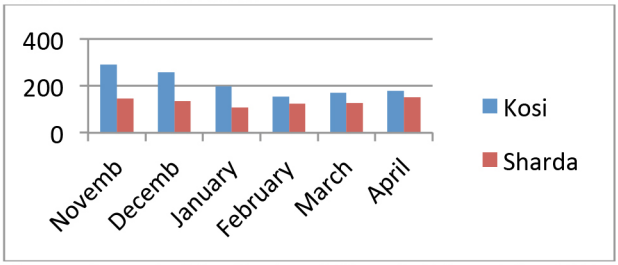


Fig-3 Monthly variation in Total Hardness during the study period in Kosi and Sharda River Fig-4 Monthly variation in Total Dissolved Solids during the study

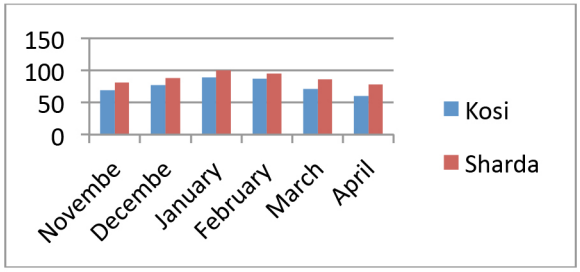


Fig-5 Monthly variation in Alkalinity during the study period in Kosi and Sharda River

NITROGEN DYNAMICS AND MICROBIAL DIVERSITY IN AN AQUAPONIC SYSTEM

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The majority of nitrogen enters an aquaponic system (AP) via the proteins in fish feed. The metabolised N is excreted by fish as ammonia, measured as total ammonia-nitrogen (TAN) [1]. TAN is oxidised by bacteria in the biofilter and on the surfaces of the system to nitrite and subsequently to nitrate. Both, nitrate and part of the TAN are taken up by the plants [2]. With this, fish toxic compounds (TAN and nitrite) are removed from the system water and plants obtain nutrients for their growth. This study aimed to quantify nitrogen dynamics between different components of the AP and connect this to microbial diversity in the system.

The experiment was carried out in three AP system replicates (whole system volume of 4.2 m^3 and each hydroponic part covering 9 m^2) and one hydroponic (HP) control (covering 9 m^2). Each AP system was composed of a fish tank (FT), solids removal unit (SRU), solids thickening unit (STU), digester, moving bed biofilter (BF), UV treatment, oxygenation cone, HP component with floating raft tables and collection tank (CT). The systems were stocked with Nile tilapia (*O. niloticus*) and lettuce (*L. sativa*, Salanova®) and run for one lettuce cycle in summer 2017. The main forms of nitrogen (NH_4, NO_2 and NO_3) in all AP system components (FT, SRU, STU, BF, HP, CT and digested sludge) were quantified and analysed with ion chromatography. Microbial diversity of same samples was determined by terminal restriction fragment length polymorphism.

NH_4 concentrations were highest in fresh and digested sludge, and lowest in hydroponic part of the system, with the opposite being observed for NO_3. There were also significant differences between the inflow and outflow of the HP table, with higher NH_4 and NO_2, and lower NO_3 levels in the inflow. Microbial diversity was similar between BF and HP, showing that the HP table functioned as a biofilter. These findings corresponded to the nitrogen changes in the samples.

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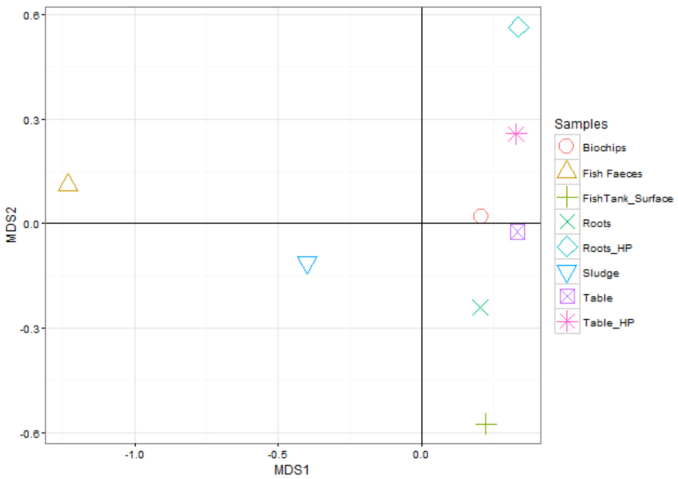

Figure 1: *Microbial community structure analysed with Bray-Curtis similarity based on the relative abundance matrix in AP.*

USING MUSSELS FROM SPAT COLLECTORS FOR RESTORATION PROJECTS: THE IMPORTANCE OF PHENOTYPIC PLASTICITY

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Understanding the capacity of organisms to adapt their phenotype to specific aspects in a new environment (generally known as 'phenotypic plasticity') is critical for restoration projects. Blue mussels (*Mytilus edulis*) are ecosystem engineers that occur in a wide range of geographical sites varying in abiotic and biotic conditions. Phenotypic plasticity allows blue mussels to inhabit a diverse range of habitats. However, overfishing and loss of suitable habitat has led to a decline of intertidal mussel beds. Efforts to restore intertidal mussel beds have been increasingly undertaken, but many attempts were only partly successful or failed completely. An important possible explanation for lack of restoration success is a limited phenotypic plasticity of the transplanted mussels to their new environment. Mussels originating from mussel seed capture installations (SMCs) are still relatively young and therefore thought to be highly plastic, forming a potential suitable donor population for restoration purposes.

In this study we tested how *Mytilus edulis* seeds originating from SMCs adapt their morphology and behavior to hydrodynamic conditions typical for the intertidal zone. Mussel seeds were exposed to three different hydrodynamic conditions. The mussels were placed (1) in tanks in which the water level followed a tidal cycle, leaving the mussels emerged a third of the time, (2) in wave tanks where they were exposed to wave energy and (3) in tanks where they were always immersed in calm water to simulate subtidal conditions.

After 5 months we found that mussels exposed to wave energy had undergone the biggest change in morphology. The shells of these mussels was found to be thicker and longer. In addition, mussels exposed to wave energy had a significant higher condition and a higher byssal attachment strength in comparison with mussels placed in tanks with a tidal cycle or subtidal conditions (Figure 1). Because mussel seeds originating from SMCs constitute a sustainable source and are able to adjust their morphology to different hydrodynamic conditions they may form a valid source for the restoration of intertidal mussel beds.

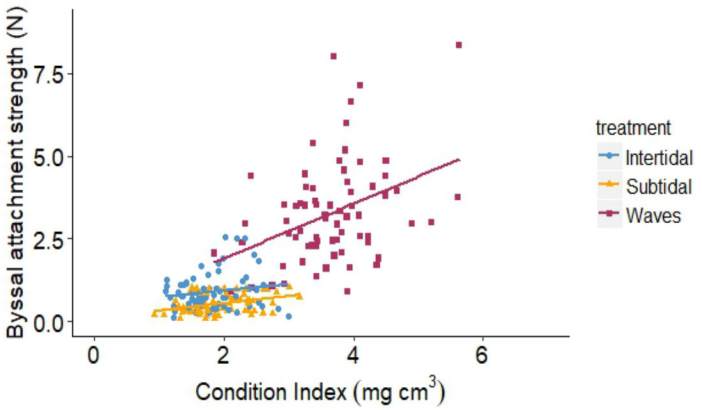

Figure 1. Correlation between byssal attachment strength and condition index for mussels exposed to three different hydrodynamic conditions: Intertidal (following a tidal cycle), subtidal and wave energy.

HOW TO COMMUNICATE AQUAPONICS TO PROMOTE PUBLIC ACCEPTANCE? RESULTS OF A PILOT STUDY USING A MIXED METHOD APPROACH

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Although sustainable food production is considered to be of great importance in public perception, aquaponics as a food production method with a high potential for sustainability is not well known. Public's requirements and information needs regarding aquaponics have to be taken into account to ensure a successful diffusion of this innovation. Because little attention has been paid to these topics yet, the current pilot study was conducted. A multi method approach was used to gain first insights into public's requirements in relation to aquaponics, and to analyse what information should be given to the public to generate trust and acceptance.

For the pilot study information material about the operation principles of aquaponics and about two fictional aquaponics farms was created. While viewing this information at a screen, the gaze behaviour of 18 participants, aged 18 to 48 years, was recorded by a remote eye-tracker (SMI RED 250). Subsequently the participants were asked in a questionnaire whether they had missed any information. Additionally they were requested to rank not less than three of 14 criteria referring to aquaponics (Fig. 1). The goal was to obtain first indications of the requirements and information needs of the general public in relation to aquaponics. A deeper understanding of information needs provide the basis for the development of communication strategies that foster acceptance.

In line with a general trend in Germany, sustainability and local production were rated clearly more often among the top three with 68 percent each, than organic certification with 12 percent only. Food safety and hygienic production as criteria which affect human health were important to one third of the participants followed by production close to nature and fish welfare. The interest in the latter was reflected by the fact that 44 percent of the participants stated, that they had missed information about the aquaponics-grown fish. 33 percent would have liked to see an image with the fish. This special information need is confirmed by the eye-tracking measurements. Participants that had missed information about fish on average fixated a window, which was integrated in the front of a fish basin, significantly longer than the other participants, $t(16) = 2.32$, $p = .034$. Probably they were looking for the fish in order to evaluate the fish welfare.

As a result, when developing communication strategies on aquaponics we recommend to pay special attention to ethical and health issues. Last but not least, it also seems to be important to provide comprehensive information on the fish.

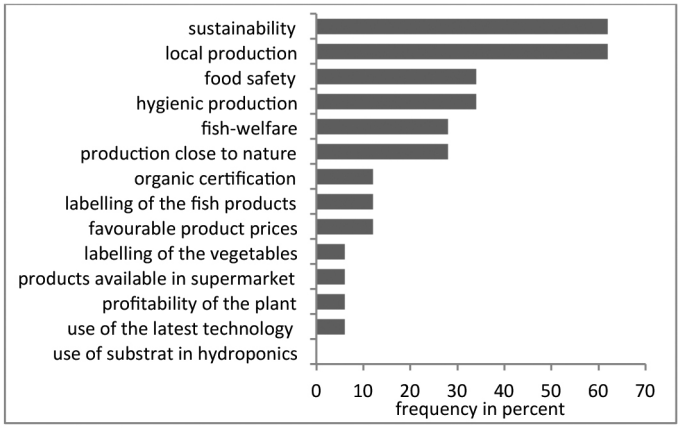

FIGURE 1: Frequency by which the criteria were ranked among the top three; Cochran's Q (13) = 52.61, p < .001

ONTOGENETIC TRANSITION FROM ECTOTHERMY TO ENDOTHERMY IN PACIFIC BLUEFIN TUNA *Thunnus Orientalis*

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Tuna aquaculture depends predominantly on the on-growing of individuals caught from the wild. Genuine tuna aquaculture, in which juveniles are raised from fertilized eggs, has met with only limited success. A major impediment is the sensitivity of very young tunas to cold water. Tunas begin life as ectotherms but later become regional endotherms. During the ectothermic phase, they require warm water temperatures (~25°C) whereas during the endothermic phase they can tolerate much cooler water temperatures (~15°C). Due to their large ultimate size, tunas raised in captivity must ultimately be transferred from the warm water of the hatchery to the often cooler water of sea cages in the ocean. This transfer is accompanied by high mortality rates and a possible cause could be underdeveloped capacity for regional endothermy. Thus, this study aimed to determine the size/age at which Pacific Bluefin tuna (PBT, *Thunnus orientalis*) makes the transition from ectothermy to regional endothermy.

Three different size cohorts of young juvenile PBT were caught in Japanese waters and the maximum temperatures in their red (slow-twitch, oxidative) muscle (T_{RM}), cranium (T_C) and viscera (T_V) were measured and compared with the ambient water temperature (T_a). Subsequently, marker enzymes for mitochondrial (aerobic) and glycolytic (anaerobic) metabolism were assayed in the red muscle (RM) and in the white muscle (WM) and gene expression for these enzymes was also quantified.

T_{RM} was, on average, 4.9°C > T_a for the PBT cohort measuring ~38 cm fork length (FL) (Fig. 1). This compared with 11.1°C for the cohort measuring ~60 cm FL but only 1.1°C for the cohort measuring ~20 cm FL. Thus, ~38 cm FL (~8 months of age) is the minimum size for significant RM temperature elevation above T_a in PBT. In contrast, there was little elevation of either T_C or T_V above T_a. Mitochondrial marker enzyme activity was ~10-fold greater in RM compared with WM and generally decreased with increasing body size. In contrast, glycolytic marker enzyme activity was greater in WM than RM and generally increased with increasing body size. Glycolytic gene expression reflected glycolytic enzyme activity but mitochondrial gene expression did not reflect mitochondrial enzyme activity.

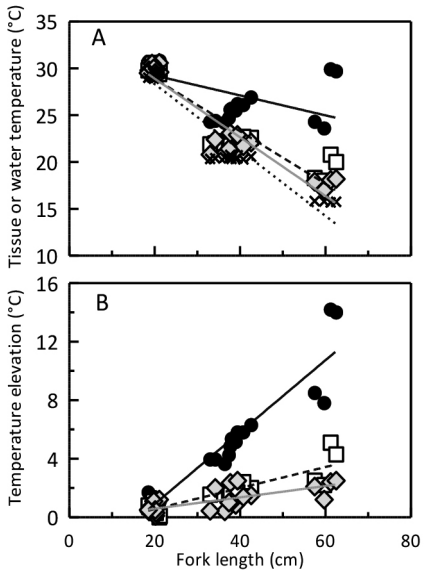

Fig. 1. Effects of increasing FL and decreasing T_a (x with dotted line) on T_{RM} (□ with solid black line), T_V (□ with dashed line) and T_C (◆ with solid grey line) in young juvenile PBT.

A TYPOLOGY OF MARINE MULTI-USE SCNENARIOS

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The increasing human use of the sea over the last decades is putting manifold pressures onto ocean and coastal ecosystems but also puts strains on relationships between users. In an attempt to curb negative impacts and minimize conflicts, different management systems that act on different spatial realms and with different objectives, i.e. from integrated coastal zone management (ICZM) to marine spatial planning (MSP) and Ecosystem Based Management (EBM), have arisen.

These often build on single use zoning approaches and have been applied, in some cases, for decades. Marine multi-use as a concept that addresses spatial efficiency of marine resource uses, has only entered the discussion at the onset of the 2000s. It marks a departure from the concept of singular exclusive resource rights, to the inclusive and synergistic sharing of resources and space between different actors. To date, the public and scientific discourse, however, suffers from the wide variety of terms and concepts in use. Terminology such as “co-use”, “co-location”, “multi-functional” or “multi-purpose” all carry normative varied meaning to the original knowledge holder, however, these remain somewhat skewed to all involved actors.

Applying a grounded theory approach, this study outlines a unified typology by refining and explaining the concept of marine multi-use of space by presenting central key types and examples. These elaborations represent findings of the trans-European H2020 Research Project MUSES: Multi-Use in European Seas. Based on this common understanding of the typology, relationships of users and uses alike are critically analysed in order to attain a complete understanding of the underlying processes, drivers and actors, thereby helping to shape future management approaches and guidelines.

LARVAL FEEDS FOR HIGH-VALUE FOODFISH AND ORNAMENTAL/CONSERVATION AQUACULTURE: RESEARCH NEEDS TO INCREASE LIVE FEEDS QUALITY, SAFETY, BIOSECURITY, AND OPTIMIZATION OF LARVAL PRODUCTION PROTOCOLS

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Aquaculture, often referred to as the blue revolution, continues to expand as one of the fastest growing agricultural sectors globally. Expanding aquaculture includes all of the freshwater, brackish, and marine environments. These expanding sectors include but are not limited to food production, pharmaceuticals/nutraceuticals, ornamentals, and stock enhancement/conservation. With specific regard to high-value foodfish and ornamental/conservation aquaculture, typically broodstock husbandry, live feeds, and larval production protocols are production-limiting factors/bottlenecks. This presentation will discuss some of these current industrial research needs as well as some implementation strategies.

EFFECTS OF MAGNESIUM CHLORIDE SUPPLEMENTS ON THE LIFE HISTORY CHARACTERISTICS OF POSTLARVAE AND JUVENILES OF WHITE SHRIMP *Penaeus vannamei*

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Introduction
The white shrimp *Penaeus vannamei* Boone, 1931 is one of the most important commercial species in the shrimp farming industry worldwide as it can tolerate a wide range of salinities (1–40 ‰) (Bray *et al*. 1994). In aquacultures with artificial seawater supply, key ions such as magnesium (Mg^{2+}) are often deficient. These deficiencies have been shown to negatively impact shrimp growth and survival (Davis *et al*. 2005; Saoud *et al*. 2003). Furthermore, lower amounts of these ions might lead to soft shells, size variation and mortality during molting (Chien 1989).

The purpose of the present study was to determine the effect of magnesium chloride concentration on the growth and survival of postlarvae and juvenile white shrimp *P. vannamei*.

Materials & Methods
Groups of postlarval (G1) and juvenile (G2) *Penaeus vannamei* were exposed to different concentrations (300, 800, 1300 mg/L) of magnesium chloride ($MgCl_2$) using artificial seawater (20 ‰).

In each setup 30 shrimps were held in triplicates in aquaria at 28°C for 4 weeks.
Molting frequency and mortality rate were noted and the weight gain was recorded at the end of the experiment. Furthermore, the hepatosomatic index (HSI, %) as an indicator of energy reserves were determined

Results
The experiment is still in progress and the results are not available at time of abstract submission. The final outcome will be presented at the resubmitted version of this abstract and provided on the poster.

INOSINE-5-MONOPHOSPHATE AS A FEEDING STIMULANT CAN ENHANCE GROWTH PERFORMANCE IN ATLANTIC SALMON (*Salmo salar*), EXPOSED TO HANDLING STRESS

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Introduction

The use of inosine-5-monophosphate (IMP) as feeding stimulance is used widely as a feed ingredient for fish. Benefits, that make it a useful tool for aquaculture, are enhancement of growth performance, especially to compensate delimited palatability due to alternative protein resources such as soy bean meal as fish meal replacement. Although these traits were observed in many fish, including large turbots (*Scophthalmus maximus*) and rainbow trouts (*Oncorhynchus mykiss*), they are yet to be established in diets, fed to Atlantic salmon (*Salmo salar*). Furthermore, IMP can help fish, to deal better with hanling stress, which makes it useful in diets fed to Atlantic salmon. This species is known to be very sensitive to stress, significanlty decreasing food uptake and growth performance upon (McCormick et al. 1998). Therefore, handling stress, in combination with delimited palatability of fish fed diets with high contents of plant derived protein sources, can be a challenge to the industry (Pratoomyot, et al. 2010). IMP, as a nucleotide in combination with other nucleotides as supplementation in diets have been confirmed to have positive effects on palatability and stress restistance in Atlantic salmon. However, no data on IMP as an individual feeding stimulant has yet been performed with this species.

Materials & Methods

Aim of the study was to identify favorable effects of IMP in terms of enhancing resistance against handling stress and improving palatability of soy bean meal based diets for the Atlantic salmon. The trial lasted 62.5 feeding days and juvenile Atlantic salmon were hand fed until apparent satiation with two diets containing 25% soy bean meal inclusion, with either addition of IMP, or no supplementation. Two additional groups were exposed to handling stress twice a day for one continous minute while being fed the named diets. Growth parameters (SGR, FCR) were measured as indication for performance rates. The trial was conducted in a recirculating aquaculture system at the Gesellschaft für Marine Aquakultur mbH (GMA) in Büsum, Germany.

Results & Discussion

Growth parameters were significantly inferior in stressed groups (SGR: 0.93±0.07; FCR: 1.48±0.05), compared to those, without stress when IMP was absent (SGR 1.15±0.08; FCR:1.29 ±0.06). Fish that encountered handling stress but fed with an IMP supplemented diet, showed no significantly lower growth performance rates (SGR: 1.02 ±0.02; FCR: 1.41±0.05), compared to low stress groups (SGR: 1.22 ±0.07; FCR: 1.26 ±0.05). In conclusion, fish fed with a IMP supplemented diet, showed decreased negative effects by handling stress and improved growth. These results suggest, that IMP supplementation in salmon diets, can enhance stress tolerance and compensate negative effects of heavy handling and improve growth, especially when alternative, plant derived protein sources are being used. This shows a potential benefit of IMP for diets, fed to Atlantic salmon, with special recognition of stress stiuations and the use of sustainable resources in industrial aquaculture.

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RNAi ANTIVIRALS FOR SHRIMP AQUACULTURE

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Pathogens cause billion dollar losses annually to shrimp aquaculture. To lessen these losses, RNA interference (RNAi) approaches employing pathogen-specific long double-stranded (ds)RNA molecules have been the focus of intense research interest over the past 2 decades. While shown experimentally to be highly effective at reducing virus replication levels in shrimp with chronic virus infections and protecting shrimp against disease following virus challenge, except for anecdotal reports of RNAi approaches being used in Thailand and South America, commercial applications have yet to materialize. This is in-part a result of the only fail-proof administration method being tail-muscle injection with all efforts to introduce the antivirals via feed not delivering a commercial outcome.

We have been examining the potential use of RNAi approaches in shrimp hatcheries as a means of reducing virus infection loads in broodstock and thus limiting or mitigating virus being transmitted vertically to seedstock. While the ability of injected dsRNA to reduce virus loads is well known, and injection of virus-specific dsRNA does not impede female broodstock spawning viable eggs, Government regulatory approvals from bodies such as the Australian Pesticides and Veterinary Medicines Authority (APVMA) will be required to further assess the value of this approach in commercial trials generating seedstock to be farmed for human consumption.

To gather scientific evidence on the human health risks of the approach, a study was undertaken to determine whether dsRNA injected into tail muscle of female *Penaeus monodon* broodstock at the time they were eyestalk ablated would persist in various tissues including ovary until the time they spawned, be carried with eggs and persist in progeny larval stages as they develop. In 3 females co-injected with dsRNA sequences (0.5 μg each dsRNA/10 g shrimp weight) specific to the gill-associated virus (954 bp) RNA genome and the firefly luciferase (Luc) gene (464 bp), both dsRNAs were detected by RT-qPCR in pleopods, lymphoid organ, ovary tissue pieces sampled immediately after each spawned between 6 and 12 days post-injection. However, RT-qPCR analyses failed to unequivocally detect either dsRNA in pools of 50 embryos, 50 nauplii, 50 protozoea, 2 x 50 mysis and 32 individual post-larvae (PL7 to PL11) reared separately for each female. These findings suggest, at least at the dsRNA dosages injected and even though dsRNA was detected in the female's ovaries, that dsRNA is not transmitted effectively in their eggs, and not amplified as these eggs developed through the various larval stages to PL that would be stocked into commercial ponds. As such, muscle injection of purified dsRNA into female broodstock used in hatcheries to generate seedstock appears to pose no human health risks in their spawned progeny.

MOLECULAR CHARACTERIZATION AND EXPRESSION ANALYSIS OF CATALASE FROM BIG BELLY SEAHORSE *Hippocampus abdominalis*

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Among enzymatic components, catalase is a key enzyme plays an indispensable role in protecting cells and tissues against oxidative damage and also has an influence in the signal transduction mechanism by detoxifying hydrogen peroxide to non-toxic form of water and oxygen. Hydrogen peroxide is a relative oxygen species (ROS) plays an important role in anti-defense against invading pathogens and also a signal transduction molecule, while it is toxic to the host organism by inducing oxidative stress and cell damage when it's become accumulate. In the current work, we molecularly characterized a homology of catalase identified from Seahorse (*Hippocampus abdominalis)* and evaluated the immune response using challenge experiment.

The cDNA sequence of catalase in *Hippocampus abdominalis* (*ShCat*) consists of 1578bp open reading frame, encoding 526 amino acids with a molecular mass of 59.33kDa and estimated PI of 7.7. No signal peptide was obtained. The protein sequence of ShCat has similar domain to already known catalases, containing proximal active site signature "FDRERIPERVVHAKGAG" and proximal heme ligand signature "RLFSYPDTH". Sequence comparison showed that the deduced amino acid sequence of ShCat shares 88.2%, 87.1%, 77.8%, 76.9%, 76.2% and 62.4% identity with that of *Oplegnathus fasciatus, Maylandia zebra, Bos taurus, Homo sapiens, Columba livia,* and *Drosophila melanogaster*, respectively. Catalase mRNA expression were detected in blood, brain, gill, heart, intestine, liver, kidney, muscle, ovary, pouch, skin, spleen, stomach and testis tissues by real-time PCR. Kidney and liver tissues showed highest mRNA expression compare to other tissues. Expression variation of mRNA in blood after immune challenge was also quantified by real-time PCR. Catalase expression showed significant up-regulation at 3h post injection, down-regulation at 12h post injection for *Edwardasiella tarda*, polyinosinic:polycytidylic, and lipopolysaccharide and 24h post injection for *Streptococcus iniae* and again up-regulated in 72h post injection at all. Collectively, the results obtained in this study suggested that ShCAT may participate in the host immune response.

SEAFOOD HACCP INSPECTION PROCESS BY THE UNITED STATES FOOD AND DRUG ADMINISTRATION

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Ensuring the safety of the food supply continues to be a top priority for the United States Food and Drug Administration (FDA). To determine compliance with FDA laws and regulations, the FDA conducts seafood HACCP (Hazard Analysis Critical Control Point) inspections before these products may enter commerce in the United States. The goal of the foreign inspection program is to help ensure that foods exported to the United States are safe and meet USFDA regulatory requirements. Countries are selected based on several factors including the volume of imports, the nature of the product (high or low risk) and the violation history of the firm/country.

The components for a seafood HACCP inspection are as follows:

1. Conduct an initial interview
2. Observe facility Operations and sanitation, with a walk-thru
3. Conduct a Hazard Analysis
4. Evaluate the processor's HACCP plan
5. Assess the implementation of the firms HACCP plan
6. Assess the firms Sanitation and Good Manufacturing Practices (GMPs)
7. Review the firms Record keeping
8. Document and firm Violations (FDA from 483)
9. Conduct Close-out Interview
10. Write Establishment Inspection Report (EIR)

In addition to the international food inspection program, the FDA has a border surveillance and sampling program intended to promote cooperative relationships with foreign regulatory authorities and assist in building confidence in their seafood regulatory programs.

AN INTEGRATED ULVA-PERIPHYTON BIOFILTER FOR BOTH AMMONIA AND NITRATE IN FISHPONDS EFFLUENT

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Periphyton- based biofilters for aquaculture effluent possess multiple advantages, as water oxygenation, CO_2 reduction and production of useful biomass. Performances of a marine periphyton biofilter in removing different forms of nitrogen was investigated. A periphyton biofilter was paired with an upstream macroalgae biofilter stocked with *Ulva sp*. to expose the periphyton to ammonia- depleted, but nitrate- rich effluent. In another system, the downstream periphyton biofilter was replaced by *Ulva sp*. to indicate which of these two plant- based biofilters is more efficient in nutrient polishing. The biofiltration and growth performance of periphyton and *Ulva sp*. were measured at different areal loads of Total ammonia nitrogen (TAN) and nitrate (NO_3-N). While the *Ulva sp*. preferred uptake of TAN over NO_3-N, the periphyton showed no preference between them, demonstrating flexible shifts between TAN and NO_3-N uptake. TAN uptake rate by periphyton was not influenced by the effluent composition. However, periphyton NO_3-N uptake rate and efficiency rose about fivefold, up to 1.4 g NO_3-N m^{-2} d^{-1} and 63%, respectively (fig. 1), upon depletion of TAN areal load below 0.18 g N m^{-2} d^{-1} (<0.3 mg L^{-1}) by the Ulva pre-treatment. Normalizing nutrient uptake rate to biomass revealed similar uptake rate of TAN and phosphorus by periphyton and *Ulva sp*., while the periphyton took up nitrate much faster. By removing up to 76% of the total nitrogen, with specific removal efficiency of 97% of the TAN and of 67% of the NO3-N, the novel dual Ulva-periphyton biofilter revealed a synergistic potential in treatment of nutrient-rich mariculture effluents.

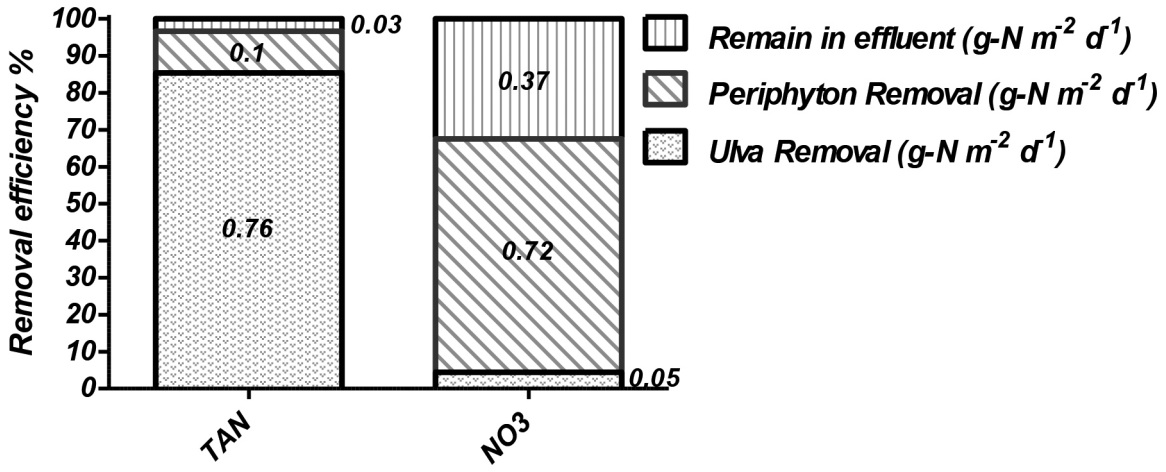

Figure 1. Distribution of the partition of the Ulva and the periphyton in the combined Ulva-periphyton biofilter and of effluent of this biofilter (i.e. discharged water) in removal of TAN, NO_3-N. The partition of each of the above components in nutrient removal was calculated according to the daily reduction in nutrient as percentage of the daily load in the influent to biofilter and is illustrated as it partition from a 100% removal. Values on columns represent the net uptake (in g m^{-2} d^{-1}) by each compartment. Net uptake in effluent was calculated by subscribing the daily uptake of Ulva and of periphyton from the daily load in influent to the combined biofilter. Values are mean, N=3.

EFFECT OF USING MARINE MICROALGAE (*Nannochloropsis oculata*) IN RAINBOW TROUT DIETS ON SOME HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS

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This study aimed to assess the effect of four dietary oven-dried *Nannochloropsis oculata* powder levels (0, 7.5, 15, and 22.5 mg algae/kg of feed) on some haematological and serum biochemical parameters for rainbow trout fry. Fish (n = 120; 2±0.3 g) were randomly divided into 12 fiberglass tanks (20 L) and reared for 60 days. There were no significant differences in some hematological parameters such as hematocrit, red blood cells, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration values ($p>0.05$). The results showed that all the serum biochemical parameters were decreased significantly with increasing dietary algae up to levels 15 mg algae/kg of diet. However, the highest and lowest high density lipoprotein was observed in and fish fed with 15 mg algae/kg of diet (164.3mg/dl) and control group (104.3 mg/dl), respectively. To conclude, we can suggest *N.oculata* powder at 15 mg algae/kg of feed in rainbow trout fry diet.

POSSIBILITY FOR NON-DRUG CONTROL OF BLOOD FLUKE INFECTIONS IN FARMED BLUEFIN TUNA

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Blood flukes of the genus *Cardicola* are important pathogens of farmed/ranched tuna around the world. These blood flukes infect the cardiovascular system and their eggs accumulate in gill capillaries causing tuna to suffocate. Oral administration of praziquantel is an effective treatment and widely used in tuna farms. It is important**,** however, to develop a non-drug control measures to ensure food safety and to prevent the appearance of drug resistant strain. Recently, terebellid polychaetes have been identified as the intermediate host of the tuna blood flukes. The present study is a recent trial of a new non-drug blood fluke control strategy by reducing the density of the intermediate host on farming cages.

A long-term monthly sampling of polychaete was being conducted at a tuna farming site to determine the temporal and spatial distribution of infected intermediate host around tuna cages. The results indicated that the ropes attached to the farming cages are the “hot spot” for infected *Nicolea gracilibranchis*, the terebellid intermediate host of *C. orientalis*. In 2017, the ropes were cleaned and the terebellids were removed before juvenile tuna had been transferred to the cages. Subsequently, blood fluke infection in juvenile tuna in cleaned cages was monitored and their infection level was compared with those from previous years.

The infection rate of *N. gracilibranchis* lived on the ropes were significantly higher than those from other cage structures. Before 2017, the infection of *C. orientalis* typically reached 100% within a few mo after juvenile tuna had been transferred to cages. After removing *N. gracilibranchis* from the ropes, *C. orientalis* infections were not detected until 9 mo after fish transfer, and the infection rate was as low as 20%. Moreover, the infection rate of *N. gracilibranchis* which has been newly established in ropes also significantly reduced. Although we need more solid evidence, cleaning of cage structures can be a simple and good strategy to reduce *C. orientalis* infection in farmed tuna.

SUITABILITY OF USING TUNA HYDROLYSATE FROM TUNA, *Thunnusmaccoyii* PROCESSING INDUSTRY AS AN ALTERNATIVE FISHMEAL PROTEIN SOURCE FOR JUVENILE BARRAMUNDI, *Latescalcarifer* (Bloch, 1790)

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There is growing interest in aquaculture industry, to identify new alternatives that not only support growth but immunomodulating capacity of the host fish. Past literature has shown that tuna hydrolysate (TH) yields a large number of biologically active immunomodulating peptides used as a growth promoter, immune modulator and immunostimulants to defence against stress and pathogens in fish. Here, we evaluated TH produced from tuna processing as a substitute for fishmeal (FM) to assess growth performance, antioxidant capacity and biochemical response of juvenile barramundi, *Lates calcarifer*.

TH was produced from Tuna, *Thunnus maccoyii* processing includes head, guts and frames through enzymatic hydrolysis. The TH then further bioprocessed by incorporating 10 % of *Saccharomyces cereviceae* (Instant dried yeast, Lowan®) and 5 % of *Lactobacillus casei*skim milk product (Yakult®) under anaerobic conditions for four days. Four experimental diets either fermented ($FTH_{50,}$ FTH_{75}) or non-fermented ($TH_{50,}$ TH_{75}) were fed to triplicate tanks (250-L) containing 20 fish (6.75±0.16 g) each for 56 days.

Growth performance regardless of fermentation was negatively affected by TH and FTH in the diets compared to control (Table 1). The apparent digestibility coefficients (ADCs) of dry matter, protein and lipid were the highest in control group and decreased with increase of dietary TH level.However, whole body proximate compositions were not affected by diet formulation. Dietary inclusion of TH had no effect on haemoglobin, haematocrit and leucocrit level but aspartate aminotransferase (AST) level was significantly increased than the control. With the exception of FTH_{75}, enzymatic glutathione peroxidase (GPx) activity was lower in all tested diets than control. Furthermore, fish fed fermented TH had higher GPx activity than non-fermented in 75% replacement level but the difference was not significant between the fermented and non-fermented in the 50% replacement diets. The TH included diets of TH_{75} and FTH_{75} caused hepatic alterations including cytoplasmic vacuolization with an increased amount of lipid accumulation and necrosis in the liver tissue of juvenile barramundi. Further trials involving fermentation are needed to optimise TH level and improve the fish growth, feed utilization, and biochemical response of juvenile barramundi.

TABLE 1. Growth performance and feed utilization of juvenile barramundi fed different levels of fermented and non-fermented TH diets for 56 days

	Experimental diets					*P*-value
	Control	TH_{50}	TH_{75}	FTH_{50}	FTH_{75}	
IBW	6.36	6.96	7.02	6.78	6.67	0.259
FBW	31.53^{a}	26.36^{b}	19.05^{c}	27.69^{b}	21.25^{c}	<0.001
SGR	2.87^{a}	2.38^{b}	1.78^{c}	2.51^{b}	2.07^{c}	<0.001
FCR	2.46^{c}	2.69^{c}	4.36^{a}	2.97^{bc}	3.64^{ab}	<0.05
SR	98.33	95.00	93.33	96.67	93.33	0.364

IBW (g), initial body weight; FBW, final body weight (g); SGR, specific growth rate (% day^{-1}); SR, survival (%). Means values of triplicate groups of fish in the same row with different superscripts are significantly different ($p<0.05$).

PREDICTING EUROPEAN SEABASS SURVIVAL TO A BACTERIAL CHALLENGE USING HEAD-KIDNEY GENE EXPRESSION MEASUREMENTS

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An increasingly important aspect of fish nutrition research is to evaluate how formulation changes affect fish immune status, given the possibility of downstream effects on disease susceptibility and growth performance. In this sense, feeding trials can sometimes be followed by an induced immune challenge that elicits a measurable phenotype (e.g. survival, degree of inflammation) that can be used to infer fish immune status. In this presentation, we explore the possibility of predicting fish survival after a bacterial challenge using head-kidney gene expression data, which would facilitate rapid screening of dietary effects on fish immune status.

For this trial, European seabass juveniles (IBW = 9 g) were reared in 12 tanks, being fed one of four experimental diets over the course of 2 weeks. Head-kidney samples were obtained for gene expression measurements using RT-qPCR (30 measured transcripts). Fish were then subjected to a bacterial challenge (*Photobacterium damselae piscicida* injection) after 4 weeks of feeding and returned to the tanks, with mortalities being recorded over the course of the following 3 weeks. Models for prediction of final tank-wise survival were then calibrated based on the head-kidney gene expression measurements performed before the challenge.

The results indicate that it is possible to predict ($R^2 = 0.49$) tank-wise survival after a bacterial challenge based on a reduced number of gene expression measurements performed prior to the challenge (Figure 1), supporting the relevance of using gene expression measurements to evaluate fish immune status.

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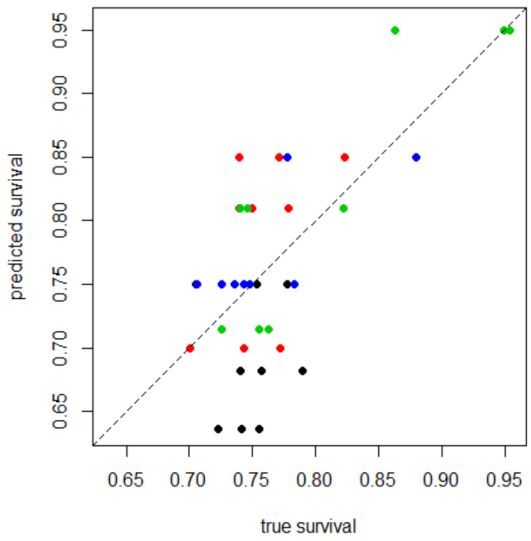

FIGURE 1. Scatter plot showing relationship between true/measured survival vs. estimate based on the expression of four genes (each color represents a distinct diet).

STUDY OF SOCIAL STATUS OF FISHING COMMUNITIES IN PATNA (INDIA)

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Bihar is one of the most populated (approx 99 million) and is one of the fastest growing States (17.3%) in Indian republic. The state majorly depends on agriculture for its economy. However, the state is very rich in terms of river area (approx 3200 sq km), floodplain wetlands (1000 sq km), Oxbow lakes (90 sq km), water reservoirs (72 sq km) and ponds (690 sq km). This provides suitable environment for fisheries and the huge population provides continuously demand of fish products in the markets. Additionally it generates ample employment opportunities. However, the socio-economic growth of fishermen/women involved in the fish markets and the supply chain is lagging behind persons involved in other agro industries.

Our current study was carried out to appraise the social status of traditional fishing communities of Patna (the capital of Bihar, India) fish markets by conducting an extensive survey. During our survey, we collected data using a well regulated questionnaire focused on: educational qualification, their age, technical ideas, price negotiation skill etc. as well as observation. We visited main fish whole sale market as well as multiple local fish markets and interviewed both male and female fish vendors. From every market, approximately a quarter of fishermen/women were randomly chosen for interview totaling to an approx 100 people.

It has been noticed that the age distribution is almost uniform, and majority of persons were uneducated, except the stockiest and contractors. However, they were very skillful in processing and marketing. It's a skill which has been passed from generations to generation. Consequence of illiteracy among both genders comes manifold. They are not aware about the modern techniques of fish processing and preservation. Even they are not aware about the Government benefits.

A majority of fish venders are keeping their children in fish markets along with them instead of sending them to schools. Another interesting observation was in the wholesale market that males have an active

participation (100%) and decision making in fish contractor ship and merchants. In contrast, the local fish markets showed > 50% women participation and decision making. Females were noticed as more dynamic participant in fish marketing as compared to the males. The trend was the same for street side and door to door fish vendors.

This study shows that, in Patna fish markets, regardless of uneven gender distribution among different market related activities, and schemes lack of proper education is the major cause of declining social status of fishermen/women and this can be significantly improved by imparting education and training among them.

Pic: Educational qualification and Age distribution of fishermen/women.

OPTIMIZATION OF NUTRIENT INPUT TO INTEGRATED AQUAPONICS SYSTEMS THROUGH MINERAL SUPPLEMENTATION BY WAY POTASSIUM FEED ADDITIVES

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This study focused on developing feed for aquaponics that would be beneficial to both plant and fish production. Aquaponics is an integrated production system combining aquaculture and hydroponics. However, it is difficult to maintain synchrony between fish and plants because they have different biological and nutritional requirements, making it difficult to produce both fish and plants optimally, and reduced productivity is frequently encountered. Therefore, there is a need to develop feed uniquely for aquaponics system, to meet the demands of both fish and plants. The first phase was a standard feeding trial to evaluate potassium as a dietary feed additive on the African catfish. Experimental diets including potassium from dietary sources at different inclusion levels were formulated and compared with a control diet. In the trial, potassium was added from two different sources, potassium diformate (KDF) and potassium chloride (KCl). KDF was added at 3, 6, and 9 gkg^{-1} and KCl at 1.7, 3.4, and 5.1 gkg^{-1}. Furthermore, in separate trials, the effect of water quality and digestibility of feed additives were evaluated. Results showed no significant differences in production parameter; weight gain, FCR, SGR and survival ($p>0.05$). Proximate body composition of the fish was significantly affected by the feed additives, except for the ash content ($p>0.0.5$). Moisture content significantly differed between the control diet (79 ±1.16) and diets containing KCl 1.7 and KCl 5.1(76 ±1.44; 76 ±0.37). Haematology parameters showed significant differences in haematocrit (HCT) levels, differing significantly from 36.7 ± 1.84 % for KDF 9 to 32.1 ± 2.30 % KCl 1.7, while there were no differences in red and white blood cell counts. The feed additives significantly affected liver mineral composition; with fish fed the control diet having significantly higher iron than fish fed KDF 6, KCl 1.7 and KCl 5.1 diets. In the water quality trial, parameters tested; K, Fe, P, DO, TAN, TSS,TDS, were significantly affected by feed additives over the two day trial period. K, P, TAN and TDS levels in the water increased significantly with time ($p<0.05$). These results will be used as a basis to determine the best additive and the optimum inclusion level for aquaponics.

PRODUCTION OF XY FEMALES AND YY MALES ASSISTED BY SEX CHROMOSOMIC MARKERS IN A NILE TILAPIA *Oreochromis niloticus* WILD POPULATION FROM BURKINA FASO

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Nile tilapia, *Oreochromis niloticus,* is a major farmed fish in which the farming of all-male offspring is the best way to avoid unwanted reproduction during the grow-out period and to optimize the production since males grow faster than females. Currently, the most sustainable method for producing all-male offspring is based on the use of YY males. However, the classic procedure to produce YY males is tedious because XY females and YY males cannot be identified before sexual maturity, and consequently YY males have been produced in very few strains. The recent identified sex markers could optimize this process. Nevertheless studies have shown that sex is linked to at least 2 linkage groups LG1 and LG23, depending on the strains as well as the wild populations. Therefore, it is necessary to develop a specific protocol for YY-males' production assisted by these markers for each farmed strain. The aim of the present study was to analyse and apply these sex markers to produce YY males in a wild population from Burkina Faso (Bama, Lake Kou) during its domestication process.

In our study, we used the *amh* genes (located on LG23) identified in a Japanese *Oreochromis niloticus* strain containing several insertions and deletions located in the promoter, exon VI and exon VII. Ninety one *O. niloticus* wild caught breeders from Lake Kou were genotyped using these *amh* genes' markers. According to progeny testing results, *amh* markers correctly assigned the genotype for 82% of the G0 XY males. This suggests that in the remaining males, sex was not linked to LG23. The markers formally identified all G0 females including XX and XY genotypes which were also confirmed by progeny testing.

To obtain G1 XY females, 34 progenies were treated with ethynyl-estradiol during the sex differentiating period. We subsequently identified precociously with the *amh* markers 58 XY females sampled amongst these progenies. The genotypes of three of the XY females out of 58 were confirmed by progeny testing (XY males crossed with XY females). We obtained 68 to 83% male proportions which were not significantly different from the theoretical expected proportion. However, the markers failed to clearly indicate the absence of an X chromosome in the possible YY males. Nevertheless, these *amh* markers allowed us to develop a rapid procedure to obtain functional XY-females. This is a major step of the ongoing YY-males' production assisted by chromosomic markers for the domestication of the Kou *O. niloticus* population. Additional sex-linked markers need to be searched with others approaches such as RAD sequencing and allelic variations including the analyses of both LG1 and LG23 sex-linked markers.

Table1. Validation of *amh* markers by progeny testing

	amh markers				Progeny testing			
Males	*amh* Y^+	*amhY*$^+$	*amhX*$^+$	XY	31	XY	38
	amh Y^-	*amhY*$^-$	*amhX*$^+$	XX	8	XX	1
Females	*amh* Y^-	*amhY*$^-$	*amhX*$^+$	XX	44	XX	44
	amh Y^-	*amhY*$^+$	*amhX*$^+$	XY	1	XY	1

NEW SUSTAINABLE PROTEIN SOURCES IN RAINBOW TROUT PLANT-BASED DIETS: CONSEQUENCES ON GROWTH PERFORMANCES, FATTY ACID CONTENT AND IMMUNE-STATUS

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The aquaculture sector has been subjected to a huge and sustained development during the last decades. The production of aquafeeds based on fishmeal and fish oils has been a key element in this development. However, the stability of the fishmeal and fish oil production is compromising the sustainable development of aquaculture. To face the decreasing availability of fishmeal and fish oil, the development of the aquaculture sector implied a search for new ingredient sources. As such, plant ingredients are nowadays being commonly incorporated in fish feed, allowing to spare consequent amount of fish meal and fish oil. However, the total replacement of fishmeal and fish oil has not been reached due to negative impacts on feed intake, metabolic and health status, flesh quality and growth performance slowdown.

Single cell protein sources such as yeast protein products could be an alternative solution to complement and mitigate detrimental effects of plant proteins. This was tested in a trial in which rainbow trout juveniles were fed with a plant-based diet (devoid of fishmeal and fish oil), containing 0, 5, 10 or 15% of a yeast protein product (from Phileo Lesaffre Animal Care) for 12 weeks. In addition to yeast, microalgae containing high level of dososahexaenoic acid (DHA, 22:6 n-3) were added to each diet to cover n-3 long chain highly unsaturated fatty acids requirement of the fish.

Mean bodyweight of fish was determined every 3 weeks and at the end of the feeding trial, whole body composition and fatty profiles were determined. Results indicated that yeast protein supplementation may upgrade growth performance of trout fed plant-based diets totally devoid of fishmeal and fish oil by improving feed efficiency and nitrogen retention. Surprisingly, it seems that yeast protein may also improve flesh quality by increasing DHA retention. Studies of cellular and molecular mechanisms are in progress to clarify the role of yeast protein on long chain highly unsaturated fatty acid biosynthesis. Immune status and gut health of the fish were also assessed through histological analyses of distal intestine and mRNA expression level measurements of immune-related genes in distal intestine. Those analyses were complemented by measurement of some plasma immune-related parameters. Results of this study revealed that inclusion of yeast proteins in a plant-based diet enables to improve rainbow trout gut health and global immune status.

In conclusion, the present study highlights the potential benefits of adding yeast protein products in rainbow trout diet devoid of fishmeal and fish oil.

OPTIMIZING OF WINGED KELP Alaria esculenta HATCHERY CONDITIONS

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Norway has large sea areas with good potentials for seaweed cultivation and an increasing number of companies establish now commercial farming of kelp along the coast. Kelp biomass may have many applications and there is an increasing interest for diversification to new products, but due to high production costs today's farmers produce biomass mainly at small scale for the food market. To increase the biomass yield and lower the overall production costs industrial scale is needed, including optimizing of the cultivation protocols as well as mechanization and automation of the processes. In this study we focused on the hatchery phase and how optimizing of different steps in the protocol used for seedlings production of winged kelp *Alaria esculenta* can improve the efficiency.

As the commercial seedlings production takes place during autumn and winter, unlike the natural cycle with spore production at spring, gametophyte cultures are maintained under red light throughout the year to have access to *A. esculenta* seedlings. Fertility needs to be induced in white light before the seedlings production can start and an experiment, testing photoperiods of 16, 23 and 24 hours light per day, demonstrated that 23 hours light gives the fastest transition from vegetative tissue to reproductive growth, with cultures ready for seeding after 6 days. Dense cultures may however need a longer induction period for optimum development due to higher light absorption.

Little is known about the phlorotannin content and mode of action in *A. esculenta* but as phlorotannin production in macroalgae often is related to stress the phlorotannin content was analyzed to examine whether this can be used as a quality parameter for gametophytes and seedlings in optimization of hatchery conditions. This experiment also included addition of a plant growth stimulant to the gametophyte cultures. The phlorotannin concentration in non-fertile gametophytes was on average 0.4±0.1 mg/g DW with no differences between cultures with or without addition of the growth stimulant. After fertility induction the concentration increased significantly, to 1.4±0.2 mg/g DW tissue in the control condition and 2.6±0.4 mg/g DW tissue in cultures added growth stimulant. Phloroglucinol and dihydrophloroglucinol were identified. More work is needed to explain the phlorotannin dynamics in *A. esculenta* seedlings.

The study also demonstrated estimation of the gametophyte culture density by use of optical density measurement (OD_{750}) and estimation of seedlings growth by image analysis, both techniques that can be developed further and implemented in automatized macroalgae seedlings production to replace time-consuming manual monitoring methods.

This research was funded by the Research Council of Norway (grant no. 267536 "MACROSEA").

CHOOSING FLOATING MACROPHYTES FOR ECOLOGICAL INTENSIFICATION OF SMALL-SCALE FISH FARMING IN TROPICAL AREAS: A METHODOLOGICAL APPROACH

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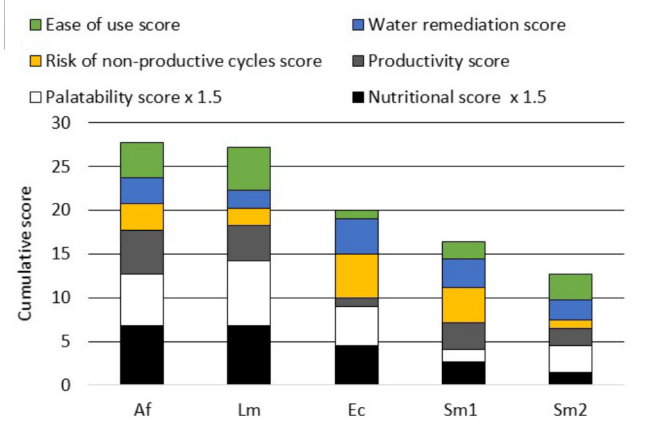
Several ecological services and functions are attributed to macrophytes, which may represent valuable resource for the ecological intensification of tropical fish farming. However, considering the multiple potential eco-services provided by macrophytes, the choice of the most appropriate species requires multiple criteria to assess these eco-services. Five floating macrophytes (*Eichhornia crassipes, Lemna minor, Azolla filiculoides, Salvinia molesta* and *Salvinia* spp.) were selected for this study. The six eco-services parameters compared among these macrophytes were: productivity (in % of seeded macrophytes), risk of non-productive cycles, nutritional value, impact on water quality, palatability (expressed as the percentage of plant ingested in 4 h) and ease of use. Experiments to determine these indicators were carried out in simple devices (net cages and plastic tanks) partially immerged in earthen ponds.

The palatability of fresh macrophytes (whole or chopped) was studied in ponds using *Osphronemus goramy* juveniles. All indicators were scored from 1 to 5, and the highest score was assigned to the highest performer.

These scores corresponded either to the evaluation of a single parameter (e.g. productivity) or to the average from scores of several parameters (e.g. nutritional score). Multiplier coefficients were used for nutritional value and palatability. *A. filiculoides* showed the best scores for productivity; *L. minor* for nutritional value and palatability, and *E. crassipes* for the lowest risk of non-productive cycles and a positive impact on water quality. After integrating the scores in a multi-parameter matrix, *A. filiculoides* showed the best overall score. The results of this approach to select macrophytes are discussed in light of the eco-services provided by macrophytes which may, in turn, promote the ecological intensification of tropical small-scale aquaculture.



MORE WITH MOLLUSCS

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Marine bivalves like oysters, clams and mussels have been cultivated for ages and are recognised as a sustainable low food chain resource that acquires feed from natural resource in their environment. They provide a rich source for human nutrition and an associated economic value for local communities. Total bivalve aquaculture production amounted 15 million tons in 2015 with a market value of 15 billion US $.

Besides human nutrition, they provide food for birds and benthos, a habitat for a large number of species, they regulate water quality and sequester carbon and nitrogen. As eco-engineers, epibenthic bivalve beds are used for coastal defence and nature conservation. Shellfish restoration is a worldwide issue that not only aims to bring back the bivalves but also restore the facilitation functions of bivalve beds. They also produce significant amounts of shell material that has many applications. These functions can be defined as ecological goods and services. This concept provides a framework for description and analysis of the role of bivalves in the ecosystem, and a basis for addressing a wide range of topics, benefits and controversies related to the use of bivalves for production, habitat restoration, water quality and coastal management. This analysis also includes cultural services like the methods of shell collection, the use of shells to trace back (pre)historic events and the local culture of shellfish gardening as a community issue.

Understanding goods and services may improve management decisions. A way to improve decision making is to valorise the goods and services. The economic revenues of aquaculture are based on the market value of harvestable products, while the economic values of ecosystem services are often not adequately quantified and not fully captured in commercial markets. Both for market and political decisions, techniques are needed that can be used to make economic values of mollusc ecosystem services explicit, provided full knowledge of the goods and services is available. A comprehensive analysis of their ecosystem functions is needed to better make use of the good and services. In the presentation, the state of the art in multiple use of molluscs will be reviewed on the basis of a number of case studies.

BIVALVE AQUACULTURE, GLOBAL CHALLENGES

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The global production of marine bivalves for human consumption has increased from 5 to more than 15 million tonnes per year over the period 1995 – 2015. It now comprised about 14 % of the total marine production in the world. Most of the bivalve production (89%) comes from aquaculture and only 11% comes from the wild fishery. China is by far the largest producer of marine bivalves, accounting for 85% of the world production, with a steady increase. This is in contrast to other continents, where the production is stabilizing or – in Europe – decreasing in the last decades. Given the need to secure food production for the growing world population in a sustainable way, there is a challenge for low food chain aquaculture. Marine bivalves are a renowned resource of protein and healthy fatty acids that feed from their natural environment. Like seaweeds, bivalve aquaculture has the potential to fulfil future food requirements without adverse ecological effects. Yet, in many areas bivalve aquaculture faces different kinds of limitations: space availability, carrying capacity for production in relation to ecological and social carrying capacities, competing claims with other users, sufficient water quality control including biotoxins, invasive species, spreading of diseases, and the provisioning of resource material. Impacts of climate change often makes these limitations more severe. Yet new technologies and methods are being developed to cope with these problems, and there are quite some examples of innovations applied by industry, science and governments that deliver promising results. In addition, better understanding of the functions of bivalves in the ecosystem paves new ways for integrated exploitation, such as in the IMTA framework. In how far can bivalve aquaculture contribute to other functions such as water quality control, nature restoration and enhancing coastal resilience ? The main global challenge for bivalve aquaculture is to explore the production of healthy food and in the meantime improve the environment. How this can be done will be discussed.

ASSESSING THE COMMERCIAL POTENTIAL FOR COLLECTION OF GREENSHELL™ MUSSEL SEED IN THE HAURAKI GULF, NEW ZEALAND

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Greenshell™ mussel production in New Zealand is constrained by three main factors. The first being the highly seasonal nature of Greenshell™ mussel production. In northern New Zealand mussels spawn mostly from July to November and during much of this period mussels cannot be harvested due to their poor condition. The second is the industry's heavy reliance on a single source of beachcast wild seed which supplies around 80% of the spat used in the country's mussel aquaculture industry. Finally, the loss of mussel seed from nursery ropes can be as high as 100%, but is usually between 50 and 70%. This wasteful use of mussel seed is thought to cost the industry between NZD$6-10 million annually. Anecdotal evidence suggests that mussels from the Hauraki Gulf region may develop and spawn at a different time of the year to seed from the main commercial seed source. If this were the case and mussel seed could be collected commercially in the Hauraki Gulf this could potentially extend the harvesting season as well as provide an alternative source of mussel seed. This study focused on the following aims; 1) determining whether seed mussels obtained from two different sources have different annual condition cycles, and growth rates, 2) determining the yearly pattern of seed arrival in the Hauraki Gulf, and 3) comparing the retention rates between the two seed sources.

GENOMIC FACTORS UNDERLYING RESISTANCE TO BROWN RING DISEASE IN THE MANILA CLAM *Ruditapes philippinarum*

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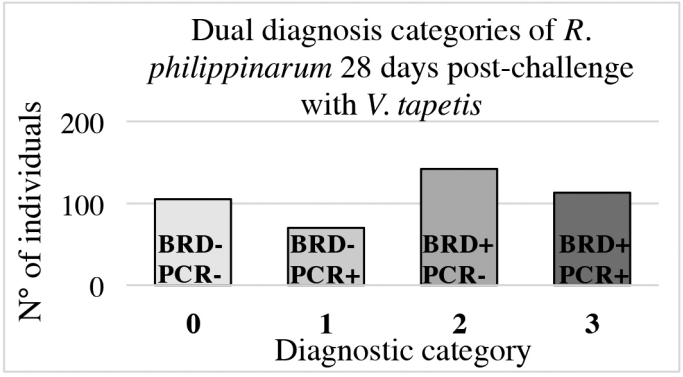
Molluscs represent over a fifth of the global aquaculture market, accounting for USD 29.2 billion in 2016. Infectious disease is one of the main limiting factors to the development of mollusk aquaculture, and the difficulties inherent to combating pathogens through antibiotic therapies or disinfection have led to extensive research on host defense mechanisms and host-pathogen relationships. It has become increasingly clear that innate immunity and genetic variability are key factors underlying disease resistance, and that genetic selection for resistance is essential for effective disease control.

The Manila clam, *Ruditapes philippinarum*, is a main cultured bivalve species of economic interest produced on a global scale. While the species is originally from Japan, intentional and accidental imports since the 1930s introduced it to the Pacific coast of America, as well as to the Atlantic and Mediterranean coasts of Europe. In 1987, mass mortality events in clam landings on the Atlantic coast of France lead to the identification of Brown Ring Disease (BRD) and of its etiological agent, *Vibrio tapetis*. This chronic disease is characterized by bacterial colonization of the extrapallial compartment which induces abnormal conchiolin deposits on the inner surface of the shell. As the disease progresses, certain individuals are capable of effectively combating the pathogen, while in others the bacteria may penetrate the host tissues, leading to rapid septicemia and death. BRD continues to impact clam production in both Europe and Asia, and though a significant body of research has allowed us to gain a better understanding the disease kinetics and host response mechanisms, uncovering the genetic host factors responsible for resistance remains imperative for the selection of resistant strains for aquaculture.

Within the context of the European H2020 project Vivaldi, an experimental population of mixed Manila clam families was produced through a factorial mating design. Over 1000 juvenile individuals from this population were subjected to a 28-day controlled challenge with *Vibrio tapetis* strain CECT4600 and sampled for shells and whole-body tissues. Dual diagnosis was carried out individually to distinguish between healthy and diseased individuals post-challenge: shells were visually diagnosed for presence of BRD, and a PCR method was adapted to detect *V. tapetis* DNA. A pooled-sequencing approach (Illumina) is used for a genome-wide comparison that highlights major genomic differences between pools of total DNA from 100 healthy individuals (BRD-/PCR-) and 100 more heavily infected individuals (BRD+/PCR+). In addition, total protein analysis is carried out by nano-liquid chromatography coupled to mass spectrometry, providing information regarding the related functional modifications that play a role in the resistance of *R. philippinarum* to BRD.



STANDARDIZED PRODUCTION OF KELP; FROM DEVELOPMENT OF HATCHERY TOOLS TO THE SEAFARM CONCEPT SPOKe

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The MACROSEA (macrosea.no) project goal is to facilitate predictable production of high quality biomass, thereby making significant steps towards industrial macroalgae cultivation in Norway. To accomplish this, new research in all the steps of the cultivation process is needed, from large scale seeding to industrial harvesting. The macroalgae industry is today highly labour intensive and depends mainly on manual tools. Development of new industrial tools and standardization of processes are key in order to build a sustainable industry.

Today macroalgae is for the most part cultivated on 1D substrate, i.e. ropes. 1D substrates have proven to be easy to handle, but are not area efficient. This argues for development of solutions of cultivating macroalgae on 2D substrates, e.g. sheets or nets. The main challenges with a 2D substrate is handling during deployment and harvesting, and cultivating the spores on a large substrate. One solution to these problems is that the substrate is in a 1D form during deployment and harvesting, and in a 2D form during the growth period, hence turning 1D substrate into a 2D structure form may provide an efficient solution.

In MACROSEA we have developed automation technology and new production concepts. The machine shown in Figure 1 automates both spinning of seedling strings onto plastic cylinders (for hatchery use), and spinning of the same strings with cultivated seedlings onto carrier ropes (for deployment at sea).

We also propose a concept for Standardized Production of Kelp (SPOKe), a module-based design of circular production units, with a specialized robotic rail for automation of deployment and harvesting operations (Figure 2).

Figure 1: Machines for spinning of seedling strings (left and right), and test result (middle)

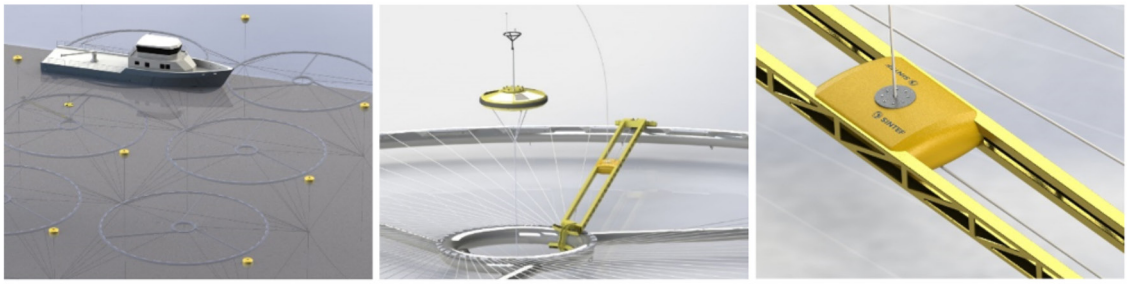

Figure 2: Standardized Production of Kelp (SPOKe)

COMPREHENSIVE BENEFITS OF DEEPCHILL COOLING FOR SUPERIOR PRESERVATION AND YIELD GAIN IN SHRIMPS

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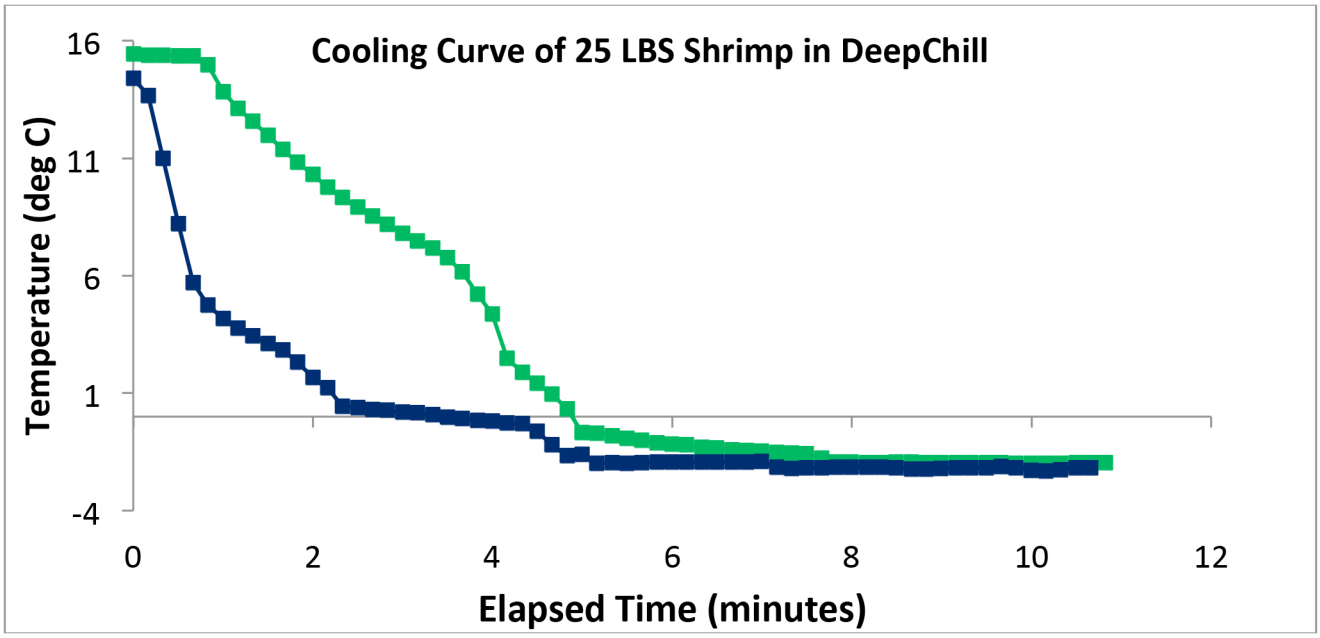
Effective and rapid seafood preservation is an important concern for aqua culturists. With better seafood preservation comes higher quality to consumers and higher premium to farmers. The use of flake ice, refrigerated sea water (RSW) or tube ice offers cooling that is fairly laborious, does not provide effective cooling below subzero temperatures, and involves fairly significant drip losses. In addition, handling of any form of crushed ice needs manual intervention thereby increasing the risk of contamination

DeepChill® is a proprietary ice slurry technology that is be able to address these concerns. As a true variable state ice slurry, it is pump able and the amount of DeepChill crystals in the slurry can be varied depending on heat transfer requirement. This paper presents the DeepChill® advantages for cooling farm raised shrimps. It must be noted that DeepChill is also effective for salmon, mussels and many other aqua cultured species.



GREEN FEED IN THE MARINE FISH FARMING

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Global catch fishery is said to been oppressed to its limit (Boyd & Schmittou, 1999), further implying aquaculture might be the only solution to the world demand for fishery products. The applied term aquaculture in this paper refers to the one used by NOAA (2008); breeding, rearing, and harvesting of plants and animals in all kind of water environments, including but not limited to ponds, rivers, lakes, and the ocean.

According to Shamshak & Anderson (2008, p. 74) aquaculture has over the past 20 years been the fastest growing food sector with an average annual growth rate of 8.7%. It further represents approximately 37 % (Shamshak & Anderson, 2008, p. 73) of total fisheries production worldwide. But even though aquaculture has taken off, the practice has its critics. The industry must counter criticism about the lack of sustainability. If the industry is able to successfully do this, the farming technique can more easily fulfill its potential role as a world food supplier (Boyd &, Schmittou, 1999). But first the aquaculture industry needs better environmental management for a continued growth.

One practice that needs to be curtailed is the choice of using unsustainable (limited) and expensive fishmeal and fishoil. The challenge is to identify more environmentally friendly and cheap substitutes for the unsustainably fishmeal and fishoil. Several trials have been made to reduce the quota of unsustainable fishmeal in farming the deep blue, and where e.g. feed has been substituted to one extend by soybeanmeal. Nonetheless, it is crucial that the substitutes for fishmeal and fishoil maintain both the quality and quantity of production that the original products achieve. Also, further importance and essentials must be paid to make these practices transparent to the industry's stakeholders.

Pittenger et al., (2007, p. 98) has shown that advances in both feed formulation and feed management on a farm level have led to increased fish growth, reduced production costs, and reduced feed conversion ratios but where research is still in progress to continue developing alternative feed ingredients. Of importance is to note that even though progress has been made in identifying substitutes for fishmeal and fish oil, there is currently no commercially available product that can completely substitute for fishmeal and fishoil (Ibid).

A fish farm needs to efficiently deal with the environmental issues it causes, or the effects will be deleterious. This thesis shows that less use of fishmeal (substituted by Soybean Protein Concentrate) can improve waterquality in some parameters used in this thesis. Further this thesis shows how sustainable benchmarks (with respect to watermetrics) efficiently can be managed and communicated to the industry's stakeholders by business managers.

A farm managed with environmental awareness and a willingness to share the experiences in the process of finding a more sustainable production method (also referred as the case farm in this thesis) is; Kona Blue Water Farm, HI, USA

TRADITIONAL AND INTEGRATED AQUACULTURE TODAY'S ENVIRONMENTAL CHALLENGES AND SOLUTIONS OF TOMORROW

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The Norwegian aquaculture industry has seen significant growth since the 1970s, with Norway now the world's largest exporter of farmed salmon. Aquaculture in Norway is based on monocultures, which means that only one species is farmed at each marine site. The industry wishes to see a trebling of production by 2025, but this is an unsustainable target following current practices. There is, however, great potential for growth by opening the industry up to Integrated Multi-Trophic Aquaculture (IMTA), which can be run in a sustainable manner.

The trend at the moment is towards fewer units with larger production, something that may be pushing at the boundaries of what the ecosystems are able to tolerate. Although the aquaculture industry in general has made improvements in a number of areas, there remain considerable challenges. Escaping fish are a particular problem that can threaten wild populations of salmon and trout. Measures that are being tested include enclosed sites, more effective marking of fish, sterilizing farmed fish to avoid dilution of the genetic integrity of wild salmon, and better nets in the aquafarms.

Salmon lice are another challenge. Large aquafarms produce considerable quantities of louse larvae, which are spread with the current. The same is true of the chemicals used to combat lice, which can threaten the ecosystems surrounding large farms. Several years have been spent trying to find good alternatives to chemicals, but the use of chemicals remains disturbingly high.

It is important to clear up environmental problems that can transfer to wild salmon. Impacts from the farms should be better monitored, different measures should be coordinated, and the results should be collated in order to identify gaps in knowledge. Norwegian farming is a major consumer of fish oil and fish meal for feed. This contributes to pressure on wild fish stocks, which has led to increased use of vegetable ingredients. It is a challenge to source sufficient omega 3, which is necessary for the fish's immune system. One possible reason for an increase in infectious diseases may be reduced marine content in the fish feed.

This problem can be solved with IMTA and algae cultivation. Integrated Multi-Trophic Aquaculture marks a new approach to fish farming. IMTA can combat a number of the problems in today's fish farming industry, and is a sustainable system. Several species are put together in a way that creates an ecosystem, such that waste from one species becomes a resource for another. There are many IMTA projects underway around the world, but Norway is lagging behind. One concern is a loss of profitability in the short term. There is also a lack of important knowledge, not least concerning IMTA's possible effects on the surrounding environment. Despite the challenges, Bellona is in no doubt that IMTA represents the future of aquaculture. The potential is enormous, but Norway ought to make better preparations for the transition. We require greater investment in research, pilot projects and eventually start-ups of commercial operations.

Algae are good sources of protein and omega 3, as well as being highly suitable as an ingredient in fish feed. Algae are also extremely interesting as biomass for the production of energy. The majority of what is produced from petroleum today, including plastics, could potentially be replaced by algae in the long term. Algae also absorb CO2 and combat ocean acidification. The speed of growth can make algaculture an important climate measure.

In the long term, Bellona believes that a transition from monoculture to IMTA will increase profitability. It will be more viable to convert existing aquaculture than to start from scratch. In this respect, we should look to countries that have come much further than Norway, such as Canada, the USA and China. Bellona works with several research institutes in Norway to develop IMTA pilot projects, with partners including the Institute of Marine Research, the Norwegian University of Science and Technology (NTNU) and research organization SINTEF.

REAL-TIME REMOTE MONITORING SYSTEM FOR AQUACULTURE WATER QUALITY

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A multi-parameter monitoring system based on wireless network was set up to achieve remote real-time monitoring of aquaculture water quality, in order to improve the quality of aquaculture products and solve such problems as being difficult in wiring and high costs in current monitoring system. In the system solar cells and lithium cells were used for power supply. The YCS-2000 dissolved oxygen sensor, pH electrode, Pt1000 temperature sensor and ammonia nitrogen sensor were used to monitor the parameters of aquaculture water quality; STM32F103 chip was used for data processing; Zigbee and GPRS modules were used for data transmission to the remote monitoring center, where the data were stored and displayed. The system was connected with aerator to realize automatic control of dissolved oxygen concentration. The test results showed high confidence level of data transmission with a packet loss rate of 0.43%. Therefore, the system could fulfill the real-time remote monitoring of aquaculture water quality and had great practical significance in reduction of labor intensity, improvement of quality of aquatic products and protection of water environment.

INTESTINAL HEALTH OF ATLANTIC SALMON *Salmo salar* FED WITH MICROALGAE *Nannochloropsis* AND *Tetraselmis*

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A variety of microalgae species have been studied as replacements for fish meal or fish oil in feeds for Atlantic salmon (*Salmo salar*). The quality of feed ingredients is often assessed based on their nutrient composition and potential to support fish growth. Their effects on fish health and product quality are also important. Intestinal mucus and mucins play vital roles in protection of fish. Mucins, the glycoproteins responsible for viscoelastic properties and functions of mucus, are produced and secreted by specialized cells. We hypothesize that differently processed microalgae may affect the intestinal health of salmon.

We examined the effect of pre-extrusion processing of two microalgae on growth and health of Atlantic salmon. The fish were fed one of the following five diets for nine weeks: (1) CO: a fish meal-based control (basal) diet, (2) NU: a *Nannochloropsis* diet, (3) NE: a pre-extruded *Nannochloropsis* diet, (4) TU: a *Tetraselmis* diet, and (5) TE: a pre-extruded *Tetraselmis* diet. The pre-extruded or untreated microalgae were included at 30% in the respective diets.

The weight gain of the fish ranged from 128.5 g to 153.3 g. The final weight of the fish fed the control diet was significantly higher compared to the fish fed diets other than the pre-extruded *Nannochloropsis* diet (NE). Pre-extrusion of *Nannochloropsis* tended to have a positive effect on fish growth compared to the other algae-fed fish groups (Figure 1). Pre-extrusion of *Tetraselmis* significantly improved the antioxidant response based on the higher expression of the related genes in the liver. Further information on the intestinal health including histology and the expression of mucin genes will be presented.

This study, funded by the Research Council of Norway (Project No. 260190, Alger4laks), is part of the COFASP ERA-NET project MARINALGAE4aqua.

NEED FOR MORE RESEARCH IN MICROBIAL MANAGEMENT TO MAKE INTENSIVE AQUACULTURE MORE SUSTAINABLE

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At the last FAO Global Conference on Aquaculture in Phuket (Thailand) in 2010 it was already postulated that modern aquaculture requires a turning point: although the farming of aquatic plants and animals has evolved into a mature industry, too much of the technology is still based on empirical approaches and more knowledge-based developments are needed to make aquaculture the blue biotechnology of the future. “More microbial management for more sustainable production” was identified as a top priority.

Today we can report about significant progress that has been achieved in this field, mainly based on new insights regarding the diversity and different functions of bacteria in aquatic systems. Management of the mutual and reciprocal interactions between bacterial populations and the farmed fish/shellfish can be used to improve the viability and robustness of our aquaculture systems. This ‘join them’ approach is contradictory to the traditional ‘beat them’ strategy generally applied in microbial management in human medicine, agriculture as well as aquaculture.

Based on these new insights operational suggestions can be formulated to achieve non-selective reduction of unwanted microbes (disinfection, increased removal of organic matter,…), as well as selective enhancement of wanted microbes (K-strategist selection with mature water and RAS systems; use of pre- and probiotics). This improved microbial management technique is exemplified in the new concept of intensive shrimp farming integrated with better waste management, including co-culture with Tilapia fish and Caulerpa seaweed. Here, the opportunistic pathogen *Vibrio parahaemolyticus* – known causative agent of AHPND - is still present in the shrimp ponds but cannot as easily reach critical densities to express virulence. The same phenomena with regard to microbial ecological status and stability likely explain why RAS systems with over-dimensioned biofilters yield improved results in terms of survival, growth and disease resistance of fish and shrimp. Using modern research (omics) tools further research can contribute to better understanding of underlying mechanisms and to develop more sustainable farming practices.

SALMON FARMING VULNERABILITY TO CLIMATE CHANGE IN SOUTHERN CHILE AND MANAGEMENT OPTIONS FOR A SUSTAINABLE FUTURE

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An assessment of salmon farming vulnerability to climate-change in southern Chile has been done using a model that combines semi-quantitative measures of Exposure (risks), Sensitivity (economic and social dependence), and Adaptation Capacity (measures that prevent and mitigate impacts). The evaluation was carried out in 8 relevant salmon farming (marine growth-out) communes in fjords and inner seas of southern Chile from 42oS to 53oS. Exposure was estimated with a semi-quantitative risk assessment based on oceanographic, meteorological and hydrological information. We also used mortality-by-cause databases, local oceanographic information from 783 farm sites and extended consultations with experts and relevant stakeholders.

Investigated threats included relevant changes in water temperature and salinity, declines in dissolved oxygen, occurrence of HABs, and diseases that could be associated with climate change. We also used the PRECIS model that addresses climatic variability in Chile through the XXI Century on a 25x25 km^2 grid and with capacity for dynamical downscaling. The climate trends were projected under a scenario of strong greenhouse gas (GHG) emissions. The combined analysis of information allowed to divide the farming regions into four sub-regions with distinctive oceanographic properties over which the sea surface warming trend and the spatial pattern of mortality by cause were superimposed.

Reduction of precipitation and the increase of air and sea surface temperature are the most relevant foreseen climate change drivers, especially for the political regions of Los Lagos and Aysen. The resulting vulnerability matrix indicated that communes with higher salmon production concentration were more exposed, this in some cases coincided with higher sensitivity and lower adaptation capacity. Thus smaller and more remote communes such as Puerto Cisnes in Aysen region that are socially more dependent from salmon farming were more vulnerable than larger communes such as Puerto Montt in Los Lagos region.

Modelling with 4 management scenarios allowed us to explore the changes in vulnerability associated with a southward movement of salmon production towards the Magallanes region, and considering a group of specific adaptation measures. This allows the identification of key aspects to reduce vulnerability; for example, decreasing exposure by reducing farming concentration and increase adaptation measures such as improving public –private coordination and establishing risk based planning of farms in a spatially explicit fashion. Model results to support policy recommendations aimed at increasing climate change adaptation and sustainability of the sector are further discussed.

THE RESPONSE OF FLUORESCENT ORGANIC MATTER TO OZONE TREATMENT IN FRESHWATER PILOT RECIRCULATING AQUACULTURE SYSTEMS

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Water quality in intense recirculating aquaculture systems (RAS) is characterised by the accumulation of organic and inorganic compounds, potentially deteriorating the water quality. Ozone has been implemented in RAS as a continuous water treatment technology improving water quality by oxidizing organic matter and reducing bacterial abundance. The analytical efforts to detect and monitor organic matter (DOM) in aquatic systems have included absorption spectroscopy of the coloured fraction (CDOM). A part of CDOM also fluoresces (FDOM). FDOM fraction has been widely used in aquatic environments as a quantitative and qualitative measure of DOM. FDOM can be characterised by the fluorescence excitation-emission matrix (EEM) spectroscopy which can be further decomposed mathematically with methods such as parallel factor (PARAFAC) analysis to identify the independent FDOM fractions. This provides both a quantitative and qualitative measure of DOM.

Recently, it was shown that the organic matter fluorescence of RAS water is highly sensitive to ozonation and fluorescence spectroscopy could therefore be used as an indirect method to determine ozone delivery within these systems. Therefore, three ozone dosages, including a control, were injected in pilot freshwater RAS where trouts were farmed. The ozonation trial lasted eight days utilising one RAS per dosage. The test levels ranged from 52-130 mg O_3/h, equivalent to 10-25 g O_3/kg feed.

The DOM consisted of four components (Fig. 1) which differed in their fluorescence characteristics and response to ozonation. A UV wavelength fluorescent fraction (C4) typical of proteinaceous material removed by 13-20% immediately after ozone initiation. The remaining fractions that exhibited visible wavelength fluorescence, at first were unaffected but during the following days, were gradually degraded, reaching a removal of 34-66%. By the end of the experiment the fluorescence intensities of all fractions were diminished up to 60% in all applied dosages.

This study provides the first application of fluorescent EEM spectroscopy and PARAFAC analysis to determine the effect of ozone on FDOM character in aquaculture water, more specifically within continuously treated RAS. The results indicate that the response in organic matter fluorescence can be used to depict the impact of ozone dosage.

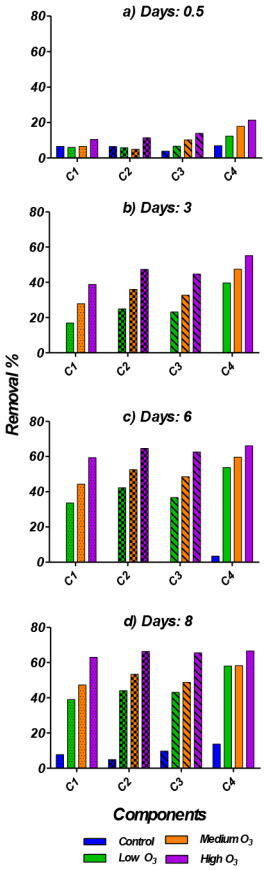

Figure 1: Effect of ozone on FDOM over time.

EFFECT OF PROBIOTICS ON PATHOGENIC *Vibrio* BACTERIAL POPULATIONS IN CULTURE PONDS OF *Litopenaeus vannamei* AT NAUPADA, SRIKAKULAM DISTRICT, ANDHRA PRADESH, INDIA

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The current study was carried out for 147 days to assess the pathogenic *Vibrio* count of *Litopenaeus vannamei* culture ponds and the influence of probiotics from Naupada, Srikakulam District, Visakhapatnam District, Andhra Pradesh, India. Three ponds were selected, one is control and other two were experimental (Pond A and B). The physico-chemical parameters of the culture ponds were recorded by adopting standard methods. During summer crop the control ponds were harvested at 24.0 gm on 107th day and at 31.0 gm on 121st day for the year 2016 and 2017 respectively due to the incidence of *Vibriosis* disease. Where as in winter crop during 2016 the control pond was harvested at 5.5 gm on 47th day because of *Vibriosis* but in 2017 the control pond was harvested normally at 32.5 gm on 145th day, as this pond is free of *Vibriosis*. Experimental ponds were harvested normally in summer season at 30.5 gm on 124th day & 31.0 gm on 127th day during 2016 and 34.0 gm on 127th day & 35.5 gm on 127th day in the year 2017 respectively.

Where as in winter season of 2016 the experimental ponds were harvested normally at 28.0 gm on 124th day as well as on 127th day. In the year 2017 winter, the experimental ponds were harvested at 36.0 gm at 146th day and 35.5 gm at 147th day respectively. This study suggests that the probiotics are the key agents which have a great impact on the reduction of total pathogenic *Vibrio* count in culture ponds of *Litopenaeus vannamei*.

SEAWEED AS AN AQUACULTURE GATEWAY-CROP FOR DIVERSIFICATION OF OCEAN-FOODS-BASED MARINE ECONOMIES IN RURAL COASTAL COMMUNITIES: A TEMPERATE TO TROPICAL CASE STUDY

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Wild capture fisheries across the globe have plateaued (FAO 2016) and in many cases declined, especially in tropical nations. The social and economic impacts of declining capture fisheries on coastal communities are not equal. Large scale, mobile, high-seas, fisheries are somewhat insulated from regionally declining fish stocks and the resulting stringent management measures. Their mobility, capital, and technologies allow them this luxury. Socioeconomic impacts of declining fisheries are disproportionately foisted upon small scale coastal fisheries as are typified by rural coastal communities across the globe. Often these fisheries form the bedrock of their coastal communities with regards to both economy and cultural identity.

Aquaculture has been proposed as a diversification tool for fishers, yet its successful adoption has been limited. For our purposes, we propose that adoption and integration of aquaculture into rural fisheries-based economies has been limited mainly due to 5 key factors: culture, capital, equipment, knowledge, and time. Our evolutionary approach to aquaculture development assumes that new entrants will continue to work in capture fisheries as their primary source of revenue; and that aquaculture will be developed to supplement, not supplant, their fisheries-based livelihood. We propose the adoption of seaweed farming as an ideal candidate, a "gateway-crop" to adoption of ocean farming and diversification of the ocean food systems of rural coastal communities worldwide. Seaweed farming, as opposed to other forms of aquaculture has low requirements for each of the key factors thus lowering the barriers to the adoption of aquaculture in contrast to other forms of ocean farming such as finfish and off-bottom culture of bivalves.

We compare and contrast adoption of seaweed aquaculture in two very different social-cultural-environmental contexts where seaweed farming is developing as a new industry: coastal Belize and coastal Maine, USA. These seaweed farming projects have been developed via the ***Nature Conservancy Belize***, ***UNE North: The Institute for North Atlantic Studies,*** and ***The Island Institute*** and highlight the similarities and differences between the two developing seaweed industries with a focus on history, successes and challenges in order to frame the broader potential of seaweed to diversify rural marine economies and preserve maritime heritage.

Sources:

FAO. 2016. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

TROUT (*Oncorhynchus mykiss*)?

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Fenugreek, *Trigonella foenum-graecum*, is a well known plant strongly utilized in the Indian cuisine as major ingredient in curry and other spices. Fenugreek contains different secondary plant compounds, among them a variety of different saponins, flavonoids and mannan. Fenugreek is known for a variety of beneficial effects and crude methanol extracts have resulted in 22-fold higher GH-secretion in rat pituitary cells *in vitro* compared to a control. In Switzerland, the two most important cultured fish species are rainbow trout (*O. mykiss*) and European perch (*P. fluviatilis*), which together comprise more than 90% of the annual production.

In two different experiments, a low (10.7 g/kg; FG 50) and a high (53.4 g/kg; FG250) level of powdered fenugreek seeds were included into formulated diets and compared with a control diet (C) without fenugreek. A total of 240 perch (2.96 ± 0.11 g) and 180 trout (5.98 ± 0.25 g) were stocked into 55 L aquaria and hand fed twice a day over a period of 6 weeks with 4 replicated aquaria per treatment. Effect of both fenugreek levels on growth, feed and nutrient utilization (Table 1) and for European perch also the effect on intestinal histology were evaluated.

Overall, growth in both experiments was good but not significantly different between treatment groups and control in either fish species, although there was a tendency, more pronounced in perch than in trout, that FG50 fed fish had a superior performance. In European perch, feeding with the low level of fenugreek resulted in a significantly reduced feed conversion ratio. The numerical differences between control and fenugreek fed fish were more pronounced in perch compared to rainbow trout. No negative effect on intestinal histology was observed in perch, therefore it could be assumed, that low levels of dietary fenugreek inclusion might be used to improve feed conversion and thus production efficiency in European perch. However, these results were achieved by meat-grinder pelleted feed and extrusion cooking could have a totally different outcome.

Table I: Growth, feed and protein utilization of European perch and rainbow trout fed with control and fenugreek (FG 50 and FG 250) diets.

	European perch			Rainbow trout		
	Control	FG 50	FG 250	Control	FG 50	FG 250
Initial body mass (g/fish)	2.95 ± 0.19	2.96 ± 0.07	2.98 ± 0.15	6.13 ± 0.24	5.89 ± 0.08	6.02 ± 0.26
Final body mass (g/fish)	7.10 ± 0.43	7.72 ± 0.30	7.10 ± 0.40	12.7 ± 0.76	12.6 ± 0.30	12.4 ± 0.68
Relative body mass gain (%)	141 ± 12.6	161 ± 6.8	138 ± 13.8	207 ± 8.61	214 ± 6.97	206 ± 7.11
FCR	1.16 ± 0.03	1.11 ± 0.01*	1.15 ± 0.04	1.04 ± 0.06	1.00 ± 0.04	1.08 ± 0.07
SGR (% day^{-1})	1.79 ± 0.10	1.95 ± 0.05	1.77 ± 0.09	1.48 ± 0.09	1.55 ± 0.07	1.48 ± 0.07
PPV	21.5 ± 1.55	23.3 ± 0.86	21.7 ± 3.70	52.5 ± 1.71	52.7 ± 1.33	49.8 ± 2.48

Values = mean (N = 4) ± SD, * = statistical different to control ($P < 0.05$)

ARE ESSENTIAL OILS REALLY ESSENTIAL; THE FISH MEAL DILEMMA

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Fish meal (FM) is decreasing in supply and increasing in price. Consequently, the industry is moving away from this material as a protein source and becoming more dependent on alternative proteins. Although novel proteins are being investigated (insects, single cell proteins etc.), the industry is focused on plant derived materials. These proteins come from a number of different sources including soy, corn, wheat, rice bran and rapeseed, to name a few. These materials are generally lower in digestibility and can introduce a number of anti-nutritional factors to the animal. Consequently, they can negatively affect fish health and growth performance.

Essential oils are the most concentrated form of phytogenic feed additives (PFAs). Certain PFAs have anti-inflammatory properties and can stimulate the secretion of digestive enzymes, improving feed digestibility and reducing feed conversion ratio (FCR) and improved growth performance. Furthermore, they can help bridge the 'performance gap' when it comes to FM substitution.

In a series of trials conducted in important cultured species, a commercially available PFA demonstrated its ability to improve growth performance in optimum and nutritionally challenged aquafeeds. For example, in seabream (*Sparus aurata*) the inclusion of the PFA demonstrated better diet digestibility through improved nitrogen retention (29% vs 26%) and higher pepsin activity. This resulted in significantly improved FCR (1.12 vs 1.28) after nine weeks of experimental feeding ($P < 0.05$) in PFA and control treatments, respectively. Furthermore, when used in European seabass (*Dicentrarchus labrax*) diets with varying FM levels (22.5% and 10%) diets, performance parameters (final weight, FCR, PER) were all numerically improved, regardless of FM inclusion. Interestingly, the largest improvements were observed when comparing treatments with low FM, indicating their importance when reformulating aqua feeds.

The benefits can also be observed in important tropical species. In a multifactorial design, Nile tilapia (*Oreochromis niloticus*; 15.4 ± 0.2g) were fed one of four diets; 15% FM, with and without PFA or 5% FM, with and without PFA. After 10 weeks of experimental feeding, the PFA supplemented diets appeared to improve final weight, survival and FCR of fish, regardless of FM level. Most notable was the improvement in FCR. Fish fed with 5% FM + PFA had an FCR value of 1.96 ± 0.01, compared to 2.14 ± 0.14 in the equivalent FM diet (without PFA), and 2.04 ± 0.11 in the 15% diet FM without PFA ($P > 0.05$).

These results demonstrate that PFAs offer feed formulators the option of increasing fish performance with existing formulations, or alternatively they can be an integral component in FM reduced aqua feeds, without compromising growth performance.

IDENTIFICATION OF THE MOST FAVOURABLE COMBINATION OF ENVIRONMENTAL PARAMETERS FOR GROW-OUT OF PIKEPERCH (*Sander lucioperca*) IN RAS

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Introduction

Farming pikeperch (Sander lucioperca) in RAS is a relatively new industry while rearing pikeperch in extensive pond systems has been done in Europe for decades. Hitherto only few commercial RAS farms exist as pikeperch are difficult to farm and detailed knowledge on optimal water quality parameters is still scarce. The identification of the most favourable combination of environmental parameters for grow-out of healthy pikeperch in RAS is the fundamental tool to develop sustainable and economically viable pikeperch aquaculture. Environmental factors are likely to cause growth retardation in adult pikeperch and detailed research on the effects of different water quality parameters is needed in order to adequately address size specific impacts and develop profitable RAS.

Materials and Methods

Two separate single factor experiments in a recirculating aquaculture respirometer system (RARS) on the effect of carbon dioxide and nitrate were followed by a multifactorial experiment were the cumulative feed burden (CFB) was used as an impact factor. Results of the three separate experiments were analysed and compared in order to identify the best combination of environmental factors for adult pikeperch.

Results and Discussion

The results of the single factor experiments showed a linear correlation between CO_2 concentration and several fish physiology parameters suggesting that there is no CO_2 concentration for pikeperch where no effects subsist. In contrast, the single factor experiment on the effects of nitrate on pikeperch metabolism showed that pikeperch can survive concentrations of up to 240 mg L^{-1} NO_3-N but energy requirements were most favourable at NO_3-N concentrations of 30 mg L^{-1}. The multifactorial CFB approach identified a CFB threshold value for pikeperch in RAS at 1953 ± 613 mg L^{-1} and it was possible to assign the relationship between CFB and fish performance to individual water quality parameters that were hitherto only considered individually. As a consequence it is recommended to re-evaluate common thresholds based on single factorial laboratory studies as they most likely underestimate the true adverse effects of these compounds under complex (realistic) RAS water quality conditions.

FEEDING BEHAVIOR AND PSEUDO-FAECES PRODUCTION OF MEDITERRANEAN MUSSEL *Mytilus galloprovincialis* AT INDOOR CONDITIONS

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The Mediterranean mussel, *Mytilus galloprovincialis* (Lamark 1819) as a filter-feeding bivalve play a significant ecological role in the marine ecosystems because it removes phytoplankton and other suspended particles from the water. The high concentrations suspended matter leads to the activation of the pseudo-faeces production mechanism in order to get rid of grit which cannot be used as food and which have been rejected by the animal. The rejected particles are wrapped in mucus, and are then expelled without having passed through the digestive tract.

The effect of ten (10) phytoplankton's (*Chlorella sp*.) gradual increased concentration in a range from 0.025 to 246.0 mg/l (0.025, 0.05, 0.5, 2.5, 5.0, 12.5, 25.0, 37.5, 50.0, 246.0) on pseudo-faeces production of *M. galloprovincialis* for 48H, in a closed circulation system under controlled conditions was investigated. Nine (9) individual replicates for each concentration at 2L volume were used while the phytoplankton concentration of 1 mg/l used as a blank. The photoperiod (12L:12D) and temperature (18.0±1.0 C) remained stable. The pseudo-faeces collected in 24 and 48 H and their wet and dry weight were measured (mg/l). The phytoplankton's density (concentration in cells/ml and spectrophotometric measurements) and their volume (CPV, cellule package volume) were checked twice a day during the experiment.

The results show that the production of dry pseudo-faeces was the same or higher in 24H compared to 48H. The higher values were observed at the phytoplankton's concentration of 2.5 mg/l and they were reached 0.0028 and 0.0024 mg/l, at 24H and 48H respectively. In addition, an increase was observed of the pseudo-faeces production up to this phytoplankton concentration (2.5 mg/l). After this concentration (2.5 mg/l), a decrease of the increase rate was noticed. The knowledge of the filtration rates as well as the ability of this bivalve mussel to collect the suspended particles in an enclosed system, like an aquarium, discussed as an organic removal mechanism.

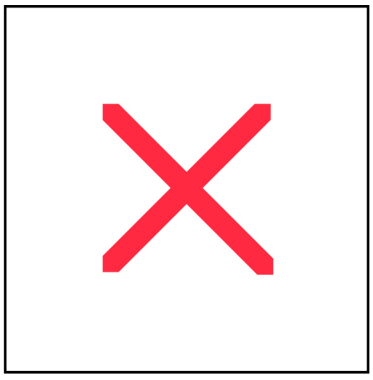

FIGURE 1. Pseudo-faeces production (in dry weight) of the Mediterranean mussel at the gradual increased concentration of phytoplankton (*Chlorella sp.)* in experimental conditions.

TOTAL RESIDUAL OXIDANTS IN ATLANTIC SALMON POST-SMOLT IN BRACKISH WATER

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Ozone is a strong oxidant which helps in recirculating aquaculture systems (RAS) to improve water quality (reduces organic matter, chemical oxygen demand, dissolved organic carbon, nitrite, water color) and fish health (pathogen inactivation). However, ozone usage is mostly restricted to freshwater systems. In brackish and seawater harmful so called "total residual oxidants (TROs)" are formed. The oxygen reduction potential (ORP) and the colorimetric N,N-diethyl-p-phenylenediamine (DPD) method can be used as proxy-measurements of water ozonation. ORP values are only a rough estimate for the real TRO concentrations. One cannot calculate one value by measuring the other. For precise and accurate measurements both methods are still challenging to use in aquaculture. One reason is the influence on the measured values by different water variables (e.g. different salinities). The available commercial TRO test-kits are not designed for aquaculture applications and can only be used on the low end of the measurement specifications with generally very low precision. For rainbow trout in freshwater a 50% lethal TRO concentration of ~60 µg/L as Cl_2 was measured. Less severe effects were shown in chronically exposure studies of marine organisms to TROs. Here concentrations >60 µg/L as Cl_2 (Turbot), 30-50 µg/L as Cl_2 (European seabass), 16 – 23 µg/L as Cl_2 (Atlantic Halibut) and 14 – 20 µg/L as Cl_2 (European lobster) affect growth performance, gill physiology and increase mortality. For now, this information is not available for Atlantic salmon post-smolts.

The effect of TRO on Atlantic salmon post-smolts will be presented for a 11-day experimental period. Fifteen tanks were stocked with 65 fish each. After a three-week adoption period five ORP (TRO) values in triplicate were adjusted in the test tanks. Ozone was dosed into the water of a header tang (end ORP set point ~500 mV). The ozonated flow through water was mixed with non-ozonated water to achieve the treatment values before it reached the fish. The used values were 230 (0), 280 (~10), 350 (~10), 425 (20) and 488 (40) mV (µg/L as Cl_2). Water quality variables were: dissolved oxygen >85% saturation, temperature ~7˚C, salinity ~12 ppt, pH 7 – 7.4 and photoperiod 24L:00D. Fish were fed ad libitum (120% overfed). The focus of this presentation will be to show ozone associated gill damage through histology.

The fish were very susceptible to ozone induced gill damage. Already at 280 mV there was increased but not severe hyperplasia and lamellar clubbing. The ORP values for the two highest treatments were already over the threshold values given in the literature. However, the measured TRO values indicated that these levels could be accepted. Both highest treatments showed severe gill damage. The highest treatment had 70-80% to the gills already damaged. Severe hyperplasia, hypertrophy and lamellar clubbing with early to advanced stage of lamellar fusion was found. Necrosis was quite severe in more than half of the samples.

EFFECTS OF ORTHO-PHOSPHATE IN THE REARING WATER OF JUVENILE AFRICAN CATFISH (*Clarias gariepinus*) ON GROWTH PERFORMANCE, WELFARE AND PRODUCT QUALITY

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Ortho-phosphate (ortho-P) inside recirculation aquaculture systems (RAS) accumulates as a consequence of low water exchange rates. To achieve better plant growth under fish aquaculture in coupled aquaponics, phosphate fertilizer addition can increase ortho-P concentrations, artificially increasing P-levels inside the process water. We investigated four ortho-P concentrations (0, 40, 80, 120 mg L^{-1}) in the rearing water on growth performance, feed efficiency, and welfare traits of juvenile African catfish (*Clarias gariepinus* Burchell, 1822). By trend, optimum specific growth rate (SGR) of 2.66 % d^{-1} and feed conversion ratio (FRC) of 0.71 were observed at 40 and 80 mg L^{-1} ortho-P. (Fig. 1)

Higher ortho-P significantly affected skin coloration, swimming activity and external injuries, with palest and inactive fish combined with most external injuries (5.4 per fish) in the P120 group (P0=2.6, P40=4.5, P80=4.0). Most product quality parameters remained unaffected, while fat contents inside the fillets enriched with increasing ortho-P. Inorganic P in blood plasma increased significantly while phosphate concentrations inside the fillet remained unchanged. We suggest that ortho-P concentrations of 40 to max. 80 mg L^{-1} do not reduce performance of African catfish while increased values of 120 mg L^{-1} affect fish welfare. This allows limited addition of ortho-P fertilizer in coupled aquaponics with African catfish to increase plant growth.

Table 1: Fish performance of African catfish under four different levels of ortho-P

		Group												
Parameter	**unit**	**P 0**			**P40**			**P80**			**P120**			**p-value**
Final weight (W_t)	[g $fish^{-1}$]	132.94	±	34.14	148.93	±	51.35	161.25	±	44.02	159.62	±	46.75	0.822
Total length	[cm]	27.30	±	2.35	28.01	±	3.31	28.87	±	2.50	28.28	±	2.26	0.615
Standard length	[cm]	24.43	±	2.07	25.14	±	2.98	25.92	±	2.30	25.37	±	2.39	0.627
Growth (G)	[g $fish^{-1}$]	105.78	±	3.45	118.76	±	1.12	128.50	±	3.04	126.38	±	4.47	0.153
Fillet ratio	%	33.28	±	2.30	32.31	±	3.40	33.92	±	1.86	33.51	±	2.21	0.155
SGR	[%]	2.64	±	0.05	2.66	±	0.03	2.66	±	0.05	2.61	±	0.03	0.172
FCR	-	0.72	±	0.03	0.71	±	0.01	0.71	±	0.03	0.73	±	0.02	0.450
TFI	[g $fish^{-1}$]	75.95	±	1.14	84.12	±	1.94	91.30	±	5.19	92.79	±	2.24	0.266

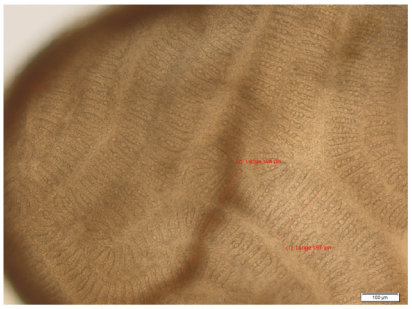

Figure 2: Gill filaments of African catfish under four different levels of ortho-P

PERACETIC ACID - A GREENER SOLUTION FOR DISINFECTING AQUACULTURE SYSTEMS

Dave Straus*, Thomas Meinelt, Dibo Liu, Lars-Flemming Pedersen, Manuel Gesto, John Davidson and Chris Good

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Peracetic acid (PAA) has recently been introduced as a green disinfectant for biosecurity in the US aquaculture industry to prevent disease outbreaks from fish pathogens. It is increasingly being used to replace chlorine in many industries. PAA is approved for use in Denmark, Germany and Norway as a water disinfectant in aquaculture. Our international collaborations have studied its applications to aquaculture including its effectiveness to pathogens such as *Ichthyophthirius multifiliis*, *Saprolegnia* spp., and various bacteria, as well as its degradation in water, toxicity to fish, and effect on fish stress. Our research studies will be reviewed.

PAA is a stabilized mixture of acetic acid, hydrogen peroxide and water that breaks down quickly to water and a small amount of dilute acetic acid (i.e., vinegar). The U.S. Environmental Protection Agency (EPA) first registered PAA as an antimicrobial in 1985 for indoor use on hard surfaces (e.g., hospitals). Registrations have been expanded to include: sanitation in food/beverage facilities, agricultural premises, wineries/breweries, greenhouse equipment, animal housing, meat and poultry processing, commercial laundries, prevention of bio-film formation in pulp/paper industries, and as a disinfectant for wastewater treatment.

EFFECT OF TANK COLOR ON SURVIVAL AND GROWTH PERFORMANCE OF PIKEPERCH *Sander lucioperca* (L.) LARVAE UNDER CONTROLLED CONDITIONS

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The production of pikeperch larvae poses many obstacles that need to be overcome to produce cost-efficient good quality fish. To improve the larval quality, more knowledge on the influence of reproduction and rearing methods has to be conducted.
In the present study, two batches of pikeperch larvae were reared in 30 liter (L) black, blue and white colored aquaria, at densities of 100 larvae per liter from 0 - 20 days post hatch (dph) and 32 larvae per liter from 21 - 36 dph to investigate the tank colour effect on larval development individually. The hypotheses for this study was, that the tank color effects the health and growth parameters of pikeperch larvae. The larval performance in standard length, wet weight, swim bladder inflation, feed consumption, mortality, deformity, and cannibalism were analyzed. In the first part of the study (0 - 20 dph) larvae reared in black aquaria showed significantly better ($p < 0.05$) growth, swim bladder inflation and feed consumption. From 21 - 36 dph the tank colour only affected the mortality rate, showing significantly better ($p < 0.05$) larval survival in black aquaria. Thus, the data suggested that the black tank colour was most suited for pike-perch larvae during the first 36 dph. Similar to the findings for other fish species, it was confirmed that larval development can be improved by adapting the rearing conditions to the natural environment.

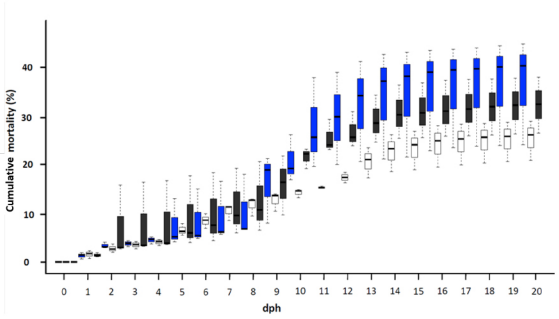

Figure 1: Boxplot of cumulative mortality over 20 days post hatch (dph), showing median, quartiles, and whiskers. Boxplot colours illustrated as aquaria colour.

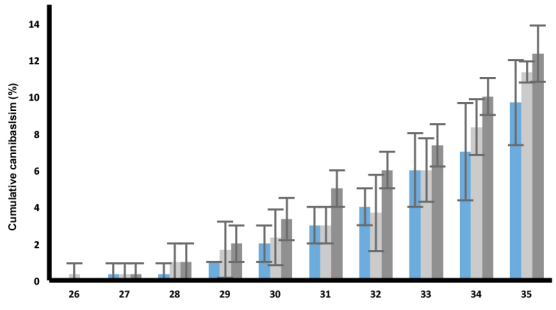

Figure 2: Cumulative cannibalism over 15 days post hatch (dph). Error bars visualize standard deviation of the data. White aquaria (light grey), blue aquaria (blue) and black aquaria (dark grey)

A FULLY AUTOMATED SAMPLE-TO-RESULT RT-PCR SYSTEM FOR QUICK AND SIMPLE DETECTION OF TILAPIA LAKE VIRUS

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Tilapia lake virus (TiLV) is a recently identified orthomyxo-like virus associated with significant mortalities in farmed tilapia. The virus represents a huge threat and may lead to catastrophic economic losses to the global tilapia industry. Detection of TiLV is important for disease management and control and has been relying mostly on conventional RT-PCR methods; application of these methods have been limited to laboratory settings due to requirement of well-trained personnel and complex processes. The aim of this study was to evaluate a recently developed quick and easy TiLV RT-PCR detection system on a compact walk-away benchtop device (POCKIT™ Central Nucleic Acid Analyzer, GeneReach) to facilitate timely identification of TiLV at points of need. The automated POCKIT™ Central system integrates magnetic bead-based NA extraction and fluorescence-based insulated isothermal PCR technology[1] to provide qualitative PCR results with minimal hands-on steps, minimizing the needs in human resources and the risks of human errors.

The TiLV RT-PCR has a limit of detection 95% of 12 genome equivalents, determined with an IVT RNA containing the target sequences. Performance of the all-in-one TiLV RT-PCR/POCKIT Central system was compared to a semi-nested RT-PCR[2] system using nucleic acids prepared by the *taco*™ *Preloaded* DNA/RNA Extraction Kit on a *taco*™ mini Nucleic Acid Automatic Extraction System (GeneReach). Test with serial dilutions of a TiLV-positive gill sample showed that the detection limits of the TiLV RT-PCR/POCKIT Central system (10^2) and the reference system (10^3) were comparable (Table 1). The TiLV RT-PCR/POCKIT Central system did not react with three *Streptococcus agalactiae* strains and a *Aeromonas sp*.. Performance of the TiLV RT-PCR/POCKIT™ Central system to detect TiLV RNA in tilapia was compared to the reference RT-PCR system with 92 brain, liver, gill, muscle, or mixtures of above tissue samples. The results indicated that the TiLV RT-PCR/POCKIT Central system and reference RT-PCR system had excellent agreement (95.65%, CI 95%: 90.73 - 100%; $\varkappa = 0.87$).

Available in a convenient lyophilized and single-dose format, the sample-in-answer-out TiLV RT-PCR/POCKIT™ Central system can serve as a point-of-need bio-detection tool to aid diagnosis and screening of TiLV infection at laboratory, pond side, and customs with limited qualified human resources for molecular diagnosis.

Table 1 Analytical sensitivity of the TiLV RT-PCR on POCKIT Central and the reference TiLV RT-PCR was comparable

Dilution fold	RT-iiPCR POCKIT Central			semi-nested RT-PCR		
	1	2	3	1	2	3
0	+	+	+	+	+	+
10^1	+	+	+	+	+	+
10^2	+	+	+	+	+	+
10^3	+	-	+	+	+	+
10^4	-	-	+	-	-	-

Table 2. TiLV RT-PCR on POCKIT Central and reference TiLV RT-PCR had great agreement

		semi-nested RT-PCR		
		Positive	**Negative**	**Total**
RT-iiPCR POCKIT Central	**Positive**	17	3	20
	Negative	1	71	72
	Total	18	74	92

Agreement 95.65%; CI (95%): 90.73 - 100%

1. Y. L. Tsai, et al. 2012. PLoS One. 7(9):e45278.
2. HT Dong, et.al. 2017. Aquaculture 479:579-83.

BIOAVAILABILITY OF CRUDE PROTEIN AND LIPID FROM BIOFLOC BASED FEED NUTROFLOC IN INTENSIVE NURSERY CULTURE OF *Penaeus vannamei*

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The present study was undertaken to asses the bioavailability of crude protein and lipid from biofloc nursery culture of *P.vannamei*. The experimental study includes control and two treatments such as Insitu (T1) and Exsitu (T2). Exsitu treatment animal were fed with biofloc meal incorporated feed –NUTRIFLOC and other treatment animals were fed with a commercial feed. The crude protein of NUTRIFLOC and Commercial feed was 35 % /The shrimp PL was reared in a raceway system with a stocking density of 1500 Pl/m3 for the period of 30 days at a C: N ratio of 10:1 for the growth of heterotrophic bacteria and removal of ammonium nitrogen from the water. Growth parameters obtained from the study clearly indicates that shrimp reared in Exsitu and Insitu system exhibited higher growth compared to control treatment.

Shrimps recorded the significantly higher SGR (% day^{-1}), in exsitu (13.15) treatment followed by insitu compared to control. The lowest FCR was observed in the exsitu (1.044) treatment followed by the insitu (1.047) and control (1.076). PER of the present study indicates that exsitu based system showed improved performance followed by insitu and control treatments..Amylase, lipase and cellulose activity was higher in exsitu treatment animals followed by insitu and control. Protease enzyme activity was higher in exsitu treatment compared to other treatments.Extracellular enzyme activity (protease, amylase and lipase) was also higher in the exsitu treatment and lower in control. NUTRIFLOC feed has a higher value of apparent digestibility (98-99%) and bioavailability, it is suitable for the shrimp diet.

Comparison of specific activity of enzymes among three treatment and part on two way repeated measures ANOVA.

Enzyme activity	Means of turkey test						
	Treatment			Part			Interaction Treatment * Part
	Insitu	Exsitu	Control	H	S	I	
PROTEASE	1.041 [a]	2.966 [a]	1.791 [a]	1.04011 [a]	1.825 [a]	1.436 [a]	NS
AMYLASE	209 [b]	463 [c]	33.373 [a]	1.54 [a]	2.39 [b]	3.12 [c]	**
LIPASE	2.387 [a]	6.13 [b]	1.52 [a]	3.158 [a]	3.214 [a]	3.673 [a]	*
CELLULASE	0.163 [c]	-0.376 [a]	0.047 [b]	0.045 [b]	0.534 [c]	-0.7498 [a]	**

* P< 0.05 ** P<0.01 NS – Not significant

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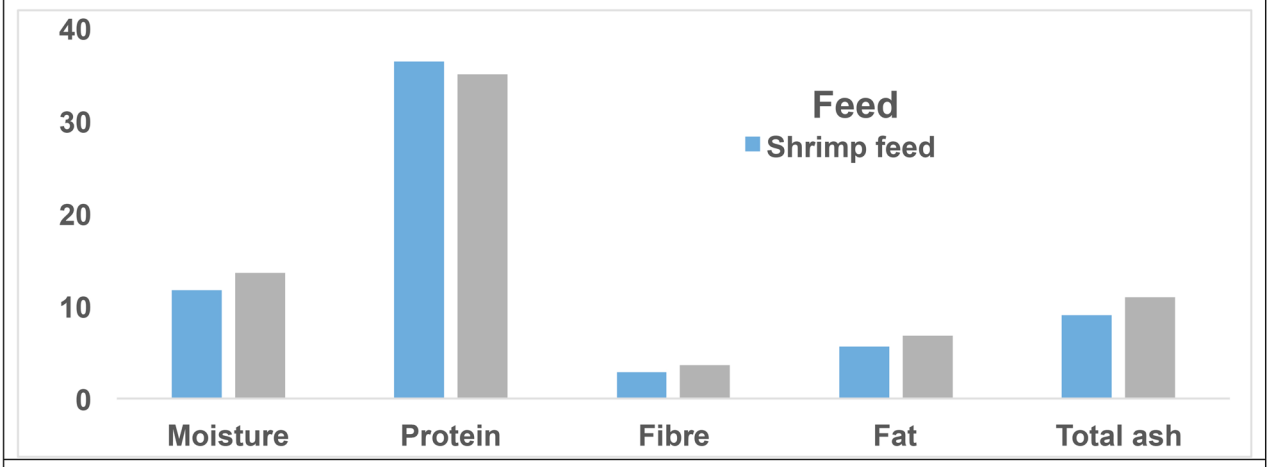
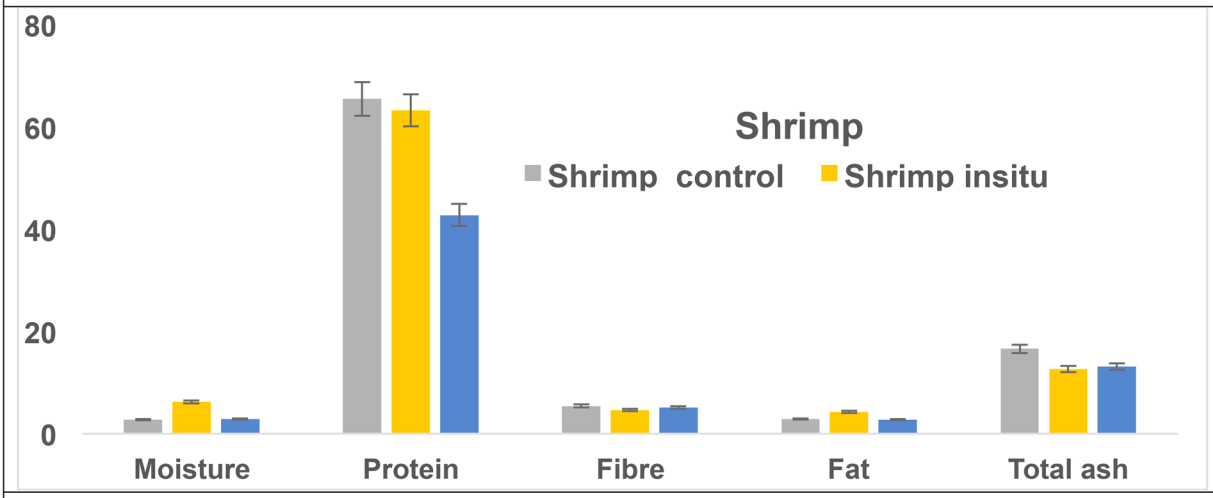
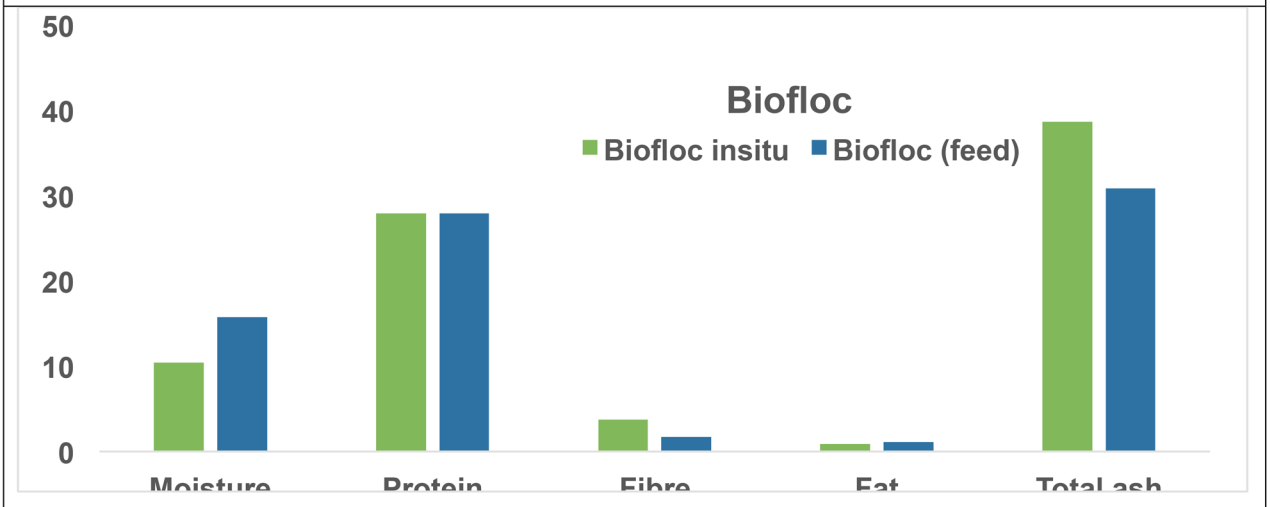
Nutrient Profile of feed, animal whole body tissue and floc from different treatments in the experimental trial.







Nutrient Profile of feed, animal whole body tissue and floc from different treatments in the experimental trial.

SUPPORTING THE SUSTAINABLE DEVELOPMENT OF THE IRISH FRESHWATER AQUACULTURE INDUSTRY

Alexandre Tahar*, Alan Kennedy, Ronan Cooney, Sarah Naughton, Conor Behan, Emer O'Neill, Siobhan Kavanagh, Neil Rowan, Eoghan Clifford

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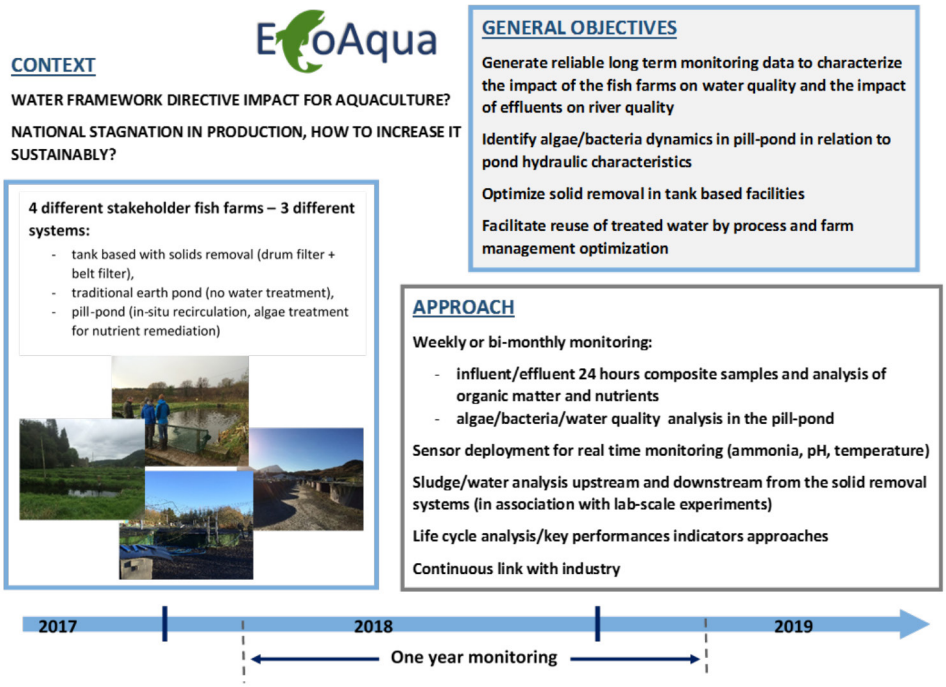
EcoAqua is BIM funded project (2017-2019). The main objectives of the project is to implement a water quality and energy monitoring program on each of the four Irish freshwater stakeholder fish farms. The facilities chosen have various configurations (i.e. pond/tank based, trout/salmon smolt/perch production) that are highly representative of the whole freshwater aquaculture industry and will be first benchmarked during year-1 of the program. This will involve extensive and regular water quality monitoring – influent and effluent – of the four aquaculture sites to assess the performance of each and subsequent identification/benchmarking of their performance. Monitoring will take the form of periodic 24 hour sampling campaigns augmented by remote monitoring using various sensors (e.g. pH, conductivity, dissolved oxygen, ammonium). This analysis will have 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) to 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. If accepted a presentation stating the general layout, objectives and approach and first results of the EcoAqua project will be given.

A graphical abstract of the project is presented below:



HYDROLYZED MEALWORM *Tenebrio molitor* AND SUPERWORM *Zophobas morio* LARVAE MEALS IN JUVENILE SEA TROUT *Salmo trutta morpha trutta* DIETS. EFFECTS ON GROWTH PERFORMANCE AND FEED UTILIZATION

Sanan Talibov[1,2*], Jan Mazurkiewicz, Silvia N. Mérida, Mateusz Rawski, Paola Gobbi, Bartosz Kierończyk, Abdelbasset Benzertiha, Damian Józefiak

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Limited availability and increasing price of fishmeal provoked a strong need for replacing it with more sustainable alternatives. Due to the high-quality protein composition, *Tenebrio molitor* larvae (mealworm) have shown an immense potential as a fishmeal substitute in aquatic diets. However, the use of *Zophobas morio* larvae (superworm) in fish diets is yet severely underexplored. The aim of the present study was to evaluate the effect of hydrolyzed *T. molitor* and *Z. morio* larvae meals as partial replacement of fishmeal on growth and feed utilization of sea trout juveniles.

Sea trout fingerlings were randomly allocated into 9 tanks and 25 fish per tank. Fish received three diets in triplicate tanks for 8 weeks. A control diet (CD) contained no insect meal, and the two tested diets contained 10% of hydrolyzed mealworm (MWD) and superworm meals (SWD). At the end of the trial fish from each tank were bulk weighed, numbers were counted, and mean body weight of fish was measured. 10 fish from each tank were dissected for determination of somatic indices.

Some results from the trial are summarized in Table 1. No significant difference was observed among the experimental groups in terms of growth performance at the end of the trial. However, protein efficiency ratio was significantly ($P < 0.05$) lower in the insect meal groups compared to the control group. Significantly higher hepatosomatic and viscerosomatic indices were noted for the group fed superworm diet. Histomorphology analysis of liver and intestinal samples are in progress and will be presented. Results of our study indicate that hydrolyzed meals of mealworm and superworm can replace fishmeal at 10% inclusion level in sea trout diets with no negative effects on growth performance. Reduction in protein efficiency ratio could be due to the chitin-derived non-protein nitrogen from the exoskeleton of insects which can potentially overestimate the amount of protein in isoproteic diets. Further histomorphology analysis is needed to elucidate alterations in the liver and intestinal tissues of the fish fed the experimental diets.

TABLE 1. Growth performance, feed utilization and somatic indices of sea trouts. Values in the same row having different superscript letters are significantly different at $P < 0.05$

Items	CD	MWD	SWD
Initial body weight (g)	5.75	5.84	5.85
Final body weight g)	21.20	20.97	20.60
Weight gain (g)	15.47	15.10	14.77
Specific growth rate (%/d)	2.33	2.28	2.25
Daily intake rate (%/d)	1.39	1.44	1.48
Feed conversion ratio	0.99	1.04	1.07
Protein efficiency ratio	2.10^{a}	1.88^{b}	1.87^{b}
Survival (%)	100	99	99
Hepatosomatic index (%)	1.45^{b}	1.47^{b}	1.82^{a}
Viscerosomatic index (%)	7.78^{b}	7.94^{b}	8.62^{a}

EFFECT OF HYDROLYZED MEALWORM *Tenebrio molitor* AND SUPERWORM *Zophobas morio* LARVAE MEALS ON SERUM BIOCHEMICAL INDICES OF SEA TROUT *Salmo trutta morpha trutta* JUVENILES

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Numerous attempts have been made to replace fishmeal with insect meals since they are not only a rich source of proteins, but also lipids, vitamins, minerals, chitin and antimicrobial peptides. However, little research has been devoted to the potential effects of insect meals on fish health. Blood parameters are important markers indicating general health condition of fish. Therefore, the study was conducted to evaluate the impact of hydrolyzed *T. molitor* and *Z. morio* larvae meals as partial replacement of fishmeal on blood biochemical indices and immune parameters of sea trout juveniles.

Sea trout fingerlings (mean weight 5.81g) were randomly allocated into 9 tanks and 25 fish per tank. Fish received three diets in triplicate tanks for 8 weeks. A control diet contained no insect meal, and the two tested diets contained 10% of hydrolyzed mealworm and superworm larvae meals. The insect meals and the diets were analyzed for proximate composition, amino acid and fatty acid profiles were determined. At the end of the trial blood samples were collected from 8 fish per tank, serum was isolated and later analyzed for biochemical indices and immune parameters.

Serum biochemical indices of sea trouts are summarized in table 1. Elevated levels of aspartate transaminase in the serum of superworm diet (SWD) fed fish may indicate hepatic cell injury. Hypolipidemic effect of dietary insects can be associated with the presence of chitin which may interfere with lipid absorption in the intestine. The effect was more pronounced in the fish fed mealworm diet since chitin level was about two-fold higher in the mealworm diet than the superworm diet. No adverse effect of dietary insect meals on fish immune parameters was seen in this experiment. The results of the present study show that mealworm can successfully replace fishmeal in juvenile sea trout diet at 10% inclusion level with no negative effect on liver functions. However, results also suggest that the inclusion of superworm in juvenile sea trout diet may impair liver function to a certain degree. Histological assessment of the liver samples would be helpful to explain the differences in a cell level.

TABLE 1. Serum biochemistry values of sea trouts. Values in the same row having different superscript letters are significantly different at $P < 0.05$.

Items	CD	MWD	SWD
ALT (IU/L)	4.56	4.54	3.92
AST (IU/L)	34.96^{a}	34.24^{a}	55.46^{b}
ALP (IU/L)	90.49^{a}	79.80^{a}	65.81^{b}
Total protein (g/l)	55.6	57.2	54.4
Albumin (g/l)	25.7^{b}	27.3^{a}	27.4^{a}
FFA (mmol/l)	0.60	0.60	0.58
Triglycerides (mg/dl)	493.19^{a}	384.25^{b}	412.04^{a}
Cholestrol (mg/dl)	290.41^{b}	365.12^{a}	342.86^{a}
IgM (mg/ml)	0.40	0.37	0.45
Lysozyme (µg/ml)	15.48	14.11	15.05

ALT = alanine transaminase, AST = aspartate transaminase, ALP = alkaline phosphatase, FFA = free fatty acids

STRUCTURE AND PERFORMANCE OF THE UK MARINE NET-CAGE SALMONID AQUACULTURE INDUSTRY

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After emerging, most industries undergo a life cycle comprising the stages of growth, maturity and decline, each characterised by a different structure. A central element of the structure of an industry is the level of competitive rivalry within it – the number of companies forming the industry and their market shares. The different structures - ranging from perfect competition to monopoly – have distinct impacts on the profitability potential and overall competitive performance of the industry. While commercial aquaculture is still a young industry, composed by a high number of small companies, its structure is rapidly evolving. A process of restructuring towards consolidation is most apparent in the marine net-cage farming of salmonids, arguably the most mature aquaculture sector globally. This study combines elements of industry organisation and strategic management school concepts in analysing the structure and the inter-firm variation in competitive performance and strategic positioning of the UK net-cage aquaculture covering more than 90% of the marine-grown Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) in the country. The study uses a mixed-methods approach based mostly on secondary, publicly available, company-level data supplemented by semi-structured interviews with industry representatives and other stakeholders. The analysis begins at the sectoral level by examining the horizontal and vertical structure of the salmon industry and the level of concentration in the context of other aquaculture sectors. This is followed by analysis of company-level generic competitive strategies and productivity and efficiency measures using financial indicators. The conclusions of this analysis provide insight into the future development of other aquaculture industries anticipating restructuring.

ZONING AND CARRYING CAPACITY OF PEIXE ANGICAL RESERVOIR FOR TAMBAQUI PRODUCTION

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Considering the recent changes in the regulatory aspects about the environmental laws related to aquaculture practice in public federal waters, it was observed an increase in the number of net cages installed in freshwater reservoirs, contributing with the fish production status in Brazil. However, since this technology may affect the environment, regarding limonogical characteristics mainly in the surrounding areas next to the net cages, it is always advisable to carry out zoning studies for selection of aquaculture areas and determine the environment maximum carrying capacity for fish production. The Dillon & Rigler (1974) methodology used do estimate the reservoirs carrying capacity was developed for temperate waters, besides considered outdated, it shows conservative results, being at present the only method accepted by the Brazilian Water National Agency/ANA for licensing purposes. In this context, the development of methods and technics for the carrying capacity calculation in water bodies are extremely relevant to guarantee the activity sustainability, reduce environmental impacts and minimize water use conflicts. Thus, the present study was conducted in Peixe Angical reservoir and aimed at selecting suitable areas for aquaculture and estimate the carrying capacity for fish production in net cages. The project was developed by Embrapa Fisheries and Aquaculture and funded by Enerpeixe, the hydroeletric power organization responsible for the reservoir administration.

The methodology employed for zoning these aquaculture areas consisted into two consecutive stages that involved the generation of thematic maps and scenarios to facilitate the process of understanding the regional peculiarities and taking the decisions to identify the most appropriate areas for the installation of aquaculture farms. The first stage aimed to exclude unsuitable areas for fish production practice as follows: depletion zone, which is defined as the minimum elevation operated by the power plant during 95% of the energy generating period; depth (areas shallower than 4 m); conservation units; effluent emission areas; water supply plants proximity, safety areas, underwater vegetation presence and tourism and traffic routes. After the data organization, the exclusion areas maps were presented with the restriction sites. For the second stage, water quality parameters (pH, conductivity, dissolved oxygen, COT, BOD, total solids, ammonium, nitrite, nitrate, ortophosphate and clorophyll-a were analyzed in order to generate the suitability maps. Besides that, it was conducted a tambaqui production cycle in net cages aiming at measuring the environmental impacts caused by the fish production, through the analysis of water quality parameters, feed digestibility and phosphorous excretion by fish during the one-year production cycle. The results from five aquaculture zones were defined, totalizing a surface area of 283 ha. Considering the maximum fish production, the bionergetic modeling approach (based on the factorial bioenergetic model Fish-PrFEQ® adapted to tropical species) and a mass balance was applied to estimate the particulate (WP), dissolved (WD) wastes, total phosphorous (P) and total nitrogen (N) discharged by tambaqui (*Colossoma macropomum*) in net-cages. From that scenario, it was concluded that a maximum tambaqui production of 15 thousand tons could be practiced within these aquaculture zones in Peixe Angical Reservoir without any major environmental impacts.

VARIOUS INTENSIVE SHRIMP FARMING SYSTEMS IN ASIA: COMMERCIAL IMPLEMENTATION OF BIOFLOC AND RAS PRODUCTION SYSTEMS HELP CONTROL SHRIMP FARMING DISEASES

Nyan Taw

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Before the mid-1990s, the major diseases affecting the farmed shrimp industry were of bacterial origin. But in Asia and from late 1994, the appearance of various, major viral diseases – like White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV), Infectious Myonecrosis Virus (IMNV) and others – led to changes in the design and operation of some shrimp farms to help prevent outbreaks and dissemination of viral diseases.

More recently, WSSV outbreaks in Saudi Arabia in farming operations for Indian white shrimp (*Penaeus indicus*) provided more evidence that additional biosecurity was needed. And since 2009, outbreaks of a new bacterial disease, Acute Hepatopancreatic Necrosis Syndrome (AHPNS). – which started in China and spread to Vietnam, Malaysia and Thailand, and spread Central America in 2015.. In Australia WSSV outbreak in early 2017 at black tiger shrimp (*Penaeus monodon*) farms provide additional evidence for the need to change shrimp production systems.

Because of old and newly emerging viral and bacterial diseases affecting their farmed shrimp industry, some years ago various Asian countries started developing and using biofloc and recirculation aquaculture system (RAS) production technologies and/or treating incoming water for culture operations and wastewater treatment as biosecurity measures for disease prevention and control. Many production systems are used by Asian shrimp growers – from single-pond base management to larger RAS systems – but many have yet to reduce their environmental impact due to their wastewater discharges.

This was discussed with examples of different biofloc and recirculation aquaculture (RAS) shrimp farming systems used in Asia in the last two decades, including some of the projects I have personally been involved with and provided technical expertise, as well as some perspectives on these technologies. Large integrated shrimp farms in Indonesia: PT DCD in Lampung, PT CPB in Lampung and PT SAJ in Sumbawa and Blue Archipelago Bhd projects in Kedah and Terengganu in Malaysia.

In any aquaculture business, sustainability of a system can improve profits. With emerging disease problems, treating wastewater discharged from farming facility or RAS is of utmost important. What investors, shrimp farmers and technicians need to be aware of is that, whatever waste is discharged into the environment, it will likely com/e back to you in the form of disease sooner or later.

GROWTH HETEROGENEITY IN PIKEPERCH (*Sander lucioperca*) PRODUCTION: FROM LARVAE TO TARGET WEIGHT

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Size heterogeneity is a central problem in aquaculture and especially in predatory species, as it is known that size heterogeneity induces cannibalism. Growth rate differences may be the product of multiple factors including parental, biotic, as well as abiotic factors. Recent studies showed that in pikeperch larvae, the vast majority of growth variability can be explained by differences in feed consumption. As an increased variation in the fish size leads to higher rates of cannibalism, sorting of pikeperch larvae has a significant impact on the rearing success.

In this study, we investigated the accumulation of size variability among pikeperch from post-larvae over juveniles to harvest weight. Between batches, pikeperch may demonstrate different growth rates that can be related to their genetic provenance. Within batches, pikeperch show identical size heterogeneity. As expected, we notice an increase in size variation throughout the rearing process. However the coefficient of variation (CV; which is the standard deviation relative to the mean) shows a more modest increase that is comparable to other fish species. Size grading results in a significant decrease of the CV.

Various studies demonstrate that post-larval size heterogeneity does not affect growth or survival in different fish species, and suggest that size grading may be not necessary or even have a negative impact. On the other hand, this seems in contradiction with observations that the intensity of cannibalism is generally determined by the ratio between predator gape size and prey size. From our experience at Inagro, it seems that cannibalism among pikeperch above 5-10 g is not as severe a phenomenon as it is among larvae. Yet, we experience size grading as an important and positive measure to prevent too high or too low stocking densities, and principally to restore social interactions associated with access to feed.

HOW TO PROMOTE AQUACULTURE DEVELOPMENT IN EUROPEAN OVERSEAS: THE CASE OF SAINT-PIERRE AND MIQUELON

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Saint-Pierre and Miquelon is one of the twenty-five European Overseas Countries and Territories (OCTs). The OCTs are all islands, of which three have no permanent population. They are located all around the world, spreading from the Poles to the Tropics. The OCTs are dependent territories that share constitutional relationships with four member states of the European Union: Denmark (1), France (6), the Netherlands (6) and the United Kingdom (12). The OCTs have large autonomy in several areas, including economic affairs, employment market, public health, and customs.

Saint-Pierre and Miquelon is the only French OCT in the North Atlantic Ocean. The archipelago is located approximately 25 kilometers (km) from the coast of Newfoundland, and 4,300 km from France's capital (Paris). It is also the smallest French overseas islands with around 6,000 inhabitants. For centuries, the fishery of the Atlantic cod (*Gadus morhua*) was the sole economic activity, employing nearly half of the private sector in the 1980s. Yet, in 1992, the Canadian government declared a moratorium on the commercial cod fishery due to the collapse of the stocks. More than 25 years later, stocks have not rebuilt and capture fisheries are less than 10% of their peak (Figure 1), with now only a few dozens people involved in the fishery sector. Besides, both aquaculture and agriculture remain nearly inexistent, mostly due to the harsh climate and soil conditions; thus nearly all food products are imported and the archipelago's economy is strongly depending on the French government subsidies (more than half of the active population is government employees).

In this context, the present project funded by the local administration is to promote the development of innovative projects in aquaculture (including aquaponics and hydroponics). Those new projects will require the technical and scientific assistance of people outside the archipelago as well as the development of tight links with both the local and national administrations and schools. Preliminary achievements will be presented during the congress. More generally, the workflow developed within this project might serve as an example to promote the highly needed development of aquaculture in similar remote islands.

The author is thankful to the Conseil Territorial de Saint-Pierre et Miquelon for funding this project.

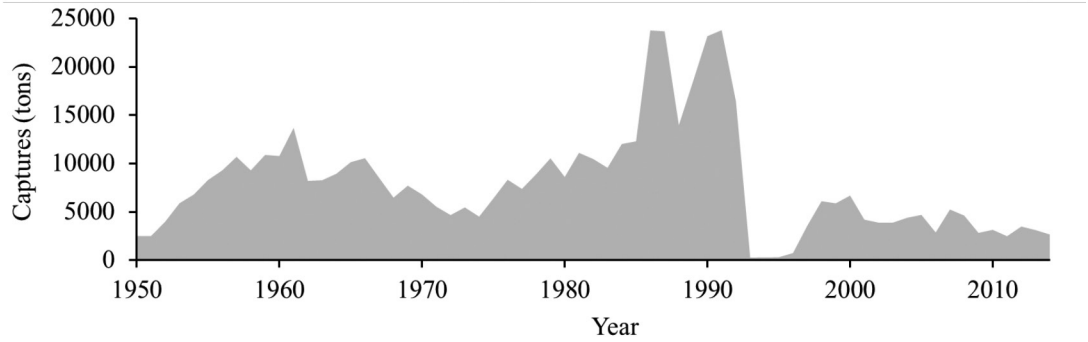

Figure 1. Captures from the Saint-Pierre et Miquelon fleet (based on the FAO database)

HOW FISH AQUACULTURE PRODUCTION HAS EVOLVED IN THE PAST DECADES: TRUE SPECIES DIVERSIFICATION?

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Aquaculture, the farming of aquatic animals and plants (not considered here), has increased tremendously in the past decades, particularly since the early 1980s. This has resulted in 2014, and this for the first time ever in human history, that more than half of seafood come from farmed rather than wild species. The strong rise of global aquaculture production has been based partly on the domestication, which by definition is a long and endless process, of an increasing number of finfish species: from 33 in 1950 to ca. 200 in 2016. Yet, as illustrated for instance for the year 2009, the production is heavily skewed (Figure 1). Among the 184 farmed species that year, only 20 contributed for more than 90% of the total production, among which several has been introduced almost globally, such as common carp (*Cyprinus carpio*) or Nile tilapia (*Oreochromis nilocitus*).

Based on the FAO database (http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en), the main aim of the present study is to evaluate precisely how fish species diversity production has evolved since 1950 both at global scale and within each of the five main geographic regions defined by the FAO: Africa, Americas, Asia, Europe, and Oceania. Here, species diversity was analyzed with four of the most informative indices in ecology: species richness (*S*), Shannon index (*H'*), Simpson index (*D*) and Hurlbert evenness (*J'*). We applied them on quantitative data from fishbase (www.fishbase.org) to analyze both the species diversification in a taxonomic sense and the diversification of species profiles in terms of trophic ecology or bio/ecological profile. We complement our analyses with comparisons using species/profiles similarity indices across geographical areas.

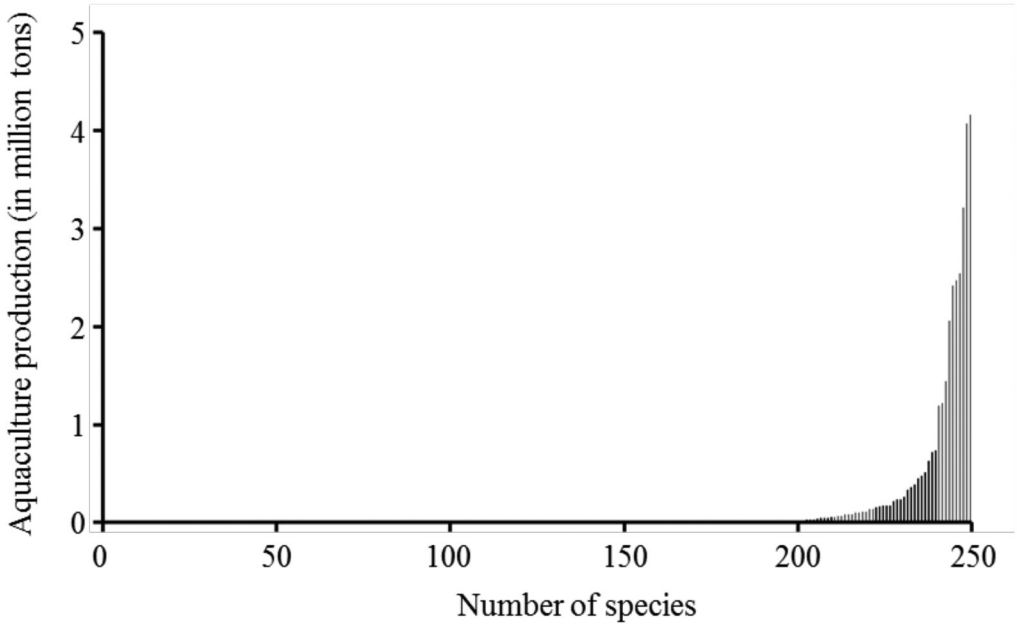

Figure 1. Aquaculture production per finfish species listed in the FAO database in 2009

AQUARIUM FISH AQUACULTURE: OVERVIEW AND EMERGING QUESTIONS

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Ornamental fishkeeping has become one of the most popular hobbies globally, particularly in developed countries, exemplified by the United States of America, Germany, France, Belgium, Italy, Japan, and Australia. In total, several million households now possess at least one aquarium. The entire industry is valued between US$ 15 and 30 billion per year and the international market may involve more than 100 countries. It has been estimated that over a billion fish belonging to 6,000 species are sold annually. Historically, this hobby has relied on wild-caught animals. The recent trend is that an increasing number of species are being bred in captivity. Today, 9 out of 10 freshwater fish are farmed. Conversely, marine aquarium trade is still largely based on the capture of wild fish in nature, even though more and more species are being produced in captivity.

In 2002, Tlusty reviewed the risks and benefits of the aquaculture production of ornamental fish. Here, our two main objectives are to: (i) reassess how aquaculture production of both freshwater and marine aquarium species has evolved during the last two decades, and (ii) discuss how the risks (e.g., invasive species) and benefits (e.g., production of endangered species) of ornamental aquaculture have changed, and to define new emerging questions such as welfare issues (e.g., domestication effects, fish feeds). Numerous examples from both freshwater and marine species will be used to illustrate these issues.

TOOLS, MODELS AND DECISION SUPPORT SYSTEMS FOR POLICY AND GOVERNANCE: THE 'TAPAS' PROJECT

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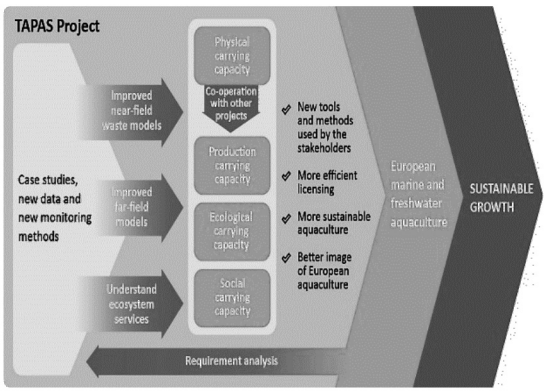
Establishing and operating an aquaculture site can be a complicated process as there are biological, environmental, socio-economic, technical and legal aspects that must be considered at all stages of planning and production. Depending on the species, system and area, there will be regulatory requirements for planning, monitoring and management that aim to minimise negative impacts on the environment and other stakeholders. These requirements can range from simple to complex and may involve the use of predictive models to provide information that would otherwise be difficult to obtain. Predictive models can simulate existing conditions, forecast future activities and show alternative scenarios which can inform any decision making process. Models, tools and approaches can be used individually or they can feed into or be part of a larger decision support system. However, the term decision support system can mean different things to different people so it is important to consult not only with stakeholders but also the developers of tools, approaches and models, to ensure the final product is useful and useable.

The work presented here includes an overview of why tools, models and decision support systems are needed for aquaculture environmental regulation, planning and governance. Some initial results for European-wide aquaculture show considerable inconsistency in policy, planning and approaches to environmental management for different aquaculture systems, and shows a need for more coherence and consistency of policy implementation.

This work is part of the EU H2020 TAPAS (Tools for Assessment and Planning of Aquaculture Sustainability) project which aims to support aquaculture development in Europe by developing tools, approaches, models and a decision support system that can be used in regulation and development of EU policy for sustainable aquaculture. Covering the key production systems throughout Europe - including freshwater ponds, re-circulating systems, shellfish farms, marine cages and emerging technologies such as Integrated Multi-Trophic Aquaculture (IMTA) - the project uses a combination of fieldwork case-studies, real-time monitoring, lab-work, computer-based modelling, policy reviews and stakeholder consultation to develop approaches to strengthen planning and management strategies and to understand the influence and contribution of aquaculture to ecosystem services.

Acknowledgements
This work has been conducted as part of the Tools for Assessment and Planning of Aquaculture Sustainability (TAPAS) project which has received funding from the EU H2020 research and innovation programme under Grant Agreement No 678396.

Webpage: tapas-h2020.eu, Twitter: @tapas_h2020



EFFECTS OF *Hermetia illucens* MEAL ON THE RAINBOW TROUT (*Oncorhynchus mykiss*) INTESTINAL MICROBIOTA COMPOSITION

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FAO indicates insects as an innovative and high potential source of protein to be employed in aqua feed manufacturing, due to their high nutritional value, especially in terms of crude protein content. Insect-based meal could thus become a sustainable and commercially viable alternative to fishmeal (FM) in aquaculture. One of the most promising insect species for commercial exploitation is the black soldier fly, *Hermetia illucens* (HI).

We conducted a 12-weeks feeding trial with 4 diets with partial replacement of FM with insect-meal. Three fish groups were fed with increasing replacement levels of FM with HI meal [10% (HI10), 20% (HI20) and 30% (HI30)] and a control group was fed a FM-based diet (HI0). The aim of the study was to investigate the effect of insect meal on gut microbiota of rainbow trout (*Oncorhynchus mykiss*). High-throughput 16S rRNA gene amplicon sequencing (MiSeq platform, Illumina) was utilized to study the gut microbial community profile.

Insect meal inclusion significantly affected the number of observed species as well as the species richness index (Chao1). In addition, the Shannon's diversity value, increased in the groups that received insect meal based diet. The most abundant phyla in all groups were *Firmicutes* (not affected by diet), *Proteobacteria* that decreased in fish fed HI20 and HI30 diet in comparison to control (HI0) and HI10 groups, and *Actinobacteria*, that was significantly ($P < 0.05$) lower in fish fed with HI0 diet in comparison to fish fed the HI-based diets. At genus level, insect-based diet positively influenced the abundance of *Aerococcus*, *Enterococcus*, *Lactobacillus* and *Pediococcus* genera (lactic acid bacteria). Beta diversity metrics showed a strong separation between control groups (HI 0) and the three HI-based dietary groups for both, the presence/absence (unweighted beta diversity) and relative abundance (weighted beta diversity) of microbial taxa (Fig 1).

In conclusion, our results showed that HI meal increased intestinal microbial diversity without affecting fish growth performances. *H. illucens* meal could thus be a promising feed ingredient to replace FM in trout diets.

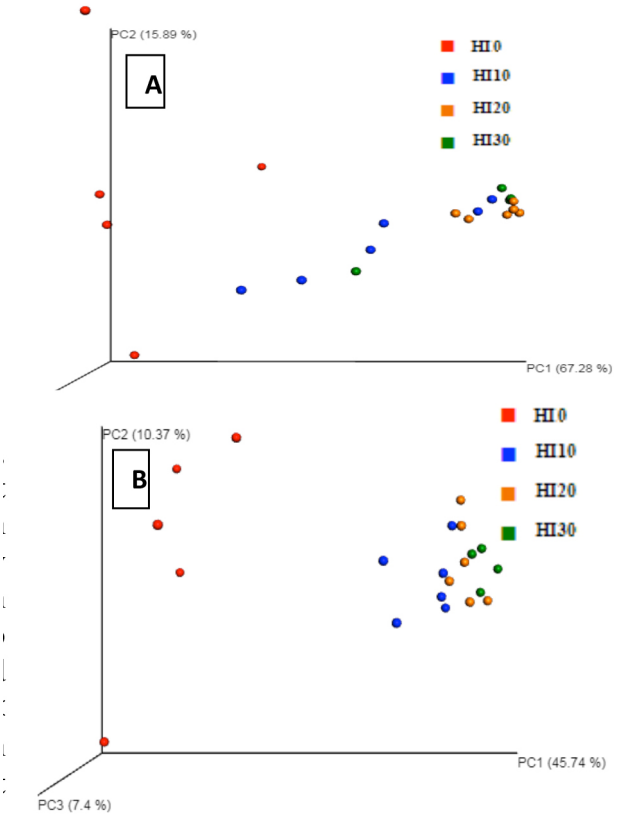

Fig 1. Principal Coordinate Analysis (PCoA) of Unweighted (**A**) and Weighted (**B**) Unifrac distances of gut microbial communities associated to four experimental diets. Each dot represents an individual sample plots according to its microbial profile at genus level.

CHARACTERIZATION AND EXPRESSION ANALYSIS UNDER IMMUNOGENIC STIMULATIONS OF BIG BELLY SEAHORSE INTERLEUKIN-8 *Hippocampus abdominalis*

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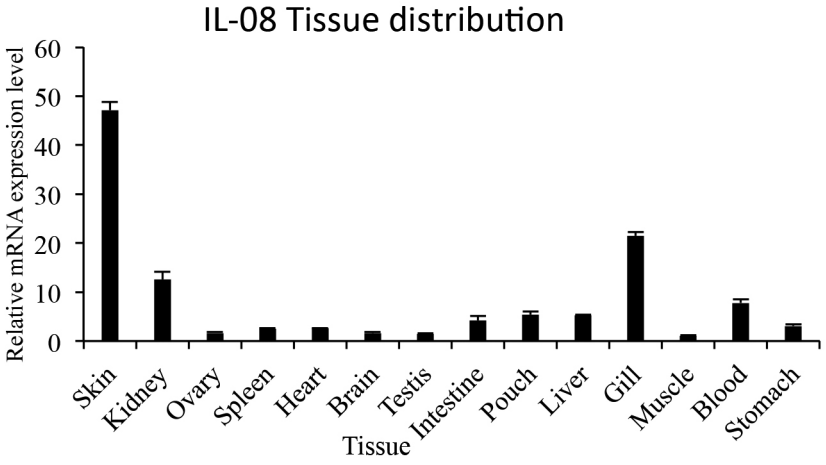
Interleukin- 8 (IL- 8) is a multi-functional chemokine, which play a key role in the inflammatory response cascade by their capability to activate of different leukocyte to the place of injury or inflammation. IL-8 belongs to the group that contain ELR motif (ELR^+) signaling. Mainly, IL-8 attracts neutrophils and ELR^- (without ELR motif) chemokine attracts lymphocytes and monocytes. IL- 8 can be produced in response to both exogenous (LPS) and endogenous (IL-1, TNF) stimuli by numerous cell types including neutrophil, monocyte/macrophage, epithelial cell, mast cell, vascular endothelial cell and dendritic cell. In this study, IL- 8 of big belly seahorse (*Hippocampus abdominalis; HaIL8*) was identified and characterized at sequence and transcriptional levels. *HaIL8* comprises coding sequence of 300 bp which encode respective protein with 99 amino acids in length. Predicted molecular weight and theoretical pH values are 11.01 kDa and 9.13 respectively. The HaIL-8 3D structure by homology modeling was similar to that of the human IL- 8 monomer. Phylogenetic analysis revealed a close evolutionary relationship for HaIL- 8 vertebrate counterparts, with close clustering to the fish homologs.

IL- 8 was expressed in all analyzed tissues with the highest in skin then in gill, while lowest expressions were observed in muscle and testis. The mRNA expression of HaIL-8 in the blood and the kidney upon lipopolysaccharide (LPS), poly (I:C), *S. iniae* challenges shown a significant up regulated expression in first few hours and later down regulated with poly (I: C) and LPS challenge. In addition, HaIL-8 expression has been increased significantly upon poly (I: C), LPS and *S. iniae* challenges in gill indicating involvement in host immune defense mechanism.



OPTIMIZATION OF FEEDING PERIOD IN JUVENILE PIKE-PERCH *Sander lucioperca*

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For pike-perch (*Sander lucioperca*) RAS based out-of-season reproduction and grow out is a promising way to produce a high amount of larvae and to quickly grow the fish to marketable size. Unfortunately, in RAS's operation costs for technology and staff are typically high and for a cost effective production cultured species need to obtain high market prices and a fast grow out. Pike-perch meets those criteria but production is economically risky since profit depends on fluctuating prices and high production costs. Further increase in productivity i.e. decreases of production cost is necessary to decrease economic risks of a RAS based pike-perch culture and to provide incentives for initiating new aquaculture ventures. One possibility to reduce production costs is an optimized feeding regime to attain maximal feed conversion and minimal wastage. For a specific adjustment i.e. optimization of feeding period the present study compared growth performance of juvenile pike-perch fed with variations in feeding events, duration of feeding events and temporal allocation.

In a first experiment juvenile pike-perch with an average weight of 30g were fed for 6 weeks with a feeding intensity of 1.2 % with a 12h day-time feeding for treatment one and a 12 h night-time feeding for treatment two. Results showed no significant differences between growth performances of both treatments (Fig.1).

Additionally in a second experiment pike-perch with an average weight of 100g were fed continuously every 5 min, continuously every 30 min, during night-times (12h) every 5 min and continuously every 5 min with longer feeding duration. The second experiment is still in progress and results will be obtained within the next month.

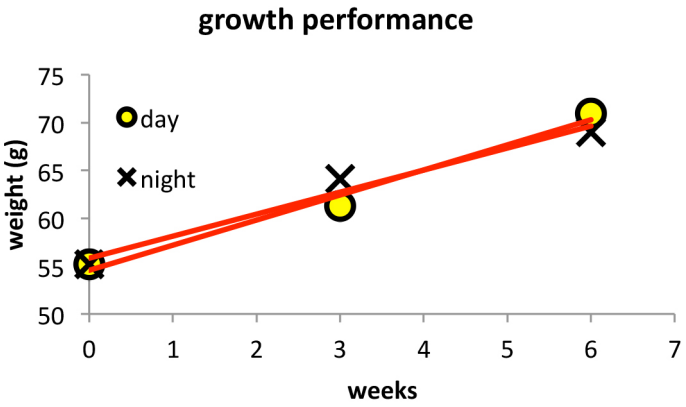

Figure 1. Growth performance of pike-perch fed during day-time (yellow) and during night-time (black)

GROWTH AND HEALTH PROMOTING FEEDS FOR STURGEON

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To optimize production, sturgeon farmers are in the need of adapted feeds for sensitive periods in the production cycle. Starting with sturgeon fry, our results have shown that several components enhance production performance in this delicate developmental stage. Fry feeds include raw materials of marine origin which ensures highest digestibility and fast growth. Furthermore, high contents of omega-3 fatty acids guarantee optimal development of cell membranes in the fast-growing fry. The inclusion of β-glucans, Vitamins C & E supports the immune system and improves stress resistance. Nucleotides in the feed support cell signalling and energy metabolism.

In the grower stage, fast growth and optimal water quality are in the focus of feed development. In this respect, the selection of marine raw materials ensures highest palatability and digestibility. Low levels of carbohydrates in the feeds and improved mixture of minerals secure optimal development and growth. In addition, careful selection of raw materials is a prerequisite to establish firm faeces structure for effective water cleaning in the production systems.

Production of caviar requires highest quality of the product which requires highest quality and performance of the feed used in this period of production. By including best fish meal and using only fish oil in broodstock feeds our results and farmers' experience show optimal delivery of omega-3 fatty acids to the fish which results in optimal egg quality and high caviar gain. The inclusion of β-glucans, Vitamins C & E supports the immune system and improve egg size and development.

During a production cycle, sturgeon may experience stressful situations, such as grading, transport, vaccination, suboptimal water quality and changes in water temperature. Active ingredients in our functional feeds have proven to naturally enhance the immune system of the fish to cope with stressful events.

When environmental conditions become unfavourable, pathogenic bacteria take advantage of the weakened organism and eventually infect the fish. This is especially true for bacteria of the genus *Aeromonas* and *Vibrio*. Extensive research and farm trials have shown that the addition of short chain monoglycerides (SCM) help the fish to alleviate the symptoms caused by pathogenic bacteria. This results in significantly reduced mortality and recovery of infected fish without restrictions compared to the use of antibiotics.

DEVELOPING SUSTAINABLE AQUA-FEEDS TO PROMOTE HUMAN HEALTH USING NOVEL, INDIGENOUS, NON-CONVENTIONAL INGREDIENTS

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The Omega-3 (n-3) polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential dietary nutrients for human health. However, increasing demand, finite supplies, and conflicting interests maintain inflated prices of these nutrients. Consequently, socioeconomic status influences access to these beneficial nutrients, with effects of chronic deficiency associated with disproportionate low-health burdens in developing countries.

Carps and tilapia are the main cultured freshwater species in both India and sub-Saharan Africa respectively. These species have an endogenous capability to convert alpha-linolenic acid (ALA) to EPA and DHA. Additionally, some species of freshwater macrophytes, seaweeds, and microalgae can be rich in ALA and do not compete with extant human food resources or current animal feedstuffs. Therefore, this represents a possible opportunity to redress this imbalance in an ethical, sustainable and environmentally sensitive way. Thus, the primary objective of the Sustainable New Ingredients to Promote Health (SNIPH) project was to investigate the potential to include feed ingredients with locally-sourced indigenous raw materials containing significant levels of ALA/EPA/DHA, with the aim of improving the nutritional quality of farmed fish, and ultimately, the health of consumers.

Preliminary screening of 'wild'-sourced candidates was based on availability and abundance in regions of interest. Some species were also cultured using a range of substrates to investigate effects of controlled growth on productivity. Nutritional quality of the materials was determined by biochemical proximate analysis and PUFA profile. Results demonstrated numerous suitable candidates including the freshwater macrophytes *Lemna minor*, *Spirodela polyrhiza* and *Ipomoea aquatica*, particularly when cultured using optimised substrates. Macroalgae (seaweed) nutritional quality was lower than that of freshwater macrophytes, but *Ulva lactuca*, *Turbinaria ornata* and *Eucheuma denticulatum* were the best candidates from the green, brown and red phyla, respectively. From this analysis, the most suitable candidates are being included in nutritionally balanced manufactured feeds, and assessed in a series of fish feeding trials.

The SNIPH project will ultimately provide an evidence base to inform aquaculture nutritionists, commercial fish feed companies, fish farmers, consumers and government policy makers of the potential for improving the Omega-3 content, and thus health benefit, of farmed fish fed on aquafeeds including locally sourced and cultivated low cost novel ingredients.

This research was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) Newton Fund, the Department for International Development (DFID) in the UK, and Department of Biotechnology (DBT) in India.

PARTIAL REPLACEMENT OF FISHMEAL BY *Daphnia sp*. AS PROTEIN SOURCE IN FORMULATED DIETS FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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Daphnia sp. is widely used as live zooplankton to feed the larvae fish, for their nutritional value and healthy benefits, but few studies have evaluated their use as ingredient in extruded diets. The aim of this study was to evaluate the viability of *Daphnia sp*. biomass as source of protein in formulated diets for rainbow trout.

For that three isoproteic (43.6%) and isolipic (18.5%) diets were formulated at increasing levels of replacement of the fishmeal by lyophilized *Daphnia sp*. Biomass (DB): 0 (control; D0), 20 (D20) and 40% (D40), corresponding to an inclusion of 0, 7 and 14% in feed formulation. The DB was recollected from a natural lake during several months and frozen until its lyophilisation. Trout with an initial weight of 29 g were cultured during 70 days in a recirculating aquaculture system and hand-fed to satiety once a day. At the end of the trial growth performance, protein utilization and fatty acids content in the fillet were analysed.

The trout fed with the diets DB20 and DB40 reached higher weight than trout fed with control diet. This increase was statically significant in the DB20 ($p<0.05$). This improvement could be related with the higher feed intake and the better digestibility of the protein observed. Regarding fatty acid composition of the fillet, DB increased the proportion of unsaturated fatty acids fillet improving the nutritional value.

The inclusion of *Daphnia sp*. biomass from a natural lake in formulation feeds for rainbow trout is presented in this study as a viable and sustainable source of protein for fish. More studies are necessary to optimize the inclusion in aquafeeds and to exclude possible negative effects on health fish.

Acknowledgements: This study was supported by FEDER funds (ACUI-SOSTENIBLE; Ref. 2015/000214).

	DB0	DB20	DB40	SEM
Growth performance				
Initial weight (g)	29.11	29.06	29.3	0.75
Final weight (g)	87.6^{b}	96.1^{a}	91.04^{ab}	2.25
WG (%)	196.2	221.4	210.6	7.76
SGR (% day^{-1})	1.57^{b}	1.70^{a}	1.61^{ab}	0.03
DFI (g100g $fish^{-1}day^{-1}$)	1.23^{b}	1.31^{a}	1.33^{a}	0.02
FCR	0.87	0.87	0.91	0.01
Protein utilization				
PER	2.57	2.39	2.51	0.06
PPV	0.51	0.48	0.47	0.02
$ADC_{protein}$ (%)	0.92^{a}	0.96^{b}	0.95^{b}	0.03
Fillet composition				
Protein (% dm)	80.97	79.1	79	0.90
Fat (% dm)	11.34	12.4	12.39	0.71
Moisture	76.5	75.6	76.63	0.35
Ash	6.18	5.79	6.21	0.17
Fatty acids (%)				
C14:0	4	4.02	3.54	0.19
C16:0	26.00^{b}	22.73^{ab}	19.84^{a}	1.3
C18:0	5.39	4.69	4.19	0.3
C15:1	1.92	0.85	0.91	0.55
C16:1	10.73	9.02	9.78	1.6
C18:1n9	36.64^{a}	40.15^{b}	41.27^{b}	0.54
C18:2	13.51	16.4	17.77	0.72
C18:3	1.81^{a}	2.13^{ab}	2.7^{b}	0.19
Saturated	35.4^{b}	31.45^{ab}	27.57^{a}	1.5
Unsaturated	64.6^{a}	68.55^{ab}	72.44^{b}	1.51

WG: weight gain; SGR: Specific Growth Rate; DFI: Daily Feed Intake; FCR: Feed Conversion Ratio; PER: Protein Eficiency Retention; PPV: Productive Protein Value; ADC: Apparent Digestibility Coefficient. $P<0.05$.

USE OF INSECT MEAL AS PROTEIN SOURCE IN FORMULATED DIETS FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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In recent years, there has been a growing interest in the use of insect meal as protein source in formulated diets for aquaculture. And after the European Commission Regulation (2017/893/EU) regulated their use, in the next years will become an usual ingredient in aquafeeds. The aim of this study was to evaluate the effect of the inclusion of two different insect meals, *Tenebrio molitor* and *Hermetia Illucens*, at different levels on the growth efficiency of rainbow trout in the initial growing period.

For that, a total of 5 different diets were formulated to contain 46% of crude protein and 17% lipid, and two different insect meals from *T. molitor* (T) and *H. illucens* (H) were used to replace 0 (C; control), 15 (H15 and T15) and 30% (H30 and T30) of dietary fishmeal (corresponding to 5 and 10 % in feed formulation). Trout with an initial weight of 55 g were cultured during 46 days in a recirculating aquaculture system and hand-fed to satiety once a day. At the end of the trial, growth performance and protein utilization were analyzed. The growth efficiency was good for all diets, and non-differences were observed in the final weight. Despite the FCR had good values for all diets, T15 showed better FCR than diets containing *H. illucens* (H15 and H30). Respect to protein utilization was not a clear effect in the inclusion level, but the diets containing insect meal in the formulation trended to improve the protein digestibility, finding significant difference in T30. Similar results have been obtained by other authors where the fishmeal was replaced up to 25% and 67% by *H. Illucens* (Hilari et al., 2007*)* and *T. molitor* (Belforti et al., 2015) respectively in diets for rainbow trout, without negative effects on the growth or even improving performance. In conclusion, the insect meal from *T. molitor* and *H. illucens* could be utilized in formulated diets for rainbow trout without negative effects on the growth efficiency in the initial growing period, nevertheless more studies are required to ensure their safety on the fish, and to stablish which is the optimal inclusion level depending on the insect and fish species. Acknowledgements: This study was supported by INIA (Spanish Agricultural and Agro-Food Research Institute), project: RTA 2015-00021-C03-01.

	C	H15	H30	T15	T30	SEM
Growth performance						
Initial weight (g)	55.44	54.89	56.46	53.7	55.98	0.51
Final weight (g)	141.32	139.97	139.73	141.9	139.82	0.83
Weight gain (%)	155.67	153.17	152.83	156.9	152.97	1.62
SGR (% day^{-1})	2.04	2.02	2.02	2.05	2.02	0.01
DFI (g100g $fish^{-1}$ day^{-1})	1.46	1.47	1.47	1.44	1.46	0.01
FCR	0.768^{ab}	0.778^{b}	0.782^{b}	0.754^{a}	0.773^{ab}	0.004
Proteinutilization						
PER	2.86	2.80	2.80	2.87	2.84	0.02
$ADC_{protein}$ (%)	86.11^{a}	89.04^{ab}	88.73^{ab}	88.47^{ab}	90.64^{b}	0.87

SGR: specific growth rate; DFI: Daily Feed Intake; FCR: Feed Conversion Ratio;
PER: Protein Efficiency Ratio; $ADC_{protein}$: Apparent Digestibility of the protein. $P<0.05$.

IMPROVING *Perca fluviatilis* AQUACULTURE BY CONSIDERING POPULATION-SPECIFIC PERFORMANCES IN RECIRCULATING AQUACULTURE SYSTEM

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Despite a growing economic interest, European perch aquaculture is still limited by several bottlenecks, especially at first life stages (e.g., slow growth rate, high cannibalism rate). Yet, first pieces of evidence suggest that geographic differentiation (i.e. differentiation between wild allopatric populations) can lead to population-specific potential for aquaculture. Some of the European perch aquaculture bottlenecks could thus be overcome by selecting the most efficient population(s) for fish production. This places a premium on the assessment of (i) the likely geographic differentiation in a species of concern and (ii) the potential population-specific performances in captive environment. Here, we investigate potential divergences in aquaculture performances between genetically differentiated allopatric populations of *Perca fluviatilis* through a multi-trait assessment approach in standardized RAS (re-circulating aquaculture system) environment.

First, we use genetic information as a proxy to infer geographic differentiations within the range of *Perca fluviatilis*. We analyze the genetic variability across 84 West-Palearctic sampling sites using four mitochondrial sequences (1,893 bp) and eight microsatellites using spatial analyses of molecular variance and STRUCTURE analyses. Second, we compare the performances between groups of genetically differentiated populations for several traits important in aquaculture (growth, behaviour, stress resistance, and immunity) through univariate and multivariate statistical analyses.

Our genetic analyses show an uneven distribution of the genetic variability of *P. fluviatilis* across the West-Palearctic. We highlight a two-level population structure: (i) a population structure dividing the species European range in five population groups occurring in five different large geographic areas (Figure 1) based on mitochondrial sequences and (ii) a subdivision of these five groups into several subgroups according to microsatellites' analyses. Our preliminary and ongoing analyses show performance differentiations between populations from the five different large geographic areas.

Our findings will be helpful to improve rearing programs, overcome bottlenecks in *P. fluviatilis* aquaculture by considering intraspecific differentiation of the species. This should allow positioning future developments of the species production to the most adapted population(s) to RAS conditions.

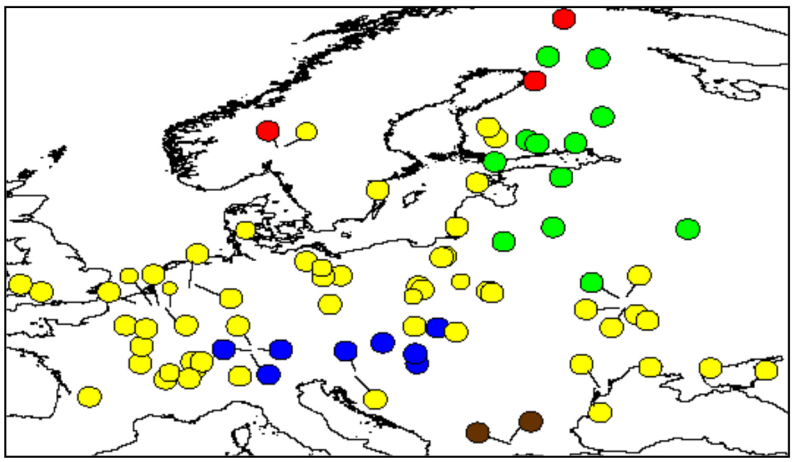

Figure 1: Mitochondrial genetic structure. Each circle represents a sampling site and each colour refers to a group. Circle sizes are relative to the number of samples analysed.

THE AQUACULTURE GOVERNANCE INDEX: AN INTRODUCTION

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Private certification and wallet cards provide a set assessment tools focused on the environmental and social (labour) dimensions of fish production at the scale of individual farms or idealised species-based production systems (e.g. intensive or extensive shrimp farming). While giving a snapshot of the current performance of production and setting technical criteria for improvement, these assessments tools say little about the adaptive capacity of both private and public actors in the industry to continually identify and resolve sustainability challenges. In partnership with Monterrey Bay Seafood watch, we introduce the Aquaculture Governance Indicators (AGIs) which aims to identify potential gaps in existing governance systems and provide guidance to the organisation and regulation of aquaculture improvement in any given country. The AGIs are based on four governance dimensions on three levels. On the first level, the performance of legislation and prescriptive codes and standards are assessed. On the second level, collaborative arrangements between public and private actors. On level three, the capabilities of these actors to engage in governance are assessed. In assessing the governance of the aquaculture industry the AGIs provide a framework for moving beyond the performance of production to instead assess the capacity of public and private rules and actors to organise the identification, assessment, and responses to a range of sustainability challenges.

DIETARY PREBIOTICS AND PHYTOGENICS IN LOW FISH MEAL AND FISH OIL BASED DIETS FOR EUROPEAN SEABASS *Dicentrarchus labrax*: AN EFFECTIVE TOOL TO BOOST MUCOSAL TISSUES HEALTH AND DISEASE RESISTANCE

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The successful replacement of fish meal (FM) and fish oil (FO) by land-based meals and oils in feeds for marine fish species is a determining factor to achieve a sustainable aquaculture sector development. However, their use has been associated with variable side-effects on fish growth performance and health. For European sea bass (*Dicentrarchus labrax*) gut in particular, replacement of dietary marine raw materials causes gut lamina propria and submucosa engrossment, increases mucus production and gut bacterial translocation rates, up-regulates several inflammation related genes and alters microbiota populations. Therefore, it is necessary to develop functional diets as part of a management strategy targeting mucosal health and disease incidence when low FM/FO formulations are used. In this sense, prebiotics and phytogenics may be potential candidates to safeguard fish mucosal tissues health and increase disease resistance. In the present study, one control and three experimental diets containing galactooligosaccharides (GOS) and a mixture of essential oils (PHYTO) or their combination (GOSPHYTO) were fed to European sea bass for 9 weeks before challenging the fish via gut inoculation with *Vibrio anguillarum* in combination with a stress panel by confinement. At the end of the feeding trial, growth performance, systemic immunocompetence, gut and gill morphology and associated lymphoid tissue (GALT and GIALT) status were evaluated by immunological, morphological and immunohistochemical methods via an automated image analysis software. Gut morphological pattern and anti-immunopositivity patterns for several immune related molecules (anti-iNOS, anti-TNFα, anti-PCNA) were studied along the challenge test. Dietary GOS, PHYTO and GOSPHYTO reduced gut *V. anguillarum* translocation rates and fish mortality after 7 days of challenge test. Gut morphological and immunopositivity patterns as well as gene expression of several gut junctional complexes-related genes varied depending on the product supplemented and the area of the mucosal tissue studied, whereas gill was less affected. Fish fed GOS and PHYTO diet presented less ($P<0.05$) posterior gut (preileorectal valve) fold area covered by goblet cells, due to a smaller ($P<0.05$) goblet cell area compared to fish fed non-supplemented diets. A similar pattern was observed in rectum (postileorectal valve) for fish fed GOS diet. Indeed, for this intestinal region fish fed GOS based diets presented a thinner ($P<0.05$) submucosa, higher density of rodlet cells and lesser anti-PCNA immunopositivity compared to fish control diet. On the other hand, fish fed PHYTO based diets, presented an upregulation of several genes related with junctional complexes, in relation to the lower gut bacterial translocation rates found. The pattern of response after intestinal bacterial challenge in terms of fold area covered by mucus, goblet cell area and immunohistochemical positivity patterns varied among dietary treatments, presenting fish fed PHYTO diet the higher and longer capacity of response. Finally, a comparison among the morphometric parameters evaluated within all the intestinal segments evaluated as well as between both mucosal tissues (gut and gill) revealed variations on the goblet cells size, distribution and density, regardless of the dietary treatment supplemented.

RESEARCH PRIORITIES TO SUPPORT SUSTAINABLE DEVELOPMENT OF ATLANTIC SALMON AQUACULTURE IN NORWAY

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Global demand for food increase with increasing world population, and modern aquaculture is part of the solution for global food security. Today, fish and seafood account for less than 10% of the global protein sources produced. This stands in contrast to that 70% of the earth is oceans. Seafood is a source of protein, but in addition seafood contribute with essential minerals, vitamins and omega-3 lipids necessary for good human health. In the context of global aquaculture, only 36% of total aquaculture is in the marine environment and less than 15% of production is aquaculture of fin fish in the oceans and coastal areas. Thus, we need to explore sustainable growth and development of aquaculture in the oceans to meet future demand for safe and nutritious seafood also to contribute to the realization of the UN's Sustainable Development Goals.

Research and development support sustainable growth and development of salmon aquaculture. This require priorities with the fields of technology, fish health and welfare, feed development and nutrition, breeding and genetics utilizing new technology, and understanding the interaction between environment and aquaculture. The presentation will give the outlook of research priorities to support a sustainable growth and development of salmon aquaculture in Norway.

THE VARIABILITY OF STRESS AND IMMUNE RESPONSES IN FISH SUBJECTED TO STRESSORS. EVIDENCES FROM PHYSIOLOGICAL AND GENOMIC DATA IN THREE MODEL SPECIES

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Fish show a high degree of variability in many functional mechanisms as a result of both genetic variation and adaptation to a wide range of environmental conditions including depth, temperature , salinity, oxygen availablity or life-style. In the present work we studied whether this variability is also observed in the physiological and genomic indicators in three species when subjected to the same type of stressors. We subjected trout (*Oncorhynchus mykiss*), seabream (*Sparus aurata*) and zebrafish (*Danio rerio*) to either an abiotic stressor (air exposure), and a biotic stressor (*Vibrio anguillarum* bacterin bath).

After acclimation to laboratory conditions fish were either subjected to air exposure hypoxia by maintaining the fish 3 minutes out of water or to a *Vibrio anguillarum* bacterin bath. After treatments fish were killed by deep anesthesia and samples were taken from blood and liver. On these samples the following variables were determined: Cortisol, glucose and Immunoglobulin in blood, gene inflammatory cytokines and innate response genes in mucus and liver and kidney.

The results showed that the physiological responses such as cortisol show a consistent increase at short term after air exposure and a different recovery depending on the species, and that only trout and zebrafish show increases after vaccination. Regarding the overall gene expression in liver, seabream shows a higher responsiveness after air exposure whereas trout shows the highest susceptibility to vaccine exposure (Table 1).

These results are discussed in terms of the genetic background of each species and the respective adaptation to their specific biotic (the relationship with the microorganism load and variability) and abiotic (salinity, temperature, water chemistry) environment.

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Table 1: Plasma cortisol (ng/ml) and overall gene expression (normalized relative fold expression) in the three species of fish subjected to either air exposure and vaccine exposure at 1h, 6h and 24h. (+ indicates significant increase, - indicates small or no changes).

	Rainbow trout			Gilthead seabream			Zebrafish		
AIR EXPOSURE	1h	6h	24h	1h	6h	24h	1h	6h	24h
Plasma cortisol	+	-	-	+	+	-	+	-	-
Liver gene expression	+	-	-	+	+	+	-	+	+
VACCINE EXPOSURE	1h	6h	24h	1h	6h	24h	1h	6h	24h
Plasma cortisol	-	+	-	-	-	-	-	+	-
Liver gene expression	-	+	+	-	-	+	-	-	-

ECOLOGICALLY REALISTIC MODEL OF INFECTION FOR EXPLORING THE HOST DAMAGE CAUSED BY *Vibrio aestuarianus*

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Although vibrios are frequently associated with marine organisms mortality outbreaks, knowledge on their ecology and pathogenicity is sparse, thus limiting disease management and prophylactic strategies. Here, we investigated *V. aestuarianus* infection onset and progression in the wild, taking advantage of a "claire" pond: a semi-closed system with limited seawater renewal, theoretically more adapted to disease transmission. We showed a positive association of the bacteria with oysters, which can constitute a reservoir for the bacteria in the winter. Moreover, passage through oysters was found to be necessary for experimental disease reproduction as vibrios shedding from diseased oysters has higher infectivity than from *in vitro* grown. We next developed an experimental "ecologically realistic" infection model in a mesocosm, allowing infection by natural route. By means of this non-invasive protocol, we analysed the pathogenesis of the bacteria and demonstrated the importance of haemolymph for initial colonisation and the septicaemic nature of this disease.

SETTLEMENT TRIGGER IN BLUE MUSSELS

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Like the majority of benthic invertebrates, the blue mussel *Mytilus edulis* has a bentho-pelagic cycle and its larval settlement is a complex phenomenon involving numerous factors. The success of this phenomenon is at the basis of the spat collections of the majority of mussel farmers. Among them, both underwater noise and pelagic trophic conditions have been weakly studied in previous research. Here, we tested in the laboratory conditions the hypothesis that phytoplankton assimilation by the pediveliger blue mussel larvae may act as a food cue interacting with anthropic underwater sound and stimulates the settlement. We used 13C-labelling microalgae to validate the assimilation of different phytoplankton species in the tissues of pediveliger larvae. Our results clearly confirm our hypothesis with a significant synergies effect of these two factors. However, only the picoeukaryotes strains assimilated by larvae stimulated the settlement whereas the non-ingested picocyanobacteria did not. Similar positive responses were observed with underwater sound characterized by low frequency vessel noises. The combination of both factors (trophic and vessel noise) increased the mussel settlement from a 4 order comparatively to the control (without phytoplankton and noise) with settlement levels ranging from 16.5 to 67% in 67 h.

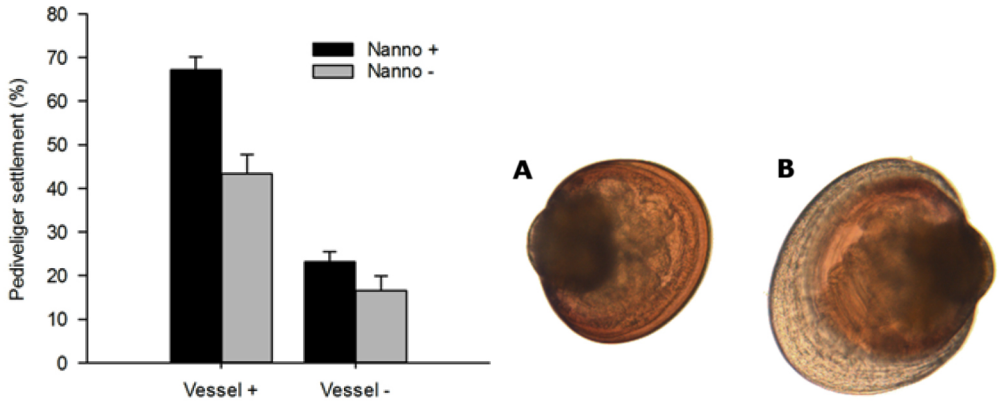

Figure. Settlement success (mean ± SE) for each condition: sound treatment (noted Vessel + / -) and trophic treatment with *Nannochloropsis oculata* (noted Nanno + / -). Photograph showing pediveliger larvae (A, 267 µm) and post-larvae with the prodissoconch II (B, 346 µm) of *Mytilus edulis*.

COMPARATIVE TRANSCRIPTOMICS USING RNA SEQUENCING TO IDENTIFY GENE EXPRESSION SEX-BIAS PATTERNS IN FIVE SPARIDS

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Among the animals that reproduce sexually, teleost fishes show a very wide repertoire of reproductive modes. The Sparidae family in particular is considered to be one of the most diversified ones regarding reproductive systems. To date, studies on sex-specific differences in gene expression have been conducted mainly in sex determination (SD) systems of model fish species. In aquaculture conditions, understanding and controlling reproduction is key issues for the efficient management of fish. In this study, we explored the sex-specific expression patterns in five sparids which have an important role in the Mediterranean aquaculture production and show different reproductive styles (protogynous, protandrous, and gonoschorist).

Using RNA sequencing, we generated millions of reads in five sparid species, for male and female gonads and brains, the tissues with the most profound role in sexual development and reproduction. Then, we analyzed and compared differences between male and female transcriptomes of the fish under study and investigated gene expression differences (data analysis pipeline Fig. 1).

We consider that a global view of sex-biased expression in these tissues was obtained, and through comparative analysis we revealed common male and female- specific genes/pathways that are implicated in sex differentiation and sex maintenance. We detected genes involved also in sex determination/differentiation mechanisms in other fish and vertebrates, and compared their expression patterns in our five species. The present study permitted us to reveal the "basic" molecular toolkit establishing the sex phenotype in the studied organisms (Fig. 2).

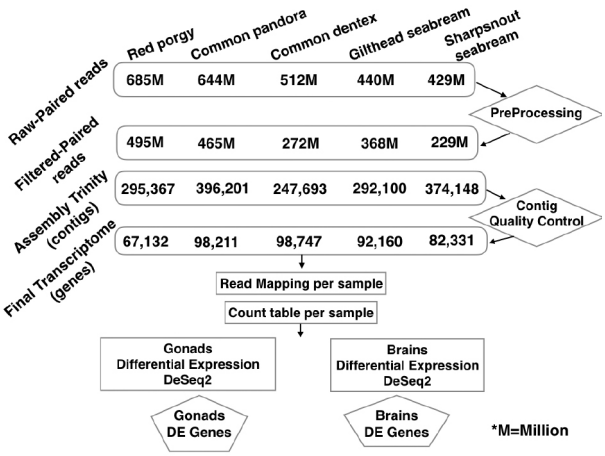

Figure 1. The pipeline followed to build assemblies and differential expression profiles in brains and gonads of the five studied sparids.

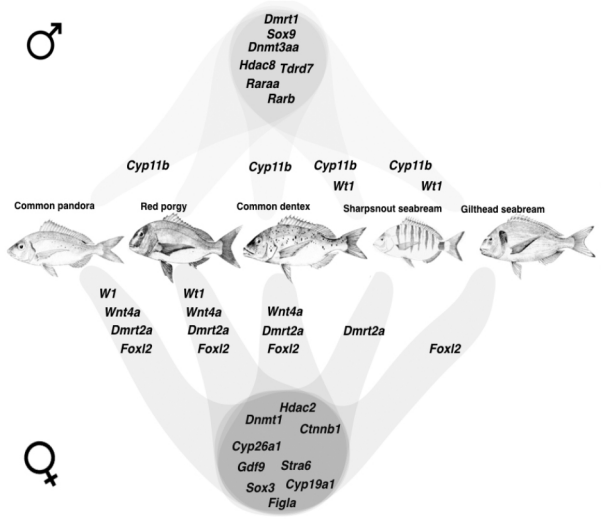

Figure 2. Comparative view of known sex-related genes, commonly (intersections) and uniquely expressed in each sparid species.

COMPARING THE SENSITIVITY OF DIFFERENT FISH SPECIES TO OPTIMIZE THE ASSESSMENT OF MYCOTOXIN TOXICITY

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Introduction: Today often wheat, soy and corn are used to produce fish feed, however, the inclusion of these plants leads to the introduction of undesired mycotoxins. Over 400 of these toxic fungal metabolites have been described up to now and their detection is often laborious and expensive.

Methods: In order to evaluate the threat mycotoxins pose, a literature search on the average and maximum contamination of feed ingredients was conducted. Based on these values the contamination risk (with the 10 most commonly present mycotoxins) in more than 70 commercially available fish feeds was calculated. Furthermore, the literature search yielded toxicity information for 7 mycotoxins (aflatoxin B1, zearalenone (ZEN), deoxynivalenol (DON), ochratoxin, fumonisin B1, moniliformin and T2 toxin). Based on this data the risk for mycotoxin effects in aquaculture fish was estimated and the species sensitivity for the different fish species was assessed.

Results: For most mycotoxins, no critical concentration in the feeds was estimated with respect to the currently recommended maximum allowable contamination levels in fish feeds. However, this risk assessment remains incomplete as the yearly differences in the quality of the ingredients and the storage of the feed may be relevant for the final contamination. Nevertheless, the assessment can result in recommendations of certain feed ingredients that show moderate mycotoxin contaminations in general, and thus, appear to be less problematic for fish.

For assessing toxicological effects in fish, it is recommended to use several fish species, as the comparison of sensitivities to different mycotoxins revealed considerable differences among fish species (e.g. Figure 1).

Discussion: The current knowledge of the effects of mycotoxins in fish feed is not sufficient to calculate robust risk estimations. Furthermore, future experiments on toxicological effects of mycotoxins should be conducted with various fish species, as they may show considerable variance in their sensitivity towards different mycotoxins, probably due to the structural diversity of these substances.

References:

Elmegaard, N. and Jagers op Akkerhuis, G.A.J.M. (2000). Safety Factors in Pesticide Risk Assessment. Differences in Species Sensitivity and Acute-Chronic Relations. NERI Technical Report 325. National Environmental Research Institute, Silkeborg, Denmark, 60pp.

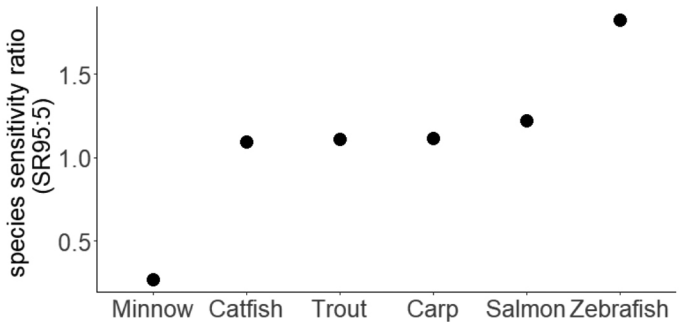

Fig.1: Example of calculated species sensitivity ratios 95:5 according to Elmegaard and Jagers (2000) for the mycotoxins DON and ZEN.

DOMESTICATION AND FITNESS IN THE WILD: A MULTIVARIATE VIEW

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Domesticated species continually escaping and interbreeding with wild relatives impose a detrimental migration load on wild populations. As domesticated stocks become increasingly different as a result of artificial and natural selection in captivity, fitness of escapees in the wild is expected to decline, reducing the effective rate of migration into wild populations. Recent theory suggest that this may alleviate and eventually eliminate the resulting migration load. I present a multivariate model (Tufto 2017) showing that this view is likely to be too simplistic. The model considers trait and wild fitness evolution resulting from the joint effects of artificial selection and natural multivariate correlational Gaussian selection in the captive environment. I assume some alignment between the adaptive landscapes in the wild and in captivity (Fig. 1a, fitness contours shown as green and blue ellipses, respectively) reflecting the assumption that similar functional constraints and optimization principles are likely to apply in both environments. Observable evolutionary changes (Fig. 1c and d) are shown to involve fast and slow phases over different characteristic time scales associated with the strength of stabilizing natural selection acting on a set of decoupled, independently evolving linear transformations (Fig. 1b) of the original traits (Fig. 1a). Initially, the evolutionary trajectory (Fig. 1a, blue dotted curve) is dominated by the effects of artificial selection causing a fast initial decline in fitness of escapees in the wild (Fig. 1d, blue dotted curve, generations 1-20). In later phases, through the counteracting effects of correlational stabilizing natural selection in captivity (Fig. 1a, blue ellipse), the mean phenotype is pushed in directions of weak counteracting stabilizing selection, allowing a sustained response in the trait subject to artificial selection (Fig. 1c, blue dotted curve). These phases are associated with slower rates of decline in wild fitness of the domesticated stock (Fig. 1d, generations 20-150), suggesting that escapees are likely to impose a considerable migration load on wild populations in the foreseeable future. The model also provide a novel interpretation of patterns often seen in artificial selection experiments after relaxation of selection (Fig. 1c, green dotted curve).

References

Tufto, J. (2017) Evolution, 71: 2262–2270. doi:10.1111/evo.13307

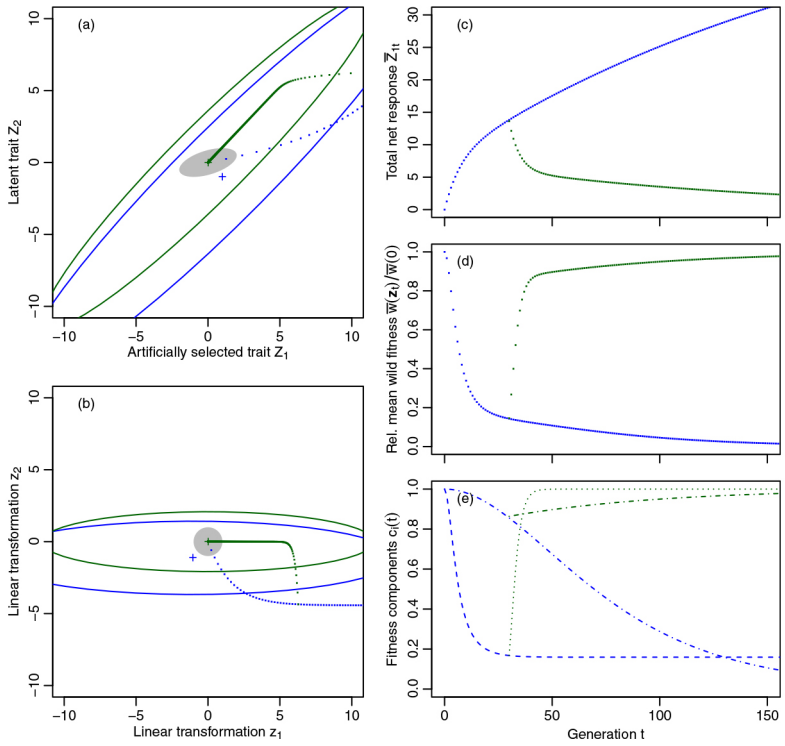

Figure 1. Trait trajectories and evolution of relative mean population fitness for a bivariate version of the model.

LATEST RESULTS OF WELS (*Silurus glanis*) CATFISH SPERM CRYOPRESERVATION AND POSSIBILITIES OF PRACTICAL APPLICATION

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Introduction

First laboratory experiments on the cryopreservation of wels catfish (*Silurus glanis*) sperm were successful (Krasznai and Márian 1985; Linhart et al. 1993) and were followed by further research in order to refine the different methodologies and for increasing the size of the storage capacity (straws, cryotubes etc.) during cryopreservation (Linhart et al. 2005; Ogier de Baulny et al. 2008). Nonetheless, a well-standardized methodology with high-volume sperm storage capacity that can be used in practical applications is not yet available.

Material and Methods

Due to the problems of catfish sperm stripping, our research team based the research on the removal of the testis, and cryopreservation was carried out in the vapour of liquid nitrogen with a simple Styrofoam box. At the beginning of our work the possible storage time of fresh sperm in different glucose-based extenders (glucose, fructose etc.) and freezing large quantity of sperm were tested. Later on, the cryopreservation of large volume of sperm (4 ml straw) was experimentally developed where thawed sperm was used for fertilisation 250-500 g of eggs (the used quantity is one that is usually applied in practice) at the hatchery (Bokor et al. 2010). Cryopreservation experiments were supplemented by the viability test of juveniles originating from artificial fertilisation using thawed sperm (condition factor, SGR, survival/ rate) as well (Bokor et al. 2015). Nowadays the possibilities of increasing the size of the sperm volume (10 ml cryotube) and the improvement of the different parameters: i.e. sperm concentration, determination of the activating solution, storability of fresh sperm etc.) and the application possibilities of a programmable deep-freezing equipment are investigated.

Results

The 6% fructose extender and 10% methanol cryoprotectant was the most suitable freezing media in the case of wels catfish according to our preliminary results. Large scale artificial fertilisation of eggs with cryopreserved sperm showed excellent fertilisation rate which was not lower than the control native sperm. Viability parameters of juveniles from cryopreserved sperm were the same as juveniles from native sperm. The application of 10 mL cryotube was successful where thawed sperm resulted similar high fertilisation rate than the control fresh sperm at hatchery conditions.

Conclusion

A reproducible catfish propagation technology, applicable in practice will be the result of the series of our experiments. Large-scale cryopreservation (4 ml straw and 10 ml cryotube) proved promising, further increasing the opportunity of the application of the cryopreservation in factory practice.

Acknowledgements

Our work was supported by the GINOP-2.1.1-15-2015-00645 project. The publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

MANAGEMENT OF TEMPERATURE AND FOOD ON PARALARVAL CULTURE OF PATAGONIAN RED OCTOPUS *Enteroctopus megalocyathus*

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The rearing of *Enteroctopus megalocyathus* paralarvae is currently the biggest bottleneck for the massive juvenile production of this species with the aim of its ongrowing. To address this issue, this study aims to understand the effect of different temperatures in interaction with the feed quality on paralarval performance. Three factorial experiments were carried out with three replicates per each combination of treatments. Experiment 1: Paralarvae from 1 day after hatching (DAH) until 15 DAH were reared at 10, 12 and 14°C and they were fed on three diets: Artemia enrichment 1 (Nannochloropsis), Artemia enrichment 2 (Origold ® Skretting), and Artemia enrichment 3 (an emulsion using LC60 Phosphotech). Experiment 2: Paralarvae of 21 DAH were subjected to the same combinations of diets and temperatures of experiment 1, for 30 days. Experiment 3: Paralarvae of 1 DAH were reared in the combinations of three temperatures: 10, 12 and 14°C and three Artemia sizes: 3, 5 and 7 mm length with enrichment 3. Survival, gravimetric and morphometric measurements of paralarvae were evaluated each 15 days. Samples were taken for bichemistry and enzyme activity of trypsin, chymotrypsin, leucine-aminopeptidase and lipase.

The main results showed interaction between diet and temperature on survival, growth, and digestive activities of paralarvae. Between 1 and 21 DAH, paralarvae presented the best growth at 10 °C fed with Artemia 3mm, while there was not a significant growth at any temperature when paralarvae fed with Artemia 5 or 7mm (Fig.1). In experiment 1, cannibalism was very low. In experiment 2, cannibalism was lower when paralarvae fed with Artemia enrichment 2, specially at 12 °C (Fig.2). In Experiment 3, no cannibalism was observed when paralarvae were fed with 5 mm Artemia in any of the temperatures, stressing the importance of the prey size for these paralarvae. The greatest enzyme activities were observed at temperatures of 10 and 12 °C, when paralarvae were fed Artemia enrichment 2 and Artemia enrichment 3. With Artemia enrichment 1 the enzyme activities of paralarvae not showed the effect of temperature.

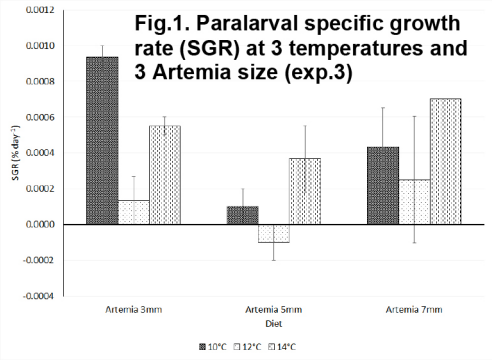

Fig.1. Paralarval specific growth rate (SGR) at 3 temperatures and 3 Artemia size (exp.3)

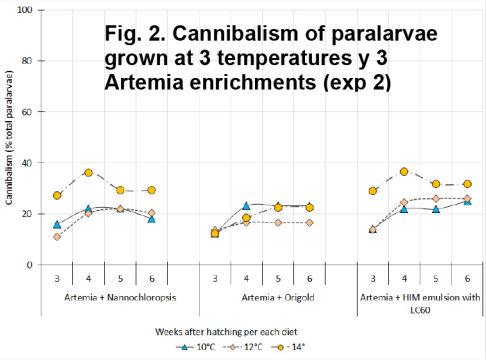

Fig. 2. Cannibalism of paralarvae grown at 3 temperatures y 3 Artemia enrichments (exp 2)

COMPARISON OF GONADS FROM SEA URCHIN *Paracentrotus lividus* FED A COMMERCIAL DIET AND WILD INDIVIDUALS

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In the Mediterranean Sea, the sea urchin (*Paracentrotus lividus*) gonads have been considered a prized seafood product and a delicacy due to its flavour and texture. The increasing market demand for sea urchin roe has resulted in the depletion of wild stocks becoming an interesting target species for aquaculture production. A practical low-fat diet, with 6% lipids and 30% crude protein (D30), was formulated and fed to three homogeneous groups of 15 individuals (35 g mass; 4.5 cm diameter) harvested in an intertidal zone of North Portugal. Sea urchins were distributed by cages (45 urchins/m^2) in a saltwater recirculation system (salinity 35‰, 18ºC) and fed the experimental diets for 15 weeks. At the end of the trial, wild animals were harvested from the sampling site and compared with those fed the experimental diet.

Irrespectively of the genre, the gonadal somatic index (GSI) of sea urchins fed the experimental diet was higher (20-23%) than average values observed in wild specimens (6-7%), with similar stage of maturation. Final gonad composition of sea urchins fed diet D30 had higher protein but lower lipid content, than their wild counterparts.
Gonads of wild males and females had significantly higher total carotenoid content mainly resulting from higher levels of echinenone, β-carotene and β-cryptoxanthin. Echinenone was the dominant carotenoid in gonads from both sexes, but males had higher levels in both wild and cultivated sea urchins (Table 1). Gonads redness (a* value) was significantly higher in wild urchins, and females always exhibited reddish gonads compared to males. Wild specimens had firmer gonads than those fed the experimental diet, but resilience remained similar among animals.

The experimental diet significantly improved gonad size but resulted in gonads with a significantly different colour from wild specimens. Further studies are required to evaluate diets rich in pigments able to enhance gonads redness in farmed sea urchins.

Work supported by Project INNOVMAR (NORTE-01-0145-FEDER-000035) within the line "INSEAFOOD - Innovation and valorization of seafood products", founded by NORTE2020 through the ERDF.

Table 1 - Gonads carotenoid content and colour in sea urchin male and female fed an experimental diet under captivity (D30) or harvest in the wild.

	Male		Female		Two-way ANOVA *P*-value		
	D30	Wild	D30	Wild	Genre	Diet	G x D
Carotenoids							
Total carotenoid (µg/g WW)	11.9 ± 3.9	89.1 ± 45.1	15.3 ± 1.0	86.6 ± 25.1	1.0	0.001	0.9
α-carotene	0.2 ± 0.01^{c}	1.3 ± 0.05^{a}	0.2 ± 0.02^{c}	0.4 ± 0.02^{b}	<0.001	<0.001	<0.001
β- carotene	1.0 ± 0.02^{c}	19.4 ± 0.1^{a}	2.1 ± 0.01^{c}	10.1 ± 0.6^{b}	<0.001	<0.001	<0.001
Echinenone	22.0 ± 0.1^{c}	93.8 ± 0.2^{a}	8.0 ± 0.04^{d}	26.5 ± 0.01^{b}	<0.001	<0.001	<0.001
Lutein	0.2 ± 0.00^{d}	0.7 ± 0.01^{c}	1.8 ± 0.04^{b}	4.6 ± 0.04^{a}	<0.001	<0.001	<0.001
β-cryptoxantin	5.1 ± 0.00^{b}	20.3 ± 0.01^{a}	4.6 ± 0.00^{c}	20.3 ± 0.01^{a}	<0.001	<0.001	<0.001
Colour and texture							
L* (Brightness)	59.0 ± 6.6	47.8 ± 4.7	46.3 ± 3.8	38.2 ± 4.0	0.004	0.01	0.6
a* (Redness)	7.1 ± 3.2	14.7 ± 2.9	1825 ± 0.2	25.4 ± 3.2	<0.001	0.001	0.9
b* (Yellowness)	25.1 ± 4.0	25.1 ± 0.5	34.3 ± 3.3	34.3 ± 5.5	0.03	1.0	1.0

BIO-UTILISATION OF MACRO- AND MICROALGAE IN EUROPEAN SEABASS AFTER TECHNOLOGICAL PROCESSING

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Two macroalgae (*Gracilaria* sp., 36% of crude protein (CP) and 1% of crude lipid (CL) and *Ulva* sp., 15% CP and 1% CL) and three microalgae (*Nannochloropsis* sp., 36% CP and 11% CL; *Chlorella* sp., 56% CP and 10% CL and *Tetraselmis* sp., 27% CP and 7% CL) were subjected to two technological processes (physic and enzymatic) before dried.

Microscopy observation and the determination of soluble protein (Bradford method) of samples were used to verify and confirm the algae cell wall disruption after processing. For determination of protein apparent digestibility coefficient (ADC) in European sea bass, a commercial-based extruded diet with 49% CP and 13% CL was used as reference diet (REF) and 1% chromic oxide was added as inert marker. The test diets were obtained by replacing 30% of the REF diet by each algae biomass, either before (whole alga) or after physic (PHY) or enzymatic (ENZ) processing. Homogeneous groups of 12 European seabass (62 ± 8.6g), subjected to a natural photoperiod were kept in a recirculating salt water system (35‰, 21 ± 1.8ºC) with a Guelph system to collect feces. Each diet was randomly assigned to three tanks, for replication of results. The apparent digestibility coefficients (ADCs) of the experimental diets were calculated according to Maynard et al. (1979) and the protein ADC of the test ingredients were estimated according to NRC (2011).

Both disruption methods promoted cell disintegration and increased the soluble protein fraction (SPF). The enzymatic process was very effective in *Nannochloropsis* sp. resulting in an increase in the SPF value from 0% in the non-processed to 4.3% in the processed biomass. In the case of *Chlorella* sp. and *Gracilaria* sp. the physic process also increased SPF value from 0% in the non-processed algae to 5.1% and 2.5%, respectively, in the processed counterparts.

Among microalgae, the diet containing 30% ENZ *Nannochloropsis* sp. showed the highest protein ADC that was similar to the REF diet (>90%); concerning ingredients ADC, the highest protein ADC value was also observed for ENZ-*Nannochloropsis* (>80%). Unprocessed microalgae were generally well digested (>70%), having the *Chlorella* sp. the highest protein ADC value. *Nannochloropsis* sp. protein ADC can be further improved after enzymatic processing. The diet containing 30% PHY *Gracilaria* sp. showed the highest protein ADC that was similar to the REF diet (>90%); the highest protein ADC value among test ingredients was also observed for PHY- *Gracilaria* (>90%). *Gracilaria* sp. is a better protein source than *Ulva* sp. to be incorporated in sea bass diets, irrespectively of processing, and can be further improved by physic processing.

Work supported by Project "MARINALGAE4AQUA - Improving bio-utilisation of marine algae as sustainable feed ingredients to increase efficiency and quality of aquaculture production", financed by FCT-Portugal through the research project ERA-NET COFASP/004/2015.

EFFECTS OF IONIC COMPOSITION ON GROWTH AND SURVIVAL OF WHITE SHRIMP *Penaeus vannamei* CULTURE AT LOW-SALINITY WELL WATER

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The culture of white shrimp *Litopenaeus vannamei* in low salinity waters (1.2 ± 0.5 g L^{-1}) presents challenges, the deviation of the ionic composition of water is known that influences the overall condition of the cultured organisms. The effect of water of low salinity with different ion content on the growth and survival of shrimp was determined. First, water was extracted from 4 wells with a depth of 5 to 7 m, located in Sinaloa River Basin in Guasave, Mexico at: ($T_{1)}$ 25.43°N, 108.44°W; $_{(T2}$) 25.48°N, 108.37°W; (T_3) 25.60°N, 108.40°W and (T_4) 25.64°N, 108.51°W and were selected on base the different ionic water composition. The experimental design was 4 independent treatments with 3 replicate. Shrimp were grown at sea water ($T_m = 34 \pm 1.4$ g $L^{-1)}$ used as a control. Shrimps grown in T_m and T_1 had averaged weight (over 12 ± 0.61 g) and survival rates (78%), with statistical differences to others low salinity water treatments. The condition factor obtained with low-salinity water ($T_1 = 0.654$) was like that recorded for T_m (0.670), where ionic ratios (Na/K and Mg/K) were similar to that of seawater. This observation strongly suggests that the ratios of the major ions ($Na^{+,K+}$, Mg^{2+} and $Ca^{2+)}$ play a relevant role for development of organisms under cultivation. Shrimp cultured in water of low salinity with an ionic ratio similar to that of sea water were organisms with the best development under cultivation, therefore indicating that the proportion of major ions were important for shrimp.

TECHNO-ECONOMIC AND LIFE-CYCLE ASSESSMENT OF TWO NEW VALUE CHAINS FOR EUROPEAN SEAWEEDS

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Seaweed has long been recognised as a valuable source of diverse bioactive compounds, and has great potential to be used in pharmaceuticals, nutraceuticals and functional foods, yet these markets needs to develop further before commercial, large-scale seaweed value chain will come to mature.

As of now, the global seaweed market is dominated by few countries – most notably China, Indonesia and the Philippines, producing for direct human consumption and processed food industry. Production of seaweeds in Europe is still in its infancy, with promising first experiences with cultivation. Among the challenges are much needed costs reduction, increased scales of production need to be increased, quality improved, and development of successfully refinery processes to make into multiple useful products. These challenges are at the core of the Horizon 2020 Blue Growth project GENIALG.

The future European seaweed sector faces the challenge to produce cost-effectively, in the face of competition from foreign producers and processors. High sustainability standards and product quality are deemed necessary to stand out. Next to the development of technologies for growing, storing and processing seaweeds, GENIALG therefore also assess future value chain from an economic and environmental perspective.

This paper describes first results from the techno-economic and life-cycle assessment of two seaweed value-chains: the production of bio-stimulants from *Ulva spp* and the production of fucoidans from *Saccharina spp*.

We present the techno-economic model that is used to take stock of production costs, operating costs and revenues, and present results of sensitivity analyses. The first results of the LCA are presented to identify the hotspots in environmental impacts. The results of this assessment and use to adjust production and biorefinery processes and inform the discussion on the potential of the European seaweed sector.

USING EARTH OBSERVATION DATA AS A TOOL FOR THE OPTIMIZATION OF GROWTH ON MUSSEL BOTTOM CULTURE FARMS

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(4) Deltares, The Netherlands

Good and reliable data on quality and quantity of food for shellfish are important tools in the optimization of shellfish culture in coastal waters. Spatial and temporal fluctuations in food quantity and quality influence biomass production and meat yield. This case study aims to identify and collect the environmental variables that influence mussel growth on bottom culture mussel farms in the Netherlands using Earth Observation data, with the aim to improve culture practices. In a first step, satellite remote sensing data is combined with field data on mussel growth and meat content and with environmental data on mussel plot scale. Therefore, Sentinel 3 EO data were processed into spatiotemporal information on chlorophyll-a, turbidity, and sea surface temperature. Growth of mussels (*Mytilus edulis*) on bottom culture sites was measured at 12 contrasting locations in the Oosterschelde and 12 contrasting locations in the Wadden Sea from April to October 2017. Similar sized mussels (30 mm ± 2mm) were placed in baskets close to the bottom and monthly subsamples were analyzed for their specific growth rate (SGR) and condition index (CI). Spatiotemporal point data of mussel performance, and EO data of water temperature, food quantity and quality, together with interpolated plot specific data of current velocity, depth and salinity were used to perform a multivariate analysis to determine the factors that explain most of the spatiotemporal variation in SGR and CI on the mussel culture sites. Use of earth observation data for optimizing mussel culture practices on bottom plots is discussed.

TAILOR-MADE SEAFOOD: OYSTER REFINEMENT AS A TOOL OF OVERCOMING CONSUMER BARRIERS

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The concept of tailor-made seafood is based upon consumer driven approach in new product development. A product can either by 'tailored' to meet consumer preferences or by taking away barriers for consumption. Barriers preventing consumption of seafood in general include: lack of convenience, lack of cooking skills, sensorial properties, availability and relative high price levels. More specifically the sensorial properties of the oyster (slimy texture, gulp of seawater, too salty) and the fact that it is often eaten alive seem to be the most important barriers preventing consumption.

Oyster refinement is aimed to: 1. Increase the condition index (measurement for the tissue content of the oyster shell) and 2. Change sensorial properties of the oysters. Oyster refinement therefore might be a method in overcoming some of the consumer barriers to oyster consumption.

In the refinement process market sized Pacific cupped oysters (*Crassostrea gigas*) were kept in basins and fed with either *Skeletonema costatum* or *Rhodomonas baltica* before bringing the oysters to the market. In this way we were able to increase the condition index of the oysters from 11.40% to 15.08% in seven weeks' time. Furthermore during this period the carbohydrate content increased from 3.87 to 4.14 g 100g WW oyster^{-1} most likely due to glycogen storage.

Sensory scores showed significant differences ($P<0.05$) between reference oysters, *Skeletonema* refined oysters and *Rhodomonas* refined oysters for marine odor, fullness of the shell, crunchy texture, chewiness, marine taste and saltiness (see figure 1).

Our results show that using oyster refinement we were able to change the sensorial properties of oysters which were regarded as barriers for oyster consumption. Nonetheless, other consumer barriers may remain as these were not addressed.

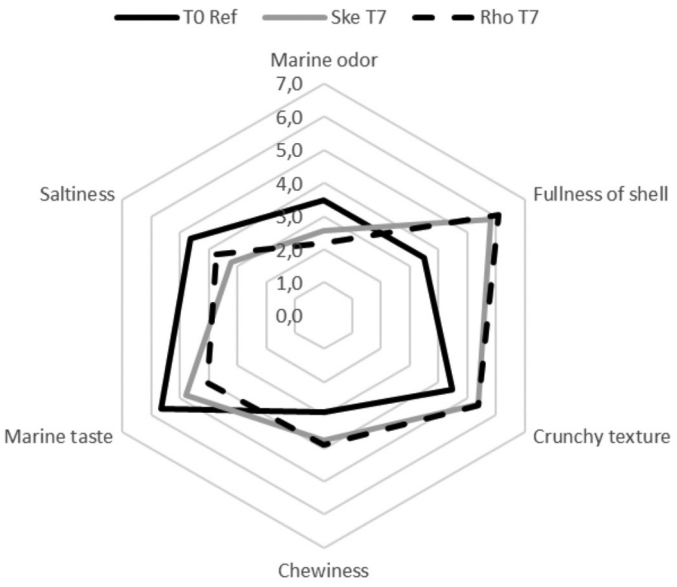

Figure 1: Sensory profile of reference oysters (T0 Ref) and *Skeletonema* (Ske T7) and *Rhodomonas* (Rho T7) refined Pacific cupped oysters.

ACCURATE GENOMIC PREDICTION OF PHENOTYPIC SEX WITHIN EUROPEAN SEA BASS FAMILIES: A UNIQUE TOOL TO UNDERSTAND AND CONTROL SEX RATIO VARIATIONS IN A SPECIES WITH POLYGENIC SEX DETERMINATION

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The European sea bass, *Dicentrarchus labrax*, a major aquaculture species in Europe, has a peculiar polygenic system of sex determination, with several QTLs determining the genetic component of sex. Temperature during the early phases of larval rearing (before 60 days post hatch, dph) also has large effects on sex-ratio: high temperatures (>17°C, HT) masculinize fish that would have developed as females in low temperature (<17°C, LT). In a polygenic system, there cannot be a diagnostic sex marker, and thus it is *a priori* impossible to identify which individuals are sex-reversed by temperature (i.e., the genotypic females that differentiate as males, called "neomales" in fish with chromosomal sex determination).

We produced two families by mating two males with the same female. The progenies were reared in common garden, half of them in a sex-neutral LT temperature protocol (16°C until 60 dph), and the other half in a masculinizing HT treatment (21°C until 60 dph). The fish were sexed at 319 dph, and assigned to their parents with 12 microsatellite markers. Among the assigned fish, 92 HT fish and 84 LT fish from sire A, as well as 78 LT fish from sire B, were selected for genotyping on a 3K SNP Illumina ISelect array. In total, 966 SNP markers were polymorphic within or between the two families, with a call rate >0.90, a sufficient minor allele frequency (MAF>0.05) and no mendelian transmission errors.

Sex was predicted by the genomic estimated breeding value of sex tendency using a genomic best linear unbiased prediction (GBLUP) model, applied to each family at each temperature and various combinations thereof. When data from two temperature treatments were applied, a fixed effect of temperature was included in the model. To the prediction of sex, a receiver operating characteristic (ROC) curve was built using all possible sex tendency thresholds to separate males from females. The quality of the prediction was assessed by the area under the curve (AUC).

The AUC was close to its maximum value of 1 in family A, HT (AUC=0.999). In family A, LT, the AUC was 0.947, and it was 0.994 in family B, LT. Thus, prediction of sex was very good in the three groups. When the data from both LT and HT were combined in family A, the AUC was 0.962, showing coherence between the models at two temperatures. However, when data from family A and family B were combined, the AUC dropped to 0.900, showing that the optimal model was not the same in both families.

We show that despite its polygenic nature, sex in The European sea bass can be predicted with very high accuracy within family using a genome-wide approach (GBLUP). This paves the way to the identification of neomales and to the possibility to identify the phenotypic sex of individual animals at the time of sex determination, which should provide major advances to our understanding of European sea bass sex determination.

This study was supported by the EU's 7th Framework Programme under grant agreement 262336 (AQUAEXCEL), TNA project 0102/06/07/20, and by the French Ministry of Environment under grant CRECHE[2016]

SUCCESSFUL REALIZED SELECTION RESPONSE FOR FILLET YIELD IN RAINBOW TROUT *Oncorhynchus mykiss*

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Fillet yield, expressed as the proportion of edible fillet relative to body weight, is a very important trait to improve in fish sold processed, as it has a direct impact on profitability and can simultaneously decrease the environmental impact of producing a given amount of fillet for human consumption. However, it is a difficult trait to improve by selective breeding, because it cannot be measured on live breeding candidates, its phenotypic variation is small, and, as a ratio, it is not normally distributed and a same change in fillet yield can be the result of different changes in its components, fillet weight and body weight. However, we showed previously that residual headless gutted carcass weight (rHGCW) was heritable and highly genetically correlated to Fillet% in rainbow trout, and that it could be predicted by E8/E23, the ratio of abdominal wall thickness (E8) to depth of the peritoneal cavity (E23), measured on live fish by ultrasound tomography.

In the present experiment, we selected broodstock from the 5th generation of the breeding programme of the Aquaculteurs Bretons breeding company, using either EBVs estimated from slaughtered sibs where rHCGW was measured, or EBVs obtained using the values of E8/E23 measured on the live breeding candidates, or a combination of the two. Seven groups of candidate broodstock were then selected:

- Fish with 15% highest and 15% lowest EBV for rHGCW (group HGC+, HGC-)
- Fish with 15% highest and 15% lowest EBV for E8/E23 (groups Echo+, Echo-)
- Fish which were both HGC+ and Echo+ or HGC- and Echo- (groups HE+, HE-)
- Fish with close to zero EBVs for both traits (group mid)

Seven corresponding groups of offspring were produced on the same day and reared communally. At harvest size (1.5 kg mean weight),they were slaughtered measured for the traits of interest and assigned to their parents by genotyping of 13 microsatellite markers.

All selected offspring from groups HE+, HGC+ and Echo + had a significantly higher EBV for rHGCW than the control group, while all down-selected groups had a significantly lower EBV. Looking at the phenotypic mean for fillet% (correlated response), up-selected fish had significantly more fillet than down-selected fish. The highest difference was between HE+ (69.4%) and HE- (68.2%), a 1.2% difference in fillet percentage. The change in fillet% was explained by an opposite change in Viscera%, while Head % remained stable. Selection using sib info on rHGCW was more efficient than selection using own E8/E23 phenotypes, and downward selection (decreasing fillet%) was more efficient than upward selection.

As a whole, our results are the first demonstration that fillet yield can be improved by selective breeding in fish, using either information from slaughtered sibs or from indirect predictors.

This study was supported by the EU's 7th Framework Programme (KBBE.2013.1.2-10) under grant agreement no. 613611 FISHBOOST (http://www.fishboost.eu/)

IS THE BENTHIC HABITAT OF OYSTER TABLES THE IDEAL SHELTER FOR THE PATHOGENIC AGENT OsHV-1 μvar PERSISTENCE : AN ENVIRONNEMENTAL STUDY

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On the French coastline, *Magallana gigas* is grown on culture tables slightly above the sediment. Thus, oyster farming is well known to create environmental variations especially during mortality outbreaks. Furthermore, since 2008 an Ostreid Herpes Virus (OsHV-1) is causing massive mortalities on oyster spats when the water-temperature exceeds 16°C leading to an organic matter and OsHV-1 flux toward the sediment. Because of the table-light-intensity-attenuation, a more muddy sediment and the protective properties of the biofilm, oyster tables represents the ideal shelter for OsHV-1 persistence which still remain unknown.

During a 41 days period covering spring and neap tide cycle we analysed physical and biological parameters beside and beneath an oyster table at two sampling times (3 hours before low tide and low tide) in the Bay des Veys.

Thus, light and surface temperature were always lower beneath the oyster table rather than beside and daily variations showed the same pattern. The water content of the sediment was higher beneath the oyster table translating a smaller eveporation and also a muddier sediment. Moreover, we also highlighted that epipelic MPB chl *a* concentrations were 1,42 times higher under the table and that the averaged photosynthetic yield was higher beneath the table (0,436 ± 0.035) rather than between the tables (0,366 ± 0.031). Thereby the oyster table habitat would be more eutrophic. These results reveals that the oyster table habitat is more benefic for the biofilm development rather than beside the oyster table. Consequently, these results reinforce the idea that the oyster table represents the ideal shelter for OsHV-1 persistence.

OsHV-1 quantities have been quantified into oysters during this sampling period. Thus, alive oyster spats showed important OsHV-1 quantities (up to $4.5.10^4$ UG/ng DNA) a few days before the mortality outbreak, then dead oysters showed high virus quantities (up to $1.8.10^6$ UG/ng DNA). The mortality of oyster spat showed a classic pattern with a sudden mortality outbreak and mortalities rates reaching 35%. The relation between the sediment and the oysters during this mortality outbreak has also been investigated to explore whether the sediment surface and microphytobenthic biofilm can play a role as reservoir in the viral transmission.

GROWTH DIFFERENCES AMONG GEOGRAPHCALLY DISTANT POPULATIONS IN EUROPEAN PERCH JUVENILES UNDER RAS CONDITIONS: PRELIMINARY DATA

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A large part of European perch aquaculture production relies on wild broodstocks inputs. However, this approach is highly unsuitable for the intensification of aquaculture (*e.g.* selection and domestication), which were shown to have positive effect on number of yield indicators. Identifying the population best adapted for RAS conditions is of crucial importance before starting any genetic manipulation. Main aim of our study was to investigate differences in growth rate among four populations of European perch with different geographic origin supported by genetic analyses.

Eggs ribbons were collected in Poland, Italy, Czech Republic and Slovakia and transported to the facility. After hatching, all populations were fed with *Artemia* nauplii followed by co-feeding (21st DPH-25th DPH). The experiment was initiated on the 31st DPH with artificial feed-trained juveniles. Juveniles were randomly distributed into 60L tanks (150 ind./tank) in triplicates in RAS with T-23°C ±0.5, O_2-90%±10 and pH at 7±0.5. Rearing experiment lasted 28 days, from the 31st to 59th DPH. On respective sampling dates, 15 individuals per tank (45 ind./population) were weighted. Specific growth rate (SGR), growth heterogeneity (GH), mortality rate were calculated from day 31st to 59th PH. To estimate genetic differentiation, fragments of mitochondrial D-loop of control region, 16S rRNA, cytochrome oxidase I and cytochrome b genes were amplified.

Preliminary results showed strong genetic differentiation between Polish population and the rest of the groups. However growing performance had different pattern (Table 1). Polish population had the lowest body weight throughout the experiment, although reaching the highest SGR. On the other hand Czech population had the most promising results with the respect to rearing in RAS, however lower recruitment of SGR. Mortality was not significantly different among populations during 28 days of rearing. The highest and lowest GH showed Slovak and Polish population, respectively, while no significant difference was found in Czech and Italian populations.

The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects „CENAKVA“(No.CZ.1.05/2.1.00/01.0024), “CENAKVA II“(No. LO1205), NAZV project (QJ1510117) and GAJU project (No. 060/2016/Z).

Table 1. Growth parameters of European perch juveniles in Slovak (SK), Czech (CZ), Italian (IT) and Polish (PL) population on the 31st DPH and the 59th DPH expressed as mean ±SD. Different letters in single rows denote statistical difference.

	SK	CZ	IT	PL
Body weight (g)				
31DPH	0.11±0.02^{c}	0.13±0.02^{b}	0.08±0.02^{d}	0.04±0.01^{a}
59DPH	0.38±0.04^{a}	0.60±0.07^{b}	0.55±0.06^{b}	0.30±0.03^{a}
SGR(%$^{-1}$)				
31DPH to 59DPH	4.29±0.31^{a}	5.41±0.25^{a}	6.81±0.56^{b}	7.26±0.61^{b}
Mortality (%)				
31DPH to 59DPH	46.67±10.35^{a}	36.89±2.78^{a}	27.11±9.05^{a}	40.00±9.26^{a}
GH				
31DPH to 59DPH	1.66±0.45^{b}	0.95±0.24^{ab}	1.10±0.26^{ab}	0.82±0.25^{a}

AQUACULTURE FEEDS AND THE RISK OF MYCOTOXINS

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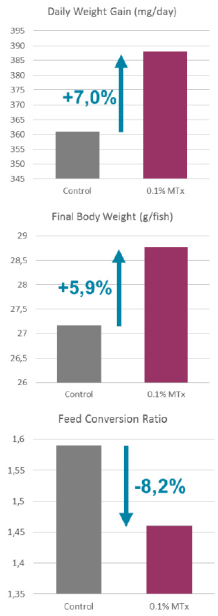
The use of plant ingredients in aquaculture feeds is continuously increasing and for some feeds and species it is the only protein source in the feed. This increasing use brings with it the need to deal with anti-nutritional factors. Some can be eliminated through processing but others are more difficult to eliminate such as potential mycotoxins in the grains used as ingredients in these aquaculture feeds.

Livestock has been dealing with this mycotoxin issue for a much longer period of time and therefore knowledge of the effects are better understood. The comparatively more recent awareness, large variety of species, formulations and the relative inexperience of mycotoxin effects in aquaculture requires a steep learning curve. It would be difficult to believe that mycotoxins affect all livestock species, but aquaculture species would be exempt from this.

Mycotoxins are secondary metabolites produced by fungi present in grain crops, and are produced when fungi are stressed. Stress in this case can be changing temperatures, changing humidity's, harvesting, poor storage conditions, etc. These mycotoxins can be found in grain from any part of the world, present as a single mycotoxin or more commonly as multiple mycotoxins (poly-contamination) in the same batch of grain.

At the farm level, clinical signs of mycotoxicosis are not easily recognized, however in aquaculture, research is being conducted on various species and under various conditions. This can also be seen in the fact that raw materials are regularly checked for only one or just a few mycotoxins. A low mycotoxin level may be considered safe but chronic low mycotoxicosis can influence the feed efficiency and therefore cause a negative economic impact on the farm's performance. Also ynergistic effects due to polycontamination can increase the impact of single mycotoxins when they act in combination with other mycotoxins. Understanding the effects of not only the individual mycotoxins but also the synergistic effects mycotoxins can have, is very important.

A trial was set up in a research facility in the Mekong Delta, with two treatments groups: a control group being fed with a standard diet and a treatment group being fed the same diet with a mycotoxin binder. The diet was tested using a HPLC for 44 different mycotoxins, and 13 mycotoxins were found. 2400 fish were divided among each treatment with 400 (5,5 gram) fish per hapa and four replicates per treatment. Duration of the trial was 8 weeks. At the end of the trial, treatment groups showed a 5,9% increase in final body weight and a 7,0% increase in average daily weight gain. The food conversion ratio (FCR) also showed a decrease of 8,2%.



There is an increase in the use of ingredients that have a risk of mycotoxin contamination and these mycotoxins can have a negative impact on the farms performance. It is therefore important to understand the potential role mycotoxins play in aquaculture feeds.

METHODICAL IMPROVEMENT OF SPERM CRYOPRESERVATION IN A HUNGARIAN COMMON CARP LANDRACE (*Cyprinus carpio carpio morpha accuminatus*)

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Introduction

The *Cyprinus carpio carpio morpha accuminatus* is a state-approved landrace. The Balaton Fish Management Non-Profit Ltd. is the maintenance breeder and owner of the variety (Udvari, 2017). The aim of our study was to improve sperm cryopreservation method. The optimized technique can support the reintroduction of the native populations and satisfy the enhancing angling demand.

Materials and methods

Progressive motility (pMOT %) was measured by using Computer-assisted Sperm Analysis (CASA). In our experiments 5mL straws and 10mL cryotubes were used. In our study, grayling (200 mM glucose, 40 mM KCl, 30 mM Tris, pH: 8.0, Horváth et al., 2012) and pike (75 mM NaCl, 30 mM KCl, 1 mM Na_2HPO_4, 1 mM $MgCl_2x6H_2O$, 1 mM $CaCl_2x2H_2O$, 20 mM Tris, and 0.5% BSA, pH: 8.0, Bernáth et al., 2017) extenders were compared and 10% methanol as cryoprotectant was used. During cryopreservation, a floating styrofoam frame (3 cm above the surface of liquid nitrogen for 7 minutes) and the CRF (Controlled-rate freezer) (from 4°C to -160°C, cooling rate: 15°C/min) were also compared. In *Experiment 1*., sperm (4mL) from 5 males was loaded into 5mL straws. Samples were cryopreserved on a floating frame and in CRF. In *Experiment 2*., sperm (5 males) was mixed with grayling and pike extenders and 6mL of diluted sperm was freezed in 10mL cryotubes using CRF.

Results

In *Experiment 1*., pMOT decreased significantly using CRF (56±1%) and with the floating frame (50±7%) compared to the fresh control (pMOT: 91±2%). No remarkable difference between the two freezing technique was observed after thawing. In *Experiment 2*., no significant difference in pMOT was observed between the grayling (28±6%) and the pike (31±9%) extenders. However both cryopreserved groups showed a significant decline compared to the fresh control (91±2%).

Discussion

Based on our result, the CRF is adaptable for the large-scale sperm cryopreservation in this carp landrace. According to our knowledge, 10mL cryotube was tested successfully at the first time for common carp sperm cryopreservation. Pike extender can be a new novel and an applicable solution in carp sperm cryopreservation beside the generally used grayling extender.

Acknowledgements

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POTENTIAL OF PIKEPERCH ONGROWING IN BALTIC SEA

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Currently, the consumer demand is exceeding the domestic supply of pikeperch in Finland, and the status of fishery stocks and their regulations are not going to make the situation easier. While sea cage ongrowing could provide solution to shortage, the growth and economical potential of pikeperch in Baltic sea conditions and temperatures are currently unknown. Furthermore, climate warming may enhance the potential of pikeperch farming in Baltic sea. This study was designed to provide values to enable evaluation of pikeperch sea cage farming potential.

During 2017, a set of pilot tests was run to determine the possibilities and challenges of transferring pikeperch from a RAS system to net cages in the Baltic Sea for on-growing. The pilot tests revealed that transfer from the RAS to brackish water sea cage rearing is feasible (SGR: 0.99-1.73). During the pilot tests, however, large proportions of non-feeding fish and extensive mortality were also encountered, due to vibriosis and handling stress during measurements.

Based on the experiences gathered from the pilots, we intitialized an experiment for the 2018 growing season to estimate biological and economical values for feasibility of sea cage ongrowing.

The specific questions of the experiment were: 1) Is the ALPHA JECT 3000® vaccine effective against vibriosis in pikeperch, 2) is it possible to gather enough data with minimal handling during the growing season by PIT-tagging individuals in the studied fish groups, and 3) what are the growth and economical parameters in sea cage ongrowing.

In April, pikeperches were transferred from the commercial RAS system to the experimental RAS & sea cage research and learning environment operated in cooperation of Natural Resources Institute Finland (Luke) and Livia College of Fisheries and Environment in Parainen.

The fish were individually measured and the effects of vaccination and PIT-tagging were first monitored in experimental RAS system until the immune response was fully developed (450 temperature days).

In May, fish were transferred to sea cages. After the growing season, all fish were individually measured. Survival, growth and feed conversion ratio were the primary traits compared between the study groups.

THE CONTRIBUTION OF MICROORGANISMS IN BIOFLOCS IN MAKING UNAVAILABLE PHOSPHATE, FIXED IN DIETARY PHYTATE, AVAILABLE FOR NILE TILAPIA

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Phytic acid is the principal storage form of phosphorus (P) in many plant tissues, especially in brans and seeds. Fish lack the intestinal digestive enzyme phytase, required to separate P from the phytate molecules. Because much of the total P in plant based ingredients used in fish diets is present in the form of phytate, diets high in plant ingredients are mostly P deficient. To address this deficiency, phytase is often included in plant-based diets to increase available P. Phytases are also widely distributed among microorganisms. The latter are present in high concentrations in biofloc systems. In aim of this project was to test if in biofloc systems fed a plant-based diet high in phytate but low in available P, Nile tilapia (*Oreochromis niloticus*) can retain more P than tilapia raised in clear water flow-through systems when fed the same diet.

Nile tilapia of approximately 60 grams were used. A 2x2 factorial design was applied, with diet (2 levels: diet with or without sufficient available P) and system (2 levels: flow through or biofloc) as main factors. Per treatment, 20 fish were stocked in 70-L aquaria (3 replicates per treatment). To house the fish in the same size aquarium in biofloc as in flow-through tanks, in the latter the aquaria were linked to a separate 700-L biofloc tank (Figure 1). The water quality of the fish held in biofloc water was controlled through the addition of corn starch aiming to maintain a carbon to nitrogen ratio of 17-18.

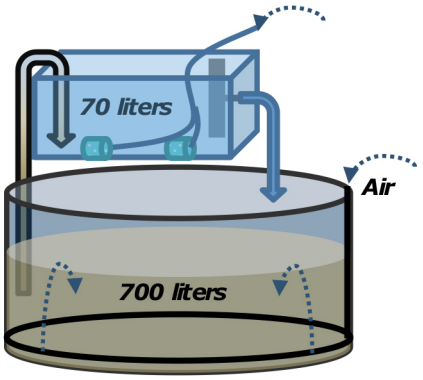

Figure 1 Biofloc system set-up. The water will be pumped from the 700-litre basin into a 70 litre aquarium on one side and leave through an airlift back into the basin from the other side of the aquarium. Each biofloc tank will be aerated and mixed by an aeration ring and each aquarium with 2 air stones

Ingredients	**g/kg**	
	High P diet	Low P diet
Total phosphorous	12.3	8.8
Digestible Phosphorous	7.1	3.5

		1-way ANOVA				2-way ANOVA		
	Unit	Bio LP	Bio HP	Flow LP	Flow HP	Diet	System	D x S
Dry matter	g/kg	284.43 ^{a}	285.93 ^{a}	276.87 ^{a}	274.48 ^{a}	0.889	**0.015**	0.546
Ash	g/kg dm	127.44 ^{b}	139.54 ^{bc}	114.32 ^{a}	140.16 ^{c}	**0.000**	**0.049**	**0.034**
Crude protein	g/kg dm	529.33 ^{a}	540.17 ^{a}	564.72 ^{ab}	582.98 ^{b}	**0.100**	**0.001**	0.648
Energy	kJ/g dm	25.23 ^{b}	24.25 ^{a}	25.27 ^{b}	24.59 ^{a}	**0.000**	0.205	0.292
Fat	g/kg dm	303.98 ^{a}	281.71 ^{a}	313.29 ^{a}	283.66 ^{a}	**0.008**	0.464	0.628
P	g/kg dm	21.62 ^{ab}	23.26 ^{b}	19.03 ^{a}	23.55 ^{b}	**0.001**	0.104	**0.050**

Proximate composition harvested Nile tilapia. Bio = biofloc; Flow = flow-through; LP = low P diet; HP = high P diet.

Two diets were formulated, only differing in digestible available P. In flow-through tanks there was low mortality (2 – 5%). In biofloc tanks the mortality was zero. In flow-through tanks, on the High P diet, Nile tilapia grew faster than on the Low P diet ($P < 0.05$). The feed conversion ratio with the Low P diet was 1.89, compared to 1.45 with the High P diet ($P < 0.05$). Less P (g/kg dm) was present in whole fish in flow-through tanks with the Low P diet than with the High P diet, while the amount of P retained in biofloc tanks with the Low P diet was intermediate between the two flow-through treatments. In conclusion, in biofloc systems Nile tilapia obtained more P from the Low P diet. To explain the high variation between replicate tanks in biofloc systems additional research is required.

FEEDING EUROPE FARMED FISH: TRENDS IN PRODUCTION, CONSUMPTION AND TRADE

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Europe remains the wealthiest seafood market in the world, but faced growing competition with other international markets. Faced with this competition we explore whether Europe maintain enough imports of farmed fish. To answer this question we review trade production, trade and consumption data for Europe and three Southeast Asian countries (Bangladesh, Thailand and Vietnam). Four important trends emerged. First, aquaculture production and consumption in the EU has stagnated over the last decade, while it has boomed in Southeast Asia. Second, Europe's consumption of seafood has levelled off, while consumption in Southeast Asia has continued to expand for both the rich and the poor. Third, the relative share of farmed fish sold from Thailand and Vietnam (the first and second largest exporter to Europe) is declining. Fourth, the EU will continue to become more dependent on seafood imports from other parts of the world to maintain its current level of fish consumption, just as it is losing market share to emerging domestic markets and new export markets such as China. The consequences of changes to global demand hold a number of consequences for Europe. Not only will competition for fish imports continue to grow, the relative power of Europe to regulate safe and sustainable production, as it has done to date, is likely to decrease. In short, as the relative market share of Europe declines, so will its regulatory and normative control (through various food safety, traceability and eco-certification) over the industry. To maintain aquaculture based consumption into the future Europe will either have to expand production, or trade agreements will be required to capture enough fish in an ever more competitive global market.

AN OVERVIEW OF PACIFIC OYSTER RESEARCH IN NEW ZEALAND

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Pacific oyster (*Crassostrea gigas)* farming in New Zealand (NZ) began in the 1970's and has grown into a NZ$ 30 million industry, producing over 2,000 t per year. NZ aquaculture is a fast-growing sector estimated to be worth over NZ$ 500 million per year, with a strategic goal of NZ$ 1 billion in sales by 2025. In this context, selective breeding, disease research and optimisation of hatchery technologies for oysters are high science priorities, with the overarching goal to improve growth and sustainability of NZ oyster production.

In response to the devastating impact of the OsHV-1 virus following its detection in 2010, the focus of NZ's oyster breeding programme switched from improving growth and yield to selecting for virus resilience and enhanced survival. Since then, four cohorts of selectively bred families have been produced and on-grown at two farm sites and a virus lab challenge was developed. On-farm OsHV-1 resilience is moderately to highly heritable and direct selection for enhanced on-farm survival has proven to be effective. This has allowed recovery of the industry to pre-virus levels, and growth beyond. Moreover, consistent strong correlations for mean survival in oyster families between the two virus-impacted farm sites indicate no evidence for strong genotype-by-environment interactions.

We sought to understand the physiological mechanisms underlying resilience to OsHV-1, specifically to determine whether reduced growth or environmental stress might be associated with OsHV-1 resilience. We also examined whether strong selection for viral resilience created unwanted trade-offs in other traits (e.g. growth, hypoxia tolerance). Selected families were raised under different food conditions to create slow and fast growth, and then exposed to hypoxic conditions for 7 days. No trade-offs were apparent, with the more OsHV-1 resilient families demonstrating increased tolerance to low oxygen environments and reduced levels of oxidative stress damage, while growth rate was unrelated to virus susceptibility under lab challenge.

Cryopreservation of oyster D-larvae has multiple advantages such as maintenance of reference families for selective breeding, storage of excess larvae from family crosses as an insurance, or preservation of excess larvae allowing year-round spat production. A protocol to freeze D-larvae was developed using a range of different cryopreservation treatments, and larvae were thawed and successfully reared up to settlement and spat. The numbers of post-thaw spat obtained are likely to be sufficient for selective breeding purposes.

Finally, we investigated the development of microbial assemblages (microbiome) during Pacific oyster larval culture and the effects of different UV seawater disinfection treatments on the microbiome and larval survival. Research to optimise hatchery triploid production, as well as the incorporation of new omics technologies, such as SNP genotyping, to further understand disease resilience, also continues.

ENTERIC BACTERIA EXPRESSING dsRNA TARGETTING ESSENTIAL WSSV GENES PREVENT WHITE SPOT SYNDROM VIRUS (WSSV) IN SHRIMP, *Litopenaeus vannamei*

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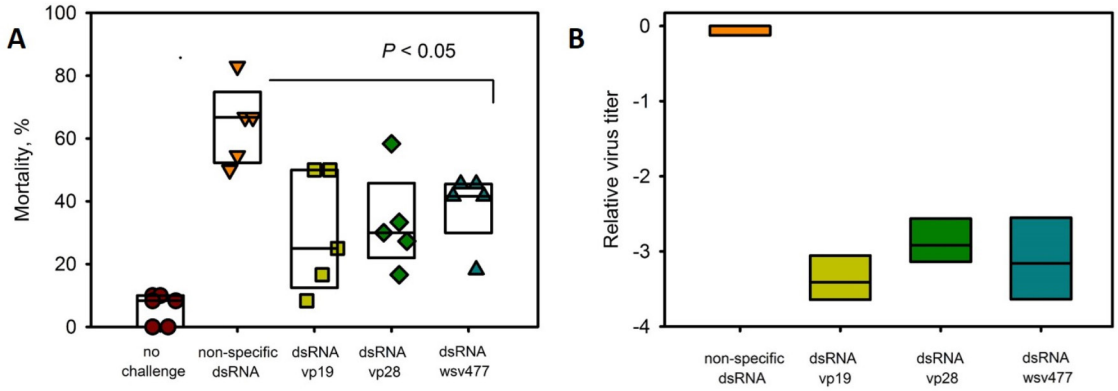
Here we report a novel approach using enteric bacterial to produce and deliver dsRNA in shrimp designed to target and silence essential WSSV genes. Antiviral effect of dsRNA for protecting shrimp against viral diseases has been well documented using intramuscular injection or by feeding formaldehyde-inactivated *E. coli* expressing dsRNA. Both methods work successfully in lab conditions but are not suitable for global application in shrimp farms or for all stages in shrimp development.

We engineered the *Bacillus subtilis* RNaseIII deficient strain BG322 to express dsRNAs targeting essential WSSV genes. *B. subtilis* is frequently found in shrimp intestines and often is used as a probiotic and therefore was an ideal bacterial delivery system for shrimp. We confirmed the ability of BG322 to colonize shrimp intestines by detection of GFP-labeled BG322 bacteria in shrimp intestines following delivery of the bacteria in feed. For virus challenge experiments, shrimp were fed BG322 bacteria expressing dsRNAs targeting essential WSSV genes for 5 days prior to WSSV challenge by injection. We observed a ~50% reduction in shrimp mortality in shrimp fed BG322 expressing anti-WSSV specific dsRNA versus shrimp fed BG322 expressing a non-WSSV targeting dsRNA. In addition, we observed by qPCR analysis a log 3- to 4-fold reduction in virus titer in shrimp fed bacteria expressing dsRNA targeting WSSV genes versus controls.

Delivery of virus-specific dsRNA by enteric intestine-colonizing bacteria is a simple and cost-efficient platform for protection of shrimp against viral infections at all stages of shrimp development. Importantly, once shrimp intestines are colonized, enteric bacteria will continuously produce and deliver the dsRNA molecules to shrimp providing life-long anti-viral protection.



SUPER INTENSIVE RAS TEST WITH CATFISH *Clarias anguillaris*

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The population growth increases the demand of protein sources for human consumption, thus highlighting the need for different production methods, even in the urban environment. New technologies in food production are developed and applied fast. Fish used for human consumption is traditionally from wild catches. However, wild stocks can't answer the increased demand for fish, so there is a need for several aquaculture techniques. According to the Finnish aquaculture strategy, the supply of new fish species to the domestic market should be encouraged. To be safe on a commercial basis for the commercialization of a new species of fish, the "know-how" of the whole fish life cycle should be acquired in theory and practice using systems that are as close as possible to commercially available systems.

Clewer Aquaculture started a three-stage project where the goal was to find and produce information on a new species and its production and to verify the performance of our aquaculture system for warm water. The first stage of the project was about selecting the species and finding information about it from the literature. A fast-growing catfish species, *Clarias anguillaris*, was chosen as the species to be studied and a small amount of fish was also reared in Clewer R&D facility.

The second stage was about verifying the on-growing potential of *Clarias anguillaris* in a super intensive RAS facility. Juveniles weighing 15 grams on average were farmed up to a slaughter size of 1,1 kg in a period of 112 days. The water consumption was around 1,1 m^3/day on average, which allowed the water temperature to be high (25-27 °C) without excessive heating costs. The total amount of fish produced was 4100 kg with a feed conversion rate of 0,8.

Such a fast growth in a species makes it important that the potential commercial farmers have a secure supply of juveniles. The third stage of the project was about getting the know-how on broodstock handling and hatchery procedures. Information was gained on the amount of eggs, survival rates, hatching, start feeding, weaning and other stages of juvenile production. The juveniles reached an average weight of 15 g in approximately 65 days, but there is room for improvement in the practices for this stage.

To summarize the results gotten so far, this species is very suitable for super intensive RAS and it is possible to get it from egg to slaughter size in less than six months. Currently we are working with the processing and slaughtering of the fish and finding the best practices for this species.

SPATIAL GROWTH PATTERNS AND NUTRIENT MITIGATION CAPACITY OF THE BLUE MUSSEL (*Mytilus edulis*) AND KELP (*Saccharina latissima*) ON THE SWEDISH WEST COAST

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The influx of nutrients from land to sea is a vital component of coastal primary production. However, excess nutrient loading can lead to eutrophication of marine coastal waters and is considered one of the largest threats to the quality and ecosystem functioning of the coastal marine environment, with high costs for society. Efforts need to be made to tackle this problem. Cultivation of extractive species has been proposed as a mean of environmentally friendly aquaculture practices and resource management. Bivalve cultivation serves as a means to mitigate eutrophic conditions through top-down biofiltration control of primary production and enhancement of biogeochemical cycling, whereas seaweed aquaculture contributes by removing dissolved inorganic nutrients from the marine environment. However, site selection in aquaculture can be challenging, especially when species from different trophic levels are cultured in an integrated setting (IMTA).

The aim of this study is to investigate spatial growth patterns and mitigation capacity of two extractive marine organisms, the blue mussel *Mytilus edulis* and kelp *Saccharina latissima*, in an open-ended fjord system on the Swedish west coast. A 50-day transplantation experiment was carried out, testing growth of both species in five administrative water bodies (Areas) and 5 Sites within each Area.

Our results show that mussel growth was higher at sheltered inland areas (Fig. 1a), whereas the exposed marine area favoured seaweed growth (Fig. 1b). For mussels, the dry soft tissue growth was up to twice as high at sheltered inland sites (Havestensfjorden) compared to exposed marine sites (Käringön). By contrast, seaweed growth showed a reversed growth pattern with up to a fivefold increase in wet weight at exposed marine sites compared to sheltered inland sites. Furthermore, the nutrient composition of the seaweed demonstrated high carbon but low nitrogen content at exposed marine sites compared to low carbon and high nitrogen at sheltered inland sites. In order to maximize nutrient mitigation our results indicate that site selection is species specific if species from different trophic levels are to synergistically contribute optimal.

In conclusion, there is a mismatch for suitable cultivation sites between *M. edulis* and *S. latissima*. This makes site selection in aquaculture challenging, especially for an IMTA setting.

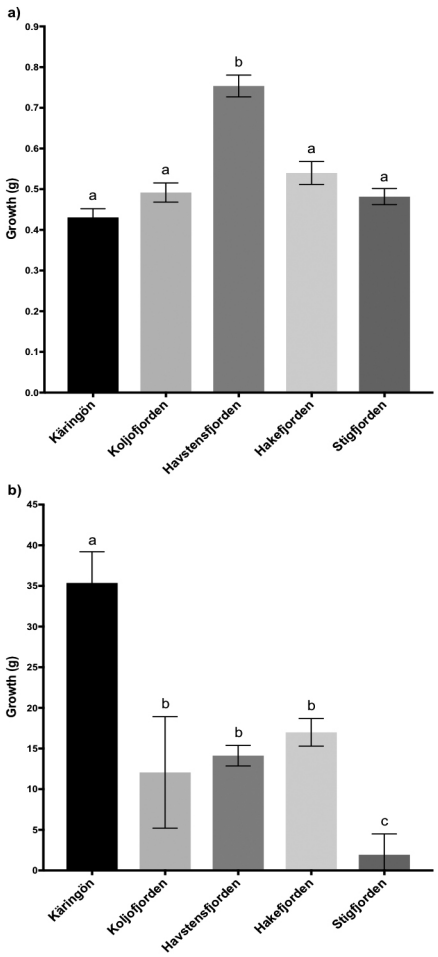

Figure 1. The average growth (g) per area of a) the individual dry tissue weight of *M. edulis*, and b) 50 cm of *S. latissima* seeded cultivation line. The letters above the bars indicate significant differences between mean values based on the SNK-test ($p < 0.05$). Error bars show SE, $n_{M.\ edulis}=75$, $n_{S.\ latissima}=5$.

EFFECTS OF LONG-CHAIN POLYUNSATURATED FATTY ACID FORTIFICATION USING *Aurantiochytrium limacinum* BCC52274 ON GROWTH AND HEALTH OF POST LARVAL PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Polyunsaturated fatty acids (PUFAs) are essential for growth and health of marine species. Especially, n-3 and n-6 PUFAs are not only sources for energy but also precursors of eicosanoids, which function as local hormones for controlling a wide range of important mechanisms e.g., growth, development, stress and immune responses. Producing high amounts of long-chain PUFAs (LC-PUFAs), thraustochytrids are heterotrophic marine protists with high potential for aquafeed application. They have been demonstrated to improve growth and health of several cultured marine animals. However, effects of thraustochytrid application had never been evaluated in larval shrimp. Here, a thraustochytrid, *Aurantiochytrium limacinum* BCC52274 (AL), isolated from a mangrove forest in Thailand and producing high amounts of docosahexaenoic acid (DHA), was fed to *Litopenaeus vannamei* post larvae (PL) from PL1 to PL19 via enrichment in instar-II *Artemia*. Its effects on growth and health of the PL were then evaluated.

Feeding experiment consisted of treatment groups fed with varied proportions (from 0-100 %) of the non-enriched and enriched instar-II *Artemia* and a reference group fed only with instar-I *Artemia*, the stage commonly used in commercial practice ($N = 3$ tank replicates). The reference group was included for practical comparison. Through gas chromatography analysis, AL enrichment replenished DHA deficiency in *Artemia* and elevated LC-PUFA content in the fed PL. Increased feeding amounts of the enriched *Artemia* resulted in positive effects on increased biomass gain, longer average length and decreased length variation of the PL ($P < 0.05$). The highest growth performance was from the group fed with the highest amount of the enriched *Artemia* and comparable to that of the instar-I reference group. AL increased tolerability of the PL under hypo-salinity. PL fed with AL were more tolerant to hypo-salinity than those fed with no AL ($P < 0.05$). In addition, swimming performance of PL fed only with the enriched *Artemia* was improved and exhibited no negative-rheotaxis swimming, which was different from the remaining groups ($P < 0.05$). PL's immunology was not affected by feeding with AL when analyzed by *Vibrio harveyi* challenge, phenoloxidase and superoxide dismutase activities ($P > 0.05$). The results indicated that AL affected on the growth and hypo-salinity tolerance aspects of the PL and suggested its potential to be used for improving performance of shrimp larviculture.

QUALIDIFF – QUALITY-BASED DIFFERENTIATION OF SALMON

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is a project with the anticipated impact to enhance the long-term competiveness and resilience of the Norwegian salmon farming industry through quality-based differentiation strategies. The project will provide novel theoretical insights and practical guidelines regarding whether – or to what extent – relevant intrinsic quality dimension such as type and amount of fat (DHA/EPA), color of the flesh, form and appearance, as well as extrinsic quality dimensions such as ecolabels, branding, and packaging might lead to profitable differentiation of Norwegian salmon.

The project will explore and examine consumers' and industrial buyers' demands and preferences for different aspects of salmon quality as well as price premiums for ecolabels and other quality related product attributes. It will also assess requirements (skills, resources and costs) for various differentiation strategies along the whole value chain. This integrated focus makes for a unique contribution and makes it possible to reveal the true competitive benefits of various differentiation strategies which increases the chances of successful implementation. Through its focus on matching quality with market demand, QUALIDFF will contribute new actionable knowledge with the potential to enhance customer satisfaction, product quality, healthiness, profitability and competitiveness. This will benefit consumers, companies and the society at large.

The production of Atlantic salmon has grown immensely since the early 1980s. Norway is the worlds' largest producer, and salmon is now the most important species in Norwegian seafood exports, accounting for almost 70% of the total export value of seafood. The main products are whole fresh gutted salmon and fresh fillets. These products are mainly commodities with standardized specifications sold in markets where price is primarily affected by supply and demand. There are relatively few differentiated products and volumes for these products are small. This effectively locks much of the Norwegian industry into highly competitive markets wherein large price fluctuations prevail. In order to shield themselves from price-based competition and add more value to its products, the companies and value chains involved, must develop differentiation strategies that can lead to competitive advantage. Value adding in Norway may also give environmental benefits with more efficient logistics, and contribute to increased use of waste and by-products, thus making the industry more sustainable.

Qualidiff

VARIATION OF DISSOLVED OXYGEN (DO) IN FISH CAGES WITH SHIELDING SKIRT FOR PREVENTION OF SALMON LICE (*Lepeophtheirus Salmonis*)

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Prevention is the main strategy to avoid infestation infestation salmon lice in Norwegian salmon farming. Many salmon farmers use different variations of lice skirts, as they have shown to reduce the infestation significantly. Still, there are concerns regarding the risks involved with using skirts, in particular reduced oxygen levels, increased mooring loads, and increased gill health risks due to faster amoebic gill disease (AGD) development and concentration of harmful algae.

Water currents affect dispersion of plankton, including early stages of salmon lice and exchange of water. In addition to oceanographic and topographic conditions, skirts affect currents and DO inside the salmon cage. If one should be able to fully utilize the skirt for safe prevention of salmon lice infestation, one should understand how different variations of skirts behave at different sea states, so that the solution could be customized for the specific site. This is investigated in the project SKJERMTEK, funded by The Norwegian Seafood Research Fund (FHF). As part of this project, measurements of dissolved oxygen (DO) have been conducted to demonstrate the DO distribution within 2 different lice skirts.

The experiments were accomplished at to different aquaculture sites, with a 5m deep impermeable tarpaulin skirt at Korsneset (Ellingsen Seafood AS), and a 10m skirt with 50% permeable tarpaulin skirt at Finnkjerka (Nordlaks AS), located in Lofoten, Northern Norway. 6 DO sensors were used in 2 depths (3 and 5m depth) in 3 different positions within the cages and one outside in 3 m depth (Fig.). A Nortek ADCP current profiler was used for the current measurements.

At site 1 the perimeter of the cage was 100m with 20m deep net, approximately flat bottom containing 83 859 salmon with average weight of 4.1kg. At site 2 the perimeter of the cage was 160m, a conical net with a depth of 50m containing 184173 fish with average weight of 3,4kg.

The result of the measurements and data analysis will be presented.

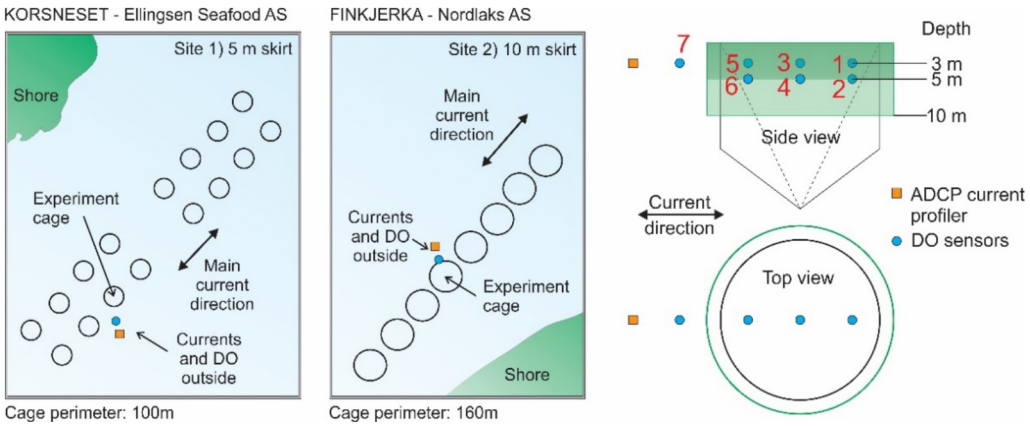

Fig. Measurement area and instrument positions at Korsneset (site 1) and Finnkjerka (site 2).

LIVE FEED COMPOSITIONS AND SELECTION BY SOME FRESHWATER FISH DYURING THE FIRST 30 DAYS IN THE NURSERY PONDS

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Live feed are very crucial to fish fry especially when their yolk sac has been exhausted. At this moment their mouth gap is very small in size and need suitably small preys. Manipulation for growth of zooplankton which are diverse in sizes to meet feed requirement of fish fry are of important to promote their growth and survival during the nursery phase. Two studies were conducted to investigate growth of zooplankton during the first 30 days of rearing in pangasius catfish (*Pangasianodon hypophthalmus*) and snakehead (*Channa striata*). Zooplankton and their densities were sampled every day starting from the day fish fry stocked until day 10 and every 3 days to day 30. Simultaneously, fish specimens were also taken to measure their mouth gap and analyze feed compositions in the gut. Rotifera, Cladocera (water fleas), Copepoda (with their nauplii) and Protozoa were the main zooplankton groups present during the nursery period. In the snakehead ponds total species of zooplankton was 96 in which rotifers were the most abundant one with 46 species (48%). Densities of zooplankton in these ponds ranged from 542,524 to 2,104,859 inds.m^{-3}. In pangasius ponds, 56 species were recorded and rotifers accounted for 33% (19 species). Densities of zooplankton in these ponds were significantly lower ranging 2,344 – 297,979 inds.m^{-3}. However, rotifers and copepod nauplii were abundant in the first 4 days and followed by water fleas and copepods in the rest period. Mouth gaps of pangasius ranged from 225 μm at the first day of hatching to 1143 μm at day 20 and 1493 μm at day 30. For snakehead the size of mouth gap was 241 μm at the first day and up to 1729 μm and 3116 μm at day 21 and 30, respectively. The live feed composition in the fish gut was recorded with 4 main groups including Nauplii, Rotifera, Cladocera, and Copepoda in which Nauplius and Rotifera were mostly abundant in the first 4 days and replaced gradually by cladocerans and copepods. It is obvious that manipulation of the zooplankton growth by fertilizing the ponds in advance to induce growth of zooplankton as live feed, especially the small sized groups to meet the feed requirement of fish fry at the first few days of rearing.

THE INCLUSION OF A GRAPE MARC EXTRACT MODULATES OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) AND BIOCHEMICAL PARAMETERS IN RAINBOW TROUT PLASMA

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Sustainability issues have consistently reduced the inclusion fishmeal in carnivorous fish diets using different plant derived protein sources as replacement. This strategy have been successful when near half of fishmeal is replaced, however higher levels of inclusion of plant protein, particularly soybean meal, are still associated with negative impacts, among these, alterations in the antioxidant defenses in different fish tissues. Many studies in mammals have shown that the intake of plant phenolic compounds have beneficial antioxidant properties, however if the inclusion of this type of compound in carnivorous fish diets have similar effects, needs to be addressed. Hence, our objective was to evaluate changes in antioxidant capacity (ORAC) and biochemical parameters in the plasma of rainbow trout (RBT) in response to the dietary inclusion of a grape marc extract. We used red wine grape pomace as raw material and evaluated different extraction conditions using phenolic compounds contents and HPLC-DAD chromatographic profiles of anthocyanic and non-anthocyanic low molecular phenolics along with ORAC to define the best extraction strategy. The optimized extract was encapsulated (Alginate) by using a spay dryer equipment and added to a control diet (no extract) at 0.5, 1, 2%. After being acclimated, four hundred RBT juveniles (80 g) were distributed to eight 200 L tanks and fed four experimental diets during 30 days. Diets were randomly distributed and delivered three times a day (2% BW). Blood samples were taken from 5 fish per tank at 0, 10, 20 and 30 days. Initial ORAC values in meq Trolox/100 g sample, for the control diet were 250.000 (n=6) and for the encapsulated grape marc extract 220.000 (n=6). Biochemical plasma parameters for the initial fish were inside the ranges described for RBT and no different among groups (Manera and Britti, 2006). We expect to see increments in ORAC values in trout plasma with increasing levels of extract and changes in plasma parameters.

Manera, M., Britti, D., 2006. Assessment of blood chemistry normal ranges in rainbow trout. J. Fish Biol. 69, 1427–1434. doi:10.1111/j.1095-8649.2006.01205.x

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CONSUMER ATTITUDES TO FISH WELFARE IN EUROPEAN SOCIETY

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Eurogroup for Animals and Compassion in World Farming are animal advocacy organisations advancing the welfare of animals through the engagement of civil society, business, and policy makers. Together they have designed and commissioned a survey exploring perceptions and preferences in society with regard to the welfare of fish. The online survey is being carried out with 1,000 respondents in each of 9 EU countries that together have over 75% of the population of the European Union. Fieldwork is taking place in April 2018 with results being delivered by May 2018.

Investigating people's opinions on and understanding of fish welfare, the survey explores a number of value positions, the perceived importance of fish welfare, the relative importance of different aspects of welfare, and includes questions on pain and emotions.

Consumer behaviour is also investigated, with questions on dietary and purchasing habits, the relative importance of different product qualities, and willingness to pay for higher welfare fish products.

The survey explores people's understanding of how welfare is, and should be, positioned for consumers. It determines the perceived benefits of higher welfare production and preferences for how the consumer is informed about welfare. It also explores how welfare relates to different aspects of sustainability and how people would like to have fish welfare regulated.

Building on a few smaller-scale and more narrowly defined studies, this is the first large-scale and in-depth look at social attitudes, preferences and perceptions regarding the welfare of fish. European citizens typically have strong views on animal welfare, and findings from this survey will shed light on their attitudes towards fish welfare - a topic gaining increasing prominence.

N.B. This abstract reflects the finalised content and methodology of a survey being carried out soon after the deadline for submission of abstracts. The results will be received by May 2018 and the presentation at conference will be of the results.

KNOW YOUR FISH: TRACING THE ORIGIN OF SALMON DIET

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The rapid expansion of the aquaculture industry with carnivorous fish such as salmon has been accompanied by an equally rapid development in alternative feed ingredients. This has outpaced the ability of prevailing authentication method to trace the diet and origins of salmon products at the retail end. To close this gap, we developed a new profiling tool based on amino acid $\delta^{13}C$ fingerprints. With this tool, we discriminated with high-accuracy among wild-caught, organically, and conventionally farmed salmon groups, as well as salmon fed alternative diets such as insects and macroalgae. Substitution of fishmeal with macroalgae was detected at 5% difference level. The $\delta^{13}C$ fingerprints of essential amino acids appear particularly well suited for tracing protein sources, and the non-essentials for tracing lipid origins (terrestrial vs. aquatic). In an industry constantly developing new feed proteins and functional additives, our method is a promising tool for tracing salmon and other seafood products

NUTRITIONAL COMPOSITION OF THE POLYCHAETE *Hediste Diversicolor* CULTIVATED WITH LAND-BASED AQUACULTURE WASTE: A POTENTIALLY SUSTAINABLE FEED

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As aquaculture is increasing to meet the demands for aquatic products, it requires more feed and produces more waste (feed leftovers and faeces). The deposit feeding polychaete *Hediste diversicolor* might effectively consume such wastes and transfer them into valuable compounds such as lipids and proteins. Therefore, the aim of this study was to evaluate the nutritional composition of *H. diversicolor* fed with aquaculture waste, and to assess its nutritional value as an ingredient in formulated feed.

H. diversicolor was cultivated for 30 days on five different diets; fish feed, smolt particulate waste, microalgae paste, and a 1:5 mixture (based on carbon content, %) of microalgae paste (Shellfish diet, Reed Mariculture, USA) and smolt waste. Worms reared on fish feed grew significantly faster than worms grown on the other diets. Worms fed with a mix of smolt waste and microalgae paste showed lowest growth, while those fed on smolt waste or microalgae showed similar growth and placed in-between these two groups (*Fig. 1*). The protein and lipid content was 54-58 % and 12-16 % of DW, respectively (**Fig. 1***Fig. 2*). The major lipid classes were found to be phospholipids, triacylglycerol, free fatty acid, and cholesterol. Phytol was found only for worms fed microalgae (*Fig. 3*).

Polyunsaturated fatty acids accounted for nearly 50% of the total fatty acids. Especially a high content of DHA was found in all feeding groups. Moreover, worms contained all ten essential amino acids (EAA) required by aquatic animals. Therefore, our results suggest that *H. diversicolor* might have a high nutritional value, and that worm biomass produced on smolt waste can be a potential protein and lipid source for formulated feeds in aquaculture.

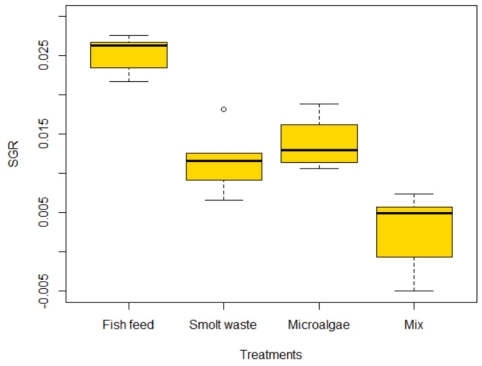

Fig. 1 The specific growth rate of *H. diversicolor* fed with different feedstuff

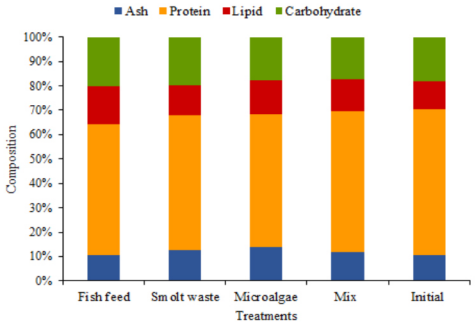

Fig. 2 The Proximate nutritional composition (mean ± SE; % dry weight) of *H. diversicolor*

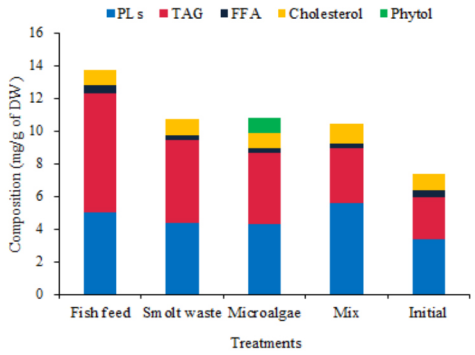

Fig. 3 The lipid classes of *H. diversicolor* with different feedstuff

SPYTAG/SPYCATCHER MOLECULAR CYCLIZATION CONFERS PROTEIN STABILITY AND RESILIENCE TO AGGREGATION

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The capability for thermal and inhibitor tolerance are critical for aquatic enzymes. Loss of enzyme activity is a major challenge in deploying natural enzymes for commercial application. During the past decade, protein engineering approaches, such as site-directed mutagenesis and directed evolution, have been devoted to modify natural enzymes. A post-translation protein engineering strategy SpyTag/SpyCatcher system was recently described. Herein, we generated a thermo- and ion-tolerance xylanase simply by fusing the SpyTag and SpyCatcher peptides to its N- and C- terminus.

The cyclized xylanase C-TFX was achieved by using the SpyTag/SpyCatcher cyclization system. Compared with the linear enzyme, the C-TFX was capable of maintaining more residual activity after heating or metal ion exposure. The results of intrinsic fluorescence and circular dichroism analysis revealed that the isopeptide bond mediated by SpyTag/SpyCatcher cyclization contributed to enhanced thermostability (Fig. 1) and ion stability, probably by stabilizing its secondary and conformational structure. Further, the heat-challenged C-TFX was observed to efficiently degrade natural lignocellulosic substrates.

In conclusion, we demonstrated that the cyclized xylanase was more stable and more resilient to denaturation and aggregation than the linear enzyme. The “superglue” SpyTag/SpyCatcher cyclization enables the cyclized enzyme to maintain its structural conformation which will be of great interests in protein engineering, especially for aquatic enzyme application.

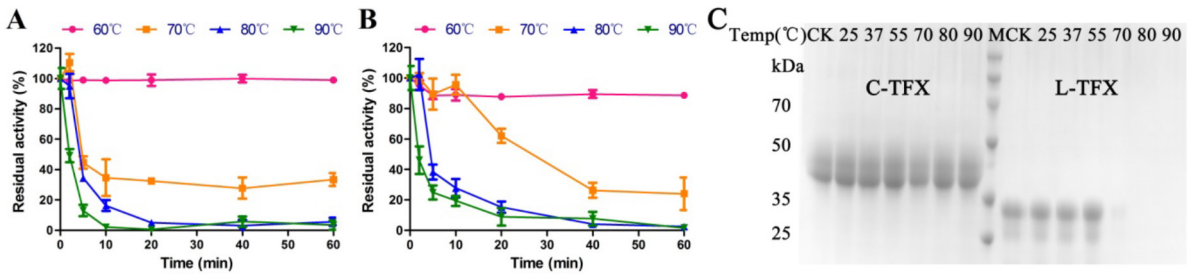

Fig. 1 Spytag/Spycatcher molecular cyclization confers protein stability and resilience to aggregation. A-B, Thermostability of linear (A) and cyclized enzyme (B). C, heat-induced protein aggregation.

GREEN DEVELOPMENT – A RATIONAL APPROACH FOR SUSTAINABLE AQUACULTURE

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Aquaculture is one of the most ancient production activities in human history. Fish culture first appeared in China in the late Shang Dynasty (1300BC-1046BC), and high level of fish culture technology was achieved in the Spring and Autumn Period (770BC-256BC). the first publication on fish culture, *FAN LI on PISCICULTURE*, was a proof of China's rich experience on pond culture of carp at that time. Generally, aquaculture is relatively a non-technology-concentrated industry. Once being closely connected with catch fishery via enhancement release and resource conservation, aquaculture may also become the real motivation for sustainable development of fishery industry.

China is one of the first countries in the world which have recognized the importance of aquaculture as a food producer thus aligned aquaculture with catch fishery. The national policy of "Developing Aquaculture and Catch Fishery Simultaneously" was presented in 1959, and Aquaculture first policy was established with the issue of "Fishery Law" in 1986. In 2016, total aquatic production in China mounted to 69.1Mt in volume and 1200 billion RMB in value. Among this, aquaculture output was 51.4Mt and with a value of 895.4 billion RMB, representing 74.51% and 74.59%in yield and value of total aquatic production respectively,. Species diversity and habitat diversity, diverse consumption habit and colorful cultural tradition have together created the richness of China's aquaculture. According to incomplete statistics, there are more than 200 aquaculture species in China, including fish, crustaceans, mollusc, algae and sea cucumber etc.. In FAO report *The State of World Fisheries and Aquaculture* (2016), global aquaculture output took over catch fishery for the first time in 2014, when aquaculture yielded 101.1Mt products and took 52% of the total fishery production. Among these, cultured animal was 73.8Mt in volume and over 160 Billion dollars in value. As such, China's contribution to global aquaculture production was 60% of the total.

China's aquaculture has been facing with a series of problems and challenges. Generally, the current level of technology has limited overall control of the aquaculture process; aquaculture has negatively affected the local ecosystems, which then affect the aquaculture industry. In technical aspects, we are still in need of more genetic improved fine varieties. Furthermore, consumers' concern of quality and safety of aquatic products, the extensive production modes, continued expansion of production scale, lack of science and technology support, as well as climate change and habitat degradation, all seriously affect aquaculture sustainability.

Green development is the only way for sustainable development. Aquaculture development should focus not only on yield, but also on quality, market demand, resource utilization, and the ecological and social benefits as a whole. We must take into consideration the ecological and environmental carrying capacity, make Ecosystem-based Management as our guiding ideology, and advance green aquaculture industry. Green development is a mode of economic growth and social progress targeting efficiency, harmony and sustainability. As an innovated development mode rooted in tradition, green development is based on ecological and resource carrying capacity, and is built upon a merged foundation of economic growth and ecosystem protection.

Transformation from output-focus to quality-focus, then to green development, symbolizes the maturation of aquaculture industry. Some new development in aquaculture in China such as Integrated Multi-Trophic Aquaculture (IMTA), Rice-Fishery Integrated System (RFIS) and Enclosed Recirculation Aquaculture System were presented as examples of green development of aquaculture in this paper.

MICROALGAE AS FEED SOURCE FOR AQUACULTURE: INFLUENCE OF NITROGEN LIMITATION ON LIPID PRODUCTION AND DISTRIBUTION OF FATTY ACIDS

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The global supply of fish oil is limited and it can not meet the future demands for aquaculture and human consumption. Microalgae are natural primary producers of n-3 LC-PUFA and are widely used in aquaculture either for direct consumption or indirectly as live feed for fish larvae.

In the present study, the effect of N-limitation on the lipid content and fatty acid profiles of *P. tricornutum*, *Isochrysis* aff. *galbana* clone T-Iso, *R. baltica* and *N. oceanica* were investigated. Microalgae cells were cultivated by two different methods, batch and semi-continuous culture to create a gradient in N limitation. The production of these fatty acids was the main focus of the present study. Furthermore, we compared the distribution of EPA and DHA in different lipid classes in the four microalgae species and evaluated the potential of using microalgae as alternative of fish oils in aquaculture feed.

The results showed significant variations in the lipid content and the fatty acids profiles in the four marine microalgae species, and also between the two cultivation methods in each microalgae species. All four species accumulated lipids mainly in the form of triacylglycerols in response to increasing nitrogen limitation, however, the degree of lipid accumulation did not increase with increased nitrogen limitation in *N. oceanica*. The highest amount of lipids was found in *N. oceanica*, indicative of the superiority of using this species for lipid production. *Isochrysis* aff. *galbana* clone T-Iso was the only species where the fraction of PUFA increased with extended N-limitation, suggesting that the stronger N-limitation gave a desired increase in PUFA, specially the content of DHA. Total lipid productivity, however, showed no increase with increased nitrogen limitation except in *N. oceanica* although the lipid content has been increased over 50%. *P. tricornutum* had the highest EPA content while *N. oceanica* showed the highest EPA productivity due to the accumulation of lipid and high biomass. The highest DHA productivity was found in T-Iso from batch culture mainly due to the high lipid and DHA content. In conclusion, *N. oceanica* and T-Iso are two promising microalgae strains for producing long-term alternative source of lipid and *n*-3 LC-PUFAs for fish feed.

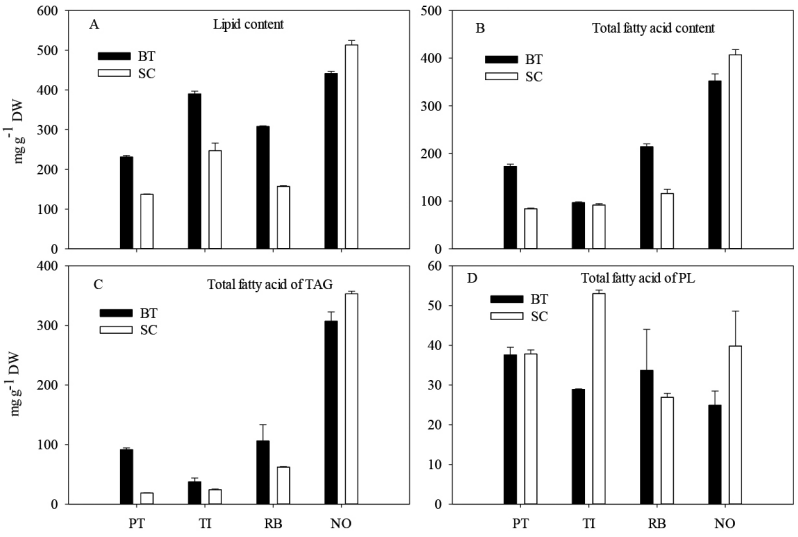

Figure 1 The total lipid content (A), total fatty acid content (B), total fatty acid content in triacylglycerol (C) and total fatty acid content in polar lipids (D) of four marine microalgae species cultured under nitrogen limitation, by batch(BT) and semi-continuous culture (SC). *Mean with SD (SE) bars.* PT: *P. tricornutum*; IT: *Isochrysis aff. galbana clone T-ISO*; RB: *R. baltica* NIVA-5/91; NO: *N. oceanica*

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

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The environmental implications of integrated multi-trophic aquaculture (IMTA) have been well studied in China but few studies have empirically investigated the potential economic benefits. This study investigated the technical and economic feasibility of physically integrating the noble scallop *Mimachlamys nobilis* (Reeve 1852) with existing fish monoculture farms in Hong Kong.

Scallops were grown for 201 days from June – December in lantern nets hung from fish farm platforms at treatment depths of 1 m, 3.5 m and 6 m at a fish culture zone that had a maximum depth of 14 m. Only the 1 m treatment attained the target shell height-at-harvest of 80 mm. Fitted von Bertalanffy growth functions showed significant differences in growth performance between depths. VBGFs projected that the 3.5 m and 6 m treatments would require an additional 26 and 59 days of culture to reach 80 mm. Mortality was significantly lower at 1 m (53 ± 12.5 %) compared to 3.5 m (70 ± 9.0 %) and 6 m (83 ± 4.5 %). The slower growth and higher mortality at 3.5 m and 6 m were probably caused by periodically low oxygen in deeper water which dropped to 4.96, 3.08 and 1.73 mg.L^{-1} at 1 m, 3.5 m and 6 m in mid-summer. Growth, mortality and financial data from the field trial were used in bioeconomic assessments of two typical farm sizes; small (45 m^2) and large (315 m^2). The initial investment, discounted payback time and 10-year net present value of the projects was US$ 5,485.51, three years and US$ 20,211.33 for the small farm and US$ 27.659.03, two years and US$ 227,406.49 for the large farm (Fig 1). Sensitivity analysis revealed that the profitability of operations was sensitive to changes in mortality and sales price.

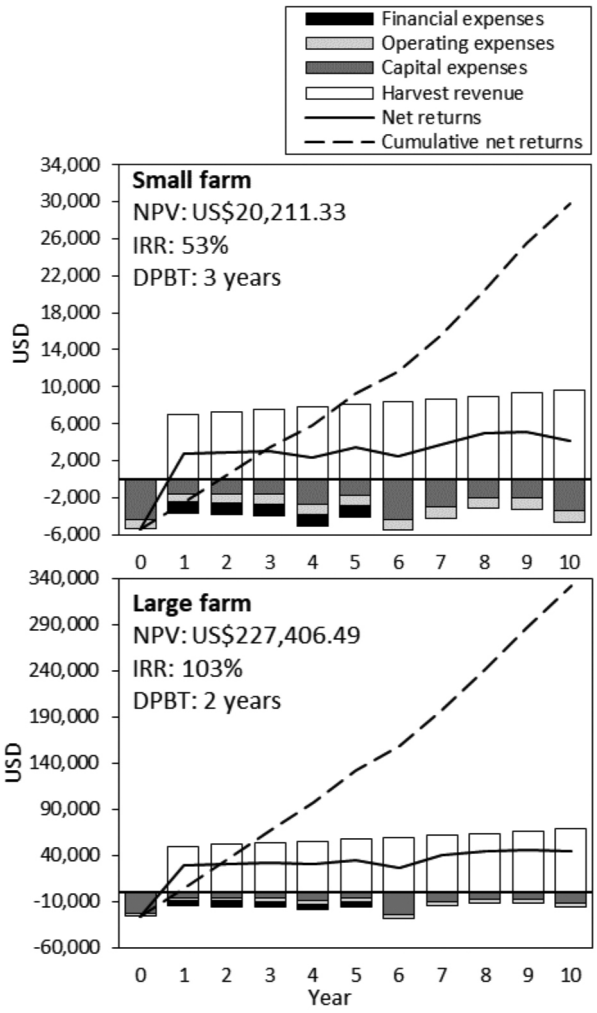

Fig. 1 10-year cash flows for the integration of scallop *Mimachlamys nobilis* at existing fish farms in Hong Kong.

EVALUATION OF THE USE OF BIOBALLS IN THE NITRIFICATION PROCESS OF PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*, BIOFLOC TECHNOLOGY CULTURE SYSTEM

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Litopenaeus vannamei is the most cultured marine shrimp in all types of systems including the Bioflocs Technology System (BFT). Bioflocs are formed by microorganisms (bacteria, ciliates, diatoms, nematodes, etc.), feces, feed remains, and carapace, among others. Among these, autotrophic bacteria are responsible for the nitrification process. This step is crucial in this type of culture since the nitrogenous compounds in high concentrations are toxic to the shrimp. This study aimed to identify and promote the development of nitrifying bacteria by adding artificial substrates in a *L. vannamei* BFT culture.

The experiment consisted of three treatments with three repetitions (3x3) as follows: (1) control: clear water in which bioflocs were formed; (2) IB: clear water with substrate (immature "bioballs"); and (3) MB: clear water with mature "bioball" inoculum from a recirculation system. All treatments were stocked with shrimp juveniles (4.92±0.45 g) in 9 tanks with 200 L for a stocking density of 200 shrimp/m^3. The shrimps were fed 2x/day with a commercial feed containing 38% crude protein (Guabi-potimar 38% CP) following a feeding table, and daily observations of food intake were made over the four weeks of the experiment (28 days). Biofloc and "bioball" samples were collected to detect the growth of the population of nitrifying and heterotrophic (pseudomonas spp.) bacteria by FISH (Fluorescent in situ Hybridization).

There was no significant difference between treatments ($P>0.05$) for survival, obtaining mean values greater than 88%. The MB treatment presented higher final weight, final biomass, productivity, weekly weight gain and lower FCR ($P<0.05$). The use substrates like bioballs that already contain nitrifying bacteria is an effective option for avoiding high concentrations of ammonia and nitrite and to provide a better quality of water throughout the crop.

BLUE LUPIN FOR WHITE SHRIMP (*Litopenaeus vannamei*)?

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Formulated feeds used for the growing production of carnivorous fish and crustaceans generally have a high protein content still met mostly by fish meal supply. Maintaining the steady and enormous growth of aquaculture production requires practical alternative protein sources. Terrestrial protein sources have been the focus of feed research for more than twenty years. Many studies test legumes like field peas, lupin and faba bean. Several different cultivars of lupin have been tested with different fish species mainly in Australia. The results of these studies are promising for example for salmon and seabass but. Lupin diets with a relevant replacement level for high value crustaceans are tested in the present study.

An experimental diet (Basis) with 30% fish meal as main protein source was formulated. *Lupinus angustifolius* kernel meal was added as 10, 20 and 30 % of the diet, incrementally replacing fishmeal. All diets were balanced to meet the requirements of *L. vannamei* in the grow out phase, concerning energy content, protein and amino acid profile, lipid and fatty acid composition, vitamins and minerals. These diets were compared with a commercial control.

Feeding experiments were conducted over 8 weeks in a RAS device, with 18 separate 50 l tanks.
Mean survival rate of the shrimps was 65% across all treatments. Mean biomass of animals fed with high lupine levels (L30) was significantly lower than in control diets (Basis and Commercial control) and L10 after 8 weeks. Metabolic analyses showed malnutrition of animals fed the L30 diet concerning glucose and triglyceride levels, although appropriate protein provision was attained with all experimental feeds.

The total haemocyte count showed significantly higher counts in animals fed the L10 diet, which hints at an elevated immune capacity of these animals. This is an intriguing result concerning immunostimulation in cultured animals and needs further investigation. Untreated lupine meal can be used as an alternative protein source at rates of up to 10% (-20%) of the total feed (= **30-40% of animal protein**).

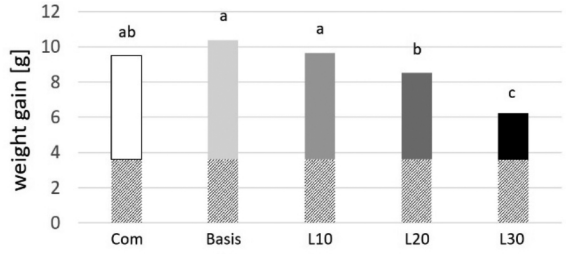

Figure 01: Weight gain of L. vannamei *within 8 weeks*

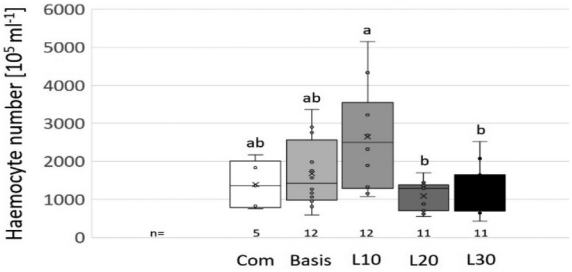

Figure 02: Total haemocyte counts show feed dependent variation

COMMERCIAL AFRICAN CATFISH *Clarias gariepinus* RECIRCULATING AQUACULTURE SYSTEMS: ASSESSMENT OF ELEMENT AND ENERGY PATHWAYS WITH SPECIAL FOCUS ON THE PHOSPHORUS CYCLE

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The sustainable reuse of effluent waters and deposited solids from African catfish (*Clarias gariepinus*) recirculation aquaculture systems requires a deeper understanding of the nutrient and energy flows and material pathways. Three commercial systems, differing in stocking density and feeding regime, were sampled for nutritive and pollutant elements of the input (tap water, feed) and output (fillet, carcass, process water, solid wastes) by ICP-OES/MS and calorimetry. Highly water-soluble elements, e.g. potassium, accumulated in the water, while iron, copper, chromium and uranium where abundant inside the solids. 100.0% of feed derived phosphorous was accounted for, 58.3-64.2% inside the fish, 9.7 – 19.3% in solid wastes, and low concentrations 9.6-14.5% in the process waters (Fig. 1).

About 7.1-9.9% of the fish feed accumulated as dry matter in the deposited solids, including 5.5-8.7% total organic carbon, 3.7-5.2% N. 44.5-47.1% of the input energy were in the fish and 5.7-7-7% in the deposited solids (Fig. 2). The macro-nutrients potassium, nitrate, phosphate and the micro-nutrient iron were deficient with respect to aquaponics production.

Low energy contents and adverse C/N-ratio restrict the solely use of African catfish solids for biogas production or vermiculture. Combined use of the outputs as biogas supplement, effluents and solids for aquaponics farming or in aquaponics *s.s.* combined with additional nutrients appears possible.

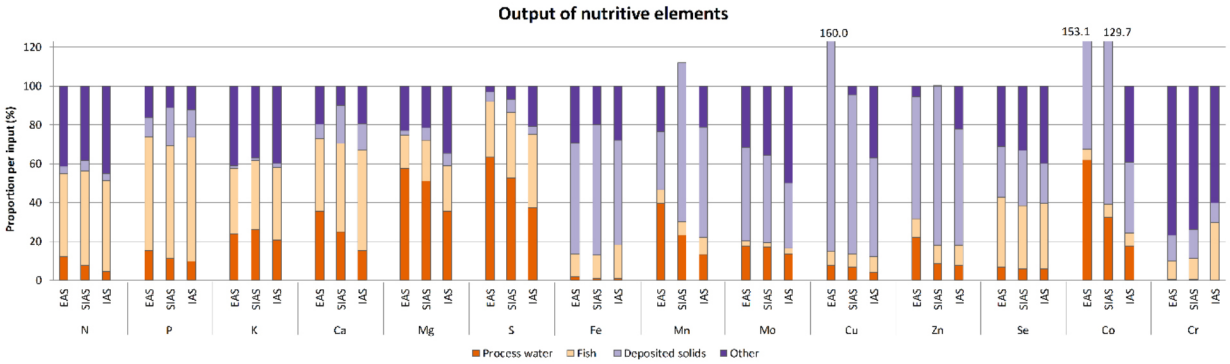

Figure 1: Nutrient allocation

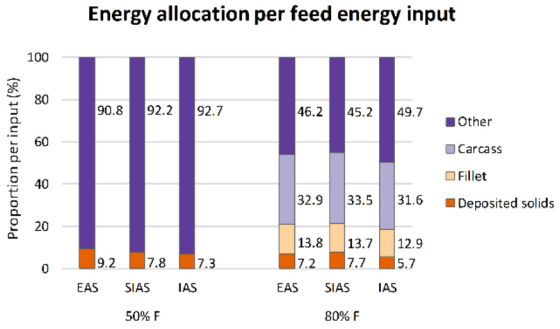

Figure 2: Energy allocation

PROMOTING THE CLUSTERING OF SMALL TO MEDIUM SIZED AQUACULTURE ENTERPRISES AND AREA MANAGEMENT OF ZONES THROUGH FURTHER DEVELOPMENT OF THE AQUACULTURE PARK CONCEPT

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Many countries have allowed ad hoc aquaculture development based on the application of licenses for farm sites selected by developers. This has led to increasing conflict with other users of the coast and made management and control of the industry difficult.

Many countries are now organising aquaculture development into aquaculture zones (for example aquaculture zones in Turkey and Mariculture Parks in the Philippines) where aquaculture takes place in a suitable site where conflict with other coastal users is minimised and there are long term legal rights for production in those areas.

Clustering of farms also allows the use of shared infrastructure for example jetties for loading/unloading feed and fish, boat mooring areas, coastguard security etc. Clustering also encourages the clustering of the aquaculture support industries in the same area for example, hatcheries, cage construction, net cleaning, net mending, packing, processing and marketing.

There are top down organisation of clusters where a company, committee or agency organises and manages production from SME producers (Mariculture Parks in the Philippines, Nucleus Estates in Indonesia, contract growing, etc.) or there are bottom up organisation of clusters where the small scale producers agree to work together to manage themselves as a cluster (farmer associations).

Area management of small to medium aquaculture enteprises has developed in different forms in different parts of the world with different levels of success and failure. Many countries new to aquaculture are now considering the use of the aquaculture park concept to facilitate and control aquaculture development (for example Uganda and Zambia) but the concept needs to be adapted to the different social and cultural situation and production needs.

The aquaculture park concept needs to be adapted to the locals laws and regulations on the allocation of the aquatic space, the management objectives and needs (biosecurity, environmental, marketing, etc.), the local organisational model for cooperating producers (farmer associations, clubs, etc.) and the technical and financial support required until the aquaculture park is self supporting.

This paper discusses these adaptations and potential solutions.

STURGEON CONSERVATION AND CULTURE ISSUES: THE EXAMPLE OF THE SIBERIAN STURGEON, *Acipenser baerii*, BRANDT, 1869

Patrick Williot *, Mikhail Chebanov and Guy Nonnotte

In contrast of being endangered within its entire natural geographical area, the species is the most widely spread farmed sturgeon. However, most of the farming of this species for both caviar and meat, has been developed outside of its natural range. As a result of this very brief panorama on the status of the species, cultured issues are mobilized for the production of young specimen (mostly fingerlings) for either stock enhancement or for the initiation of complete biological production cycle under farming conditions. Two additional lines belong to the subject. The first deals with the use of the species as a biological model to help develop conservation strategies for another species (e.g. the European sturgeon, *Acipenser sturio* Linneé 1758), including the techniques of reproduction, some physiological approaches, genetics, feeding, tagging and/or marking, the study on the stomach contents, and welfare issues. The second line of investigations relates to ecological issues related to the risks of introducing a new species (such the Siberian sturgeon) as specimens of this species may escape and then colonize a non native environment.

This presentation aims at using both the experience of the authors and the literature to document these issues.

Some key conclusions point to the complementarity between Siberian sturgeon conservation and culture issues and the absolute necessity to openmindly embrace these matters.

VALUATING POTENTIAL ECOSYSTEM SERVICES OF A FRENCH POND FARM WITH A LIFE-CYCLE ORIENTED METHODOLOGY

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Since the 80's, aquaculture has increased and will supply nearly two thirds of fish consumption worldwide by 2050 (FAO, 2016). This tendency is not observed everywhere. French pond farms are jeopardised with a weak and declining production. Moreover, they have multiple roles used by different stakeholders, such as hunting, angling, water reservoir, as well as fish production. The concept of ecosystem services is able to take this whole multi-functionality into account. Numerous methods exist for valuating ecosystem services, notably contingent methods. An objectivised method could better assess the ecosystem services.

We developed a method based on Life Cycle Assessment and Emergy Accounting (Odum, 1996) following the four steps of LCA (ISO, 2006). (i) *System boundary definition* consists of defining boundaries of the aquaecosystem. (ii) *Ecosystem flux inventory* consists mainly of quantifying emissions captures of matter and resource use, as well as emergy flows. (iii) *Potential ecosystem services valuation* consists in relying each flow to ecosystem services and then aggregates flows into ecosystem services. Two types of metrics are used, the potential value of ecosystem services and the potential environmental work done to produce the service. (iv) *Valuation interpretation* follows the classic LCA step.

We assessed a bundle of 20 ecosystem services (from the Common International Classification of Ecosystem Services, (Haines-Youngand Potschin, 2013)) for a fish farm pond located in the Dombes area (France). We modelled the pond ecosystem using EcoPath with EcoSim, in order to detail the flows in the trophic chain. Preliminary results indicate that the pond has a great potential supply of ecosystem services. Our method permitted to assess the level of provisioning services (fish production), and also regulation services, such as climate change regulation, and water depuration. Both metrics permitted to estimate ecosystem services and associated environmental works, giving perspectives in management optimisation. It was difficult to take cultural ecosystem services into account, because of the environmental methodologies used.

The proposed methodology is a step toward a practical valuation of bundles of ecosystem services of aquaculture. Despite the weak inclusion of cultural ecosystem services, it is able to consider different categories of ecosystem services. We hope that the both metrics can help to give us keys for a better management of ponds, and to enhance the recognition of ponds and their multi-functionality in France.

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EFFECT OF ORGANIC AND INORGANIC DIETARY SELENIUM SUPPLEMENTATION IN RAINBOW TROUT *Oncorhynchus mykiss* BROODSTOCK ON THE OXIDATIVE STATUS IN PROGENY

Pauline Wischhusen*, Maroussia Parailloux, Mickael Briens, Pierre-André Geraert, Antony Jesu Prabhu, Sadasivam J. Kaushik, Benoit Fauconneau, Stéphanie Fontagné-Dicharry

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The replacement of marine derived ingredients by terrestrial plant based feedstuffs in fish diets usually leads to a reduction of total selenium (Se) content. Se is an important micronutrient needed to preserve the oxidative status in cells. This study was aimed to assess the impact of different forms of dietary Se supplementation in a plant-ingredient based diet on the antioxidant status in rainbow trout (*Oncorhynchus mykiss*). The fish (initial mean weight: 1100 ± 990 g) were divided into three groups consisting of 15 males and 25 females each. Each group was fed one of the three test diets (Se1, no selenium supplementation (basal Se level: 0.3 ppm); Se2, supplemented with 0.3 ppm sodium selenite or Se3, supplemented with 0.3 ppm hydroxy-selenomethionine) over a 6 month period prior to spawning.

We did not detect any effect of dietary Se supplementation on growth or survival in the different groups. There was no effect on glutathione peroxidase (GPX) or other antioxidant enzyme activity in the liver of female or male broodstock, whereas in the swim-up fry, Se-dependent GPX activity was significantly higher in both Se supplemented treatments with highest activity in the Se3 group (data not shown). Gene expression of Se-dependent selenoprotein SePP1x1 was significantly higher in Se2 treatment of female liver than in the other groups, while in the swim-up fry, gene expression of the Se3 group was enhanced not only for SePP1, but also for the glutathione peroxidases GPX1a1 and GPX1b2, as well as MsrB2 (Figure 1).

Overall, these results show that dietary selenium supplementation in rainbow trout broodstock can improve the antioxidant status in the progeny in a different way depending on Se source.

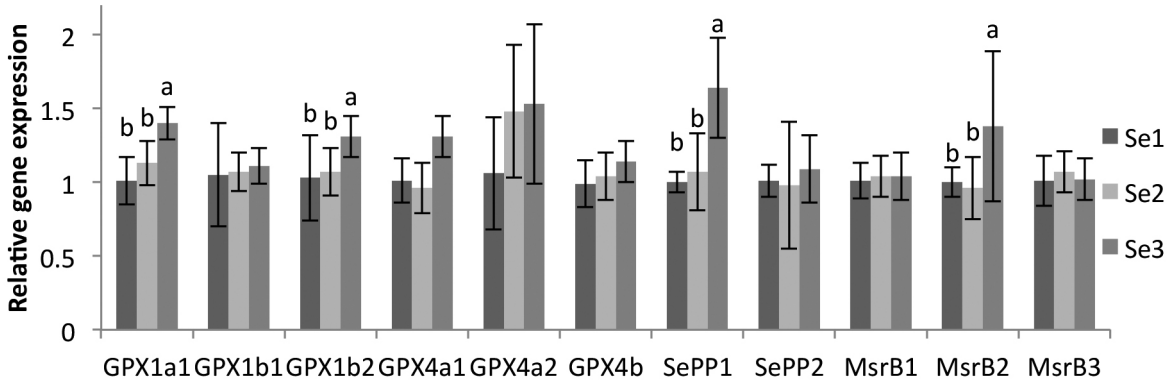

Figure 1 Relative mRNA expression of Se-dependent proteins normalized to ß-actine RNA in whole body swim-up fry. For each gene, means not sharing the same letter are significant different according to ANOVA ($p<0.05$) followed by Newman-Keuls test.

ESTIMATION THE WASTES LOAD FROM COMMERCIAL PRODUCTION OF NILE TILAPIA (*Oreochromis niloticus*) IN CAGES BY MEANS OF BIONERGETIC MODEL

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The culture of tilapia using net-cages in reservoirs has been overspread in tropical areas. Nevertheless, the nutrients and organic matter release in the body of water remain an environment issue. This study aimed to determine the feasibility to use the bionergetic modeling approach and mass balance to estimate the particulated (WP) and dissolved (WD) wastes, total phosphorous (P) and total nitrogen (N) discharged in reservoirs by commercial tilapia (*Oreochromis niloticus*) net-cage farms.

A farm comprised of 18 net-cages (43 m^3 each) located in Chavantes reservoir, São Paulo, Brazil (644.481 E, 7.413.975 N) was used as a model. Each cage was stocked with juveniles I (31.0 ± 3.5 g) at 70 kg/m^3. After 45 days, the stocking density decreased to 40 kg/m^3 for 170 days, when the cages were harvested (mean harvested size = 810 ± 7 g) The culture was divided in three phases: juvenile I (JI), from 30 to 100 g; juvenile II (JII), from 100 to 500 g; market weight (MW), above 500 g. Fish growth performance was monitored and the body composition was analyzed monthly. At the same time, the digestibility of commercial diets used was assessed for each phase of culture. The relationships of body weight and body content of water, protein, fat, ash, gross energy, phosphorus and nitrogen were determined by regression analysis. Based on these equations the factorial bioenergetic model Fish-PrFEQ® (adapted to tropical species) was determined.

The diets used in each of phases JI, JII, MW have, respectively, 32, 29 and 29% of digestible protein; 15, 14 and 12 MJ kg^{-1} of digestible energy; and 85, 70, 68% of digestible phosphorus. The average of retained energy during grow was 6.15 MJ kg^{-1} of fish. The mean value of solid wastes discharged in the environment was 295 kg for metric tons of produced fish. The total load by tone of fish produced of particulated nitrogen was 11kg, particulated phosphorous was 6 kg, dissolved nitrogen was 46 kg dissolved phosphorous was 4 kg.

These preliminary results indicate that bionergetic model may be used to estimate the wastes produced by net-cage fish culture in tropical regions.

(FAPESP, CNPq, FINEP)

FEASIBILITY STUDY OF MUSSEL *Mytilus trossulus* FARMING IN THE POLISH ZONE OF SOUTHERN BALTIC SEA

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Poland does not have a tradition of marine aquaculture. Traditionally only inland farms are present. In spite of this, marine aquaculture was identified by the European Commisson's Blue Growth agenda for the Baltic Sea Region as one of the most promising sectors of the region's maritime economy in terms of growth and job potential. It may be however challenging to perform marine farming in Southern Baltic Sea because of low salinity and high eutrophication. Among the few candidate organisms for aquaculture in this region, blue mussels possess several characteristics making them especially useful for this purpose. In the present study we report results of experimental farming of mussels in the Puck Bay together with an assessment of the practicality and cost-effectiveness evaluation, implemented as part of the project InnoAquaTech (EC, Interreg South Baltic).

POTENTIAL USE OF DIETARY TWO SINGLE PROBIOTICS AND THEIR MIXTURE AS THE ANTIBIOTIC REPLACERS IN RAINBOW TROUT *Oncorhynchus mykiss* AND STARRY FLOUNCER *Platichthys stellatus*

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Two feeding trials were conducted to investigate the potential use of dietary two single probiotics (*Bacillus subtilis* and *Bacillus licheniformis*) and their mixture as the antibiotic replacers in rainbow trout and starry flounder. Results of the first feeding trial indicated that single (*B. subtilis* or *B. licheniformis*; 2 × 109 CFU kg -1 diet) and their mixture (*B. subtilis* + *B. licheniformis*; 2 × 109 CFU kg -1 diet) had equal beneficial effects as the antibiotic replacers in terms of growth performance, non-specific immune responses and disease resistance against *Aeromas salmonicida* in rainbow trout. In the second feeding trial, also results showed same beneficial effects of single (*B. subtilis*, 2 × 109 CFU kg -1 diet) and their mixture (*B. subtilis* + *B. licheniformis*, 2 × 109 CFU kg -1 diet) as antibiotic replacers on growth performance, non-specific immune responses and disease resistance against *Edwardsiella tarda* in starry flounder. Therefore, these results indicated that *B. subtilis*, *B. licheniformis* and the mixture of *B. subtilis* and *B. licheniformis*, and *B. subtilis* and the mixture could have the potential use as the dietary antibiotics replacers in rainbow trout and starry flounder respectively

TAGGING EFFECTS ON ATLANTIC SALMON IN DEPTH-MODIFIED CAGES

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Electronic tags are widely used to study farmed fish behaviour. However, tags may alter the behaviours being measured and alter welfare outcomes for studied individuals. We conducted an experiment to assess Atlantic salmon behaviour in different cage types and observed negative tagging effects on salmon in depth-modified cages, which confounded tag data.

The experiment used both standard and depth-modified "snorkel" cages that pushed salmon below a 4 m net roof or into a restricted parasitic sea lice barrier tube space to the surface that had freshwater or seawater pumped into it. Implanted data storage tags monitored swimming depths of 6 Atlantic salmon in triplicate cages of each type over 2 weeks. All tagged individuals survived in standard cages, whereas tagged fish survival fell to 66 % in depth-modified cages. Surviving individuals swam deeper in depth-modified than standard cages, and dying fish in depth-modified cages were detected moving into shallower depths.

We calculated how the excess weight in water and reduction in peritoneal cavity volume of fish, owing to the implanted tag, reduced their maximum depth of neutral buoyancy. These effects likely impacted on the behaviour and survival of tagged fish in our study. We recommend that future fish tagging studies use tags with excess weight in water and internal implantation methods with caution, and carefully consider the incorporation of data from tagged fish that are known to have perished. This is particularly pertinent when using tags to examine the behaviour of fish under different depth treatments.

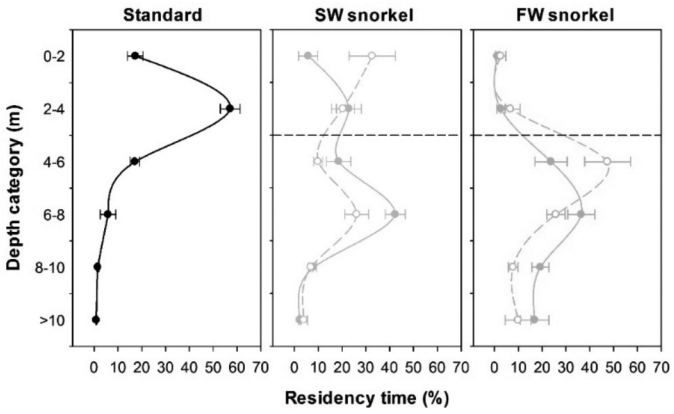

Figure 1. Residency times of tagged Atlantic salmon in different depth categories within standard (black lines), seawater snorkel (grey lines) and freshwater snorkel cages (blue lines). Bold solid and dashed lines represent surviving and dying fish (only observed in snorkel cages), respectively. Unbolded black dashed lines indicate the 4 m snorkel depth. Seawater and freshwater are abbreviated to SW and FW.

IDENTIFICATION OF AQUATIC SPECIES THROUGH qPCR

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EU regulation 1143/2014 aims to avoid, reduce as much as possible and mitigate the adverse effects of the introduction and spread of invasive alien species in the European Union. To do so requires early detection and rapid eradication. Article 14 requests a surveillance system be put in place, which, among other things, will determine the presence and distribution of new invasive alien species considered to be of Union concern, along with those already established. This system needs to be sufficiently dynamic so as to quickly detect the appearance of any invasive alien species considered to be of Union concern. To do so, it is essential tools be made available to quickly and reliably inform us of the presence of these species.

Currently, our work team is combining eDNA (environmental DNA) techniques with qPCR (quantitative PCR) in order to quickly identify aquatic species in water samples. The eDNA identification process is as follows: a water sample is taken, from which DNA is extracted and purified. The DNA sample is amplified via the qPCR process. In order to only amplify the necessary DNA, primers characteristic to the species we are looking for will be included in the mix. A fluorophore sensor, which emits light when added to DNA, will be included in the sample. Hence, as DNA duplicates, the former will emit increasing levels of light; the more DNA in the sample, the quicker the pre-set light intensity levels will be reached. For this reason, if we establish a calibration curve, relating biomass with luminescence, we can estimate the biomass of the sample. One of the great advantages of this method is that it does not require a prior gel electrophoresis process, since the results will already be available once the PCR is over.

The purification process takes five minutes and there is no need to use any standard purification laboratory apparatus, such as centrifuges, electromagnets, etc. PCR is carried out in a thermal cycler which weighs less than one kilo, and is fully mobile. The 45 recommended cycles in the protocol take 50 minutes. Consequently, results are available within an hour. Furthermore, all work can be carried out in the field.

Our work team is currently developing primers for invasive species, protected species and also to detect species with a high commercial value. In standard conditions, a primer for a new species can be developed in six months.

This results in an invasive species detection tool that can be adapted to the needs of each handler within a reasonable time frame. This technique can also be applied to protected species, as we do not need to capture them to know where they are. This is extremely important when the number of individuals in a population is low. It can also be used with parasites or other infectious diseases in aquaculture, with the aim of detecting the presence of these species before they attack fish, enabling us to take preventive measures.

EXPANSION AND DIVERSIFICATION OF EUROPEAN AQUACULTURE MARKET: INDUSTRIALIZING GREATER AMBERJACK *(Seriola dumerili)*. THE FIRST INITIATIVE OF INDUSTRIAL FINGERLINS PRODUCTION IN SPAIN

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Aquaculture is set to be one of the most important activities for food industry in the nearly future. However, it has to face two main issues that threaten aquaculture's growth. First of all, aquaculture needs a significant increase in the use of technology. Secondly, the diversification of species grown is a need to reach a higher development of the market of aquaculture products. Currently, it mainly depends on salmon, shrimp, carp, tilapia and bivalve mollusks, although sea bream, sea bass, trout, sole and turbot are also important in Europe and in Spain.

Some species of amberjack (Genus *Seriola*) are common fishing and aquaculture products. *Seriola lalandi* is broadly consumed in South America and its aquaculture is developing fast. *Seriola quinqueradiata* is a highly appreciated fish in Japan, both fished and farmed, although hatcheries are not yet developed. However, *Seriola dumerili* is the most appreciated fish of this genus and reaches very higher prices when sold, due to its excellent organoleptic properties.

Aquaculture of greater amberjack is currently rare, since it is not easy to produce fingerlings and wild caught fish is very competitive mainly in far east markets. However, as other species of this genus, it is said to be a very suitable fish for aquaculture. Cage culture is proven to perform well for this species, but juvenile supply is a handicap for the development of this industry. To reach a profitable amberjack aquaculture, it is first necessary to work in the production of fingerlings.

Futuna Blue España, S.L., where Everis Ingenieria SLU is a shareholder, is a hatchery established in southern Spain and it is the first in the world to produce greater amberjack juveniles industrially (100.000 fingerlings the current year). However, Futuna's production capacity is not yet significant for European aquaculture market and needs to be expanded. Everis Ingeniería is committed to help amberjack aquaculture overcoming this challenge by using its expertise in water engineering and Recirculating Aquaculture Systems (RAS), allowing Futuna Blue to produce as much as 1 million fingerlings per year, causing greater amberjack to become an increasingly important species in European aquaculture, which will be soon common to find in most European homes.

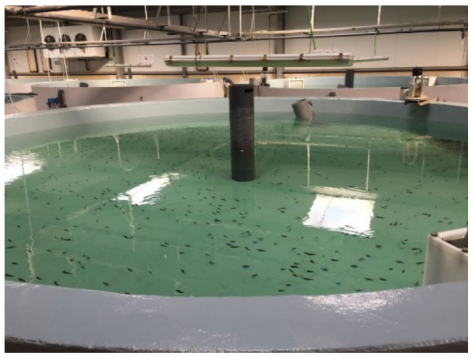

Figure 1. Greater amberjack juveniles in Futuna Blue

THE EXPRESSION AND TRANSMISSION OF IMMUNE FACTORS FROM MATERNAL IMMUNIZED GRASS CARP *Ctenopharyngodon idella* TO ITS PROGENY

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The grass carp (*Ctenopharyngodon idella*) is an economically important freshwater fish species in China, which is susceptible to death due to the infection of a grass carp reovirus. In order to investigate the expression levels and transmission effects of immune factors from the female parent (immunized with GCRV attenuated vaccine or Astragalus polysaccharides) to the progeny of *C. idella*, protein activities and mRNA expression levels of IgM, C3 and LSZ were detected. The protein activities of IgM in the blood of female parent (immunized with GCRV attenuated vaccine), and in the early embryos and the juvenile fish were significantly higher than those in the unimmunized group. The protein activity of C3 and LSZ were significantly higher in immunized group (the female parent, and stages from the egg cell to three days after hatchling) than those in unimmunized group. On the whole, transcription levels of *IgM*, *C3* and *LSZ* were significantly higher than those in the unimmunized group (Fig.1).

For female parent immunized by Astragalus polysaccharides, protein activities of IgM, C3 and LSZ in the blood of female parent were significantly higher than those in the unimmunized group. In samples of egg cell, 5 and 28 day after hatchling, the activities of IgM protein in immunized group were 2.2 times, 1.7 times and 1.8 times of those in unimmunized group, separately. After GCRV infection, the expression levels of immune factors and the antiviral ability of the two-month-old fish were significantly higher than those of the unimmunized group. The results indicated that maternal immunization could enhance the transmit efficiency of immune factors, and play important roles in immune protection against grass carp reovirus of progeny.

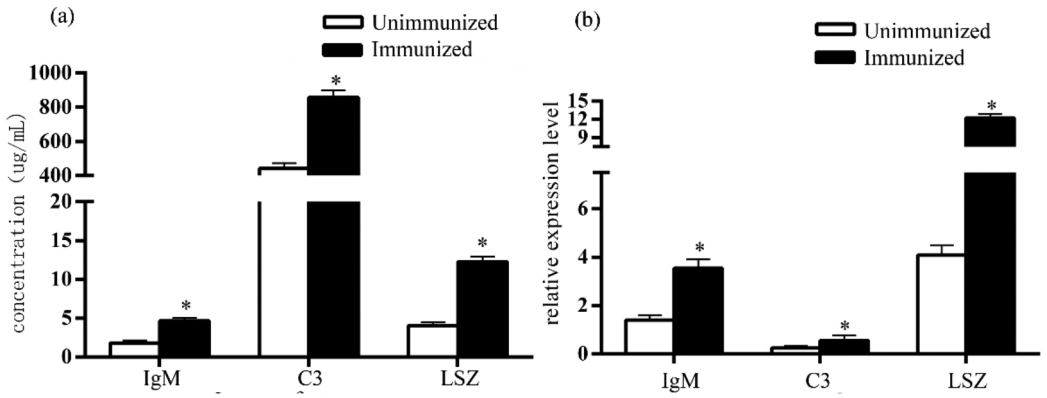

Figure 1 Protein activity and transcription level of immune factor in the blood of female parent.
a. Protein activity; b. mRNA expression level

OYSTER INSULIN-LIKE GENES AND THEIR POSSIBLE FUNCTIONS

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Insulin superfamily is considered to be an evolutionary ancient gene family, and has been identified in most animal groups. Vertebrates insulin superfamily includes four main groups: insulin (INS), insulin-like growth factor (IGF), relaxin (RLN) and vertebrate insulin-like peptide (INSL), all characterize by the highly conserved insulin-type disulfide bonds (six cysteine residues form two inter-chain and one intra-chain disulfide bonds). Members from invertebrate insulin-like genes were also identified. These include amphioxus insulin-like peptides (ILP), Ciona insulin-like peptides (INSL), sea urchin insulin-like peptides (ILP), Drosophila insulin-like peptides (IlP), Caenorhabditis insulin-related peptides (ins), Bombyxin (Bxn) of silk worm, locust insulin-related peptide (LIRP), and molluscan insulin-related peptides (MIP). Thus far, the functions of these protostomes insulin-like peptides are yet to be studied.

We report that oyster has a diversity of *insulin*-related genes (ILPs) including three (ILP1~3) expressed in neurons and one (ILP4) co-expressed with *Pdx* in the endodermal layer of oyster hepatopancreas (Figure 1). Transcriptome analysis reveals functional similarity of this tissue to vertebrate pancreas. Using ATAC-seq, we identify a Pdx homeodomain binding site upstream of the endodermally-expressed oyster *insulin*-related gene and using cell culture demonstrate that oyster Pdx acts as a transcriptional activator through this site (Figure 2). These data argue that ILP1~3 genes should play roles in neuroendocrine regulation system, while ILP4 should play similar roles with vertebrate insulin. At the same time, the results suggested a classic homeodomain-target gene interaction dates back to the origin of Bilateria.

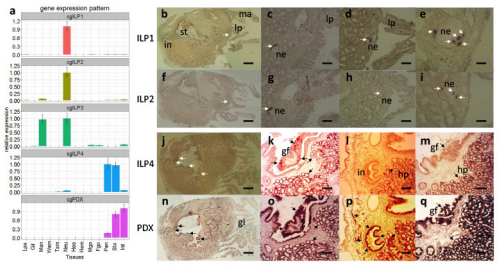

Figure 1 Analysis of oyster ILPs and Pdx genes expression.

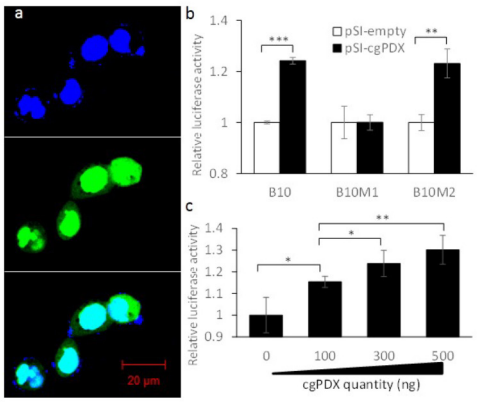

Figure 2 Nuclear localization and transcriptional activity of oyster Pdx.

TEMPORAL AND SPECTRAL VOLATILITY SPILLOVER DYNAMICS IN AQUACULTURE FISH MARKETS

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The global aquaculture production and trade has increased significantly over the last decades. According to Food and Agricultural Organization (FAO) (2016), the global trade has more than doubled in the seafood market from USD 72 billion during 2004 to USD 148 billion in 2014. The increased trade and consumption of seafood can be attributed to the growth in aquaculture (Asche, 2008). The increased globalization and connectedness of international seafood markets is a crucial factor leading to this upsurge. This indicates that the price uncertainty and supply disruptions in one region might influence the price dynamics of seafood species in other markets. Therefore, in this paper, we evaluate and quantify the temporal and spectral volatility spillover between the seafood species across different markets: the EU, the U.S., and Japan. There is considerable uncertainty in the volatility of seafood prices. Several studies argue the advantage of aquaculture production over wild capture provides stability in supply thus reducing price volatility.

In this paper, we study the price and volatility spillover between seafood prices across three major markets: the EU, the US, and Japan. Table 1 provides an overview of the markets and species considered in our study. We utilize monthly trade data spanning over 01.1990 to 12.2016. Furthermore, we aggregate a value-weighted index per species and per region. We evaluate the temporal and spectral dependence spillover by employing Barunik and Krehlik (2017) framework of frequency connectedness. This method enables us to evaluate and quantify the spillover over time and across different frequencies (short-, medium-, and long-run). We contribute to the existing literature by estimating the spectral dependence dynamics of the aquaculture fish markets. The empirical findings of our study corroborate with the previous research in terms of temporal and spectral dependence between the species and markets.

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Table 1. Aquaculture species, product forms, and markets in the study. Source: Import prices in the US, Japan, the EU provided by the Norwegian Seafood Council.

Species	Product forms	Markets
Salmon	Frozen and fresh	EU, Japan, US
Trout	Frozen and fresh	EU, Japan, US
Catfish	Frozen	US
Tilapia	Frozen and fresh	US
Putterfish	Frozen	Japan
Seabass	Fresh	EU

HIGH-THROUGHPUT SINGLE NUCLEOTIDE POLYMORPHISM DISCOVERY IN NILE TILAPIA USING WHOLE GENOME SEQUENCING OF HUNDREDS OF ANIMALS

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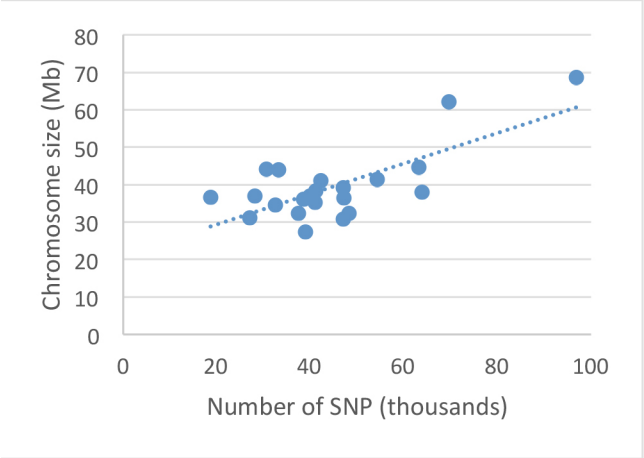
Nile Tilapia (*Oreochromis niloticus*) is the second most farmed fish in the world and a sustainable source of protein for human consumption. Several genetic improvement programs are established for this species in the world and so far they are mainly based on conventional artificial selection using genealogical and phenotypic information to estimate the genetic merit of breeders and make selection decisions. The incorporation of genomic information, in which has been called genomic selection, can take genetic improvement of farmed tilapia to the next level. Genome-wide information can be exploited to efficiently incorporate traits that are difficult to measure in the breeding goal (i.e. carcass quality and disease resistance traits). A substantial number of single nucleotide polymorphisms (SNPs) are required to investigate phenotype–genotype associations and determine the genomic basis of economically important traits. In this study, we performed *de novo* SNP discovery in three different populations of farmed tilapias. A total of 21,267,048 non-redundant SNPs were identified across a set of 350 fish by Illumina (HiSeq 2500) whole-genome resequencing of individual samples.

After filtering by Minor allele Frequency (MAF < 0.05), Call-rate (CR < 0.8) and Hardy-Weinberg equilibrium (bonferroni corrected p-value < 0.05) a total of 9,826,779 high quality SNPs were available. A total of ~1 million SNPs were showed medium to high MAF values across all the populations analysed and they were positioned on the genome assembly O_niloticus_UMD1. The relationship between the number of SNPs discovered and chromosome length is shown in Figure 1. A 50K Illumina BeadChip SNP panel is under development. This novel dense tilapia SNP panel will be very useful for the dissection of economically relevant traits, enhancing breeding programmes through genomic selection as well as supporting genetic studies in farmed populations Nile tilapia using high-resolution genome-wide information.

Figure 1. Relationship between the number of SNPs and chromosome length. Scatterplot of the number of SNPs per chromosome and the total chromosome length in Mb according to the assembly O_niloticus_UMD1.



A BACTERIAL TYPE IV SECRETION SYSTEM EFFECTOR NEGATIVELY REGULATES FISH MAPK SIGNALING TO RESTRAIN ANTIBACTERIAL RESPONSES

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Many bacteria employ multicomponent secretion systems to deliver macromolecules into host cells for manipulation immune systems and promote efficient infection. The type IV secretion system (T4SS) represent major bacterial virulence determinants have recently been the focus, but to characterize the effectors and identify their function during infection remain largely unknown. Here, we found that the thioredoxin-like protein encoded gene (*trxlp*) was significantly up-regulated during *Edwardsiella piscicida* infection, which might play important role in its pathogenesis. Thus, we stepped forward to analysis the crystal structure of this protein, and found that Trxlp is a new bacterial thioredoxin family protein, which shares signature WCXXC motif with human thioredoxin-1. Unlike the classical bacterial thioredoxins, Trxlp can be secreted out and translocated into host cells via the bacterial T4SS dependent manner. Furthermore, the *E. piscicida* T4SS effector Trxlp can mimic as the endogenous Trx1, and direct targets and inhibits the apoptosis signal-regulating kinase 1 (ASK1) phosphorylation. Subsequent studies revealed that Trxlp significantly suppressed the phosphorylation of Erk1/2- and p38-MAPK signaling cascades in both mammalian and zebrafish cells. Finally, Trxlp-mediated ASK1 and MAPK signaling inhibition promoted bacterial survival and replication in vivo. Collectively, this work advances our understanding of the bacterial thioredoxin as the T4SS effector in manipulating host innate immune defenses.

STUDIES ON FEEDING BEHAVIOR AND THE RELATIONSHIP BETWEEN FEEDING BEHAVIOR AND CHELIPED IN CHINESE MITTEN CRAB (*ERIOCHEIRS SINENSIS*) UNDER SINGLE REARING CONDITION

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Feeding behavior can affect individual growth and the role of species in the community. During this process, chelipeds play an important role. For example, crustaceans use chelipeds for defense, capture, manipulation, and subjugation of prey. But as we all know, such studies in the Chinese mitten crab, *Eriocheirsinensi*s, an important valuable freshwater aquacultural crustacean in China, are still few. In this study, the feeding behaviors of 12 intact male crabs (weight 22.54 ± 6.37g) in 1 hour after feeding were observed and recorded continuously by using a high-definition camera for 6 days.

We found that about 50% of the animals feed every day. The amount of the average intake was about 1.9% of the body weight, which was similar to the feeding dose in the actual aquaculture. The reaction time to food for crabs was significant longer on the first day (45.53±25.07min) than other days (average reactive time is 14.88±4.91min)□P<0.05□. It is possible that crabs need the adaption time to face the change of food. There are continuous feeding behaviors in crabs, including continuous 2d, 3d, 4d, 5d and 6d, of which the days of crabs with continuous feeding of 2d (66.7%)was the most. The reaction time to food in continuous feeding 6d was obviously longer than that of continuous feeding 3d, 4d and 5d (average reactive time is 19.88±3.56min)□P<0.05□. We speculated that the reaction of the crab to the food may be reduced because of satiety. Moreover, the frequencies of using the right chelate, the left chelate and the double chelates for crabs during the feeding were 55%, 41% and 4%, respectively. The length of 91.67% crab's right chelate was the longer than that of left chelate. There was a significant correlation between the chelate length (average value of the sum of the length of left chelate and right chelate) and body length (R^2=0.9683, P<0.01), but there was no significant correlation between the claw length and the food consumption.

THE EFFECTS OF FISH OIL REPLACEMENT BY VEGETABLE OILS ON GROWTH PERFORMANCE AND FATTY ACID PROFILE OF RAINBOW TROUT: RE-FEEDING WITH FINISHING DIET IMPROVED BODY HUFA CONTENTS

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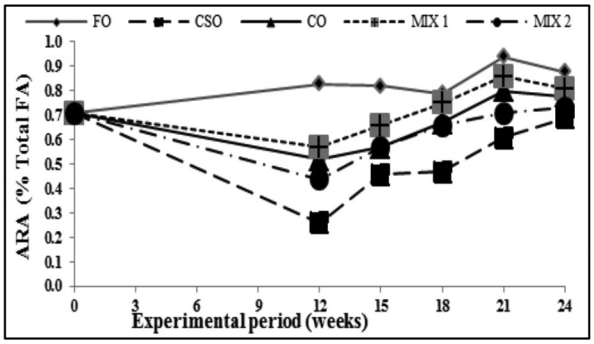
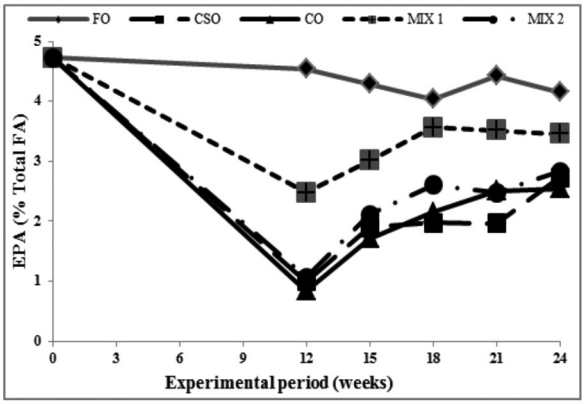
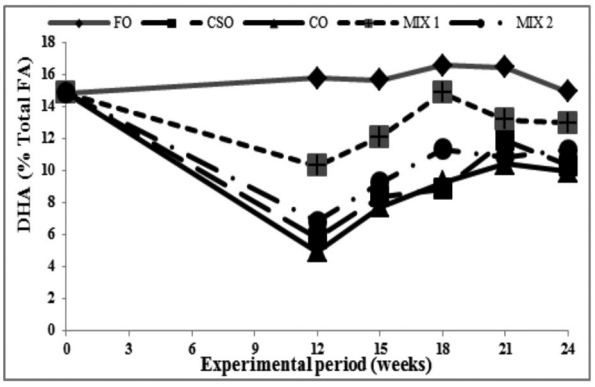
The present study investigated the progressive recovery of fatty acid (FA) profile in rainbow trout, *Oncorhynchus mykiss* previously fed vegetable oil (VO) based diets. Juveniles (≈ 15g) were fed five experimental diets in which dietary fish oil (FO) was totally replaced by cottonseed oil (CSO) and canola oil (CO), or partially in MIX1 (50% FO, 25% CSO and 25% CO) and MIX2 (50% CSO and 50% CO) in a grow-out period for 12 weeks. Afterwards, all fish were switched to a diet containing 100% FO diet for a further 12 weeks in a finishing phase to determine the progressive recovery of FA profile of rainbow trout. Results showed that total and/or partial replacement of FO did not negatively affect growth and feed utilization after in the grow-out period. Feeding VOs significantly reduced body contents of arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to a lower degree than their reduction in the diet. Re-feeding with FO-diet for 12 weeks led to significant ($P < 0.05$) improvements in highly unsaturated fatty acid (HUFA), with a full recovery in ARA contents of rainbow trout. Finally, FA profile in whole body of MIX1 fed fish had the closest resemblance to that of FO fed fish.

Alternative lipid sources such as VOs are needed to guarantee the future growth of the aquafeeds industry (Turchini et al., 2009). Although this has not had negative effects on growth, it has been shown to modify flesh FA with a resultant reduced HUFA contents (Bell et al., 2003; Yildiz et al., 2015). Finishing diet strategies have been adopted to increase nutritional value of fish previously fed FO-deprived diets (Turchini et al., 2007). The present study investigated the progressive recovery of FA profile after a 12 week-finishing phase in in rainbow trout initially fed VO-based diets and their blends in a grow-out period for 12 weeks.







IMPACTS OF EU ACCESSION PROCESS IN TURKEY FISHERIES PRODUCTS MARKETING

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As it is known, in the framework of the export promotion policy followed since 1980, Turkey's foreign trade has increased both quantitatively and qualitatively. In 1996, together with the Customs Union Treaty with the European Union, began a new era in trade between the EU and Turkey. Considering the Turkey's foreign trade with the EU, it has been observed that in especially aquaculture and animal products there is a special place for fishery products. While the most aquaculture exports made are EU countries, Turkey is positive foreign trade situation in aquatic products.

Turkey as of 2015, has made 250 million dollars in imports corresponding to 692 million dollars of exports in seafood (Table 1). As a matter of fact, the price of aquatic products exported in 2015 is $ 5.85 per kg, while the price of imported aquatic products is $ 2.55 per kg. As of 2015, Turkey's exports of aquatic products from EU countries made by the Netherlands, Italy, Germany, England, Spain and France value is approximately $ 450 million. The share of the EU countries in the top 10 countries where the export of fishery products is 65%. The production of the fisheries sector of the European Union has been on a downward trend in the recent years. However, as well as all over the world, it is an indisputable fact that the fisheries stock reached its limits in Turkey (Table 2). For this reason, fisheries policies should be based on the sustainability of production, with an ecosystem-based approach. Protection against all kinds of pollution spoiling the quality and nature of our seas and inland waters is very important for both domestic and foreign trade. As a matter of fact, the EU has the desire to affect our exports especially in these issues. Therefore the effects of EU aquatic products marketing process in Turkey were examined in this study.

Table 1. Turkey's share in world fisheries production (%)

Years	Fisheries (%)	Aquaculture (%)	Total (%)
2010	0.54	0.28	0.44
2011	0.55	0.30	0.45
2012	0.47	0.31	0.41
2013	0.40	0.25	0.37
2014	0.32	0.25	0.32
2015	0.46	0.26	0.39

Table 2. Turkey's aquatic products quantities (thousand tons)

	2011	2012	2013	2014	2015
Fisheries	514.7	432.4	374.1	302.2	431.9
Marine fish products	432.2	315.6	295.1	231.0	345.7
Other marine products	45.4	80.7	43.8	35.0	51.9
Inland water products	37.1	36.1	35.1	36.1	34.2
Aquaculture	188.8	212.4	233.3	235.1	240.3
Total	**703.5**	**644.8**	**607.5**	**537.3**	**672.2**

TURKEY'S FOREIGN TRADE TRENDS OF FISHERIES

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Turkey's foreign trade in aquaculture and animal products sector is increasing. Especially, aquaculture has more place in our exports. Thus, fish products led by sea bass, sea bream and trout have a share of 13% in trout, 24% in sea bass and 21% in sea bream in export value. Among the 10 countries with a total of $ 647 million in exports of fishery products, the EU countries have a rate of 60%, while Japan has 6.6%, Russia 5.9%, Lebanon 3.9%, USA 3.5% and other countries 15.1%.

As of 2015, Turkey's imports of aquatic products is about 111 thousand tons, 72% is met by five countries. These five countries; Norway, (49.3%), Iceland (8.1%), Morocco (5.3%), China (4.9%) and Libya (4.4%) while imports from other countries are 28%. About half of the total value of imports of aquatic products belongs to Norway.

For tuna fattening in Turkey, salmon and mackerel / Spanish mackerel are brought from Norway. In the import value of fishery products, salmon has a share of 24% and mackerel has a share of 27%. For fattening in Turkey, Bluefin tuna is brought alive from Morocco and Libya that are subject to international quotas by ICCAT. The biggest problem in Turkey's aquatic products marketing; are deficiencies in sales and operations in accordance with the international norms. Along with all these developments, especially the negativities caused by the feed prices in recent years are affecting the fisheries stocks and therefore the foreign trade (Table 1). As of 2015, about half of the anchovy with a 49% share in total production and almost all of the sprat fishes were used for fish flour and oil, thus the breeding of herbivore and omnivore species has been on the agenda. Therefore, in this study, these issues will be examined and recent trends in Turkey's foreign trade will be discussed.

Table 1. Aquaculture foreign trade (million $)

Years	Export	Import	Balance *(Exp - Imp)*	Export Change % *[(Exp2:Imp1-1)]*100*	Import Change % *[(Imp2:Exp1)-1]*100*	Export coverage of Import % *(Export *100/Import)*
2006	233.4	83.4	150.0	+13.3	+21.7	+279.8
2007	273.1	96.6	176.5	+17.0	+15.8	+282.7
2008	383.3	119.7	263.6	+40.3	+23.9	+320.2
2009	318.1	105.8	212.3	-17.0	-11.6	+300.6
2010	312.9	133.8	179.1	-1.63	+26.5	+233.8
2011	395.3	173.8	221.5	+26.3	+29.8	+227.4
2012	413.9	176.4	237.5	+4.7	+1.5	+234.6
2013	568.2	188.1	380.1	+37.3	+6.6	+302.1
2014	675.8	198.2	477.6	+18.9	+5.4	+340.9
2015	692.2	250.9	441.3	+2.4	+26.6	+275.8
2016	790.3	180.7	609.6	+14.2	-27.9	+437.3

IMPORTANCE OF PRODUCER ORGANIZATIONS IN FISHING ACTIVITIES IN TURKEY-EU PROCESS

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Organizational structures, brings individuals, who have the same aim in the existing social structure, together and enables development of responsibility and decision-making powers or a structure, that will provide more benefits to its members when done together in comparison to individual efforts. Today, organization structures are widespread and developed in the European Union (EU) countries among the most other countries. Within the European Union, organizational structures pose an important role in constitution and implementation of policies towards rural areas.

Despite the fact that there are many organizations, such as fishing cooperatives and producer associations, operating in the field of fisheries in Turkey, these organizations neither have a strong structure nor capable of fallowing innovations in the sector and protect the rights of the producers. Thus, the share of small scale fisheries in internal revenue is low (0,04%) (Table1,2).

Due to belated joint efforts to defend their common interests, producers who have not fully paid for their labour and consumers have to pay high prices for their products. As a result, despite its potency, fisheries sector couldn't thrive through the years. Meanwhile, the marketing of fishery products is mainly based on producer organizations and their top organizations in the European Union. As being a member of the producer organizations with a capability of regulating the markets is a necessity to be able to benefit from the incentives, it is important for the fisherman.

Common fisheries policy criteria;
- Protection fisheries stock,
- Structural state,
- Common market organisation,
- Relations with non- EU member countries and international organisations

As stated in the common fisheries policy in fisheries, to increase the high-quality production and increase the life quality of the fisheries labour, it is important to form organizational structures. A closer evaluation of the European Union fisheries sector reveals the developed and industrialized sector and organized fisheries labour.

Table1.EU Fishing Fleet Capacity by lenght category, 2014

Length	Number of vessels	Gross tonnage	Engine power in kW	Average age
0 - 6	28.198	23.385	352.894	27
6 – 12	45.946	162.730	2.287.848	24
12 – 18	6.955	159.505	986.749	25
18 – 24	3.330	249.700	886.491	22
24 – 30	1.729	243.883	642.124	20
30 – 36	579	193.979	311.268	22
36 – 45	433	172.689	409.750	17
45 – 60	109	93.235	164.607	19
60 – 75	76	130.994	225.889	16
>75	90	349.840	414.945	20
Total	87.445	1.725.938	6.682.574	21,3

Source: EU Fishing Fleet Registe

Table2.Turkish Fishing Fleet Capacity by lenght category, 2013

Lenght, (m)	Operation Area			
	Marine Fisheries	Marine Carrier	Inland water	Total
0–4,9	750	-	308	1.058
5–7,9	10.103	1	2.575	12.679
8–9,9	3.072	3	240	3.315
10–11,9	1.002	4	33	1.039
12-14,9	492	7	61	560
15-19,9	303	8	15	326
20-29,9	481	31		512
30-49,9	228	17		245
50+	6			6
Total (number of piece)	16.437	71	3.232	19.740

Source: General Directorate of Fisheries and Aquaculture

Continued on next page)

To form and enforce fisheries policies, to lead the market, to be capable of using modern production methods and increasing productivity in the rural areas will be possible only with the organized production structures (İnan, 2008). Fishing organization in Turkey is not sufficiently effective, although sufficient in number as in the European Union. Thus, there have serious structural and functional problems.

Main Problems of Organizing in Fisheries;

- State
- Organization Relations,
- Legal Issues,
- Financial Problem,
- Collaboration Between Producer Organizations,
- Education-Research,

and It can be summarized as the input of small businesses and the reduction of competitiveness of their products in the market, compared to companies dominating the input and product market.

Faced with these problems, it is only possible to organize cooperative and similar organizations to protect and maintain family businesses and to create sustainable fisheries policies compatible with the environment. As a result, it is important to underline that realistic solutions to organizing in fisheries, from producers' contribution to producing national and international policies.

MICROAEROPHILIC ASSIMILATION (BIOFLOC) SIDE- REACTOR, A NOVEL APPROACH FOR RECIRCULATING AQUACULTURE SYSTEMS (RAS): MODELING AND A PROOF OF CONCEPT

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Recirculating aquaculture systems (RAS) are expected to play a significant role in the next few decades based on Lux Research predictions.

In RAS, fish are grown at high densities (typically 20–150 kg/m^3) and fed with protein-rich feed (25–60%) at 1-3% of the fish biomass, thus creating a significant ammonia and solids loads that are treated via set of treatment units to allow water reuse. Fish recover only about 20-30% of the applied C and N and the rest is released into the water, producing high waste load which is toxic.

The goals of this research were to; 1) develop a quantitative model of a near-zero discharge intensive RAS in which water treatment is based on an assimilation side-reactor instead of the more traditional nitrification (with or without denitrification) reactor. 2) test the suggested model. In the suggested reactor, ammonia and carbon from the fish tank plus external C source (e.g. wheat flour) are introduced into assimilation reactor where it is assimilated into microbial biomass. Routinely part of the biomass is removed from the system and can possibly be used as protein-rich fish feed amendment (Figure 1).

Based on the model's predictions, a pilot RAS was constructed. The system was successfully operated for 101 days. Overall, fish and biomass production was 38.5 and 16.7 kg respectively, at a maximum fish density of 50 kg/m^3. Survival was 93%. Average total ammonia nitrogen, nitrite, and nitrate removal were 89.1, 69.4 and 100%, respectively. Biomass organic content was similar to aquafeed and nitrogen content was equivalent to 40% crude protein. Daily energy demand at the system's design capacity of 80 kg/m^3 would have resulted in a low energy consumption of 7.72 kW·h/kg fish.

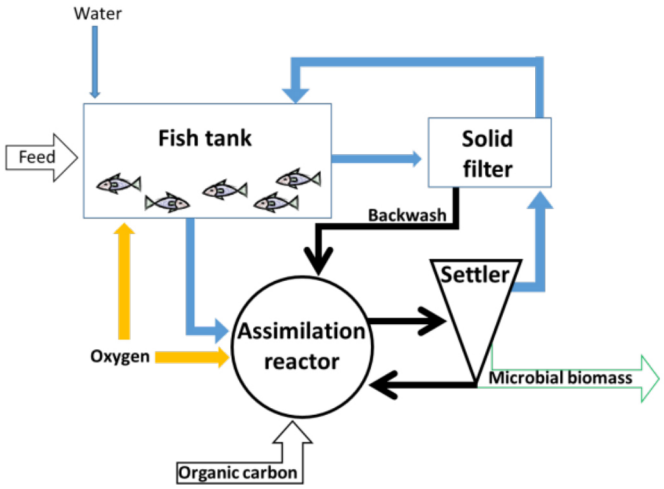

Figure 1. Conceptual scheme of the novel RAS based on TAN assimilation and production of microbial biomass (biofloc).

WORLDFISH RESEARCH IN FISH FEEDS AND NUTRITION

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The WorldFish mission is to strengthen livelihoods and enhance food and nutrition security by improving fisheries and aquaculture. In WorldFish Strategy 2017–2022, the Sustainable Aquaculture Flagship aims to enable sustainable increases in livelihoods from aquaculture production without creating adverse socio economic or environmental impacts. To achieve this objective, WorldFish will capitalize on synergies within the broader CGIAR portfolio and work collaboratively with multiple research and delivery partners on 1) fish breeds and genetics, 2) aquaculture systems, and 3) fish health, nutrition and feeds. Research on fish feed and nutrition will lead to the development of cost-effective and sustainable aqua-feeds, in order to spur the sustainable growth of aquaculture in Africa and Asia. In this presentation, the authors will first characterize the aqua-feed industry and its context in some African and Asian countries, then describe WorldFish research plans for innovation in aquaculture nutrition, and finally provide an update on action research and outreach activities for sustainable aqua-feed development.

CERTIFYING PROFESSIONAL AQUACULTURISTS TO COMPLEMENT FARM CERTIFICATION?

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Responsible growth in global aquaculture production is crucial to assure the continuous supply of quality food to the ever-increasing world population. An increasingly recognized way to spray responsibility in aquaculture practices is farm certification, in addition to strict environmental and social regulations established by some Governments. While farm certification is gaining momentum worldwide, little attention is paid on setting aquaculture as a profession, where aquaculturists receives certain recognition as true professionals. Unlike agronomist or engineers, there is currently no way to verify that a "so-called" aquaculturist hired to conduct an aquaculture production project has the training and experience necessary to reduce investment risk and to limit the short and long run environmental impacts of this project. This situation is arguably attributable to the lack of any national or international certifying body dedicated to professional aquaculturists. The well-thought out certification and continuous re-certification of aquaculturists would help setting professional standards that spur professionalism, sustainability, and ethics in aquaculture practice, and therefore reduce risks in the aquaculture venture. The professional certification of aquaculturists would therefore complement the ongoing global efforts on farm certifications, and together they would continue to stimulate investors' confidence and public trust for the sustainable development of this important and fast growing food production sector.

MANAGING AQUACULTURE PROJECTS

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A project is a sum of activities undertaken to achieve a product, result or service, with start and end dates. Like in any other project, the management of aquaculture research projects requires the use of conventional and unconventional knowledge, skills, tools, and techniques to projects activities. According to the Guide to the project management body of knowledge (PMBOK guide) published by the Project Management Institute, project management is accomplished through the appropriate application and integration of project management processes, which are categorized into five process groups, namely initiating, planning, executing, monitoring and controlling, and closing, and ten knowledge areas, which include project integration management, project scope management, project time management, project cost management, project quality management, project human resource management, project communication management, project risk management, project procurement management, and project stakeholder management.

The objective of this presentation is to introduce students and less experienced aquaculture project managers to tools and techniques that are necessary for applying the skills and capabilities sought in a project manager. The content of the presentation will include the description of the standard for project management, the application of project management tools and techniques in aquaculture research and development projects, case studies of project failures, and case studies of well-managed, successful projects.

SWIMMING CHALLENGE WITH WILD, FARMED AND HYBRIDE ATLANTIC SLAMON (SALMO SALAR) REVEAL REMARKABLE PHYSIOLOGICAL DIFFERENCES

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Invasion of escaped farmed Atlantic salmon into natural populations may compromise the genetic structure and adaptations of wild populations and reduce their viability and productivity. The empirical proof has however been limited. Experimental releases of farmed salmon and crosses with wild salmon have been instrumental in quantifying fitness of farmed salmon in the wild and also for unravelling some of the mechanisms behind their interactions with wild fish. To understand the underlying genomic causes of fitness in the wild, we have tested wild, farmed and hybrid salmon in a swimming challenge.

The aim of this study was to evaluate the differences in skin and cardiac performance and physiology in farmed, hybrid and wild salmon by comparing a range of robust-related traits using the experimental swimming flume at Nofima, Norway. Eggs from the three different strains were incubated, hatched and reared until post-smolt stage under standard conditions. At the parr stage, each strain was tested in a (duplicate) swim challenge (n=240 per strain). Water speed was stepwise increased, following a 2.5h ramp-up program up to 100 cm/s. Fish, which showed clear signs of fatigue, were removed from the flume and registered. Skin and heart were sampled before (basal resting level) and 24h post swim challenge (psc) for genomic and histological analyses.

The wild fish performed lowest in the swimming trial and had lower average heart rates, arrhythmia temperature and possibly optimum temperature. These results may suggest that wild fish were in physically worse condition than the other two strains, but most likely these results rather reflects that farmed and hybrids are genetically better adapted to live in a fish tank. Hybrids performed best in the swimming trials and reached highest arrhythmia temperature. When it comes to skin samples and mucosal response, all three groups had a skin morphology typical for stressed fish prior to the swim challenge. 24h psc, differences were more pronounced, with wild fish recovering quicker than farmed fish. This could be related to the evolutionary pressure on wild fish to be able to react to immediate threads.

These results add to our understanding of the basic biology of salmon, how to treat and handle farmed fish and the impact of hybridization between wild and farmed salmon. Here we found evidence that farmed salmon are more robust to stressful challenges, probably due to adaptation to the tank/farm environment, but wild fish recover faster from acute stress, a quality that may be more useful and important in the wild.

CLIMEFISH – CO-CREATING A DECITION SUPPORT FRAMEWORK TO ENSURE SUSTAINABLE FISH PRODUCTION IN EUROPE UNDER CLIMATE CHANGE

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The EU financed, H2020 project ClimeFish, aim at estimating the effects of climate change on fisheries and aquaculture in Europe from today until 2050. The overall goal is to help ensure that the increase in seafood production comes in areas and for species where there is a potential for sustainable growth, given the expected developments in climate, thus contributing to robust employment and sustainable development of rural and coastal communities.

ClimeFish will use two climate scenarios from the International Panel of Climate Change (IPCC), RCP4.5 and RCP8.5, to forecast the future production in 16 specific seafood producing cases. The 16 cases include the three main sectors for seafood production: fisheries, aquaculture and freshwater lake and ponds, including the most productive and the less resilient species. These sectors bring a broad aspect and diversity to the project and give a good overview of the consequences climate changes will have upon seafood production in Europe. The underlying biological models used in each case are based on single species distribution and production, as well as multispecies interactions. Forecasting models will provide production scenarios that will serve as input to socio-economic analysis where risks and opportunities are identified, and early warning methodologies are developed. Strategies to mitigate risk and utilize opportunities will be identified in co-creation with stakeholders, and will serve to strengthen the scientific advice, to improve long term production planning and the policy making process.

For 7 of the 16 cases, cases ClimeFish will develop specific management plans (MPs) coherent with the ecosystem approach and based on a results-based scheme that will allow regulators, fishers and aquaculture operators to anticipate, prepare and adapt to climate change while minimizing economic losses and social consequences. A guideline for how to make climate-enabled MPs will be produced, and published as a low-level, voluntary European standard after a consensus-based open consultation process. As a container for the models, scenarios and MPs, ClimeFish will develop the ClimeFish Decision Support Framework (DSF) which also contains the ClimeFish Decision Support System (DSS): a software application with capabilities for what-if analysis and visualization of scenarios.

RESEACH ON THE LOGIC OF FISHERY SUPPLY-SIDE STRUCTURAL REFORM: A INDUSTRIAL FRAMWORK

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It is significant for supply-side structural reform of fishery economic policy to sort out the logic and effectiveness of fishery economic reforms over the past 40 years. From perspective of mainstream economics evolution, the paper established a general analysis framework for the evolution of industrial policy. Based on the framework, using FAO and the statistical data of the Fisheries Chief of China, it thoroughly analyzed the fishery reform logic and its effects in China. Finally, put forward fishery policy based on supply-side structural reforms in future. The results showed that the continuous emergence of new problems in economic reality has led to the continuous evolution of mainstream economic theory. Economics evolves according to the logic of market-government-both hands. In the supply shortage phase, to increase supply was main goal of policy, and its path was exploitation of 'invisible hands', such as division of labor, specialization, competition etc. To 1930s, over supply became a more serious issue. In this phase, economic policy focused on stimulating demand by playing the role of the government. "Visible hands" includes of government investment, infrastructure construction and social security, etc. To 1970s, main issues was stagflation, and it was successful in balancing the economic growth and inflation through both hands together. As an important industry in the basic sector, fisheries was one of the earliest industries that have undergone market-oriented reforms in China. From 1978 to 1994, fishery was in the supply shortage phase. Supply increased rapidly and sharply benefit from production responsibility system and market reform. The problem of eating fish was solved. To the phase of sufficient fishery production, from 1995 to 2011, the fishery major contradiction were disorderly development, destruction of resources and pollution of environment. Fishery policy focused on strengthening government role to promote sustainable supply. With the release of "invisible hand" and strengthened "visible hand", China's fisheries have achieved increased supply and standardized development. At present, the fishery economy has been relative and structural excess from the shortage. Fishery functions such as society, ecology, and life rather than economic are priority and more important. To balance multiple goals is policy direction. In the future, the fishery economic policy should focus on supply-side structural reforms and adhere to use "tow hands" together. On one hand, it is necessary to give a decisive role of the market in the allocation of fishery resources, such as clarifying and reforming property rights of fishery resource, rights to entering fishery and expanding opening up, participating in the international specialization division. On the other hand, it should that give full play to the role of the government in solving external and basic issues, including of ecology priority, green development, quality and safety of aquatic products, investment in infrastructure, preferential taxation, investment in research, and brand cultivation. It concluded that the logic of China's fishery reforms basically follows the general law of the evolution of mainstream economics, and the constructed industrial policy analysis framework can be used as a logical framework for the transformation of industrial policy.

THE USAGE OF MODELLING FOR PLANNING SITE SELECTION AREA OF MARINE FISH FARM

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Inland aquaculture started in Turkey during the 1970s. But aquaculture expanded rapidly with the contribution of marine fish farms in the 1980s. It was dominated by cage farming of sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*). Turkish aquaculture production has increased from 61.000 tons in 2000 to around 253.395 tons in 2016. It has been going through a period of unprecedented growth over the past 10 years. The production of fresh water and marine water are respectively %41 and %59 in 2016. At coastal zones marine aquaculture became limited in time because of the pressure to marine and coastal ecosystems. It has also created conflicts among existing users of coastal resources. To avoid conflicts The Ministry of the Environment amended existing environmental legislation. It was then obligatory for the farms to be moved from the shallow shoreline waters to the deeper areas, particularly in gulfs and bays.

It is vital to calculate the possible risks and choose to protect the marine ecosystem during the site selection and the operation process. In this study MERAMOD model was used to investigate the carrying capacity of marine fish farms. Modelling offers the possibility to simulate and predict the environmental impact of sea bream and sea bass fish farms. Solid matter accumulation (feces and feed) and carbon distribution (footprint) around the cages were simulated using hydrographic parameters and production data (feed data). According to EIA of Gulluk Bay 2008, the effect of impact on the environment by fish farms is calculated in consideration of the amount of food and fish. FCR is 2 for sea bass and 2.2 for sea bream. Food not consumed by automatic feeding is calculated as 0.1 percent. For other feeding processes 12 percent is estimated. The amount of nitrogen (N) and phosphorous (P) are calculated according to these numbers. Within the IMST-216 project, some modules of this software (MERAMOD) were used. Carbon and solid accumulation quantities were examined in two farms that produced 370 tons/year and 1800 tons year in the cases where the discharge intensity changes in 0 cm/s, 10 cm/s and 20 cm/s currents. If the current was 10 cm/s, it was observed that the precipitate spread over an area of 200 meters, if it was 20 cm/s it spreads 300 meters. When studying the degree of change of effects in the study, horizontal distributions were used to show that the effects decreased as they moved away from the cages.

IMST-216: 2013. Project was supported by Ministry of Environment and Urban Planning Turkey. Project entitled “Environmentally Friendly Sustainable Environment Friendly Fish Farm Systems”.

THE PREVENTION OF WHITE SPOT SYNDROME VIRUS (WSSV) AND *Vibrio harveyi* CO-INFECTION ON PACIFIC WHITE SHRIMP *Litopenaeus vannamei* BY DIETARY SUPPLEMENTATION OF MICROENCAPSULATED SYNBIOTIC

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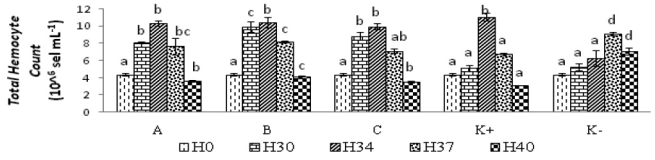
We applied microencapsulated synbiotic, a combination of probiotic and prebiotic, in shrimp feed which has proven the beneficial effects to the white shrimp. Synbiotic supplementation capable of stimulating microbial composition in the shrimp gastrointestinal tract, improving immune responses for disease protection and inducing growth promoting factor in the form of synergistic action.

The purpose of the study is to evaluate the effectiveness of dietary supplementation of synbiotic microcapsule, a combination *Bacillus* sp. NP5 Rf^R and mannan oligosaccharide (MOS), on the growth performance and innate immune responses of Pacific white shrimp infected with WSSV and *Vibrio harveyi* MR5339 Rf^R. Methodology: The synbiotic was MOS encapsulated using spray drying method. The shrimps with initial weight of 5.68±0.13 g were fed by diet supplemented with (0.5% (A), 1% (B), 2% (C) (w/w)) and without synbiotic as controls (C- and C+) for 30 days. At the end of the feeding experiment, all shrimps of each tank were first injected intramuscularly with 0.1 mL WSSV filtrate (10^1 copies mL^{-1}). After twenty-four hours post WSSV injection, the shrimps were immersed with *V. harveyi* MR5339 Rf^R (10^6 CFU mL^{-1}).

Findings (Figure 1 and Table 1): The present study showed that total hemocyte count, differential hemocyte count, phenoloxidase activity and respiratory burst (data not shown) of shrimp supplemented by 1% synbiotic microcapsule were significantly different ($p<0.05$) than those of both controls until the end of experiment. Shrimp fed with synbiotic microcapsule diet also showed significantly better specific growth rate, feed conversion ratio ($p<0.05$), and survival rate than those of both controls treatment.

Conclusion: The optimum dose of synbiotic microcapsule that significantly improved growth performance, immune reponses, survival rate and disease resistance against WSSV and *V. harveyi* MR5339 Rf^R of the white shrimp was shown by supplementation of 1% synbiotic microcapsule.

Figure 1. Total hemocyte count of shrimp, *Litopenaeus vannamei* fed with control diet (C+ and C-), 0.5% (A), 1% (B), 2% synbiotic microcapsule-supplemented diet (C), respectively after 30 days feeding experiment and 7 days co-infection test. Data represents the mean ± SD; three replicates were set for the tests, with 15 shrimps per replicate. Data at the same sampling time with different letters are significantly different ($P < 0.05$).



*H: day of culture

Table 1.
Growth performance of shrimp *Litopenaeus vannamei* fed with control diet (C+ and C-), and 0.5% (A), 1% (B), and 2% (C) synbiotic microcapsule-supplemented diet, respectively, and survival rates after 30 days of feeding experiment and after 7 days post co-infection test with WSSV and *V. harveyi* MR5339 Rf^R.

Parameter	Synbiotic microcapsule-supplemented treatment				
	A	B	C	C+	C-
Initial weight (g)	5.68±0.13^a	5.68±0.13^a	5.68±0.13^a	5.68±0.13^a	5.68±0.13^a
Final weight (g)	32.11±0.19^b	33.17±0.07^b	32.01±0.45^b	30.17±0.18^a	30.26±0.05^a
Specific growth rate (*SGR, % / day)	2.10±0.02^b	2.21±0.05^c	2.13±0.06^b	1.89±0.02^a	1.92±0.04^a
Feed conversion ratio (**FCR)	2.15±0.06^b	1.97±0.08^a	2.04±0.09^{ab}	2.47±0.08^c	2.40±0.06^c
Survival (after feeding experiment; %)	100±0^a	100±0^a	100±0^a	100±0^a	100±0^a
Survival (after co-infection test; %)	57.78±3.85^b	64.44±3.85^c	55.56±3.85^b	42.22±3.85^a	100±0^d

Values are expressed as mean ± SD; three replicates were set for the tests, with 15 shrimps per replicate. Data at the same sampling time with different letters are significantly different ($P < 0.05$).

EGG QUALITY DETERMINANTS IN PIKEPERCH *Sander lucioperca* – IMPLICATIONS FOR AQUACULTURE

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Evaluation of egg quality during controlled reproduction is among the key steps determining production effectiveness. Reliable methods of evaluation of egg quality, by discarding low quality eggs allows to reduce labor, usage of hatchery devices and improve the efficiency of larviculture. This is especially important in predatory species such as pikeperch (*Sander lucioperca*), being one of the most important candidate for intensive freshwater aquaculture.

Following the findings of our previous works on perch (*Perca fluviatilis*) and pikeperch we have determined developmental competence (on the base of: fertilization rate at 72h post fertilization, hatching rate and deformity rate of the larvae) in 82 portions of freshly stripped eggs (following controlled reproduction with the use of hormonal stimulation: hCG at a dose of 500 IU per kg of wet body weight of females) taking into account the following features:
1. Fragmentation rate of lipid droplets;
2. Cortical reaction rate between 3 and 5 min following egg activation.

This allowed to distinguish 6 different groups of eggs, as shown in Tab. 1.

The results of this study revealed, that moderate fragmentation of lipid droplets (where not more than 50% of eggs exhibited fragmented lipid droplet) in ovulated eggs of pikeperch does not affect their overall developmental competence whenever the cortical reaction occurred in all the eggs. However, in the egg portions with high lipid fragmentation rate (group No. 3 and 6) developmental success was related with cortical reaction occurrence (Fig. 1).

The results obtained, for the first time, indicates that fragmentation of lipid droplet in pikeperch eggs can be used as a biomarker of egg quality in pikeperch. However, these findings clearly shows, that more precise estimation of overall developmental competence of obtained eggs in this species can be achieved by using combined analysis of both: oil droplet fragmentation and cortical reaction rate, with the latter already reported to be credible indicator of high egg quality.

Tab. 1. Characterization of egg quality groups.

No. of group	Cortical reaction rate	Fragmentation rate of lipid droplets
1	100%	0% (single droplet)
2		<50%
3		100%
4	<90%	0% (single droplet)
5		<50%
6		100%

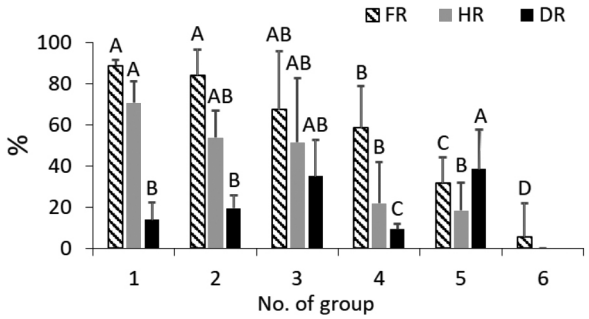

Fig. 1. Fertilization rate of embryos (FR), hatching rate (HR) and deformity rates (DR) of larvae recorded in different categories of eggs (provided on 'x' axis – see Tab. 1).

POLYMORPHISM OF GROWTH HORMONE RECEPTOR (GHR) GENE IN *Pelodiscus sinensis*

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Growth hormone receptor (GHR) is the cell surface receptor for growth hormone (GH) and plays an important role in regulating body growth and other metabolic processes in the animal. This study was conducted to detect single nucleotide polymorphisms (SNPs) of the GHR gene and explore their relationships with growth traits to assess the potential use of the GHR gene for the breeding selection. The results showed that 13 SNPs were verified and 8 SNPs were genotyped successfully in 173 individuals from the GHR gene. In Dongting population of *Pelodiscus sinensis*, the value of observed heterozygosity (Ho) and expected heterozygosity (He) varied from 0.2091 to 0.4182 and 0.2497 to 0.4325, respectively and growth correlation analysis revealed 4 SNPs (C902T, C984G, C1077G, and C2231G) associated with growth ($p < 0.05$). 8 SNPs were genotyped in other three different population and 4 SNPs in TP, 3 SNPs in HP, and 2 SNPs in JP were genotyped successfully (the alleles frequency > 0.05). According to results of χ^2-test of the genotypic and allelic frequencies between different population, it was known that the sites of G195T, C902T, and C984G were significantly different in four population ($p < 0.01$). This result suggested that GHR could use as candidate genes and C902T, C984G, C1077G, and C2231G as candidate sites for the selective breeding of *P. sinensis*. These findings provide new clues about the molecular basis and candidate markers for *P. sinensis* breeding.

EVALUATING THE GROWTH PERFORMANCE AND DIGESTIVE ENZYME CAPACITY UNDER CONSIDERATION OF NATURAL PRODUCTS FROM COPEPODS IN MICRODIETS FOR SEA BASS (*Dicentrarchus labrax*) LARVAE

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Replacement of live feed in rearing marine fish larvae is still a challenge in first feeding stages. Since marine copepods and their nauplii stages are considered as the best food source for marine fish larvae, new feed formulations were tested containing a significant amount of products from marine copepods. In addition, proteolytic enzymes were added in order to bridge the rather poor digestive enzyme capacity in the early stages of sea bass larvae and compared to traditional live feed (*Artemia nauplii*) and a standard micro diet formulation.

Over the experimental period of 45 days larvae fed diets which contain calanus powder (MD1, MD2 and MD5) had significantly better growth in weight than those fed diets with the enriched proteolytic enzymes (MD3 and MD4) or the control (MD-C) (Figure 01). At the end of the trial the highest survival rates (in %) were determined for the LF group and MD1 group with a similar value of 26% and followed by MD3, MD2 and MD4.

Furthermore, neither pancreatin (MD3) nor trypsin (MD4) supplementation alone lead better results in terms of tryptic enzyme activity compared to control groups (MD-C and live feed group). However, the combination of pancreatin and trypsin supplementation with calanus powder MD1 and MD2 showed higher tryptic enzyme activity respectively, than without calanus powder recipes (MD3 and MD4) (Figure 02).

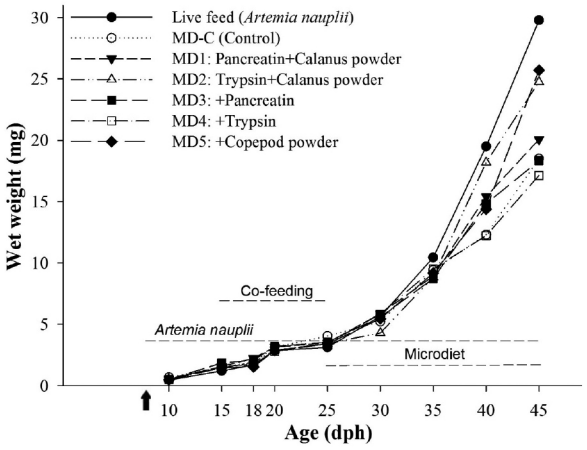

Figure 01: Wet weight with increasing age between different feeding regimes in sea bass larvae.

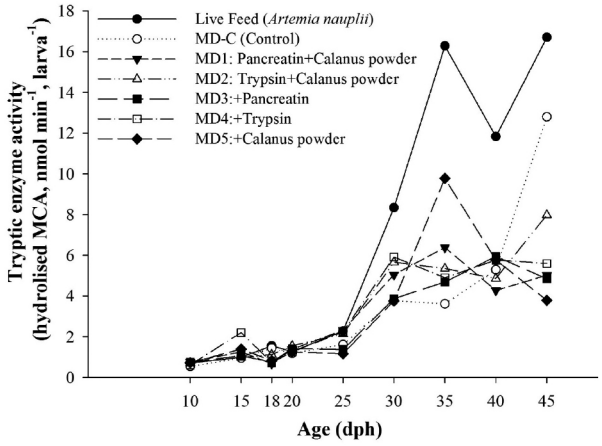

Figure 02: Tryptic enzyme activity with increasing age between different feeding regimes in sea bass larvae.

TRANSLOCATION OF MICROPLASTIC IN SEA BASS (*Dicentrarchus labrax*) FILET

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The amount of plastic waste in the oceans, and resulting microplastic (MP), has been rising steadily for years. Uptake of MP particles has already been detected in a large number of marine organisms. In particular, marine diets can be a problem because humans may also absorb MP. While mussels have already been shown to translocate particles into the edible tissue or filet, this is far from clear in more commercially important finfish.

Therefore, the overall goal of this study is to develop a suitable method for the detection of MP in the muscle tissue of food fish and to investigate the translocation of MP from feed to the tissue of fish.

A 16-week controlled feeding experiment with juvenile sea bass (*Dicentrarchus labrax*) was conducted with 120 fish per tank in quadruplicate. Fish with an initial weight of 12.5 ± 1.5 g were fed *ad libitum* with a control diet (without MP additive) and a test diet (with defined amount of fluorescent MP particles- ~1-5 μm) under controlled conditions in a recirculation system.

Growth was monitored at experiment onset and termination. Liver, intestine, gill samples were taken for further histology analyses. Muscle tissues of fish (filet) were taken for maceration, digestion and subsequent determination of MP content.

Detection of MP in sea bass fillet from experiment will be detected with fluorescence microscopy. In the case of commercial sea bass fillet: from wild and aquaculture product from market, detection of MP will be carried out with FT-IR microscopy and Raman microscopy.

Final results are pending! But we expect that the extraction method being developed for the detection of MP will be new to the industry, and can be implemented as a standardized method for MP detection and quality control in commercial fisheries.

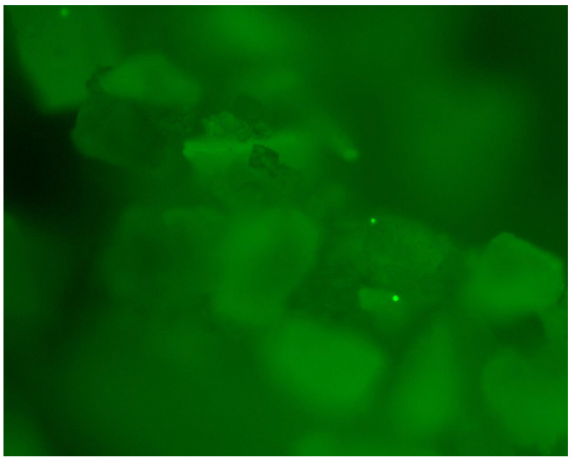

Figure 01. MP in experimental feed (Photo: Alfred-Wegener-Institut)

SEAHORSE AQUACULTURE: A SUSTAINABLE SOURCE OF MEDICINE

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The earliest record that use seahorses as medicine in China is in a book entitled “Ben Cao Jing Ji Zhu” by Tao Hong Jing in 480 - 498. Chinese medicine manuals refer to it as being “sweet in taste, warm in nature, and enters the kidney and liver channels”. In Europe, seahorses are used a medicine since 18th century. People in Asia have been using seahorses as a cure for a variety of ailments. Seahorses tonify the kidneys and fortify the Yang, and are used for impotence, urinary incontinence, wheezing and old age debilitation. Modern medical studies have shown that seahorse extracts have multiple biological activities, including anti-arthritis and its associated inflammation, anti-ageing, anti-fatigue, anti-cancers, and can strength immunity. Seahorses have been considered a power candidate species for many diseases.

Currently, the traditional Chinese medicine (TCM) market is largely depending on wild seahorse resources. It is estimated that more than 150 million seahorses are used for TCM each year. However, there is still a large gap between the supply and demand. Seahorse culture surged in 2014 in China, more than 5 million were produced. However, production declined sharply to about 1 million in 2015 and 2016 due to low profitability. Seahorse aquaculture warmed up again in 2017, and production reached to 2 million. China will produce up to 10 million seahorses by about 30 large farms in 2018. In China, farmed seahorses are becoming a sustainable source of TCM with significant progress in survival improvement and production cost cut.

In the past few years, major bottlenecks, such as disease prevention and control have been broken through, and survival has been significantly improved, resulting in higher profit margin. As production continues to rise research is needed to keep costs to a minimum so the industry can move forward farther.

About 80 countries are involved in the wild seahorse trade. Although many fisheries biologists thought that wild populations of seahorses are under threat due to the extremely high-demand-driven over-exploited, the truth is that most seahorses are by-captured, and the endangered status of seahorses resulted from multiple causes. Therefore, simply restricting seahorse trade is not a positive action to protect wild seahorse resources. Establishing marine protected areas and developing seahorse aquaculture are the best ways to conserve seahorses.

EXPERIMENTAL STUDY ON FEEDING ACCURACY OF THE PROJECTION TYPE OF QUANTITATIVE FEEDING MACHINE IN RAS

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In recent years, China's aquaculture has developed rapidly, it is a necessary trend that the use of feeding machine replaces the artificial feeding. The use of feeding machine in China has a variety of types, and can be roughly divided into the type of pneumatic pipeline, track, vibration, rotate throwing and projection.

In order to find out the factors that affect the accuracy of the feeding machine and thier influence degree, this paper takes the QC-TR-15 feeding machine which is the type of projection as the experimental object, and its cost-price is high, has representation in China's feeding model.

Then, the test platform used in the feeding precision test was designed and built, and the single factor pre-test was carried out, then the test factors and their levels were determined by pre-test. After that, 29 sets of test tests were designed by using the response surface design method of Box-Behnken Design (BBD), and in accordance with the order of experimental numbers to start the test., the effects of wind speed, feeding interval, feeding amount and feeder loading on feeding accuracy were studied, then used the Design Expert software for data analysis, and obtained a quadratic regression model for the feeding accuracy.

The variance analysis was performed on the regression model, the results show that the model is extremely significant(p<0.0001), mismatch is not significant(p=0.635>0.05), indicating that the model is appropriate. and the results also show the wind speed has a extremely significant effect on the accuracy of feeding (p <0.001) in the four factors, and other factors have no significant effect on them. Then the single factor analysis and interaction analysis were carried out, and we can get the influence degree of each factor on the accuracy of feeding: wind speed> feed amount> feeding interval> cylinder volume. The results of this study can be used as a reference for optimization and application of the type of feeding machine.

ANTIPARASITICAL EFFICACY, TOXICITY, RESIDUES ELIMINATION, AND ANTIPARASITIC MECHANISM OF 10-GINGEROL ISOLATED FROM GINGER *Zingiber officinale* AGAINST *Ichthyophthirius multifiliis*

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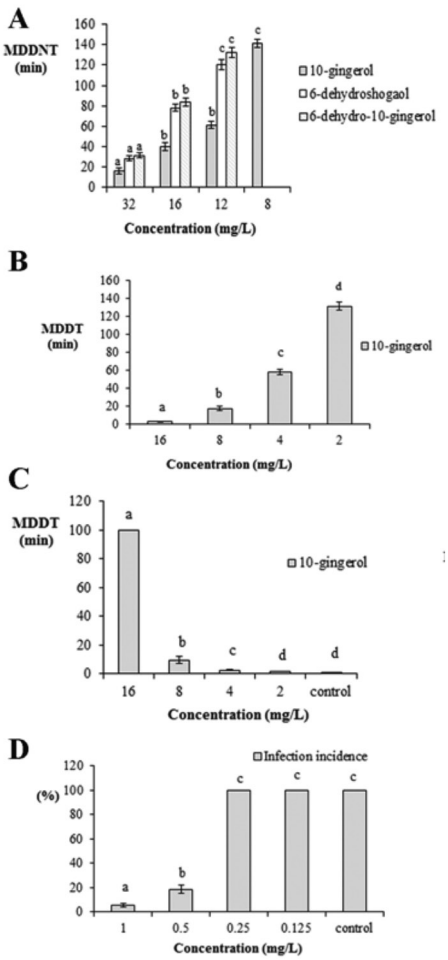
Ichthyophthirius multifiliis is a freshwater fish ciliate parasite with a global distribution and results in severe economic losses in aquaculture. The present study aimed to investigate the antiparasitical efficacy, toxicity, elimination, and mechanism of active compounds isolated from *Zingiber officinale* against *I. multifiliis*.

The results showed that 3 compounds identified as 10-gingerol, 6-dehydroshogaol, and 6-dehydro-10-gingerol were isolated from the *Z. officinale* extract. The *in vitro* tests showed that 10-gingerol demonstrated the highest antiparasitic efficacy against *I. multifiliis*. 10-gingerol at concentrations of 2, 8, and 16 mg/L resulted in 100% mortalities of theronts, nonencysted tomonts, and encysted tomonts, respectively. 10-gingerol at 1 mg/L significantly decreased theronts infectivity (p<0.05) and was effective at 4 mg/L in treating infected grass carp and protecting naïve fish from *I. multifiliis* infestation. The 96 h median lethal concentration (LC_{50}) of 10-gingerol to grass carp was 85.4 mg/L.

The antiparasitical mechanism results showed that 10-gingerol resulted in the increasing of intracellular osmotic pressure and free radicals accumulation, and damaged the membrane system of *I. multifiliis*. In addition, residues in the muscle of grass carp fell below the detection limit of ESI/MS on day 15. The study demonstrates that 10-gingerol has the potential for therapeutic development against *I. multifiliis*.



A NEW SPECIES OF *Nassarus* (GASTROPODA:NASSARIIDAE) FROM SOUTH CHINA SEA

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Nassariid species are popular food in China. Here we report a new species, *Nassarius monilius* sp. nov. of Nassariidae based on the specimens collected from the subtidal zone water depth 50-200 m in muddy and sandy bottom in the South China Sea and assigned to genus *Nassarius* Duméril, 1805. The new species has a moderate shell, a conus spire with thick axial ribs and weak spiral cords. Colour light-brown or yellowish-white, body whorl occasionally with faint reddish-brown bands. The new species was distinguished from similar species *Nassarius multivocus* Kool, 2008 in shell characteristics, its axial ribs on the dorsal of body whorl become weak or disappear. Some relative discussion was provided.

The family Nassariidae are distributed worldwide, from temperate, subtropical to tropical waters. Their habitat ranges from the intertidal zone to deeper waters. This family is represented in China by more than 70 species in three subfamilies, of which members of the genus *Nassarius* are most numerous. Nassariid species is one of the main economic groups of marine mollusks. As scavengers they play an important role in maintaining the balance of marine ecosystems, in particular, the stability of benthic communities. In China, distribution range of this family is from Bohai Sea, Yellow Sea to the South China Sea.

UNLOCKING THE FISH NON-CANONICAL INFLAMMASOME ACTIVATION IN RESPONSE TO INFECTION

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The non-canonical inflammasome is critical for cytosolic sensing of gram-negative pathogens in the innate immune responses. In the present study, we demonstrated that bacterial infection induces Caspy2 activation in zebrafish fibroblasts, which mediates significant pyroptosis via caspase-5 activity. Zebrafish Caspy2 binds directly to lipopolysaccharide via its N-terminal pyrin death domain, resulting in Caspy2 oligomerization, which correlates with the catalytic activity for pyroptosis. Furthermore, we revealed that Caspy2 is highly expressed in the zebrafish gut and is significantly activated during infection. Essentially, knockdown of caspy2 expression impaired the ability of zebrafish to restrict bacterial invasion in vivo, and also protected larvae from lethal sepsis. Collectively, our results offer a crucial event in the evolution of pattern recognition into the death domain superfamily-mediated intracellular LPS-sensing pathway in innate immunity, and also highlight the double-sided roles of the non-canonical inflammasome in modulating infection in lower vertebrates.

RETINA DEVELOPMENT OF THE MANDARRIN FISH *Siniperca chuatsi* AT THE LARVAL AND JUVENILE STAGE

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The mandarin fish, *Siniperca chuatsi*, is endemic in East Asian and important cultured species in China. It is a carnivores species with an unique feeding habitat, preying on only live fishes since the first feeding, which made its feed domestication difficult in practice. To understand the role of vision on their feed habitat, retina development of the mandarin fish from the first day to the thirtieth day after hatching (DAH) were observed using classical histology and scanning electron microscopy methods. The results showed four layers were formed in retina, the pigment epithelium layer (PEL), the immature photosensory cell layer (PCL), the inner nuclear layer (INL), and the undifferentiated ganglion cells layer (GCL) at 1DAH. Late, inner plexiform layer (IPL), nerve fibre layer (NFL), outer plexiform layer (OPL), outer nuclear layer (ONL), was differentiated at 2DAH, 3DAH, 4DAH, 5DAH, respectively. The inner limiting membrance (ILM) and out limiting membrance (OLM) was formed at 3DAH, 5DAH, respectively. The cone cells in PCL were distributed loosely at 2DAH, and became regularly arranged at 4DAH. In PEL, pigment particles were obvious at 3DAH. Since the major layers were developed incompletely at the first feeding day (3-4DAH), vision doesn't play an important role is their early feeding. During the 6-30 DAH stage, in photosensitive segment, the rod cells differentiated quickly from some neuro-epithelial cells at 10 DAH, whereas many single cone cells became fused into the double cone cells since 11DAH. With development of a large number of rod cells, together with the cone cells fusion and arrangement transformation in the retina, the significance of vision predation initiated at later stage.

EFFECT OF DIETARY LIPID AND CARBOHYDRATE ENERGY RATIO ON ONGROWING PIKEPERCH (*Sander lucioperca*) IN COMMERCIAL SCALE RAS

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Introduction

European pikeperch (*Sander lucioperca*), currently commercially farmed in traditional pond systems, become more and more relevant for economical production in recirculating aquaculture systems (RAS). Pikeperch are often fed fish feeds such as sturgeon, salmon or turbot diets. The high energy and protein levels of these feedstuffs are more or less adequate for the carnivorous pikeperch but lipid and carbohydrate levels were not sufficiently explored for ongrowing pikeperch.

Materials and Methods

To quantify effects of different lipid/carbohydrate energy ratios (L/C ratio) a feeding experiment with three different diets was conducted in 2.5 m^3 tanks connected to a RAS. The trial was carried out in a triplicate design under commercial conditions at 23°C for 101 feeding days. Wheat and corn starch was used as carbohydrate source. The diets were each composed of the same raw materials and were formulated isonitrogen and isoenergetic. The basal diet (BD) was rested upon currently used diets for pikeperch production (L/C ratio 1.88). The high carbohydrate diet (HC) had a L/C ratio of 1.16 and the low carbohydrate diet (LC) had a L/C ratio of 2.30. Fish were fed thrice a day, 1.0% of body weight (BW) at the beginning and 0.8% BW after 35 feeding days. The tanks were stocked with 30kg m^{-3} (300 fish, average weight 250g) and was set to 22 hours dim light (15-20 lux at water surface) and two hours at 100 lux for routine and maintenance. At the end of the experiment samples were taken for health parameters, liver glycogen content, growth performance and body composition. The remaining fish were fed in the same manner and finally were stripped for determination of apparent digestibility coefficients (ADC) of crude protein, crude lipid, nitrogen free extract and gross energy.

Results and Discussion

DFI was similar at about 0.81 ± 0.02 in all feeding groups. FCR decreases from 1.28 ± 0.05 in HC to 1.16 ± 0.03 in BD to 1.08 ± 0.04 in LC groups. ADCs for nitrogen-free extract (NfE) and gross energy (GE) were both calculated without crude fibre. SGR and the ADCs of crude protein (CP), crude lipid (CL), NfE and GE were regressed against the L/C ratio. SGR, NfE and GE showed significant differences. No difference could be seen in protein content of the fish and only an increasing tendency in lipid content from HC to LC groups. Splenosomatic and hepatosomatic-index showed only slight fluctuations and stayed in normal range. Liver glycogen content was very variable in all groups and therefore showed no significant difference.

According to Nyina-wamwiza et al. (2005) relatively high amount of carbohydrates (15-20%) in pikeperch fingerling feeds result in better growth and FCR. For ongrowing pikeperch high amount of carbohydrate energy in feedstuff seems to have no impact on pikeperch health parameters, feed intake and protein synthesis but it leads to decreasing feed conversion ratio and growth. The exchange of carbohydrate energy with lipid energy in feedstuff shows better growth performance but a higher visceral lipid content. It seems that diets should not exceed 20% of carbohydrates for adult pikeperch and therefore seems to be conferrable to juveniles.

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EFFECTS OF ALIPHOS® MONOCAL AND WINDMILL® AQUAPHOS ON PERFORMANCE AND PHOSPHORUS RETENTION IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Phosphorus is an essential nutritional component in the diets of farmed fish but is also linked with potential environmental impacts. Hence, optimising the use of dietary phosphorus is essential in the diets of farmed fish.

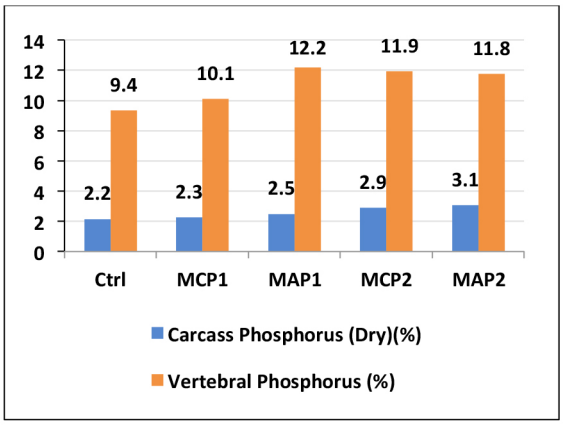
A growth trial was carried out in which 80g European sea bass, *Dicentrarchus labrax*, were fed a series of diets where a basal phosphorus-limiting diet was supplemented with two sources of phosphorus (Aliphos® Monocal and Windmill® Aquaphos) at two different doses, to determine which had the most beneficial impact on fish nutritional performance and phosphorus retention.

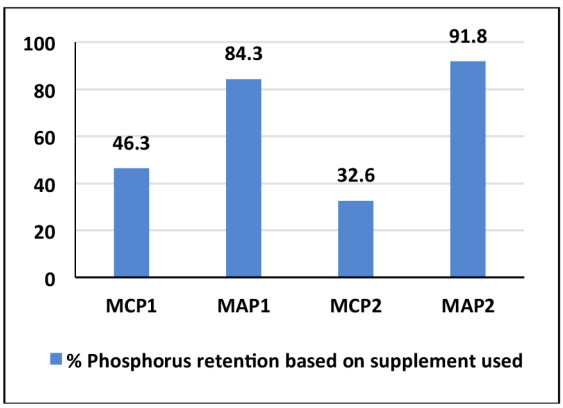
Five fish meal-free diets were used: a low-phosphorus basal diet (Ctrl), two diets in which the basal diet was supplemented with two levels of Aliphos® Monocal (MCP1, MCP2) and two diets in which the basal diet was supplemented with two levels of Windmill® Aquaphos (MAP1, MAP2).

Fish were grown in full strength sea water at 25°C in 650L tanks forming part of a 15-tank recirculating aquaculture system at the AquaBioTech Innovia facilities in Malta. Fish were fed, in triplicate, their respective diets at a fixed feeding regime four times a day. At the end of the growth phase, fish were sampled for proximate analysis.

Addition of the feed phosphates improved performance significantly compared to the control diet. Differences were obtained in carcass and vertebral mineral compositions. Phosphorus source and inclusion level were also found to affect phosphorus retention by the sea bass.

This trial demonstrated that both phosphorus source and their inclusion level affects the overall nutritional performance and retention of phosphorus in 80g European sea bass. Overall, phosphorus supplementation with Windmill® Aquaphos provided more nutritional benefits compared to Aliphos® Monocal.





ADDENDUM

HALF WAY THROUGH VIVALDI – PROGRESS TOWARDS PREVENTION AND MITIGATION OF BIVALVE DISEASE

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The European shellfish production, which mostly relies on mussels, oysters and clams, is a promising and dynamic sector, whose growth has unfortunately been hindered over the recent years by mortality events, linked to pathogen organisms. The EU project VIVALDI is a 4-years Horizon 2020 project, involving 21 partners, and aiming at better preventing and controlling marine bivalve diseases. VIVALDI brings new knowledge on the interactions between shellfish, environment and pathogens. It also develops practical tools and approaches to better prevent and control the pathogens affecting the main European farmed shellfish species: oysters (*Crassostrea gigas and Ostrea edulis*), mussels (*Mytilus edulis* and *M. galloprovincialis*), E), clams (*Venerupis philippinarum*) and scallops (*Pecten maximus*). The project addresses the most harmful pathogens affecting these species: the virus Ostreid herpesvirus 1 (OsHV-1), Vibrio species (e.g. *V. aestuarianus and V. tapetis*) as well as micro-eukaryotes such as the parasites *Perkinsus olseni* and *Bonamia ostreae*.

Key sampling sites in VIVALDI
The main sampling sites have been chosen for their contrasted features and with a view to represent at best the diversity of the EU shellfish production, allowing the partners to gather samples from the main bivalves studied in the project: Dungarvan Bay (IE), Ria de Vigo and Delta del Ebro (ES) and Rade de Brest (FR).

Progress achieved up to now
The project started in April 2016: first results have been achieved in understanding the diversity of pathogens in bivalve populations and in their detection thanks to the elaboration of passive sensors. These tools are currently being tested for the detection of OsHv-1 in the natural environment. Mathematical models for the transmission of diseases and risk levels for farming are currently developed and will be helpful to better anticipate the emergence of disease. Studies on the response of bivalves against diseases have contributed to identify key pathways (e.g. Warburg metabolism and autophagy) and novel important protein families. Microbiome is also deeply investigated during disease development mostly in *Crassostrea gigas* and *Mytilus* spp. Field experiments and monitoring are ongoing in the different key sites and allow identifying biosecurity measures contributing to avoid disease and to decrease mortality outbreaks.

Finally, in order to improve the dialogue among the parties affected by shellfish diseases and to disseminate results of the project, a mapping of stakeholders has been established, as a first step to better evaluate stakeholders' perceptions regarding disease management measures.

REPLACING SOY WITH BLACK SOLDIER FLY LARVAE IN AQUAFEEDS FOR JUVENILE ATLANTIC SALMON AFFECTS GROWTH, SWIMMING ACTIVITY AND BOLDNESS

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Increasing pressure on global wild fisheries, coupled with a need for food security for a growing population have driven expansion in the aquaculture sector. Due to scarcity and expense of fish meal, soya has become one of the most widely used protein sources in the diets of carnivorous farmed fish species, like Atlantic Salmon, *Salmo salar*. However, soy protein can cause enteritis in the distal gut of salmonids, reducing nutrient uptake and leading to physiological stress. This study aimed to investigate two key questions. Firstly, Is there a difference in growth and behavioural indicators of welfare i.e. swimming activity and boldness between a standard commercial marine protein diet and a soy protein diet? Secondly, what is the effect of replacing soy protein with the novel protein Black Soldier Fly larvae (*Hermetia illucens*) (0%, 7.5%, 15%, 22.5%, 30%) on growth and behavioural indicators of welfare i.e. swimming activity and boldness. This investigation focused on the juvenile stages of *Salmo salar* as a key period that requires high quality and quantity of protein and is influential in development of behaviour.

This study found that there was a significant difference in both the behavioural response between juvenile *Salmo salar* fed marine protein and soy protein diets (Fig.1). Individuals fed the soy protein diet swam with higher angular velocities and spent more time sheltering, both results may be indicators of impaired welfare. This study also found that with only low level (7.5%) of soy protein replacement with *Hermetia illucens* protein, behavioural indicators of impaired welfare disappeared and growth improved (Fig.2).

This study highlights the importance of behaviour as a tool in assessing dietary changes in aquafeeds and their potential consequences for welfare in intensive salmonid aquaculture.

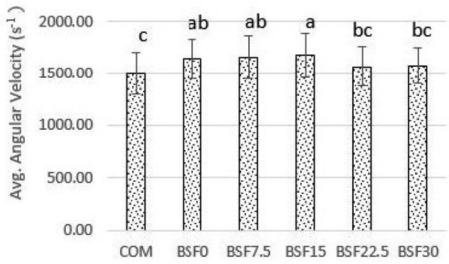

Figure 1. Swimming activity. Average Angular Velocity. Letters following means indicate statistical differences between treatments, means not sharing a common letter are significantly different.

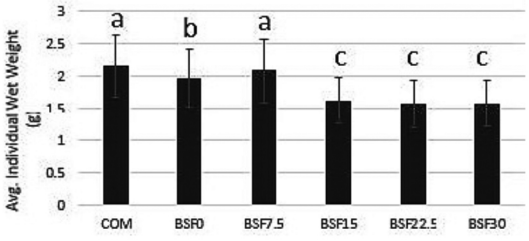

Figure 2. Final Individual Wet Weight (g) for each treatment. Letters following means indicate statistical differences between treatments, means not sharing a common letter are significantly different.

FISH CRP AND TILAPIA HEALTH RESEARCH IN RELATION TO GLOBAL BIOSECURITY GOVERNANCE

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The CGIAR Research Program on Fish Agri-Food Systems (FISH), led by WorldFish, focuses on the interlinked challenges of sustainable aquaculture and small-scale fisheries enhancing the contribution of fish to poverty reduction, with a geographical focus on Africa, Asia and the Pacific. Sustainable aquaculture research within FISH includes a cluster of research on "fish health, nutrition and feeds". WorldFish had held an international research workshop on 28–29 November 2017, which brought together 22 participants representing 16 public and private collaborating research partner institutions, to elaborate the research and partnership agenda for key health research activities on Tilapia – within the FISH Program from 2017 to 2022.

The workshop acknowledged the importance and timeliness of research into the genetic improvement of tilapia toward disease tolerance. Considering the current spread of tilapia lake virus (TiLV) and its future threats, the workshop conclusions highlighted some key recommendations. The recommendations were to: (a) improve understanding of the molecular genetic aspects of TiLV; (b) better understand the virus and its pathogenicity; and (c) make TiLV related research material virtually available to the public as community resources. The research program's impact would be enhanced by a set of community resources that would (a) be widely disseminated; (b) build human capacity development; (c) contain strong private sector participation in the prevention and control of diseases in tilapia aquaculture; and (d) create policy change at national and global levels. A key avenue for change is to establish a policy dialogue with national authorities and international networks for creating a conducive and enabling policy environment toward reducing the risks of diseases in tilapia aquaculture. Research partners saw immense value in pursuing a collaborative research agenda to find solutions to emerging tilapia health problems, with a special focus on TiLV. There was an overwhelming consensus to work closely under the FISH framework of WorldFish, communicate regularly and share experiences in the coming years.

Following the workshop WorldFish and other collaborative partners worked together and completed biosecurity assessments for tilapia breeding platforms in Bangladesh, Egypt and Malaysia. Upgradation measures were developed and implemented, including training of hatchery technicians with supporting "training manual". In addition to several research publications, WorldFish factsheet on TiLV was developed and widely disseminated to build awareness and capacity in focal and scaling countries. Fish health researchers from WorldFish and partner institutions were trained in TiLV diagnosis and surveillance in Mahidol University and Norwegian Veterinary School. With the emphasis on surveillance and diagnosis, WorldFish initiated work with NVI to develop a global assessment tool for tilapia health economics and epidemiology for implementation in 2018 and also reached an agreement with Cefas (UK) to jointly host a post-doctoral fellow to commence work in 2018 focusing on rapid diagnostics, online surveillance and epidemiology.

DEVELOPMENT OF A LOW-DENSITY SNP MARKER PANEL IN PACIFIC WHITE SHRIMP *Penaeus vannamei* FOR APPLICATION IN PARENTAGE ASSIGNMENT, DETECTION OF INBREEDING, AND DIVERSITY ASSESSMENT

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Pacific white shrimp (*Penaeus vannamei*) are the most important shrimp species in the aquaculture industry and account for more than 50% of all farmed crustaceans. Global production of *P. vannamei* has increased steadily over the years from 1 million tons in 2003 to over 3.5 million tons in 2014. The need to establish reliable genetic tools to assess parentage, relatedness and diversity among cultured populations is of increasing consequence to maintaining diverse, healthy stocks of *P. vannamei*. We report here on the development and characterization of a single nucleotide polymorphism (SNP) panel for *P. vannamei*. A panel of 192 SNP markers was established for use in genotyping and was validated in several cultured populations of *P. vannamei*. The SNP panel, with an average genotyping success rate of >98%, was able to provide measures of diversity, inbreeding and population structure within and among stocks. Use of the SNP panel to assign parentage to individuals was excellent, with an overall assignment rate of 99.99%. This genetic resource will be a valuable tool in the management of *P. vannamei* breeding programs.

GENOMIC SELECTION IN SHRIMP

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The availability of SPF (Specific Pathogen Free) shrimp broodstock has had a significant impact on the aquaculture of shrimp; from reducing the incidence of disease to facilitating more traditional family-based breeding for shrimp. From a breeding perspective, one of the shortcoming of the SPF system is that for biosecurity reasons, it does not allow for genetics from the growing ponds to be incorporated into the breeding program. Similarly, selection for other traits such as disease resistance and carcass quality prevent broodstock on which a trait was measured from returning and contributing to the breeding nucleus. Thus, the only way to use this information for genetic improvement is to rely on family and sib-ship information. This approach is not ideal as the accuracy of selection is limited because within family genetic effects are not captured. Mass selection approaches suffer from the same short comings in biosecurity, lose potential increases in genetic gain to reduced accuracy, and suffer from the risk of inbreeding depression. Genomic selection was developed to increase the accuracy of selection, accelerate genetic gain, and consequently increase genetic gain per generation while simultaneously allowing for the control of inbreeding on a whole-genome level. It relies on the measurement of genomic similarity to predict breeding values, rather than a sib-ship relationship. It is a powerful tool for many reasons: 1) it allows for increases in selection accuracy; 2) it allows for selection of breeding candidates from different genetic backgrounds that are more likely to perform well; 3) it allows for the control of inbreeding (relatedness) in a genome wide fashion; 4) it allows for selection on phenotypes that cannot be measured on the breeding candidates without depending solely on family information. This last point can have a great impact on shrimp breeding, as it would allow the accurate incorporation of genetic data from ponds without increasing biosecurity risks. Application of genomic selection is feasible when combining an economically efficient method for scanning the genome of broodstock for SNP (single nucleotide polymorphism) markers, and genomic imputation to reduce the overall burden of genotyping costs. With the careful incorporation of imputation and breeding strategies, the economic costs for incorporating genomic selection to accelerate shrimp breeding programs are no longer barriers to implementation.

RECENT ADVANCES IN THE LARVAL REARING AND FEEDING STRATEGIES FOR MARINE MEDITERRANEAN SPP. AND ITS IMPACT ON OVERALL FRY QUALITY

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During the last years, scientific and empiric evidence has shown that the feeding strategy applied during the first larval stages together with the microbial and biochemical composition of the rearing environment have a major impact on the survival and quality of the produced fry & future fish. This presentation will review the current practices in larval rearing covering new concepts of optimized use of live food and early weaning diets, the impact of the use of immunostimulants during early stages, and new concepts that aim to control the environment by means of microbial management to boost the intrinsic potential of the fish larvae to a maximum during the early phases. The effect of the different strategies on survival, growth and subsequent fry quality in terms of deformities and robustness, will be presented in this presentation.

HOW CAN WARM-WATER SHRIMP CONQUER THE COLD WORLD? A HEDONIC PRICE STUDY ON SHRIMP IN DANISH RETAIL SALE

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Abstract. The ever-growing industry of farmed warm-water shrimp in developing countries claims continued expansion of export markets in developed countries, where they form a growing share of markets traditionally supplied by wild-caught shrimp. In this market transition, developing countries can gain from knowledge regarding consumer valuation of attributes of shrimp to reasonably target product forms. In this article, the implicit price for product attributes of shrimp is analysed in Danish retail sales using a hedonic price model for 7,023 purchases of shrimp from 2011-2015. The results show that there is a price premium for warm-water shrimp, though it has a larger impact on low-income households. Freshness has a price premium for warm-water shrimp, but price discounts cold-water shrimp. Private labels have an 11-19 % price premium for warm-water shrimp. This article shows that despite traditionally eating wild-caught cold-water shrimp, Danish consumers are willing to both purchase and pay a price premium for warm-water shrimp. The article also indicates that exporters and supermarket chains together can gain by using private labels. The warm-water shrimp farmers have the potential to conquer the cold-water shrimp world.

RESEARCH NEEDS IN SUPPORT OF GROWTH OF SMALL-SCALE AQUACULTURE GLOBALLY

David Little, John Bostock, Stuart Bunting, Will Leschen,, Mahmoud Eltholth, Richard Newton and Francis Murray

Research to support further growth in small-scale aquaculture globally requires a value chain perspective and will be highly context specific. Knowledge gaps provide opportunities for local research entities to support private sector initiatives that build on global and local knowledge. Understanding and enhancing the opportunities for smallholder continuance or entry into aquaculture needs interdisciplinary capacity and inter-organisational skills. Understanding demand for aquaculture products, often ignored by promoters, resource implications and the current and potential technology options are prerequisites for framing research. Interpreting the role of aquaculture within the typical pluriactive smallholder livelihood demands specific tools and dietary norms and aspirations need understanding if nutritional outcomes of promoting aquaculture are to succeed. Capacity to understand current innovation in production, processing and marketing; and societal trends , especially the impacts of broader development are vital to framing relevant and attainable research objectives. This paper reviews the challenges for smallholder producers to achieve efficiency gains and market share to meet both local and global markets.

SYNERGISTIC EFFECTS OF DIETARY METHIONINE AND THREONINE SUPPLEMENTATION ON THE RAINBOW TROUT (*Oncorhynchus mykiss*) IMMUNE STATUS SUBMITTED TO THERMIC STRESS

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On-growing rainbow trout (*Oncorhynchus mykiss*) are usually exposed to a wide range of water temperature over the year, which in certain situations (e.g. water temperature is out of the optimal temperature range) could translate into an allostatic state and therefore compromise growth performance and immune responses in fish. In this context, dietary supplementation with key amino acids (AA) seems to be a good strategy to enhance the fish immune system. For instance, methionine (Met) is an essential AA involved in antioxidant and immune-modulatory responses, while threonine (Thr) seems to play an important role in maintaining intestinal integrity and function but also promoting antibody production in lymphocytes. The aim of this study was to assess the synergistic effect of dietary methionine and threonine supplementation on the immune status of rainbow trout exposed to thermic stress.

Fish were distributed over 24 tanks and fed with the experimental diets during a period of 2 weeks: control diet (CTRL), formulated to meet the rainbow trout AA requirements and two other diets formulated to increase Met and Thr at 1.5 and 2 times their requirement level (1.5MT and 2MT, respectively). Fish from 12 tanks were maintained at constant 15 °C whereas fish from other 12 tanks were submitted to a 10 °C increase in water temperature. At the end of the feeding period, fish (n=9) were sampled for plasma collection and further analyses of innate immune parameters.

Plasma lysozyme and alternative complement pathway activities showed no significant differences regarding dietary or temperature treatments. Despite of the observed decrease in plasma anti-protease activity at higher temperatures, the opposite trend was observed regarding plasma nitric oxide and peroxidase levels, regardless of dietary treatments. These results suggest that fish were most likely developing an innate immune response, directly or indirectly, triggered by thermic stress. Moreover, fish fed both supplement diets presented an increase in plasma peroxidase activity compared to those fed the CTRL diet, regardless of temperature. Such increase may indicate that a combined supplementation of Met and Thr could enhance an innate immune response led by neutrophils activation, which may therefore improve the rainbow trout immune status.

ARCTIC CHARR PRODUCTION IN CANADA

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Arctic Charr culture in Canada has been a modest industry for several years. Closed contained, fresh water facilities are producing charr in several provinces and territories. Investment in infrastructure, including RAS technology, has been implemented by some owners to facilitate growth of the business. Arctic Charr is a fish that performs well in culture conditions in that the species does well at higher densities and it can tolerate handling required in a production setting. The final product, to the plate, is high end and is well received by chefs and consumers across the country, and around the world.

The Arctic Charr industry is an industry poised to grow significantly in Canada. There is access to cold water, interest to grow a species other than salmon, ability to produce the fish to market on land, and access to well-developed RAS technology. Arctic Charr culture is being investigated by some First Nations communities as a venture that could provide economic development, employment, much needed food security, as well as connection to traditional food.

Whitehorse, Yukon, Canada is home to Icy Waters Ltd., an Arctic Charr company that has produced and provided certified Yukon Gold™ Ova to farms in Canada and around the world for over 25 years. Having access to proven ova twice a year is an important benefit to existing and developing Artic Charr farms.

THE CONTINUOUS DEVELOPMENT OF INNOVATIVE PRODUCTS AND CONCEPTS THAT FACILITATES THE USE OF *Artemia* IN MARINE FISH AND SHRIMP HATCHERIES

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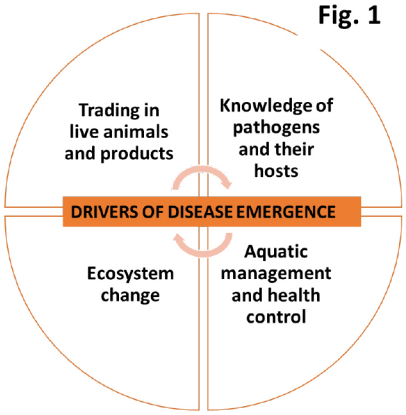
Regardless of the vast improvements in shrimp and fish nutrition, Artemia remains essential in the larval culture of most marine species. Although the use of Artemia appears to be straightforward, considerable progress has been made in the past three decades in improving and simplifying its use in marine fish and shrimp hatcheries. For more than 35 years, researchers from INVE Aquaculture have made discoveries and created innovations that have been of great significance for the aquaculture history, resulting in a more reliable performance and more efficient use of Artemia in hatcheries. The improvements include sourcing and characterization of strains, developing technologies for diapause breaking to achieve consistent and maximum hatching, developing methods and products for targeted bioencapsulation, developing technologies that allow hatcheries to obtain a perfect separation of nauplii from cysts shells. INVE Aquaculture's vision and commitment together with its continuous investment in Artemia related research have resulted in two new developments that even further facilitate and improve the use of Artemia in marine fish and shrimp hatcheries. In this presentation, we will introduce these two innovations, showing that some of the drawbacks that are inherent to the use of Artemia can now be eliminated.

Fig. 1



RESEARCH NEEDS TO ADDRESS AQUACULTURE VULNERABILITY UNDER CLIMATE CHANGE

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Climate variability and climate change are an increasing threat for aquaculture to realize its potential within the next half century and more. Direct and indirect climate change drivers can be responsible for changes in aquaculture, whether in the short- or long-term. Examples of short-term impacts include loss of production or infrastructure due to extreme events, diseases, toxic algae and parasites; and decreased productivity due to suboptimal farming conditions (temperature, salinity, hypoxia etc.). Long-term examples include scarcity of wild seed, limited access to freshwater for farming, limited access to feeds from marine and terrestrial sources, decreased productivity due to suboptimal farming conditions, eutrophication and other perturbations.

In order to address research gaps and priorities to increase aquaculture resilience to climate change, we use a global vulnerability assessment to identify most vulnerable countries and picture the main components of vulnerability i.e. Exposure, Sensitivity and Capacity to adapt. To reduce Vulnerability we must reduce sensitivity, exposure and increase adaptive capacity. In doing this analysis we realize that aquaculture research (in general) has been mainly focusing on the technical and production challenges of the sector. Such background provides a good start to address Exposure. Indeed, some relevant research gaps include -knowledge of synergistic interactions between stressors (e.g. acidification and increased water temperature and or increased salinity); understanding of species tolerance to a combination of stressors; knowledge of impacts arising from adaptation in other sectors, e.g. water for agriculture and implications for aquafeeds (for example); knowledge to improve species strains, specially of freshwater species, marine bivalves, seaweeds etc.

However, the current knowledge and understanding of the research gaps to address sensitivity (or socioeconomic dependency of the sector) and adaptation capacity (governance ability) are much scarcer. Research to enhance the adaptation to climate change of the sector should also include (among others): analyses of the social and economic consequences of climate change; knowledge of adaptation strategies and innovation at all levels of the value chain; developing and strengthening integrated monitoring systems to provide information useful to make decisions, innovative knowledge on improved inter-institutional and public private coordination mechanisms. To address vulnerability there is also a special need for interdisciplinary research linking the biophysical drivers, ecological, social and governance responses.

This presentation will use some case studies at national level and the farming system scale to address the different research needs and priorities to address each of the three components of aquaculture vulnerability to climate change.

THE ANATOMY OF THE EMERGING DISEASE

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The ability to keep aquaculture stock alive and, in a high health and welfare status, lies at centre of a sustainable and scalable aquaculture industry. However, despite this central tenet, disease is considered as *the* major constricting factor to achieving Enhanced Sustainable Production (ESP) from the aquaculture industry to 2050. Whilst sector-specific differences clearly exist, the role of infectious disease in limiting yield via direct mortality, morbidity-associated growth restriction (e.g. extending the growth period and requirement of feeding) or, spoilage of product is widely acknowledged. Overall, while difficult to estimate accurately due to the lack of official data pertaining to direct and indirect stock loss, the economic burden of disease to current the global aquaculture industry likely exceeds $6bn per annum.

Specific pathogens (in discrete aquaculture sectors) can be agents of recalcitrant and significant diseases leading to multi-$bn losses over extended time periods (e.g. White Spot Disease caused by White Spot Syndrome Virus in shrimp, from the early 1990s until present. In other scenarios, syndromic conditions, where single causative agents remain elusive, can cause ongoing production losses often without attracting industrial or political notoriety often due to an unclear strategy for managing the condition (e.g. proliferative kidney disease in trout). Elsewhere, emergent pathogens (and their associated diseases) may cause short term shock to discrete sectors but are managed out of the system via appropriate biosecurity or other intervention strategies (fish virus/vaccine). As may be expected, the array of pathogenic agents associated with known disease conditions in major farmed species is as diverse as those known to infect wild hosts from aquatic habitats; examples ranging from some of the smallest RNA viruses known through bacterial, fungi and protists, to metazoan parasites with complex on- and off-host life cycles.

Much more so than in terrestrial farming systems, a high frequency of disease emergence is considered a distinctive feature of the global aquaculture sector. In this presentation I will summarise the key drivers for disease emergence in these systems, focussing on trading in live animals and their products, changes in ecosystem status, management and control strategies and lack of knowledge of pathogens and their potential susceptible hosts (Fig. 1). Further, I will discuss the role of elimination of the pathogen threat as a basis of most aquaculture farm-, inter-farm-, national- and international-biosecurity strategies and place this in context of new data on microbial diversity and host susceptibility.

UPGRADING OF AMMONIA , CO2 AND MINERALS TO MICROBIAL PROTEIN FOR FEED PURPOSES

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Aerobic fermentation offers perspectives to the issues of a new protein supply world wide and to innovative recycling of nitrogen , carbon and minerals . Two routes are available ie the organotrophic route in which organic matter is the driving force for microbial growth and concomitant protein synthesis and also the autotrophic route in which bacteria obtain the energy to synthesize microbial biomass from CO2 by oxidizing an electron donor such as H2 gas, simply supplied by electrolysis of water . Microbial based biomass production for feed purposes dates already from the 70's ; the new elements are that now one can compose highly effective microbial teams (microbiomes) and that the pressure to achieve protein production with a low environmental footprint is steadily increasing .

At present , we are exploring both routes at a pilot scale level . The organotrophic route starts with process waters from the starch industry which are rich in carbohydrates and minerals . The latter are converted to microbial cells . These are harvested and dried . The protein content is of the order of 70% and the amino acid compostion is better than that of fish meal . Feed tests with schrimps and pigs revealed that the product yields good growth and furthermore generated increased immuno-resistance . The autotrophic route is also explored and yields a similar type of valuable biomass .

Both routes indicated major potentials to produce feed for aquaculture applications . The economics indicate that the endproducts thus obtained reach a level which becomes competitive with that of conventional protein sources . Ohter indicators such as Life Cycle Analysis , regulator authorization and public acceptance are positive . Yet , the challenges to harvest and process the microbial proteins in an efficient way are not fully resolved and possible routes to integrate aerobic microbial production of protein into aquaculture practices need to be optimized .