



COPEPODS OR ROTIFERS? EVALUATING THE USE OF DIFFERENT FEEDING PROTOCOLS FOR LARVAE OF ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*. L)

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There are still many issues that require to be solved in larval rearing of Atlantic bluefin tuna (*Thunnus thynnus*; ABT) to prevent “mass-mortality” during this developmental stage. Initial data related to the feeding sequence of ABT larvae suggested that mortality observed during the first stages of life could be due partly to nutritional deficiencies. Previous studies demonstrated that copepods appeared to be a superior live prey compared to rotifers during the first two weeks of life. Our overarching aim was to evaluate different feeding strategies during first feeding of ABT larvae from a performance, compositional and molecular perspective. In order to do so, two groups of ABT larvae were fed with either copepod (*Acartia tonsa*; C) nauplii or rotifers (*Brachionus rotundiformis*; R) enriched with Algamac 3050® from mouth opening to 13 days after hatching (dah). After this, the group C-larvae was fed either *Artemia* enriched with Algamac 3050® (CA), *Acartia* nauplii and copepodites (CC) or sea bream (*Sparus aurata*) yolk-sac larvae (CY), while the R group passed on to being fed on *Artemia* enriched with Algamac 3050® (RA) up to 18 dah. After 13 dah, larvae fed C grew more than those fed R although there were no differences in survival. ABT larvae fed R accumulated highest eicosapentaenoate (EPA) but lowest docosahexaenoate (DHA) and total n-3 long-chain polyunsaturated fatty acids (LC-PUFA) than C-fed larvae, reflecting dietary contents. Indeed, there was no activation in the expression of the enzymes involved in LC-PUFA biosynthesis. However, the different live prey elicited regulation of transcription factor, digestive enzyme, lipid metabolism and oxidative stress genes. At 18 dah larvae fed CY and CA were the largest size with larvae fed RA displaying the lowest growth with no differences in survival among the dietary treatments. The highest DHA contents were found in ABT larvae fed CC and CY, whereas the lowest contents were found in RA-fed larvae. Indeed, RA-fed larvae showed the highest level of the intermediate product n-3 docosapentaenoate, which could be reflecting up-regulation in the biosynthetic pathway although this was not supported by gene expression data.



Microalgae replacement by *Ulva rigida* in pacific oysters (*Crassostrea gigas*) diet: effects on broodstock conditioning, gonadal maturation and spawning success

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The current study evaluated the microalgae replacement by dry seaweed (*Ulva rigida*) in the reproductive success and biochemical composition of pacific oyster (*Crassostrea gigas*) during broodstock conditioning. Two trials were carried out: a feeding for 11 weeks, and thereafter a respirometry trial for 48 hours. Seven hundred and twenty oysters were divided in four dietary groups (3 tanks/diet; 60 oysters/tank). Each group were fed with one of the following diets: 100% live microalgae blend (Micro); 75% Micro + 25% seaweed (SW); 50% Micro + 50% SW or 100% SW. The Micro was composed by 33% *Isochrysis galbana* and 66% diatoms (75% *Skeletonema costatum* + 25% *Chaetoceros calcitrans*). Gonadal maturation was reflected in the physiological condition of the individuals. All treatments, except 100% SW, showed an increase in condition index and were fully matured at the end of the trial, with the best physiological condition observed in two groups: 75% Micro + 25% SW and 100% Micro. Conversely, oysters fed 100% SW showed a decrease in the reproductive condition and a delay in gonadal development. The results of the respirometry trial showed an inhibition of oxygen consumption in oysters fed 100% SW, which is in line with the condition index and gonadal maturation. Protein and total lipids content increased during the conditioning, whereas glycogen decreased. Oysters fed 75% Micro + 25% SW had higher protein and total lipids content and lower glycogen content (main energy reserve of gametogenesis) than the other treatments. In addition, this group showed the highest percentage of viable (free-living) veliger larvae after induction of spawning, even higher than the 100% Micro group. The current study demonstrated that it is possible to replace 25% of microalgae with seaweed (*U. rigida*) in the broodstock conditioning of the pacific oysters, minimizing the operative cost in bivalve hatcheries.

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FATTY ACID COMPOSITION OF OOCYTES AND EGGS FROM WRECKFISH (*Polyprion americanus*) FEMALES FED WITH DIFFERENT DIETS.

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The optimum development of broodstock diets for wreckfish will be essential for the future of the aquaculture of this species. Dietary lipids and specially fatty acids play a critical role in the successful production of high quality gametes and eggs of marine fish (Izquierdo et al., 2001; Sargent et al., 2002). Recently some studies were done about composition of wild wreckfish tissues (Roncarati et al., 2014, Linares et al., 2015).

The aim of this work is to know the influence of wreckfish broodstock diets composition on the fatty acid composition of oocytes and eggs

Three broodstock diets were used: semi-moist and dry food supplied to two batches of the IEO wreckfish broodstock and hake/squid (half and half) to the IGAFa broodstock. The semi-moisture diet was a mixture of 14.8% white fish, 14.8% oily fish, 18% mussels, 17.6% squid and 34.8% fishmeal. The dry food has 50% of fish meal, 12.5% of squid meal and 6% of krill meal. Proteins, total lipids and fatty acids of the diets were carried out. All the diets have a high amount of proteins, higher than 60%, and the lipid content is 17% in semi-moist diet, 12.5% in dry food and 8% in hake/squid (Table I). Hake/squid has the highest level of polyunsaturated (PUFA), 55% of total fatty acids (TFA) while dry food has the highest level of n-6 (16%) and ARA (7%) (Table II). Samples of oocytes and eggs were taken out by biopsies of the females. Fatty acid analysis of oocytes and eggs from females fed with the three diets were performed. The results show a high level of n-3 PUFA (41% and 43% in oocytes and eggs respectively) when hake/squid was used as diet and in the case of n-6 PUFA the highest values were found (12 and 10% TFA) in oocytes and eggs from females fed with dry food (Tables III and IV).

There is a clear relationship between the main fatty acids composition of diets, oocytes and eggs and the ratio EPA/ARA in oocytes and eggs reflected the diet and was negatively correlated to dietary ARA content.



Dietary fish oil replacement by soybean oil: Effect on plasma vitellogenin, sex steroids and ovarian steroidogenesis in Chinese Strip-necked Turtles (*Mauremys sinensis*)

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In this study, we investigated the effects of dietary fish oil replacement on plasma vitellogenin (Vtg), sex steroid levels and ovarian steroidogenesis during vitellogenesis in female Chinese strip-necked turtles. The turtles were fed four iso-nitrogenous, iso-energetic, and iso-lipidic experimental diets for 10 months: FO (100% fish oil), FSO (70% fish oil and 30% soybean oil), SFO (30% fish oil and 70% soybean oil), and SO (100% soybean oil). The results showed that plasma gonadotropin-releasing hormone (GnRH) levels were the highest pre-vitellogenesis and significantly decreased post-vitellogenesis in all four groups, which promoted the secretion of gonadotropin and sex steroids. Therefore, plasma luteinizing hormone (LH) and estrogen (E2) levels continuously increased during vitellogenesis and were significantly increased at post-vitellogenesis ($P < 0.05$), while follicle-stimulating hormone (FSH) levels increased at vitellogenesis ($P < 0.05$) and were slightly decreased at post-vitellogenesis in all four groups. The FO and FSO groups had significantly higher GnRH and E2 levels than the SFO and SO groups during vitellogenesis ($P < 0.05$). However, the SO group had the lowest plasma FSH and LH levels at vitellogenesis and post-vitellogenesis. In addition, plasma vitellogenin (Vtg) levels significantly increased at vitellogenesis and post-vitellogenesis ($P < 0.05$). The FO and FSO groups had significantly higher Vtg levels than the SO group ($P < 0.05$) during vitellogenesis. Moreover, hepatic estrogen receptor α ($Er\alpha$) mRNA levels were significantly increased at vitellogenesis and post-vitellogenesis, while ovarian Cyp19 α 1 α mRNA levels were significantly increased post-vitellogenesis ($P < 0.05$). Both Cyp19 α 1 α and $Er\alpha$ mRNA levels were decreased in the SO group during vitellogenesis. Taken together, the replacement of fish oil with 66.7% soybean oil is feasible.



Does arachidonic acid affect the maturation and reproduction performance in virgin RAS-reared pikeperch breeders?

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Role of long chain-polyunsaturated fatty acids (Lc-PUFA) in the maturation and reproduction process is well studied in several cultured fish species. Among the LC-PUFA, arachidonic acid (ARA) has been relatively neglected compared to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), although only ARA could induce the in vitro maturation of oocytes. Nevertheless, discussing individual levels of each of these Lc-PUFAs seems less vital in terms of reproduction to the ratios between them. Therefore, the present study aimed to investigate the effects of dietary ARA supplementation on the reproductive performance of pikeperch (*Sander lucioperca*), which is a promising species for intensive aquaculture in Europe.

Six months long feeding trial with virgin breeders was conducted. A commercial broodstock feed Coppens SteCo REP was selected and supplemented with arachidonic oil (DSM ARASCOTM) in laboratory condition by vacuum infusion technique. The experimental diet was developed to contain Lc-PUFA levels in the ratio DHA: EPA: ARA=1.4:1:1. Each of the diet, Control and ARA, were assigned to one of the tanks. Each tank was stocked with 35 individuals of 2-year-old pikeperch breeders. The fish were reared in 10 m³ RAS and fed to apparent satiation 3-6 times per week depending on the rearing temperature. At the end of the feeding trial 6 males and 6 females from each tank were selected for artificial propagation, when different reproduction parameters were recorded and egg samples for FA measurements were taken.

The fatty acid composition of dry eggs revealed that enrichment of the feed contributed to 3 times higher ARA level in the eggs (0.89 mg/g), however amount of all other FAs was not changed (DHA 10.60 mg/g, EPA 1.70 mg/g). Nonetheless, results of the artificial reproduction showed no improvement in any of the reproductive parameters assessed. On the contrary, although without statistical significance, reproductive parameters were impaired in ARA group.

As a conclusion, results of this preliminary study suggest that modification of ARA content in the feed may influence the reproductive parameters in pikeperch, but more comprehensive studies are needed to define the optimal levels and ratios of Lc-PUFAs for successful reproduction of RAS-reared pikeperch breeders.



Arginine influences the *Rhamdia quelen* reproduction

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Informations about broodstock nutrition are fundamental to improve the reproductive performance, gamete and progeny quality. Arginine has been studied in the nutrition of mammal breeders, but this is a pioneering study for fish. With the objective of assessing the effect of arginine on the reproductive parameters of *R. quelen*, five diets were formulated with different levels of arginine (1.37; 1.67; 1.97; 2.27 and 2.57%). After five months of experiment the fish received hormonal induction (2.5 mg.kg⁻¹ carp pituitary extract for male and 5.5 mg.kg⁻¹ for female), the semen were collected and analysed: volum, pH, concentration, velocity, motility and normality. In female the oocytes diameter and production of vitellogenin in blood plasm. The fish were euthanazied, the liver, visceral fat and gonad were collected to somatic analysis. We have observed that the addition of 2.27% arginine in the feed increased seminal volume, sperm concentration, and size of testes in this species, although these results have been contrary to those found for sperm motility and velocity. In female, the addition of 2.27% arginine to the diet resulted in larger liver. Furthermore, there was increase in the production of vitellogenin, and oocyte diameter. We concluded that 2.27% arginine in the diet of *R. quelen* broodstock improve the production and quality of gametes. Thus, arginine might be a key factor in broodstock nutrition in general and consequently raises reproductive efficiency.

KEY-WORDS: amino acids, larviculture, nutrition, semen, vitellogenin.



EFFECT OF FOOD RESTRICTION ON REPRODUCTIVE PERFORMANCES AND EGG QUALITY IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Egg production in rainbow trout is a key sector for aquaculture. This industry relies on a well-controlled reproductive cycle and extensive use of photoperiod control to obtain year-round production. However, feed management practices are very diverse between producers and not always designed to optimize egg production. Feed restriction is one of these practices. It has the advantage of limiting fish aggressive behavior and reducing egg production cost but it is not always applied in optimal conditions. The aim of the present study was to evaluate in rainbow trout the impact of feed restriction on reproductive performances, egg quality and fish lipid metabolism.

For that purpose, three feeding strategies were implemented during the last six months before the reproduction. Females were either fed (1) ad libitum, (2) 80% of the ad libitum during the first 3 months then ad libitum for 3 months (intermediate) or (3) 80% of the ad libitum (restriction). During the trial, two artificial photoperiods were applied to trigger reproduction during summer (see figure 1: Experimental design).

As expected, feed restriction during the 6 months preceding reproduction (treatment 3) resulted in smaller females in comparison with those fed ad libitum whereas absolute fecundity and gonado-somatic index were not affected by the feeding strategy (n=115 females). However, hatching rate was significantly better for eggs from restricted females.

Moreover, an analysis with visilog imaging software performed on approximately 400 eggs per females, for a total of 52143 eggs, revealed that feed restriction led to the production of bigger eggs with less size variability and lower number of white eggs (bad quality eggs) which explain the better hatching rate.

These females of treatment 3 also exhibited lower viscero-somatic index suggesting an impact of feed restriction on reserves allocation. However, lipid content in eggs and muscle as well as the expression of genes involved in lipid metabolism in liver were not different between groups. Fatty acid profile of eggs and muscle are in progress.

To sum up, these results demonstrate that feed intake of female rainbow trout broodstock can be reduced by at least 20% without impacting egg production and egg quality.



Early nutritional intervention using yeast in diets for smoltifying Atlantic salmon (*Salmo salar* L)

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The aquaculture is the fastest growing food production system in the world and plays an important role in providing the global protein supply. To secure a sustainable growth in the aquacultural industry several challenges need to be overcome. Atlantic salmon (*Salmo salar* L), the main species in Norwegian aquaculture, are anadromous fish that undergo a series of physiological, structural and functional changes in the transition from fresh water (FW) to seawater (SW). A major challenge is the high mortality during SW transfer. The use of functional feed has received increased attention as a solution to these challenges. Yeast produced from lignocellulosic biomass has shown to be a high quality protein source with health beneficial properties (Øverland and Skrede, 2016).

The main objective of this study was to investigate effect of adding yeast to diets for smoltifying salmon on growth performance and health. A control diet and a test diet containing 25% *Candida utilis* yeast were fed to Atlantic salmon for 4 weeks in FW and 4 weeks after transfer to SW. Water quality was monitored by assessing microbial load and the possible presence of dangerous pathogens in SW. At various time points, distal intestine, liver, spleen, head kidney and blood plasma were sampled from both groups. Effects on overall performance were investigated by monitoring weight gain, while the effects on health is underway using histology, morphometry, serum proteomics, western blotting and gene expression analysis. A set of genes involved in digestive function, intestinal epithelial barrier integrity, antioxidant activity and immune response will be analyzed. Results show that feeding yeast clearly enhanced growth performance during the FW phase, with subsequent improvement in growth performance during the SW phase, compared to the control. The initial western blot results demonstrated that yeast inclusion has altered the expression level of interleukin 1 β in spleen, suggesting diet induced immunomodulation. Our preliminary conclusion suggest that feeding a diet containing yeast to salmon before SW transfer can improve growth performance and robustness during smoltification. Further analysis will reveal more details of this response.



Nutritional imprinting in salmon fry

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It has become clear in recent years that several important health endpoints are impacted upon by metabolic imprinting at early life stages in mammals. These include the link between nutrition at an early developmental stage and a range of future health risks such as obesity and metabolic related diseases. However, it is not known how changes in the dietary composition of macronutrients for salmon at early life stages affect development of adipose and muscle tissues.

The aim of this project was to increase the knowledge on how different fat/protein ratios in the feed for salmon fry affect the development of adipose tissue, muscle tissue and health markers at early life stages.

Salmon fry were fed three different ratios of fat/protein from start feeding to about 5 g: Diet 1) high fat/low protein, Diet 2) medium fat/medium protein and Diet 3) low fat/high protein. Tissue samples were taken at several time points; at the yolk sac stage, at 0.2g, 1g, 2.5g and 5g sizes of salmon fry. The fish samples were examined chemical composition, histological (Oil Red O) and transcriptome (RNA sequencing) analyses.

The results showed that different fat/protein ratios of the feed significantly affected both adipose tissue and muscle tissue development of salmon fry. The histological studies showed higher levels of visceral fat deposition of fry fed high fat/low protein ratio compared to those fed the low fat/high protein diet. Transcriptome analysis of the different developmental stages showed large variation in gene expression patterns of biological processes such as fat, protein and nucleic acid metabolism, brain development and nerve signaling between the diet groups, especially in salmon fry at 1g.



Nutritional programming in juveniles of the whiteleg shrimp (*Litopenaeus vannamei*) followed by a strong early feed restriction at post-larval stage

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In this study, the concept of metabolic programming has been tested for the first time in whiteleg shrimp (*L. vannamei*). In an early stimulus phase, shrimp were raised under a 70% feed allowance restriction during the post-larval over three days and compared to a control group. After 46 days, shrimp were challenged by feeding on diets with three nitrogen free-extract: crude protein ratios (1.5, 1.0 and 0.7) for 70 days. In order to test the existence of metabolic programming, we analyzed shrimp growth performance as well as mRNA levels of different metabolic and digestive actors after the stimulus, and before and after the challenge. No direct effects of the stimulus were observed for several of the digestive and metabolic actors, except for the trypsin mRNA which was lower in the feed-restricted group. This was probably linked to a decrease in dietary protein intake. As expected, significant effects associated with the diet challenge were detected for shrimp performance, i.e., growth was lower in shrimp fed with reduced levels of dietary protein. More interestingly, some effects linked to the nutritional history were detected suggesting an improved growth performance for shrimp that had been previously fed under a restricted level. After challenge, significantly lower mRNA levels for hepatopancreatic genes involved in digestion (lipase, preamylase and trypsin), amino acid metabolism (gs), energy metabolism (cox VI b) and glucose metabolism (lvglut 1, lvglut 2 and pk) were found in early restricted shrimp. This shows the existence of metabolic programming at a molecular level. The link between an enhanced growth performance and these molecular markers in early feed restricted shrimp requires further studies. Overall, our study has demonstrated for the first time that shrimp can be programmed by an early nutritional stimulus. This will allow the development of new strategies in novel shrimp diets in a near future.



Genetic variation in digestibility in Atlantic salmon (*Salmo Salar*)

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Genetic variation in digestibility in Atlantic salmon (*Salmo Salar*)

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Abstract

A 30-day experiment were performed in the fish laboratory at the Norwegian University of Life Sciences, Aas, Norway with Atlantic salmon (n = 129) from 14 families with an initial weight between 33-338 g. The aim was to evaluate the genetic variation in individual digestibility between families. The fish had been reared together in one common tank throughout the experiment. At 5-10 g of weight, the fish were pit-tagged with a 2 x 12 mm unique glass tag (RFID Solutions, Hafslund, Norway) and genotyped. Fish were fed a fishmeal-based diet during the experiment. Faeces for determination of digestibility were collected by stripping the fish four times (once a week). Results showed significance between family effects in nitrogen (P = 0.006) and carbon (P = 0.0005) digestibility, with an estimated heritability of 49% for nitrogen and 59% for carbon. However, it seemed that digestibility has an adverse genetic correlation to growth rate (r = -0.97). A possible explanation is that a high growth rate is related to a high feed intake, which increases the passage rate in the gastro intestinal tract and reduce digestibility of the nutrients. On the other hand, growth rate is expected to improve FCR of digested nutrients after they are absorbed, because more of the absorbed nutrients are used for growth of new tissue and relatively less is used for maintenance of existing body mass. Another experiment found a significant genetic correlation between growth rate and FCR (r = -0.67) on a family level basis (two tanks per family). The favorable effects of increased growth rate outweighs the unfavorable effects of reduced digestibility. Results indicate that there is variation in FCR, which cannot be explained by growth rate or digestibility. Therefore, future research should evaluate potential phenotypes, which explain more of the variation in feed efficiency.



EMBRYONIC DEVELOPMENT IN EGGS OF OREOCHROMIS NILOTICUS: DIGESTIVE ENZYMES

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The study assessed activity of pepsin, aminopeptidase, alkaline phosphatase, trypsin, amylase, lipase, protease enzymes on the embryonic [cleavage, blastula, gastrula, hatching] and larval [7 and 10 days post-hatch] development of Nile tilapia, from broodstock fed with four levels of crude protein. The experiment was carried out from January to June/2014 (144 females and 48 males, 16 hapas), four treatments (T, Crude Protein, %, T1 (32), T2 (38), T3 (44), T4 (50)) and four replications. Eggs were collected from four females in each treatment and kept in hatcheries. For sampling of embryonic development, one sample each female in each stage was collected. After hatching, larvae were kept on fasting and were collected in each treatments, with the same samples and females. No differences for pepsin and trypsin during embryogenic, amylase exhibited difference at gastrula. Protease and lipase showed differences at blastula. During larval period, differences founded for pepsin, lipase and aminopeptidase between treatments. Differences were observed for amylase and trypsin 7 and 10 dph; for protease at 7 dph and alkaline phosphatase in 10 dph. The results evidence that treatment one influenced activity of enzymes in Nile tilapia eggs and larvae. However, needs additional studies with more information about protein for broodfish.



Quantifying the endogenous production of omega-3 (n-3) long-chain polyunsaturated fatty acids (EPA and DHA) in Atlantic salmon (*Salmo salar*)

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This study was specifically designed to provide novel insights into essential fatty acid metabolism and requirements of Atlantic salmon. Fish were fed from first feeding for 5 months on fishmeal- and fish oil-free diets completely devoid of long-chain polyunsaturated fatty acids (LC-PUFA). This made the fish totally dependent upon maternal/egg reserves and endogenous biosynthetic pathways for provision of the key biologically active fatty acids, eicosapentaenoate (EPA, 20:5n-3) and docosahexaenoate (DHA, 22:6n-3). Three diets were formulated with linseed and sunflower oils to provide α -linolenic (ALA, 18:3n-3) and linoleic (LOA, 18:2n-6) at dietary ratios of 3:1, 1:1 and 1:3, and fed to the fish in comparison with a reference diet containing fish oil. The overall aim was to quantify the in vivo production of n-3 LC-PUFA from ALA to give insight into the quantitative requirements for EPA and DHA, tissue deposition/distribution patterns, potentially "optimal" EPA/DHA ratios achieved in both whole fish and in individual tissues, and the impact of dietary LOA. Therefore, specific objectives were to: quantify total production of n-3 LC-PUFA from dietary ALA and the resultant EPA:DHA ratio achieved by endogenous biosynthetic pathways alone; define the impact of dietary LOA and ALA:LOA ratio on endogenous production of EPA and DHA from ALA; assess the relative importance of EPA, DHA and EPA:DHA ratio for different tissues (e.g. liver, gill, head kidney, intestine, brain and flesh); determine the key EPA- and DHA-containing lipid species (e.g. lipid classes and molecular species); investigate the regulatory mechanisms underpinning the critical metabolic pathways by focussed expression studies of key target genes. After 5-months, the fish fed the experimental diets were significantly smaller than fish fed the reference feed suggesting that growth was impacted by the lack of dietary n-3 LC-PUFA, but dietary ALA:LOA ratio had no major effect on growth. However, that growth was still possible in the absence of dietary n-3 LC-PUFA was a key finding. Extrapolation of the complete data set will help to establish the minimum physiological requirements of Atlantic salmon for EPA and DHA to enable formulation of feeds that maximise the efficient use of these nutrients.



Biosynthesis of very long-chain (>C24) polyunsaturated fatty acids in gilthead seabream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*): Investigating early ontogeny and nutritional regulation

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Very long-chain (>C24) polyunsaturated fatty acids (VLC-PUFA) are biosynthesized in fish through consecutive elongations of long-chain (C20-24) PUFA precursors under the control of two enzyme elongases of very long-chain fatty acids-4, namely Elov14a and Elov14b. VLC-PUFA are particularly important for the correct development and function of tissues as retina, brain and gonads of vertebrates. Our objective was to determine the spatial-temporal expression patterns of genes involved in the biosynthesis of LC-PUFA (elov15, fads2) and VLC-PUFA (elov14a, elov14b) in early stages of larval development of Senegalese sole and gilthead seabream. Moreover, we investigated the nutritional regulation of these genes in larvae fed different dietary regimes.

Fertilized eggs of both species were grown at 18°C, and samples were collected daily during early development (0 - 8 days post hatching) for quantitative PCR (qPCR) and whole mount in situ hybridization (ISH) analyses. For nutritional regulation studies, larvae of both species were fed two diets based on *Artemia* with low and high LC-PUFA contents. RNA was extracted from all samples, to evaluate relative gene expression, using qPCR.

The results show a trend towards the overexpression of fads2 and elov15 being apparent in some cases in fish fed low LC-PUFA diet, which could be indicative of the existence of an enzymatic compensatory regulatory mechanism, responding to suboptimal dietary supply of these essential nutrients. The presence of the two isoforms of elov14 was confirmed as early since the egg stage for both species. The expression levels of all genes show variations along the period studied at the inter- and intra-specific level. The expression levels of elov14 tend to increase with increasing dietary LC-PUFA, especially in elov14b. Interestingly, we observed an increase in the expression of both elov14 isoforms consistent with the timing when retinogenesis and brain development occur in both species. This evidence points at the importance of VLC-PUFA as key compounds for the normal development of eyes and brain during larval stages, when neural tissues accumulating VLC-PUFA are rapidly growing. Finally, ISH studies during the larval development of both species show clearly different spatial expression patterns for both elov14 genes, from very early stages of development.



**Effects of intraperitoneal injection of sulfur compounds on taurine synthesis in juvenile red sea bream
(Pagrus major)**

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Taurine is an important sulfur amino acid for early-stage of marine carnivorous fishes. Taurine synthesis is achieved by a series of enzymatic reaction. The three major pathways of vertebrates are identified; cysteine sulfinic acid pathway, cysteamine pathway and cysteic acid pathway. Red sea bream cannot synthesize taurine from cysteine because cysteine sulfinic acid decarboxylase (CSD) activity in liver is extremely low, but the activity of cysteamine dioxygenase (ADO) and cysteic acid decarboxylase (CAD) are higher than that of CSD. Therefore, red sea bream could be able to synthesize taurine by cysteamine pathway and/or cysteic acid pathway independent of CSD. However, it is not examined whether taurine can be synthesized from cysteamine and/or cysteic acid in red sea bream. Therefore, the present study aimed to determine effects of sulfur compounds intake on plasma and hepatic taurine content and gene expression of taurine synthesizing enzymes (cysteine dioxygenase; CDO and CSD).

48 juvenile red sea bream (initial mean weight: 74.3 ± 24.8 g) were divided into four treatments. Fish were injected one ml of sterilized saline per 100g of body weight into the peritoneal cavity as control. In the other treatment groups, fish were injected one ml of 150mM L-cysteine solution, L-cysteic acid solution and cysteamine solution per 100g of body weight, respectively (Cys-group, Cya-group and MEA-group). Fish were sampled at 2, 6, 12 and 24 hours post-injection. Plasma and liver were collected for measurement of amino acid composition and gene expression analysis of CDO and CSD by RT-qPCR.

The expression of hepatic CDO gene significantly increased in Cys-group at 2 hours post-injection than other groups ($P < 0.05$). Taurine content in plasma and liver of Cys-group did not increase, whereas higher taurine content in plasma of Cya and MEA-groups were detected at 2 and 12 hours post-injection respectively. Taurine content in liver tended to increase with time post-injection in Cya and MEA-groups.

These results suggest that juvenile red sea bream can synthesize taurine from L-cysteic acid and cysteamine, and expression of hepatic CDO gene responds elevation of plasma L-cysteine.



LONG TERM EFFECT OF DIETARY METHIONINE DEFICIENCY AT THE FIRST FEEDING ON HEPATIC METABOLISM IN JUVENILES OF RAINBOW TROUT

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Early nutritional events exerted during critical developmental windows may result in permanent changes in animals, a process termed nutritional programming. Methionine emerged as a key factor in modulating the cellular availability of the main biological methyl donor S-adenosylmethionine (SAM) needed for all biological methylation reactions including DNA and histone methylation. As such, it represents a potential critical actor in nutritional programming.

In the present study, we aimed to determine the long term consequence of dietary methionine deficiency at first feeding stage on growth and hepatic metabolism in rainbow trout (*Oncorhynchus mykiss*). Rainbow trout were exposed for 2 weeks starting at the first-feeding fry stage, to one of the three different diets: a control diet balanced in all nutrients (C), a methionine deficient diet (MD) or a methyl group donor (methionine, choline, folate, B12 vitamin) deficient diet (MGD). After this 2 weeks period, all the three groups were fed on the same commercial diet (CD) for a period of 5 months. Each group were then challenged for a 2-week period with either the MD diet or the C diet, in order to test the existence of a nutritional programming (figure 1).

The obtained results indicated that, whatever the diet offered at first feeding, the 2 weeks challenge with the MD diet increased the hepato-somatic index (HSI) of the fish. On the other hand, the diet offered at the first feeding has significantly affected the HSI and growth of fish challenged with the MD diet. Indeed, trout fed with the MGD diet at first feeding exhibited a significantly higher HSI than the control once challenged with the MD diet. Similarly, body weight of fish fed MGD but also MD diet at first feeding, was significantly higher than the C group after the 2 weeks MD-challenge.

All together, these results clearly show that a short (2 weeks) early nutritional stimulus may have long-term consequences on physiological functions and highlight the potential role of methionine to induce such effects. Currently, analyses are in progress to characterize the underlying mechanisms involved in the observed effects.



Effects of microencapsulated diets containing fish meal or plant meal and supplemented with various levels of water-soluble vitamins on growth, survival and stress resistance of common carp (*Cyprinus carpio* L.) larvae

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For most freshwater aquaculture species, one of the main bottlenecks is the stable production of high quality juveniles. As fish larvae grow rapidly, the total ingestion of nutrients must be high and the larvae are vulnerable to stress. The high and unpredictable mortality in the ponds during the first weeks after hatching remains a problem to be solved. Microencapsulated diets might be the future starter food for common carp. To determine the effects of starter diets for common carp larvae with new delivery vectors for water soluble vitamins, an experiment was carried out. Fish meal and vegetable meal based microencapsulated diets were prepared with low, medium and high levels of water-soluble vitamins (groups D1, D2, D3 and D4, D5, D6, respectively). At 10 days post-hatching, common carp larvae were distributed into six groups and fed with these feeds for 18 days. At the end, growth and survival of larvae were checked, and fish were exposed to hypoxia for one hour, during which the number of air-breathing fish were counted. Before and after the stress, larvae were collected to analyse the expression of genes related to stress response.

Growth performance was in good range in any of the groups. However, survival in groups fed with fish meal-based diets was significantly higher than in groups fed with plant-based diets. During the hypoxia stress, the number of air-breathing fish in groups D1, D2 and D3 was much lower than in groups D4, D5 and D6. However, this number was the highest in group D4 and decreased in groups D5 and D6. During the stress, expression of SOD1 significantly increased in groups D5 and D6, as well as the expression of CORT in group D2.

According to these results we concluded that both sources of protein (fish or plant meal) are usable in starter diets without any detrimental effects on growth. However, survival and stress tolerance of larvae was dependent on the protein sources of the feeds. Results of hypoxia demonstrated that stress resistances of fish fed with plant-based diets are extremely weak, however it could be enhanced by higher supplementation with water-soluble vitamins.



Effect of dietary manganese and zinc supplementation on growth and bone status of Senegalese sole post-larvae

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Essential dietary trace elements, such as zinc (Zn) and manganese (Mn), critically influence a wide range of physiological, metabolic and hormonal processes in fish larvae and post-larvae, such as survival, growth, feed utilization, skeletal formation, immune response and susceptibility to pathologies. Despite their importance for normal fish growth and skeletal development, trace mineral nutrition has not been extensively studied in early stage fish.

In this study, a 39-day feeding trial was undertaken using Senegalese sole (*Solea senegalensis*) post-larvae to determine whether the supplementation of a combination of two doses of organically bound Mn and Zn (B-TRAXIM 2C) could improve early-stage survival, growth, mineral deposition rates, and bone status.

The experimental design comprised four dietary treatments, in which an identical basal formulation was concomitantly supplemented with two levels of zinc (25 and 50 mg/kg) and two levels of Mn (50 and 100 mg/kg). Each diet was tested in triplicate. The incidence of vertebral anomalies was also assessed by x-ray analysis.

The results showed that dry weight gain, feed conversion rates (FCR), vertebral bone density and the retention rate of manganese in fish fed a diet supplemented with 50 mg Zn /kg diet were lower ($P<0.005$) than those found in fish fed a diet supplemented with 25 mg Zn /kg diet. When the diets were supplemented with 100 mg Mn/kg diet, whole fish and vertebral bone manganese content and manganese retention rates were higher ($P<0.005$), when compared to fish fed 50 mg Mn /kg diet. A preliminary analysis on vertebral malformation incidence indicates that a supplementation level of 50 mg Zn /kg feed decreased vertebral bone malformations, when compared to fish fed 25 mg Mn /kg feed. Senegalese sole post-larvae fed the different dietary treatments presented no significant differences ($P>0.05$) regarding total length, relative growth rate (RGR) and vertebral apposition rates.

A supplementation level of 50 mg Zn /kg and 50mg Mn /kg feed appears to be sufficient for normal Senegalese sole growth and may help reduce the incidence of vertebral malformations. Data generated in this trial provides new knowledge in trace mineral nutrition of early stage marine fish.



ACTIVITY OF CATALASE ENZYME IN TILAPIA LARVAE: SUPPLEMENTED DIETS WITH ORGANIC MINERALS

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Organic minerals (OM) increasingly participate in fish nutrition, besides being cofactors of oxidative stress enzymes, such as catalase. The aim of this study was to evaluate the catalase activity in Nile tilapia larvae fed with the inclusion of the minerals zinc, iron, copper, selenium and manganese in the organic form. The experiment was carried out at the Laboratory of Nutrition of the Fisheries Institute, APTA, São José do Rio Preto, São Paulo, Brazil. 11,200 larvae with 4 days post-hatch (dph) were used, with a completely randomized design with 4 treatments and 4 replicates: T1 (100% Inorganic Mineral, IN, control), T2 (33% OM), T3 (66% OM) and T4 (100% OM). Larvae were distributed in 16 tanks (310 L), disposed in a closed system with water recirculation, oxygenation, 28.0 °C and a period of 30 days. Samplings were performed 4, 10, 18 and 30 dph for catalase quantification. A two-way ANOVA (Statistic) was performed and means were compared by the Duncan test at 5%. The results show that in the 4 dph treatment 3 presented a difference ($P < 0.05$) in relation to the other treatments. However, for the other days post-hatch, treatment 5 showed the highest activity of the catalase enzyme, suggesting that the inclusion of organic minerals in the diets increases the activity of this enzyme and, consequently, a higher action against free radicals. On the other hand, analyzing the treatments in each dph, was observed that the activity of the enzyme for T1 was significantly ($P < 0.05$) in 10 dph. Regarding treatment 2, was noted that the catalase activity was higher at 10 and 30 dph. T3 showed higher activity of the enzyme at 4 and 10 dph and T4 is higher at 10 dph. The results show that the inclusion of organic minerals interferes with the enzymatic activity catalase being favorable for the animal's immune system.



INFLUENCE OF ORGANIC AND INORGANIC MINERALS IN TILAPIA LARVAE DIETS: CATALASE ENZYME

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Studies with organic minerals (OM) have been developed in order to evaluate the absorption in the intestinal tract of fish, besides its participation as cofactors of enzymes, such as catalase. This study evaluated the activity of catalase in tilapia larvae, fed with different levels of the organic and inorganic minerals zinc, iron, copper, selenium and manganese. The experiment was conducted in the Laboratory of Nutrition of the Fisheries Institute, APTA, São José do Rio Preto, São Paulo, Brazil. 14,000 larvae were used (4th days post-hatch, dph) and the treatments were: T1 (100 % inorganic mineral, IM, control), T2 (25 % IM and 75 % OM), T3 (50 % IM and 50 % OM), T4 (75 % IM and 25 % OM) and T5 (100 % OM), with four replicates. Larvae were distributed in 20 tanks (310 L), disposed in a closed system with water recirculation, oxygenation, 28.0 °C and a period of 30 days. Samplings were performed at 4, 10, 18 and 30 dph for catalase quantification. A two-way ANOVA (Statistic) was performed and means were compared by the Duncan test at 5%. By analyzing the activity of catalase among treatments in each sampling day, at 4 dph, no differences ($P > 0.05$) were observed. However, at 10 dph, the enzyme's activity increased in some treatments, with an observed difference ($P < 0.05$) between 3 and 5, in relation to 1, 2 and 4, with 5 showing the highest values. Concerning 18 dph, treatment 2 was the one that presented the highest enzyme's activity, followed by treatment 5. At 30 dph, treatments 3, 4 and 5 presented difference ($0 < 0.05$) in comparison to treatments 1 and 2, demonstrating that above 50% of inclusion of organic minerals in the diet, a significant increase of the activity of catalase occurs, evidencing an antioxidant response of the organism.



Experience of growing pikeperch (*Sander lucioperca*) larvae on artificial feeds

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As we know, there is no successful experiments with zander (*Sander lucioperca*) larvae farming using artificial starting feed only (without combining it with live feed) in world feeding practice. All attempts to grow zander larvae using exclusively artificial feed from the first days of exogenous nutrition have previously failed. In 2017 special feeds for zander were created in the laboratory of aquaculture of "GosNIORH" (Saint-Petersburg, Russia). Preliminary results of the experiment are represented in this report.

The experimental diet consisted of microbial protein (manufactured by "Giprobiosontez"), fishmeal, meat and bone meal, wheat flour, fish oil, soybean phospholipids and biologically active additives.

The experiment was conducted on zander larvae at the age of 3-4 days after hatching and with a mass of 0.35-0.4 mg. They were grown in Weiss jar with a larval density of 100 per L. Average water temperature was 21 ° C, oxygen level was 8.7 mg / L, pH 6.9.

After 34 days of feeding zander larvae on the experimental artificial forage, its length reached 26.3 mm with a mass of 153.9. Survival rate was 12.6%, while between 12 and 23 days of experiment more than 60% of larvae died. We didn't note any cases of cannibalism.

Also we should mention high growth rate of larvae fed on the experimental nutrition. During the first 23 days of feeding, the average daily weight gain was about 23.1%, which is (according to the literary data) comparable to that index for larvae fed on live and mixed (live and artificial) feed. Between day 23 and day 34 of experiment the average weight gain slowed down to 13.0% per day.

We suppose, that high growth potential of zander can be associated with the early development of its digestive system, which occurs at a mass of about 40 mg. Functional stomach development contributes to the improvement of digestibility of artificial feed components. Therefore it can be assumed that zander larvae of mentioned mass are able to adequately react to the consumed artificial feed.

Thus, today's solution of zander farming problem is in creating a high-quality artificial feed composition containing protein microbiosynthesis products which satisfies physiological needs of this



HATCHERY PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) ON A AQUAPONIC SYSTEM: EGG QUALITY, LARVAL GROWTH AND ONTOGENIC DEVELOPMENT OF THE DIGESTIVE ENZYMES ACTIVITIES IN LARVAE.

Dr. Leire Arantzamendi AZTI

Intensive tilapia farming, deserve part of their success to the hatchery performance. The aim of this study was to determine the hatchery performance of tilapia in a recirculating aquaponic system, by studying the quality of the egg produced, and the growth performance and the feed digestion capacity of tilapia larvae along ontogeny. Water quality parameters were continuously monitored and controlled [T: 26-28 °C; dissolved O₂: 3.5-6 (mg/L); pH 7-7.3; ammonia-N: 0.12-0.7 (mg/L); nitrate-NO₃: 5-15 (mg/L)] by a PROFILUX system in the hatchery facilities. In the reproduction tank natural spawning occurred in 10-15 days. The average total egg number in the egg-clutches was 404 eggs per female. Egg diameter was 2.6 ± 0.11 mm in the long axis and 1.93 ± 0.08 mm in the short axis. Eggs presented a dry weight of 2.51 ± 0.19 mg. The fertilized eggs transferred to the incubation jars hatched in 2-3 days and larvae started to feed in around 5-10 days after hatching. In the rearing period larval survival was 95%. Tilapia larvae showed an increase in length, total and furcal length, and in weight during the entire experimental period (Table 1). All the enzymes assayed were detectable at hatching (Table 2), which could indicate the maternal origin of the enzymes. In almost all enzymes studied, except for amylase, a peak of enzymatic activity was observed in the first days of feeding (5 dah-8 dah), followed by a posterior decrease by 12 dah and a final recovery of the enzymes activities by 15 dah, except for alkaline phosphatase activity which was lower at 15 dah than at 12 dah. Concluding, larvae showed a significant increase in length (total and furcal length) and weight along the entire experimental period, together with a high survival. The evolution of the digestive enzymes activities along ontogeny evidenced the capacity of tilapia larvae to digest inert feed from first feeding to 15 dah, probably contributing to the good growth performance of the larvae.



Improving fish viability under challenging temperatures

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In a scenario of global warming, fish as poikilothermic organisms face serious physiological challenges. Increases in temperature of 0.5°C have been shown to have major effects in adaptive response and fitness, in the fish larval stages. Nutrition will have a pivotal role in modulating temperature resilient fish. In this context, the objective of the present study is to contribute to determine the optimal dietary amino acid levels that may lead to maximum fitness with a positive modulation of protein accretion under challenging temperature conditions.

The experiment took place at CCMAR facilities (University of Algarve, Faro, Portugal). Triplicate groups of 100 zebrafish larvae with 15 DPF (days post fertilization) were distributed among 1L tanks for each treatment. The experiment lasted 35 days. Twelve tanks were maintained at 28°, while the remaining twelve were kept at 32° for the duration of the experiment. In addition, 28° and 32° tanks were fed 4 diets with graded levels of leucine. At the middle and end of the trial, fish were sampled for biometric measurements, performance indicators, protein metabolism, gene expression and methylation intermediates.

The overall growth performance during the first half of the experiment was significantly affected by dietary treatments and temperature. Larvae fed with LEU3 were significantly longer and heavier than larvae fed with LEU1 and LEU2. LEU3 larvae group also presented the best performance in terms of relative growth rate and condition factor K. High temperature did not damage zebrafish larvae growth performance. On the contrary, SGR and K were significantly higher in 35 GDD larvae reared at 32°. The reverse tendency was observed during the second half of the trial. Overall larvae growth performance in later stages were negatively affected by high temperatures. The dry weight of larvae reared at 28° was nearly 3-fold higher compared to larvae reared at 32°. Also, standard length and SGR were significantly higher in 28° group larvae by the end of the experiment. Analysis of gene expression and epigenetic markers is in progress, to give a better insight how to promote growth potential under challenging environments.



Water temperature differentially affects the feed transit time through stomach and intestine in Cobia fry

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Efficient macronutrients digestion depends on the time the feed is available for hydrolysis within the different sections of the digestive tract under the appropriate digestive enzymes. In fish, feed transit rates usually are estimated from gut filling or evacuation after a unique meal. Nevertheless, food residence time within the gut is dependent on the feeding frequency, as well as on the environmental conditions. In this study we have analyzed the transit rate in the stomach and intestine in early juveniles of cobia (*Rachycentron canadum*) under routine feeding and at two different temperatures.

Juveniles (3.7 ± 0.4 g wet BW) were randomly distributed to 6 experimental 200-L tanks (60 fish tank⁻¹) and reared at two temperatures (30 and 34 °C) in recirculation systems for six weeks. Fish were fed twice a day (8:00 and 16:00 h) with a diet containing 47% protein and 10% lipid. The diet included yttrium (Y) as marker only in the morning meal. At the end of the experiment, fish were sampled every 4 hours and Y-content in the stomach and intestine was analyzed by ICP-mass spectrometry. Total gut content at each sampling time was estimated from the weight of the stomach and intestine, and the transit rate from the difference of Y content between successive sampling.

In juvenile cobia, mass and volume of the stomach are much higher than in the rest of the intestine. The feed transit rate in the stomach was notably faster at 34 than at 30 °C during the first eight hours post-feeding; afterwards the rate was similar at both temperatures. Contrarily, no differences were found in the intestine, but transit rate declined drastically to residual values 12 h post-feeding. The increased temperature induced a change of filling pattern and a decrease of food residence time in the stomach.

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Food management and economic viability of tambaqui (*Colossoma macropomum*) cultivated in nurseries in Cacaulândia, Rondônia – Brazil.

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The present research had the objective to describe the food management and to evaluate the production costs of tambaqui (*Colossoma macropomum*) grown in land nurseries in the city of Cacaulândia - RO, Brazil. The study was conducted in rural property through the monitoring of biometrics, data provided by the producer and survey of prices practiced in the local market. The total production area used was 29 hectares of water mirror divided into 28 nurseries. The strategy used by the producer was the use of a biphasic system of production: rearing and fattening. In the rearing, 5 smaller nurseries were used, total area 10% in relation to fattening nurseries, where the animals were stocked for a period of 24 weeks with a feeding rate varying from 10 to 3% in relation to biomass for fish of 1,5g a 500g and protein content of 45% in the ration used. At the end of this period the fish were transferred to the fattening nurseries with stocking density of 850 g.m⁻¹ to the final weight of 3 kg. At this stage, the fish were fed twice daily with rations with a protein content of 28%. Feeding rates ranged from 300 to 750 g (2% of biomass); from 800 to 1300 g (1.5% of the biomass), from 1400 to 2100 g (1% of the biomass); and above 2200 (0.5 of the biomass) to final weight of 3000 g. In total the productive cycle for this species makes the period of 16 months of cultivation where they obtain an average apparent feed conversion of 1.8: 1, using values of 2017 as a reference. In the property were produced 195 thousand kilograms of fish in the first production cycle and marketed at US\$ 1,98 kg⁻¹ of fish. Production was sufficient to cover the effective operating cost with a net positive margin of US\$ 0,67 kg⁻¹ of fish. The ration was the item that most impacted the operating cost with 53% of the total. The results demonstrate the economic viability of the food management used for the cultivation of tambaqui in soil nurseries.



Rotifers substitution in the larval feeding of Chilean corvina *Cilus gilberti*

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The larvae of Chilean corvina *Cilus gilberti* feed the first ten days with rotifers to follow later with a mix of Rotifer plus Artemia, which then becomes exclusively of Artemia until weaning. During Rotifer period, are observed the lowest growth and survival of the larval stages. Therefore, the aim was to test a microdiet (MD) capable of substituting or complementing the Rotifers (R). A microdiet was designed in size of 100-200 μm which was distributed 5 times a day together with the Rotifers. Two experiments were carried out. The first experiment (batch noviembre 2017) had 3 treatments: 100%R, 100%R+50%MD, and 50%R+50%MD. The second experiment (batch february 2018) had 4 treatments: 100%R, 200%R, 50%R+50%MD, 100%R+50%MD. The results during the first 19 days after hatching showed that growth was similar in both batches (24.5% d⁻¹), but survival was 64% better in the batch of november 2017. At experiment 1, there was not difference between the 3 treatments for SGR or in survival. There were also no differences in experiment 2 for 4 treatments neither in SGR nor in survival. It is proposed that it is not necessary to increase the ration of rotifers (from 100 to 200%) and the 50% of the rotifers can be replaced by the microdiet. The results of diets on nutrient retention and metabolism in larvae will be discussed. Funded by INNOVA 09PDAC6912.



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Influence of dietary fatty acid profile on reproductive performance in gilthead seabream, Sparus aurata broodstock selected for high or low fads2 expression

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