Vol 6 Issue 2 2018

Hatcheryfeed

Advances in feeding early life stage and broodstock aquatic species

Do you know your microalgae?

Trends in micro diets for fish larvae

Essential nutrients and their crucial effects on reproductive performance of fish broodstock

Effects of enriched and non-enriched *Artemia* on jaw malformation in golden pompano larvae

Enrichment strategies in the early larval rearing of marine fish become a long-term investment for farmers in grow-out

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NEWS REVIEW

Replacing live feeds with formulated feed or minced fish improves survival of mud crab megalopae

Mud crab farming has long been established in the Philippines. *Scylla serrata*, *S. tranquebarica* and *S. olivacea* are the common species found in the Philippines; *S. serrata* is the preferred species for farming by crab growers.

According to Southeast Asian Fisheries Development Center (SEAFDEC), in 2015 the total production of mud crab from aquaculture was estimated at 13,720 tons, valued at US$77,025,000 and 14,437 tons valued at US$86,511,000 in 2009 and 2010, respectively.

Some 13 mud crab hatcheries have been established in the Philippines, nine of which are in the private sector, two are government owned and a further two are at state universities and colleges. A prototype commercial-scale hatchery produces about 40,000 crab instars per run. However, more hatcheries are needed since farms still depend on diminishing wild seed stock and are experiencing low survival rate during the megalopae stage due to Molt Death Syndrome (MDS), a disease due to luminescent bacteria (*Vibrio* spp.) in which zoea 5 has difficulty molting to megalopa and diseases due to bacterial and fungal infection in eggs and larvae.

The use of antibiotics as treatment for *Vibrio spp.* has been shown to improve larval survival. However, the use of antibiotics can lead to the development of resistant strains of bacteria and abnormalities that become apparent at the juvenile stage.

By replacing rotifers and *artemia* in the diet with formulated feed or minced fish or mussel, the survival rate of mud crab from zoea to megalopae has significantly improved from 3.75 percent to 22.5 percent, while the survival rate from zoea to crab instar has improved from 1.0-3.5 percent to 6 percent.

The improvement was realized under a project entitled “Improvement of Larval Rearing Protocol,” which is implemented by the Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC/AQD).

The project is part of the National Mud Crab Science and Technology (S&T) Program, which is being monitored by the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development of DOST (DOST-PCAARRD).

BioMar increases fry and RAS feed capacity at mill in Denmark

BioMar Group is increasing capacity at its feedmill in Denmark with the addition of a new production line. The new line will be highly specialized and dedicated to fry and RAS feed production, enabling the factory to increase flexibility, quality and production performance.

"BioMar has experienced a solid growth in market share on our core markets in Europe and the factory in Brande has through the last years been expanding capacity by removing bottle necks and optimizing operational processes," explained Carlos Diaz, CEO BioMar Group. "We foresee that the growth will continue and we need to take a significant leap forward to make sure we can deliver on the future demands from the customers."

The new line is expected to be ready in Q2 2019 with a budget of DKK 90 million (approx. US$14,420,927). The feedmill in Denmark will, after the capacity expansion, be able to deliver 150.000 tons of feed per year. It is BioMar's largest production unit in Europe outside the salmon markets. The factory is designed for agility with a flexible production set-up. It delivers mainly feed for species such trout, eel, sturgeon and salmon in RAS; however, in total the factory produces feed for more than 40 species.

The factory has been a pioneer within RAS feed and is highly recognized for its products as well as expertise within this segment. BioMar states that the new line enables a significant step forward in developing RAS feed with even higher growth performance and physical as well as nutritional quality.

"We are already recognized in the market for the high quality and performance of our fry and RAS feed from the factory in Denmark and the factory is often delivering feed to support other business units," added Diaz. "This is simply a step making sure we stay in front, delivering a market leading customer value proposition in terms of agility, quality and performance."

New salmon hatchery for Marine Harvest in Scotland

Marine Harvest opened a new hatchery June 1, 2018, which will grow up to 12 million fish a year and help meet the global demand for Scottish farmed salmon. The new £26.5 million hatchery at Inchmore in Glenmoriston replaces a much smaller hatchery which had been on the site more than 38 years, expanding the workforce from 5 to 18.

The hatchery team will grow salmon from eyed eggs into smolts, fry and parr which will then be transferred to the company's 49 seawater fish farms and 5 freshwater loch sites to be grown into mature fish.

Featuring one of the most technically advanced water recirculation systems in the world, which cuts down the amount of water required while ensuring the water quality, the new facility is almost identical to the Lochailort hatchery opened by Marine Harvest in 2013. It will allow Marine Harvest to stock all their Scottish farms with fish they have grown themselves from eggs.

Ben Hadfield, Managing Director of Marine Harvest (Scotland) Ltd, said: "This new hatchery is a much needed facility which will allow us to supply our expanding network of sea farms across the western Highlands and Islands and meet the increasing demand for Scottish salmon from across the world".

The new hatchery is the latest stage in Marine Harvest's expansion plans which includes the creation of new open sea fish farms in various locations in the Minch. Farms have already opened off Barra, Uist and Muck with a new site off the Isle of Rum receiving planning permission earlier this year.

Scientists make major breakthrough in omega-3 production

A major discovery that could revolutionize the understanding of omega-3 production in the ocean has been made by an international team of scientists.

Led by the University of Stirling, Scotland, research has found for the first time that omega-3 fatty acids can be created by many invertebrates inhabiting marine ecosystems, including corals, worms and molluscs.

The breakthrough challenges the generally held principle that marine microbes, such as microalgae and bacteria, are responsible for virtually all primary production of omega-3.

Lead scientist Dr. Oscar Monroig, of the Institute of Aquaculture, said that the findings strongly suggest that aquatic invertebrates may make “a very significant contribution to global omega-3 production”.

“Our study provides a significant paradigm shift, as it demonstrates that a large variety of invertebrate animals, including corals, rotifers, molluscs, polychaetes and crustaceans, possess enzymes called ‘desaturases’ of a type that enable them to produce omega-3, an ability thought to exist almost exclusively in marine microbes,” Dr. Monroig explained.

First author of the study, Dr. Naoki Kabeya, of Tokyo University of Marine Science and Technology, visited the Institute of Aquaculture after receiving a fellowship from the Marine Alliance for Science and Technology Scotland (MASTS).

Dr. Kabeya said: “Since invertebrates represent a major component of the biomass in aquatic ecosystems such as coral reefs, abyssal plains and hydrothermal vents, their contribution to the overall omega-3 production is likely to be remarkable.”

The research also involved Stirling’s Professor Douglas Tocher, and members of an international consortium of scientists, including Dr. David Ferrier, of the Scottish Oceans Institute at the University of St Andrews; Dr. Filipe Castro, of the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) - University of Porto; the Spanish National Research Council; the Australian Institute of Marine Science; and Deakin University.

Dr. Ferrier said: “It was very surprising to us to see just how widespread these genes were, particularly in animals that are so common and abundant in the sea.

“It is also intriguing that these genes seem to be jumping between very different organisms, such as from plants or fungi into an insect and a spring-tail, by a process of horizontal gene transfer. This has been a controversial idea, that genes can move around in this way, but our data looks rather convincing that these genes have done this in at least some of these species."

“These findings can revolutionize our understanding of omega-3 long-chain polyunsaturated fatty acids production on a global scale,” Dr. Monroig added.

The paper, Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals, was funded by MASTS and the European Union’s FP7 funding program.

Dr. Oscar Monroig, of the University of Stirling, said the research suggests that aquatic invertebrates may make a "very significant contribution to global omega-3 production."

Breeding for faster-growing bluegills and yellow perch

Hanping Wang, who manages The Ohio State University's Ohio Center for Aquaculture Research and Development, has succeeded in raising faster-growing fish by artificially mating them in a not so typical way. Inside cool water-filled tanks in southern Ohio, the laws of nature are being defied: female yellow perch mate with other female yellow perch; male bluegills with other male bluegills. On average, the resulting offspring reach market size six months faster than bluegills or yellow perch bred out of standard male-female mating. That's because among yellow perch, females grow quicker than males; among bluegills, males faster than females.

"We're using the animals' maximum potential to make them grow faster for human benefit," Wang said. "We have to do it this way to meet the growing need for food, specifically protein. You need to have a process to produce more animals – more chickens, cows, pigs and fish."

It seems there might be a downside to unnaturally mating fish, but Wang says that's not the case. The practice of mating females together or males together might be unusual but does not produce problem fish – that is, assuming no relatives are mated with each other, Wang said.

Among yellow perch, the females grow 60 to 70 percent faster than the males, and they grow larger than the males. As a result, it makes sense that a breeder would want to produce the fastest-growing female yellow perch. So Wang did exactly that. He mated females to females with the help of grants from the U.S. Department of Agriculture and the state-based Ohio Sea Grant program, which funds research in the Great Lakes and aquaculture.

While they remained females at the chromosome level — possessing the XX chromosome pair as opposed to the XY chromosome pair that typical males have — they still were able to produce sperm.

That allowed the females to mate with other female yellow perch. It might sound odd, perhaps, but it worked. The results were "neo-males," or "pseudo-males," as Wang calls them.

The offspring produced by the mating of a neo-male with a standard female yellow perch were all females, since there was no Y chromosome in the mix. And the female offspring grew as expected, 60 to 70 percent faster than any female offspring born out of the standard arrangement of a male and female mating with each other.

On average, it takes a farmer 16 months to raise a yellow perch to reach market size. Now it can take as little as 10 months if neo-males are mated with typical female yellow perch, Wang said.

"The farmer saves on labor, saves on feed and saves on space," he said.

With bluegills, the males grow faster and bigger than the females. So, Wang took males and mated them with males through a process similar to what was done with the yellow perch, so they became what Wang calls "neo-females." The offspring of a neo-female bluegill and a male bluegill were all male fish that could grow to 1 pound, the size needed to sell them, in about a year, cutting three to five months off the typical time needed for them to mature.

Along with mating females with females and males with males, Wang and his colleagues have conducted standard mating with yellow perch to generate the fastest-growing males and the fastest-growing females. They began with 800 yellow perch, 100 taken from eight states in the Midwest and Northeast, including Ohio. The DNA of the fish was analyzed, then the fish that were related were put in different tanks to prevent the possibility of them mating. Each fish was placed in one of a series of tanks with males and females, and they were allowed to mate as usual, males with females.

From the first round of fry, the scientists selected the 200 fastest-growing male and female fish from each cohort – then those pairs mated, and the same process occured over and over to get genetically improved fish. Across the three sites, and on average, the improved fish grew 35 percent faster than the unimproved fish, meaning the ones whose parents came together naturally without any special mating arrangements. Not only do they grow 35 percent faster, they have a higher survival rate, 20 percent higher. Whether bluegills and yellow perch can be made to grow even faster is uncertain. "We don't know," Wang said. "We're working on that."

Critical to the selective fish breeding program is a key accomplishment of Wang's research team: Completing genome sequencing of both yellow perch and bluegills. By changing the genetic makeup of the fish, researchers can select for high disease resistance and larger, faster-growing fish, Wang said. "We know long term it will have a huge impact."

Wang has also authored a book on selective fish breeding that will be available this summer.

NEW PRODUCT NEWS

INVE launches Natura diet range for early - stage marine fish larvae

INVE Aquaculture has launched 'Natura', a new diet range for early -stage marine fish larvae. The new formulation has been developed to simplify the weaning process and provides key nutritional components for the initial stages of larval development.

The Natura diets are available in 4 different sizes used from co-feeding to post-weaning. Natura is a floating, slowly sinking feed formulated to obtain satisfactory feed acquisition and feed uptake.

"Feed digestibility is of great importance, especially at the initial stages of larval development when the digestive tract is under development," stated Alessandro Moretti, Product Manager Fish Hatcheries. "Larvae fed with Natura show superior acceptance of formulated feed."

The Natura diet range has been developed using carefully selected protein sources for the early stages of the fish larvae. Not only proteins of sustainable marine origin are used, but also adequate protein sources in hydrolyzed form to obtain the most optimal uptake of amino acids and peptides in the initial stages of larval development. This together with high quality n-3 HUFA inclusion promotes larval development, juvenile growth, survival and quality.

For optimal ease of use, Natura diets can be fed daily over multiple rations according to the larval age, fish density and water temperature. They are ideally supplemented with enriched rotifers and/or Artemia.

Trends in micro diets for fish larvae

By Wilson Pinto[1*], Sofia Engrola[2], Michael Viegas[1], Jorge Dias[1] and Luís Conceição[1]

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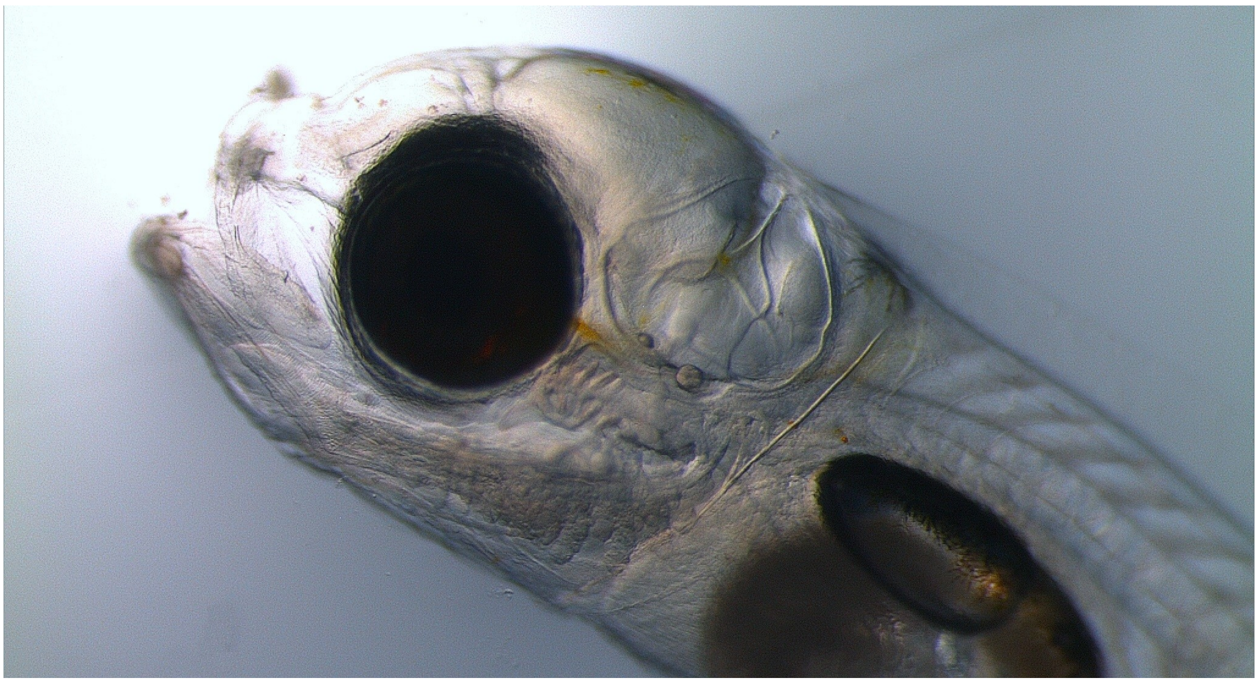

Major research efforts conducted by the industry and academia during the last decade have led to a significant evolution in the quality of commercial microdiets. This evolution reflects not only in larval growth performance and survival during weaning, but also later in the production of high quality juveniles. Nevertheless, there is still 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 should be provided by highly digestible ingredients, the exact nutritional requirement are yet poorly understood. Microdiet production technologies are also evolving day-by-day. The success of future microdiets depends on a set of technological factors like: 1) formulations that are attractive to ensure a high ingestion; and highly digestible for an easy digestion, that combine will avoid excessive nutrient losses to the surrounding water by leaching and disaggregation; 2) physical properties such as floatability, sinking speed, dispersion both in tank surface and water column; and 3) tank design and operation procedures that allow an easy cleaning of uneaten microdiets. It is also clear that early nutrition has consequences during the larval, but also later in the juvenile stage, in terms of health status, survival, skeletal deformities and growth performance.

Early weaning may bring benefits

Marine fish larvae rearing live-feed dependency and the age to start weaning have also been gradually decreasing in the last years. Beyond the benefits of off-the-shelf availability and nutritional consistency, an early introduction of microdiets may increase production predictability, avoiding live-feed nutritional and availability fluctuations and will decrease the introduction of pathogenic agents (e.g., *Vibrio* sp.) vectored by live-feeds. Even if a complete replacement of live-feeds by microdiets is still considered an utopia for the majority of cultured species, this is work in progress. A recent trial with Senegalese sole evaluated the effect of an early introduction of microdiets in larval growth and survival. Newly-hatched larvae were fed at mouth opening (2 days after hatching, DAH) with Artemia (Control) or through a combination of *Artemia* and microdiet (high replacement co-feeding strategy). Results showed that larvae from the co-feeding treatment had a lower growth performance at early stage (20 DAH) than larvae fed on Artemia alone (Figure 1). Following larval settling, larvae from both treatments were weaned and at the end of the trial (58 DAH), larvae from the co-feeding treatment had a significantly higher weight than larvae from the Control treatment. However, although not significantly different at 20 DAH (65- 70 %), survival in larvae from the co-feeding treatment was lower (34 %) than in larvae from the Control treatment (46 %) at 58 DAH. These findings indicate that an early weaning with high quality microdiets may promote an earlier maturation of the digestive system and unravel the growth potential of fish larvae, although currently this may occur at the expense of larval survival. Larvae failing to cope with an early microdiet introduction will lead to mortalities, whereas survival of the fittest larvae will prevail with large growth benefits.

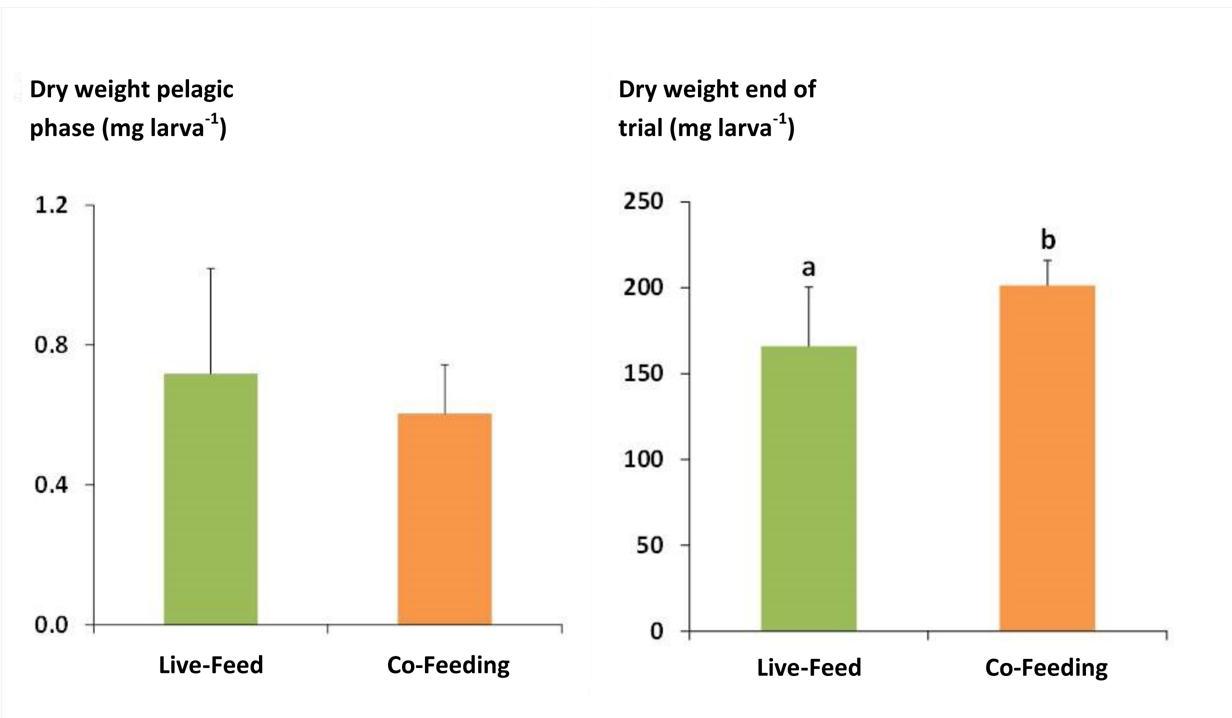

Fig. 1. Dry weight of *Solea senegalensis* fed on live-feed only or using a co-feeding strategy with a high replacement of live-feeds by microdiets from mouth opening onwards. On the left, larval dry weight at the end of the pelagic phase (18 DAH). On the right, larval dry weight at the end of the trial (58 DAH).

Microdiet formulations should be species and stage of development-specific

One of the factors contributing to a higher microdiet success within last years was the increasing perception that ingredient formulations should be adapted according to requirements and preferences of target species. Current knowledge on larval nutrition leave no choice but using an "educated guess" strategy when developing a formulation for a given species. Namely, trials using different ingredients as main protein source revealed that gilthead seabream shows preference to a squid meal-based diet, compared to a fishmeal or a mixture of high quality plant-based proteins, whereas Senegalese sole is more eclectic, dealing better with fishmeal, a mixture of high quality plant-based proteins or a mixture of all these ingredients (Figure 2). Making it even more challenging for fish larval nutritionists, it is also very likely that a given species may require different microdiet formulations at the various developmental stages. Although initially surprising, a closer look on human dietary habits reminds us that a baby´s diet changes several times during the first year of life. A study conducted by Canada et al. (2017) showed that Senegalese sole larvae (3-16 DAH) grow better when feeding on a diet including high levels of a moderately hydrolysed protein source, whereas after larval settling a higher growth performance is obtained when larvae feed on a diet composed by low levels of hydrolysed proteins and mostly with complex proteins (19-60 DAH). The most likely explanation for these findings relates to the maturation and development of the digestive tract, which may only be able to process complex proteins following a few weeks of sole development.

Diets formulations need to be optimized

Even if several commercial inert microdiets have been shown to provide good results in terms of growth and survival for species such as European seabass, gilthead seabream, red seabream and olive flounder, there is certainly still room for improvement. Dietary macro and micro-nutrient contents may be optimized, as shown recently in a study were microdiets with two different protein/lipid ratios were tested in both meagre and gilthead seabream larvae (Candeias-Mendes et al. 2016; Castanho et al. 2016). This study

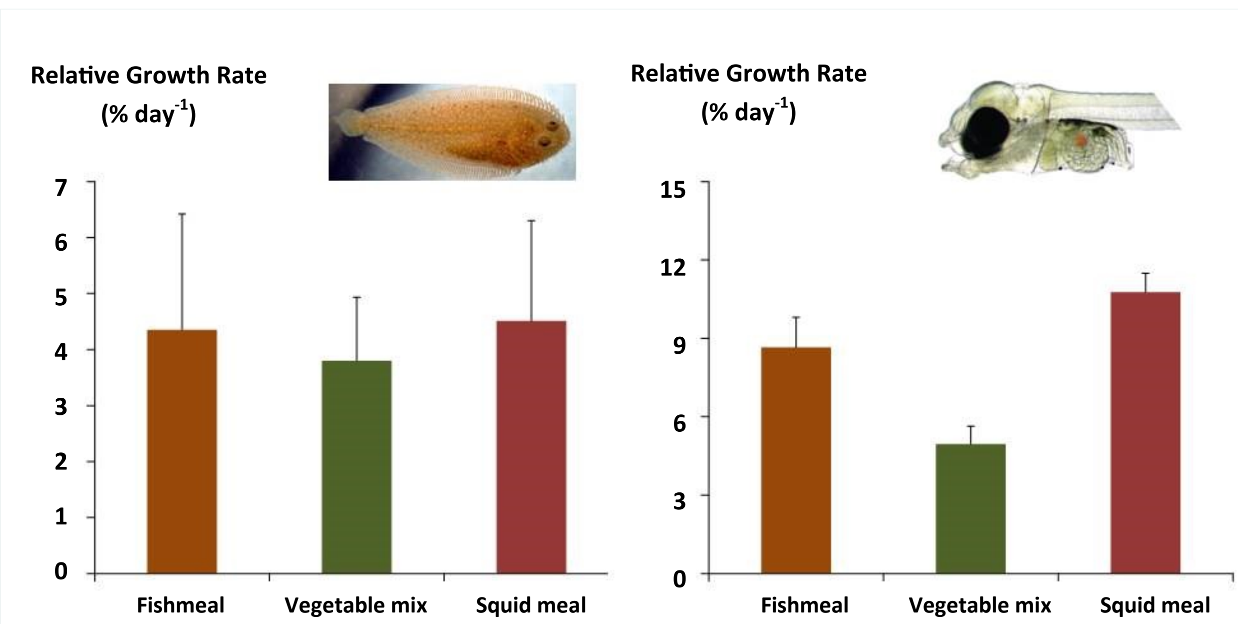

Fig. 2. Growth rates of Senegalese sole (left) and gilthead seabream (right) reared using microdiets based on ingredients from marine or plant origin.

As knowledge of larval nutrition progresses and newer technologies become available, better performing microdiets for fish will become available.

shows that both gilthead seabream and meagre larvae require a high protein/high lipid diet for an optimal performance. Diets with lower lipid contents (i.e. 15-17% crude fat), which are currently used in commercial hatcheries, may result in a lower growth in meagre. In gilthead seabream, this scenario may also occur unless a very high protein diet is used. Moreover, diets containing high lipid contents did not affect liver histology or skeletal development in both species.

Also revealing how microdiet formulations still show room for improvement, a trial conducted with Senegalese sole larvae showed that dietary phosphorous levels can have a strong impact on larval development. In this scenario, high phosphorous levels led to a depression of larval growth, whereas optimal levels increased larval growth (ranging from 4.4 to 6.4 % day $^{-1}$) and survival (from 60 to 81 %). In addition, optimal phosphorous levels also led to an increased vertebral bone density (Figure 3) and vertebral growth (Figure 4).

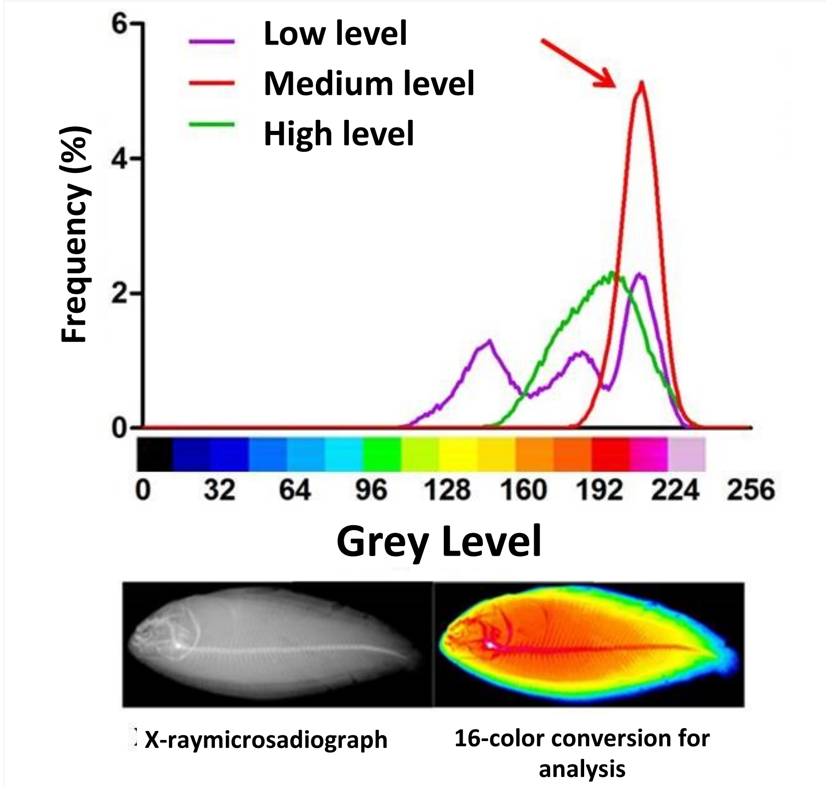

Fig. 3. Analysis of vertebral bone density by X-ray microradiograph. A higher pixel frequency, indicating a higher density of bone, is observed in sole fed a diet containing an optimal phosphorus levels.

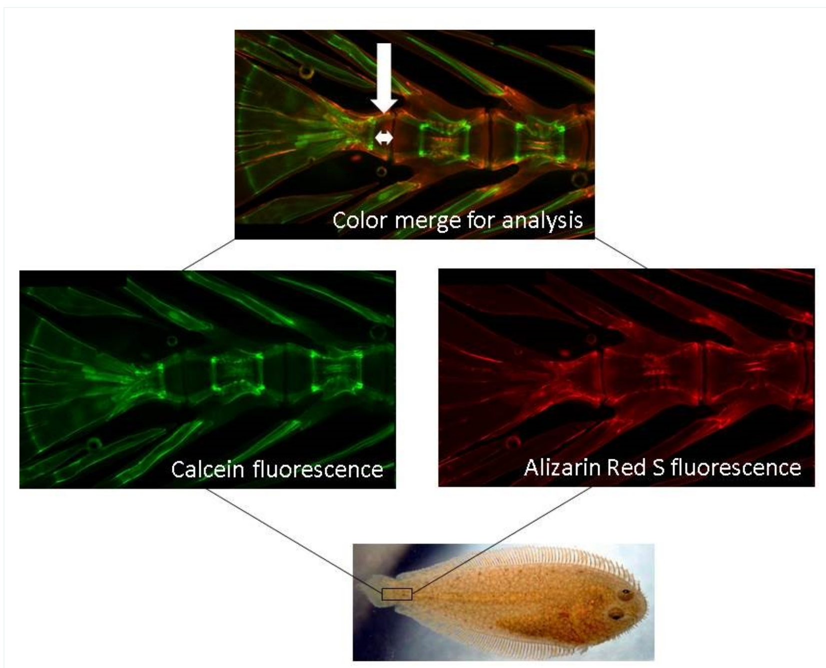

Fig. 4. Analysis of vertebral growth by double staining with Alizarin Red S and Calcein. Vertebral growth is assessed by analysing the bone formation between first (Calcein) and second (Alizarin) stainings.

Future microdiets will bring improvements

As knowledge of larval nutrition progresses and newer technologies become available, better performing microdiets for fish will become available. This will mean faster growing larvae, higher survival rates and better quality juveniles, with an early live-feed replacement increasingly being a reality.

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Do you know your microalgae?

By Emil Rue, MSc Agro and Plant Sciences, Aliga Microalgae

Breakfast is the most important meal of the day, or at least so the saying goes. Simplified logic and whether true or not, the underlying message is quite clear: make sure that you get off to a good start.

Rearing robust and high performing marine finfish larvae cultures with low mortality rates requires skills and is a multi-disciplinary field on its own. Effects of early mistakes can amplify throughout the fish's growth stages and therefore it is important that larvae are grown under good hatchery practises and feed nutritiously during its first few weeks.

The use of rotifers *B. plicatilis* and *B. rotondiformis* as well as brine shrimp *Artemia* as live prey for marine fish larvae is today commonplace since they are of major importance in the larvae's nutrition. However, these zooplankton naturally lack sufficient amounts of the essential n-3 HUFA's, yet they are rich in linolenic and linoleic acid n-6 PUFA. With the larvae's inability to synthesize the linoleic acid into essential fatty acids [1], it is required that the live prey is correctly and sufficiently enriched with eicosa-pentaenoic (EPA), docosahexaenoic (DHA) and arachidonic acid (ARA)[2.] Thus, it is pivotal that the microal-gae fed to zooplankton, either directly in its tanks or through Green Water, has the necessary essential fatty acid profile and nutritional value regardless of whether you cultivate your own microalgae or purchase concentrat-ed paste from a third party.

The most frequent microalgae genera used by hatcheries as rotifers and *Artemia* feed are *Nannochloropsis, Isochrysis, Pavlova, Tetraselmis, Dunaliella, Spirulina* and *Chlorella*. Some of these are rich in DHA, some in EPA and others in proteins. Due to their different nutritional profile, a mixture of genera is normally fed to give the best balance between growth and fatty acid enrichment of the zooplankton. A role of thumb for live prey to be used as marine larvae feed is that the DHA:EPA ratio be above 1, with a tad of ARA on top of it. It must

… fatty acid composition can change, not only when going from lab to larger scale production, but also when parameters such as light, temperature and composition within the growth media changes.

however be remembered that live prey's ability to absorb essential fatty acids from different microalgae genera varies widely between prey types, therefore the feeding rates of microalgae for the zooplankton must be analysed and adjusted accordingly [2].

Several microalgae species are capable of producing high amounts of fatty acids (see table 1) including the essential EPA, DHA and ARA [3 4]. Our findings have shown that results obtained under small scale laboratory conditions might not easily be transferrable to a larger scale production of the very same strains. Many scientific studies regarding induction of fatty acid production in different microalgae strains revolves around the application and adjustments of light, temperature, pH value, water quality, salinity, growth media, stresses and sudden shifts in culture conditions. Under laboratory conditions it is possible to adjust and control these parameters to achieve the desired fatty acid composition owning to the relatively small volumes that are used in experimental setups. Applying these parameters to a larger on-site production is often more challenging. To control the strain selection and the nutrient composition of the growth media is fairly straightforward, but to get constant pH levels, temperature levels and desired water quality, as well as induce shifts and stress on the culture for it to produce the fatty acid levels described in the scientific papers is often more challenging.

At our production facility in northern Denmark we have managed to get these parameters under control by using LED light and a software-controlled temperature and pH sensor system in our proprietary photobioreactor systems. Additionally, we formulate our own artificial sea water mainly in order to eliminate the risk of waterborne pathogens and contaminants being brought in to our cultures via collected seawater. However, in doing so we can also control exactly what is in our growth media, as we know from experience that the same strain of

Table 1: Effects on EPA and DHA content as percentage of total fatty acids in algae- fed live prey. Taken and modified from [1].

Alga	EPA	DHA	Prey	EPA	DHA	DHA: EPA	References
Nannochloris	24.9	—	Copepod nauplii	16.4	10.8	0.7	Witt *et al.* (1984)
Nannochloris	24.9	—	Copepodites	15.5	8.6	0.6	Witt *et al.* (1984)
Nannochloris	24.9	—	*Brachionus*	21.4	1.3	0.1	Witt *et al.* (1984)
Nannochloris	24.9	—	*Artemia* (fed 12 h)	6.8	—	0.0	Witt *et al.* (1984)
Chaetoceros calcitrans	33.7	1.8	*Tisbe* sp.	8.3	21.4	2.6	Nanton & Castell (1998)
Dunaliella tertiolecta	0.1	0.3	*Tisbe* sp.	6.2	12.4	2.0	Nanton & Costell (1998)
Isochrysis galbana	2.2	25.7	*Tisbe* sp.	6.7	22.9	3.4	Nanton & Costell (1998)
Dunaliella tertiolecta	0.4	0.3	*Tisbe holothuriae*	5.6	17.9	3.2	Norsker & Støttrup (1994)
Rhodomonas baltica	11.5	7.9	*Tisbe holothuriae*	17.7	32.1	1.8	Norsker & Støttrup (1994)
Tetraselmis sp.	10.8	0.5	*Brachionus plicatilis*	9.9	1.7	0.17	Reitan *et al.* (1997)
Isochrysis galbana	0.9	19.4	*Brachionus plicatilis*	4.4	13.5	3.1	Reitan *et al.* (1997)
Pavlova lutheri	28.3	15.5	*Brachionus plicatilis*	24.2	11.8	0.5	Reitan *et al.* (1997)
Cryptomonas	7.4	3.8	*Artemia* (fed 24 h)	7.1	1.5	0.2	Thinh *et al.* (1999)
Isochrysis (T-iso)	0.5	6.4	*Artemia* (fed 24 h)	2.9	0.9	0.3	Thinh *et al.* (1999)
Control (starved)	—	—	*Artemia* (unfed 24 h)	2.8	—	0.0	Thinh *et al.* (1999)

Microalgae production at Aliga Microalgae's facilities in northern Denmark.

microalgae can behave significantly differently, even under slight changes in the mineral composition of the growth media. An important aspect also lies in choosing the right microalgae strains, as some strains, even though they are from the same species, will produce higher levels of n-3 HUFA's than others [4]. Finally, it is important to remember that inducing fatty acid formation in microalgae via stress will most often be at the cost of growth, since the metabolic pathways in the cells will be redirected into energy storage. It must therefore be a balance between achieving the right fatty acid composition and at the same time maintaining acceptable culture growth of the microalgae.

Most hatcheries are today cultivating their own microalgae, to a greater or lesser extent. From our experience we have seen that the fatty acid composition can change, not only when going from lab to larger scale production, but also when parameters such as light, temperature and composition within the growth media changes. Therefore, to ensure your microalgae cultures have the fatty acid profile you think they do, profiling of your cultures on a regularly basis is advisable as without it, it is difficult to know the nutritional value your rotifer and *Artemia* provide your larvae.

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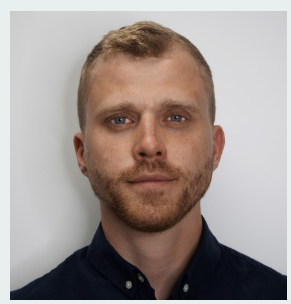
More information



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Essential nutrients and their crucial effects on reproductive performance of fish broodstock

By Chandan Haldar, ICAR - Central Institute of Fisheries Education, Mumbai, India

Broodstock nutrition is indisputably one of the most poorly understood and researched areas of finfish nutrition. To a large extent, this has been due to the necessity of suitable indoor or outdoor culture facilities for maintaining large groups of adult fish and the consequent higher cost of running and conducting extended broodstock feeding trials. However, as in human and livestock nutrition, it is clear that the dietary nutrient requirements of brood-stock will be different from those of rapidly growing juvenile animals. Moreover, as in other animals, it is also clear that many of the deficiencies and problems encountered during the early rearing phases of newly hatched finfish larvae are directly related to the feeding regime (including nutrient level and duration) of the broodstock.

Effects of food restriction

Food restriction itself can seriously affect spawning success. A reduction in feeding rate has been reported to cause an inhibition of gonadal maturation in several fish species, including goldfish, European seabass (*Dicentrachus labrax)* and male Atlantic salmon (*Salmo salar*). In seabass, after six months of feeding broodstock with a half food ration, growth rates decreased and spawning time was delayed and eggs, as well as newly hatched larvae, were smaller than those obtained from fish fed full rations. In female seabass, the detrimental effects of food restriction were associated with reduced plasma estradiol levels. However, the expression of the GtH genes was not affected by food restriction in mature female goldfish.

Effects of nutrition on fecundity of broodstock fish

Several methods have been developed to assess the egg quality of fish. One of the parameters, fecundity, has been used to determine egg quality, which is also affected by a nutritional deficiency in broodstock diets. Fecundity is the total number of eggs produced by each fish expressed either regarding eggs/spawn or eggs/body weight. Reduced fecundity, reported in several marine fish species, could be caused either by the influence of a nutrient imbalance on the brain-pituitary-gonad endocrine system or by the restriction in the availability of a biochemical component for egg formation.

The elevation of dietary lipid levels from 12% to 18% in broodstock diets for rabbitfish (*Siganus guttatus*) increased fecundity and hatching, although this effect could also be related to a gradual increase in the dietary essential fatty acid content. Indeed, one of the major nutritional factors that has been found to significantly affect reproductive performance in fish is the dietary essential fatty acid content. Fecundity in gilthead seabream (*Sparus aurata*) was found to significantly increase with an increase in dietary n−3 HUFA (polyunsaturated fatty acids with 20 or more carbon atoms, essential for marine fish) levels up to 1.6 %, and similar results have been reported in other sparids. However, studies on the reproductive performance of Nile tilapia (*Oreochromis niloticus*), as indicated by the number of females that spawn, spawning frequency, number of fry per spawning and total fry production over a 24-week period, show that the performance was much higher in fish fed a basal diet supplemented with soybean oil (high in n−6 fatty acids, essential for this fish species; and relatively low in fish fed a 5% cod liver oil supplemented diet (high in n−3 fatty acids). Fish fed the diet containing cod liver oil showed the highest weight gain.

Polyunsaturated fatty acids can also regulate eicosanoid production, particularly prostaglandins, which are involved in several reproductive processes, including the production of steroid hormones and gonadal development such as ovulation. Fish ovaries have a high capacity to generate eicosanoids, among them prostaglandin E (PGE) derived from cycloxygenase action and leukotrienes LTB4 and LTB5 derived from lipoxygenase action. Inhibitors of the latter enzyme reduced the gonadotropin-induced maturation of European seabass oocytes, suggesting that products derived from lipoxygenase action could also be involved in oocyte maturation. This fact has been demonstrated in mammals, where some leukotrienes (LTB4) enhance the steroidogenic action of LH.

Since reduced fecundity was associated with high n−3 HUFA egg content, increased EFA content alone should not be used as a criterion to assess the egg quality of gilthead seabream broodstock. High dietary n−3 HUFA levels could affect the brain-pituitary-gonad endocrine axis since both EPA and DHA have been found to reduce in vitro the steroidogenic action of gonadotropin in the ovary of teleost fish. This is similar to mammals where an increased dietary level of n−3 fatty acids delays the onset of puberty.

Other nutrients which have been shown to affect fecundity include vitamin E and ascorbic acid. An increase in dietary α-tocopherol levels up to 125 mg/kg resulted in an improvement in fecundity of gilthead seabream as expressed by the total number of eggs produced/female and egg viability. However, the reduced fecundity observed in broodstock fed a diet deficient in α-tocopherol was not associated with reduced vitamin E content of eggs, and only very high dietary vitamin E levels (2020 mg/kg) were found to increase egg α-tocopherol content. In other species such as turbot or Atlantic salmon, vitamin E was mobilized from peripheral tissues during vitellogenesis although the plasma vitellogenin content was not affected, suggesting that lipoproteins may be involved in the

Rohu fish brooder on a farm in India

transport of vitamin E during this period. Vitamin C content of rainbow trout eggs reflected the content of this nutrient in the diet and was associated with improved egg quality. Changes in the vitamin C content of cod ovaries did not significantly affect hatching rates. Dietary antioxidant requirements increase during reproduction. This may be related to the formation of oxygen radicals during steroid hormone biosynthesis as observed in higher vertebrates. For example, levels of antioxidant compounds were correlated with progesterone levels in bovine corpus luteum suggesting the activation of antioxidative mechanisms to cope with steroidogenesis dependent oxyradical formation.

Dietary tryptophan, a precursor of the neurotransmitter serotonin, may positively affect gonad maturation in both males and females. Supplementation of 0.1% tryptophan in the diets of ayu (*Plecoglossus altivelis*) resulted in a significant increase in the serum testosterone levels thus advancing the time of spermiation in males and induced maturation of females.

Effects of broodstock nutrition on fertilization

Certain dietary nutrients also exert a marked effect on fertilization. Dietary eicosapentaenoic (EPA) and arachidonic acid (AA) levels show a correlation with fertilization rates in gilthead seabream broodstock. Since sperm fatty acid composition depends upon the essential fatty acid content of broodstock diet in species such as rainbow trout and European seabass, it is possible that sperm motility and in turn fertilization would be affected. Particularly in salmonids, where cryopreservation of sperm is currently utilized, sperm fatty acid composition could be a factor that determines the membrane integrity after freeze-thawing. However, research did not find any effect of dietary fatty acids (n−3 and n−6 polyunsaturated fatty acids) on sperm freeze-thaw fertilizing ability, whereas low membrane cholesterol–phospholipid ratios were correlated with a better sperm freezing resistance.

Another hypothesis to explain the beneficial effect of EPA and AA on fertilization rates has been proposed by several investigators. Both EPA and AA are involved in cell-mediated functions and are precursors of eicosanoids. EPA is known to be a precursor of prostaglandins (PG) from series III, whereas AA is a precursor of PG from series II. In vitro AA, but not EPA or DHA, stimulates testicular testosterone in goldfish testis through its conversion to prostaglandin PGE. On the contrary, EPA or DHA blocked the steroid genic action of both arachidonic acid and PGE2. Both AA and EPA modulate steroid genesis in the goldfish testis. Thus, the timing of spermiation may be delayed and subsequently fertilization rates reduced by depressed steroid genesis caused by a broodstock EFA deficiency or imbalance. Moreover, prostaglandins are also recognized as important pheromones in some teleost fish. Some PGs produced by female goldfish, such as PGFs, have been shown to stimulate male sexual behavior and synchronize male and female spawnings, thus directly affecting them. The antioxidant function of vitamins C and E can provide an important protective role for the sperm cells during spermatogenesis and until fertilization by reducing the risk of lipid peroxidation, which is detrimental for sperm motility. The ascorbic acid concentration in the seminal fluid reflected the concentration of this vitamin in the broodstock diet, and it did not affect semen quality at the beginning of the spawning season.. However, a deficiency of ascorbic acid reduced sperm concentration and motility during the later part of the spawning period.

Effects of nutrition on embryo development

Several nutrients are essential for the normal development of the embryo, and their optimum level in broodstock diets improves egg morphology and hatching rates. The percentage of morphologically normal eggs (as a parameter to determine egg viability) has been found to increase with an increase in the n−3 HUFA levels in broodstock diets and incorporation of these fatty acids into the eggs, thus indicating the importance of EFAs for normal development of gilthead seabream eggs and embryo. Gilthead seabream fed EFA deficient diets also showed an increased number of lipid droplets in the egg as also reported in red seabream. Improved egg quality has been associated with higher total n−3 fatty acids content in European seabass fed a pelleted diet enriched with high-quality fish oil. Whereas the comparison between brackish water and seawater cod eggs showed that AA and DHA/EPA contents in the PL fraction of eggs are positively correlated with egg symmetry and viability. These fatty acids play an important structural role as components of phospholipids in fish bio membranes and are associated with the membrane fluidity and correct physiological functions for bound membrane enzymes and cell functions in marine fish. In some species, such as halibut (*Hippoglossus hippoglossus*), the n−3 PUFA (polyunsaturated fatty acids) are also regarded as major energy sources during early embryonic development. Nevertheless, fatty acid composition of fish egg lipids is not only determined by the broodstock diet but is also related to species and stock differences. Essential fatty acid requirements for sparids broodstock range between 1.5% and 2% n−3 HUFA in diet ., being higher than those determined for juveniles which range between 0.5% and 0.8% n−3 HUFA in diet. These values are higher than the optimum essential fatty acid levels determined for salmonids which are approximately around 1% n−3 HUFA.

Despite the fact that little is known about the vitamin A requirement during gonadal maturation and spawning, it is considered important for embryo and larval development due to its important role in bone development, retina

"Several nutrients are essential for the normal development of the embryo, and their optimum level in broodstock diets improves egg morphology and hatching rates"

formation and differentiation of immune cells. An increased retinol concentration in the liver of turbot has been observed during the gonad maturation as the length of the day increased, whereas retinol content in gonads was reduced during maturation.

Other dietary nutrients which have been found to affect the reproductive performance of marine fish include dietary protein intake. For example, a low protein– high-calorie diet caused a reduction in red seabream reproduction. In another sparid, gilthead seabream, a broodstock diet well balanced in essential amino acids, improved vitellogenin synthesis. Moreover, reduction of dietary protein levels from 51% to 34% together with an increase in dietary carbohydrate levels from 10% to 32% reportedly reduced egg viability in seabass. These diets have been shown to cause alterations in GnRH release in seabass broodstock during spawning and plasma hormonal levels of the gonadotropin GtH II,

Catla brood fish before breeding

the latter known to play an essential role in oocyte maturation and ovulation.

Research should also be directed to establishing the requirement of pyridoxine (vitamin B6) in broodstock diets. Vitamin B6 is known to be important in the synthesis of steroid hormones and folic acid since its deficiency may result in reduced cell division due to impaired synthesis of DNA and RNA and it has a role in hatchability of eggs. Unfortunately, there is no information available on the effect of other B vitamins on fish reproduction.

Effects of broodstock nutrition on larval quality

Few studies have been able to show the improvement of seed quality through implementation of broodstock nutrition. Increasing lipid levels from 12% to 18% in broodstock rabbitfish produced large newly hatched larvae and an increase in survival 14 days after hatching. Increased n−3 HUFA (particularly docosahexaenoic acid) levels in broodstock diets were reported to significantly enhance the weight of fish larvae and their resistance to osmotic shock. Similarly, increasing n−3 HUFA levels in broodstock diets for gilthead seabream significantly improved the percentage of live larvae after yolk reabsorption. Moreover, growth, survival and swim bladder inflation in gilthead seabream larvae were improved when fish oil was used instead of soybean oil in broodstock diets. However, excessive levels of n−3 HUFA in broodstock diets (over 2%) caused yolk sac hypertrophy in gilthead seabream larvae and a decrease in larval survival rates. This is probably associated with an increase in antioxidant nutrient requirement since an increase in dietary α tocopherol levels from 125 to 190 mg/kg prevented the appearance of yolk sac hypertrophy and larval mortality.

Effects of broodstock nutrition on reproduction

Reproduction of animals can be affected by several influences at different stages of development, which are mediated by changes in the hypothalamic-pituitary-gonadal (HPG) axis. Gonadotropins constitute an important group of pituitary regulatory hormones which comprise luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The secretion of Gonadotropin hormones is controlled by the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator that is an integrator of hormonal, metabolic and neural signals. Experimental data in mammals (and observations in humans) revealed that early life exposures influence the development and functioning of the HPG from a programming perspective. Effects of nutrition of the reproductive health of fish have not been studied until now. However, studies conducted in other vertebrates could be very useful for further focus. They have shown that, at earlier stages of fetal development, the normal ontogeny of gonadal development and function can be disrupted by under nutrition or the influence of endocrine-disrupting compounds. Any effect of under nutrition on the process of tissue differentiation, gonad formation and the establishment of associated enzyme systems is likely to have a fundamental effect on the subsequent function of these organs. Specifically, in female fetuses, the onset of meiosis is delayed, whereas, in male fetuses, testosterone synthesis is increased as a result of enhanced testicular steroid genic enzyme activity. As evoked above, most of the nutritional studies undertaken in this area have compared fasting to feeding conditions. It is then necessary to investigate the effects of different dietary components as proteins and other specific nutrients, like HUFA and vitamins. It is then well established in mammals that the reproductive performance can be influenced by factors acting during the earliest developmental periods; however, much further work is required to identify these critical developmental periods and the relationships between developmental abnormalities and adult reproductive performance.

Conclusion

These last 20 years, most of the studies on fish larvae nutrition aimed to develop a compound diet able to efficiently replace live prey in the larvae feeding sequence. It was hypothesized that fish larvae lacked a functional digestive system. Consequently, the functioning of the digestive tract during the larval period was mainly examined and, unexpectedly, revealed that larvae have a primary but efficient mode of digestion that progressively matures during development. The knowledge of these larvae digestive features allowed to develop a compound diet that can be used from the earliest larval stages. It is now possible to refine the nutritional requirements of the fish larvae and identify the potential physiological effects of some particular nutrients. Indeed, it has been shown in upper vertebrates that some nutrients, even at low dietary concentrations, may influence morphogenesis particularly by acting on bone differentiation. These nutrients, such as vitamins and HUFA, recognize nuclear receptors which are transcription factors for several genes involved in tissue differentiation and organogenesis. These nuclear receptors can link together and form heterodimers, particularly with RXR. In consequence, HUFA and the oxidative balance of lipids, itself regulated by vitamin C and E, may act on retinoid pathways and, in the same way, molecular pathways of vitamin A and D can interact together. The formation of nuclear receptor heterodimers leads to cross-talks between metabolic processes and then, may affect the quality of the future juvenile fish. It is then possible to orientate the metabolic pathways of the juvenile fish by appropriate nutritional conditioning during the larval period. This conditioning offers the possibility to have fish with an enhanced immunological system and fish able to use efficiently some vegetable diets. For this, nutritional studies on fish larvae need to take into account the global effect of nutrients on developmental processes. Important information on genome and gene expression in now available for several fish species and this information allows undertaking genomic studies on fish development. Fish could then constitute an interesting model for studying the effect of nutrition on development processes in upper vertebrates.

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PRODUCT PROFILE

Enrichment strategies in the early larval rearing of marine fish become a long-term investment for farmers in grow-out

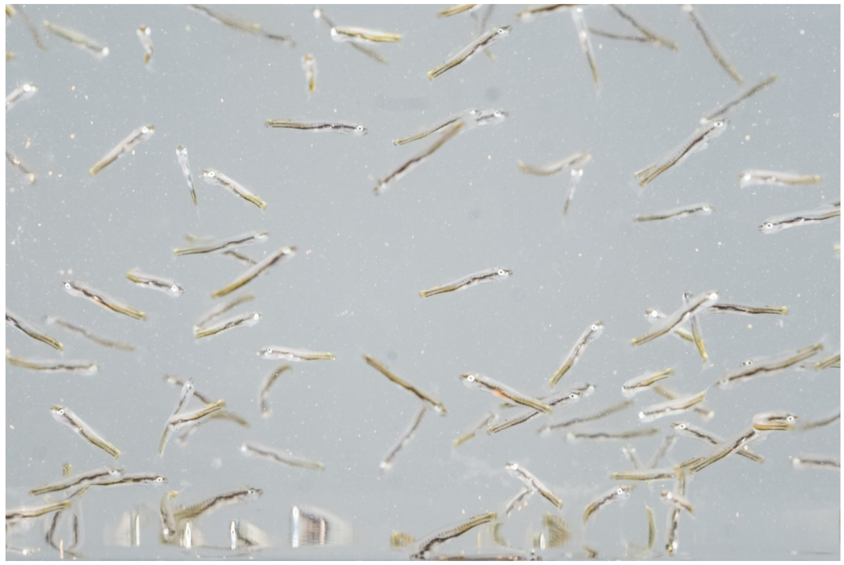
Why hatcheries should focus even more on early-stage nutrition

By Alessandro Moretti, Product Manager Fish Hatchery, Inve Aquaculture

Marine fish farming need to look for continuous improvements to keep profitability and sustainability high. Given the pressure for an intensifying production and a growing attention to cost-effectiveness, fry quality and performance measured during the whole cycle have proven to be one of the keys to success. In that respect, specialists at INVE Aquaculture see a clear and direct relation between optimized hatchery protocols and the animals' performance, growth and survival at the farm gate.

As a recognized pioneer, reference and innovator in hatchery products and protocols INVE Aquaculture – Benchmark's Advanced Nutrition Division - has always dedicated special attention to fry quality and robustness as a key success factor in aquaculture. From our vast technical experience in the industry, we can see clear correlations between nutrition in the early larval rearing and performance and robustness in the grow-out.

More specifically, the use of optimized live feed and enrichment strategies and the feeding at specific moments play a crucial role. Hatcheries who want to meet the demands of today's stringent requirements in grow-out, will need to rely on long-term solutions that are especially developed to support superior juvenile quality.



Early stimulation from 3 days post hatching onwards

INVE Aquaculture scientists are continuously developing new solutions, enrichment formulations and easy-to-use protocols to improve fry quality. Optimizing the nutritional value of live feed such as rotifers and *Artemia* is considered to be imperative in order to obtain the best possible fry quality and future fish performances and robustness.

The assumption that key factors such as the animals' immune system can only be affected after metamorphosis is probably destined to be changed in the near future. INVE researchers have now proven that lasting positive effects of stimulation can already be induced in the first month of the

This product is a dry enrichment with a formulation that boosts the rotifers and Artemia with key natural ingredients, turning it into a functional enrichment.

life cycle. These important effects of early immune system stimulation linked with other functional effects of specific nutrition and microbiological management of the tank system can actually increase production efficiency and quality.

A new, complete and smart approach to live feed enrichment

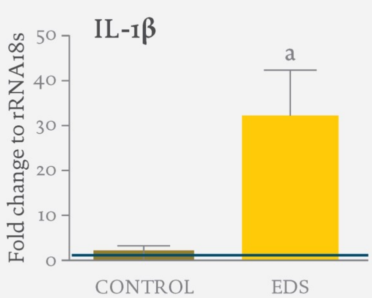
As a result of continuous research and development, INVE Aquaculture has recently expanded its enrichment portfolio with a brand-new formulation: Easy Dry Selco® (EDS).

This product is a dry enrichment with a formulation that boosts the rotifers and *Artemia* with key natural ingredients, turning it into a functional enrichment.

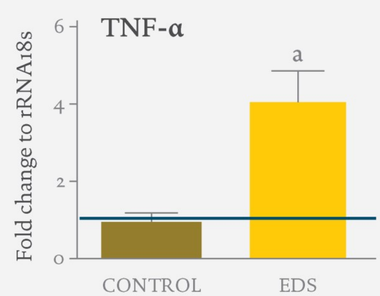
Because it adds a lot more than just selected fatty acids to the live feed, the complete and rich formula of EDS is extremely flexible and also suitable for fast growing marine fish larvae and tropical species. Completing the nutritional balance of the early-stage fish feed, this new enrichment contributes considerably to optimizing fry quality and robustness.

Remarkable results

Extensive tests and trials have shown that enriching rotifers and *Artemia* with EDS can trigger the immune system of the fish larvae in very early stages (even before 28 dph) with visible effects at 56 dph. This is the first time ever that this unique effect has been scientifically reported at such an early stage.



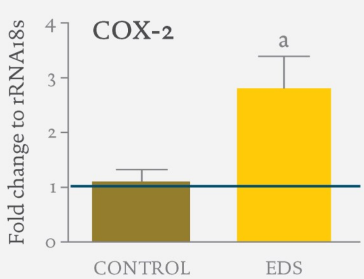
Treatments
a Significantly different from Control



Significant upregulation of both IL-1β and TNF-α revealing a stronger immunostimulat-ing effect in the EDS treatment compared to the control treatment

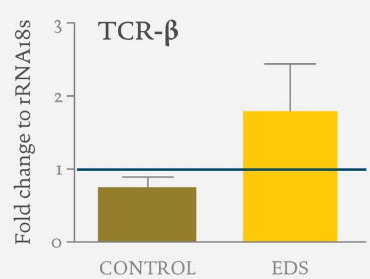
Treatments
a Significantly different from Control (P<0.05)

A significant increase of IL-1β, TNF-α and COX-2 levels mean that the fry are better prepared to fight against inflammation and pathogen threats.



Significant upregulation of COX-2 expression levels in the EDS treatment compared to the control treatment

Treatments
a Significantly different from Control (P<0.05)



Expression levels of TCR-β are slightly upregulated in the EDS group

Treatments
One-Way ANOVA ns

How to use

Despite the very complex action and the extremely high score in quality of the fry, EDS is simple and effective in use. As a rotifer enrichment product, Easy Dry Selco® can be used in the traditional way after harvesting in a separate enrichment tank, or even directly into the culture tank. This means an incredible advantage in terms of space and workload. Rotifer enrichment is finalized in 6-9 hours. For *Artemia*, INVE Aquaculture recommends harvesting 18 hours after the start of the enrichment.

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Effects of enriched and non-enriched *Artemia* on jaw malformation in golden pompano larvae

Skeleton malformation is a major bottleneck continually hindering the production of marine fish fingerlings (Sandel et al. 2010; Ma et al. 2014c). Malformed fish are usually sold at a low price or are manually removed before sale to market (Ma et al. 2014d). Moreover, deformation in fish can negatively affect fish growth, survival, food conversion ratio, and susceptibility to stress and pathogens (Andrades et al. 1996; Koumoundourous et al. 1997; Boglione et al. 2013). Although genetic factors (Ma et al. 2014c), environmental factors (Hattori et al. 2004), parasites and pesticides (Liang et al. 2012; Liu et al. 2012) can affect fish bone development, increasingly evidence has showed that nutritional factors during larval fish rearing can directly cause skeleton malformation (Villeneuve et al. 2005a; Yang et al. 2015).

Lipid is the main source of energy supply for larval fish (Sargent et al. 1999a). The morphogenesis of marine fish larvae can be altered by changing dietary lipids (Cahu et al. 2003). Among different lipid components, fatty acids are indispensable to modulate the transcription of genes involved in metabolism (Kliewer et al. 1997). Although previous studies have demonstrated that feeding with a high level of dietary lipid can improve fish growth performance and reduce skeletal malformation (Cahu et al. 2003), excessive dietary lipids and unbalanced fatty acid ratios can also lead to low survival (Fernández & Gisbert 2011) and skeleton malformation (Izquierdo et al. 2010). Nutrient enrichment for *Artemia* nauplii has been used in larval fish culture for decades, but suitable enrichment formula is not available to lower skeleton malformation in larval fish culture.

Due to high flesh quality, fast growth, and suitability for cage culture, golden pompano (*Trachinotus ovatus*) has become a good candidate species for aquaculture (Guo et al. 2014). Although several aspects pertaining to hatchery rearing of golden pompano larvae have been well studied (Ma et al. 2014a, 2014b), high malformation during early development stage of this species has continually affected its production efficiency in hatchery (Ma et al. 2014d). Furthermore, factors causing malformations on this fish are still unclear. A previous study has found that enriching formula for *Artemia* nauplii can affect the expression of retinoid X receptors (RXRs) and jaw malformation in golden pompano, but there was no clear correlation between retinoid X receptors (RXRs) expressions and malformation rates when golden pompano larvae were subjected to nutrient change (Yang et al. 2015). In order to further explore the relationships between BMPs gene expression and jaw malformation during the *Artemia* nauplii feeding phase, we cloned BMPs genes in golden pompano larvae and evaluated the correlation between gene expressions and jaw malformation. Such information would contribute to improvement of larval quality and production efficiency in the aquaculture of golden pompano and other related species.

Materials and methods

The experimental material in the present study was collected from an earlier study on nutritional trial and fish growth measurement, fatty acid analysis, and jaw malformation reported in Yang et al. (2015). Fertilized eggs of golden pompano hatched in 500-L fiberglass incubators at 26°C with a hatch rate of 97.1 ± 1.9%. On two days post hatch (DPH), larvae were stocked into four 1000-L larval rearing tanks at a density of 60 fish L−1. Rotifers *Brachionus rotundi-formis* at a density of 10–20 rotifers mL−1 were used to feed fish larvae from 2 to 12 DPH. On the morning of 11 DPH, fish larvae were restocked into 12 500-L larval rearing tanks at a density of 20 fish L−1.

The nutritional manipulation experiment included three dietary treatments with three replicates each. *Artemia* nauplii were treated in three methods (1) enriched with instant micro-algal paste, (2) enriched with Algamac 3080®, and (3) without any enrichment as control. For each treatment, three replicate tanks were used, and a total of nine tanks were used in this study. *Artemia* nauplii were fed to fish from 11 to 27 DPH. On 11 DPH, *Artemia* nauplii were first introduced at 200 nauplii L−1, and then added with a daily increment of 90% by number. For the analysis of gene expression, samples were collected from each tank. For each dietary treatment, samples in three replicates were collected.

Fish growth was determined by specific growth rate (SGR) as%/day. At the end of this experiment, 50 fish larvae from each tank were sampled for assessing growth and jaw malformation. The remaining fish in each rearing tank were harvested and counted for survival determination.

On 28 DPH, approximately 50 individuals were collected from each tank and jaws were collected for total RNA extraction. RNA was reverse-transcribed to cDNA, which was used as a template in subsequent PCR. Quantitative real-time PCR (qPCR) was used to analyze the expression levels of BMP genes in golden pompano larvae.

Fatty acid and jaw malfor-mation analysis

The nutritional content of *Artemia* nauplii was assessed when fish larvae were at 18 DPH. Fatty acids were analyzed at South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China following the method described by Ma and Qin (2014). Jaw malfor-mation was examined under a stereo microscope (Olympus SZ, Tokyo, Japan) using the criteria described by Ma et al. (2014d). Jaw malformation (%) was calculated as

In the present study, fish fed Artemia nauplii enriched with Algamac 3080 showed twofold lower jaw malformation than those fed non-enriched Artemia nauplii or Artemia nauplii enriched with Nannochloropsis.

Jaw malformation = (malformed larvae/total larvae) × 100%.

The highest SGR was obtained in fish fed *Artemia* nauplii enriched with Algamac 3080 and the lowest SGR was observed in fish fed non-enriched *Artemia* nauplii. The highest survival was achieved in fish fed non-enriched *Artemia* or *Nanochloropsis* enriched *Artemia* ($P < .05$, Table 1), and the lowest survival was observed when fish were fed with Algamac 3080 enriched *Artemia* nauplii ($P < .05$). On 28 DPH, the jaw deformity of fish larvae fed Algamac 3080 enriched *Artemia* nauplii was significantly lower than fish fed non -enriched *Artemia* nauplii or *Nannochloropsis* enriched *Artemia* nauplii ($P < .05$). However, the jaw deformity was not significantly different between fish fed non-enriched *Artemia* nauplii and *Nannochloropsis* enriched *Artemia* nauplii ($P > .05$).

Table 1. SGR, coefficients of variation, final survival rate, and jaw deformity rate of golden pompano larvae fed with enriched and non-enriched *Artemia* nauplii. Different letters represent significant differences at P < 0.05.

	Non-enriched	*Nannochloropsis*	Algamac 3080
SGR (%/day)	5.68 ± 0.22a	6.25 ± 0.08b	6.49 ± 0.05c
Final survival rate (%)	31.83 ± 8.60b	29.33 ± 5.32b	10.33 ± 0.90a
Jaw deformity rate (%)	15.91 ± 0.68b	15.39 ± 0.69b	8.33 ± 0.74a

Cloning and expressions of the BMP genes

Partial sequences of BMP1, BMP2, BMP4, BMP5, and BMP10 genes were obtained after sequencing analysis. The BMP1 gene exhibited high identities with other fish species. Nutrient enhancements significantly affected the gene expressions of BMP4 and BMP10 (P < .05, Figure 1). The expression of BMP4 in fish fed non-enriched *Artemia* nauplii or *Nannochloropsis* enriched *Artemia* nauplii was significantly higher than fish fed Algamac 3080 enriched *Artemia* nauplii (P < .05, Figure 1). The expression of BMP4 was not significantly different between fish fed non-enriched *Artemia* nauplii and *Nannochloropsis* enriched *Artemia* nauplii (P > .05). The lowest level of BMP10 expression was observed when fish were fed with non-enriched *Artemia* nauplii, and the highest BMP10 expression was found in fish fed Algamac 3080 enriched *Artemia* nauplii (Figure 1).

Nutrient enhancements were correlated to SGR, jaw deformity, and expressions of BMP4 and BMP10.

Discussion

Dietary n-3 highly unsaturated fatty acids such as DHA and EPA are essential to growth of fish larvae (Rezek et al. 2010), and their requirements are species-specific (Dantagnan et al. 2010). Fish growth rates are related to dietary DHA in many fish. Furthermore, the growth response of fish larvae to different enrichment products is also varied among species. For example, the growth rates of striped bass *Morone saxatilis* and gilthead seabream *S. aurata* larvae are not affected by feeding the *Artemia* nauplii enriched with Algamac 2000 or PL-Cr (DHA-rich phospholipid extract of *Crypthecodinium* sp.), but the growth rate of halibut *Hippoglossus hippoglossus* larvae fed *Artemia* nauplii enriched with DHA Seleco is lower than the larvae fed PL-Cr (Harel et al. 2002).

In this study, fish growth was improved when fish larvae were fed with *Artemia* nauplii enriched with Algamac 3080 or *Nannochloropsis*. The best fish SGR was achieved in the treatment of Algamac 3080, which is consistent with the high dietary DHA levels in *Artemia* nauplii. Nevertheless, the low survival and high coefficients of variation of fish length in the treatment of Algamac 3080 could also contribute to the high SGR due to the death of small larvae in this treatment.

Although a high content of dietary lipid can improve fish survival, overdosed dietary lipid or unbalanced lipid class composition can also lead to poor growth and abnormal development in fish larvae (Salhi et al. 1999). For instance, Ma and Qin (2014) reported that a high DHA/EPA ratio in live feed could lead to low survival in yellowtail kingfish Seriola lalandi. In the present study, a higher DHA/EPA ratio (0.36:1) was achieved by enriching *Artemia* nauplii with Algamac 3080. The high DHA/EPA ratio in the Algamac 3080 treatment led to fast fish growth but low survival. In contrast, better survival was obtained in the non-enriched and *Nannochloropsis* treatments where the DHA/EPA ratio was 0.07:1–0.22:1. Low fish survival in the Algamac 3080 treatment supports the claim that a high DHA content and a high DHA/EPA ratio may reduce larval fish survival (Planas & Cunha 1999) as unbalanced lipid classes in the diet affect digestion and absorption of fatty acids in fish larvae (Salhi et al. 1999).

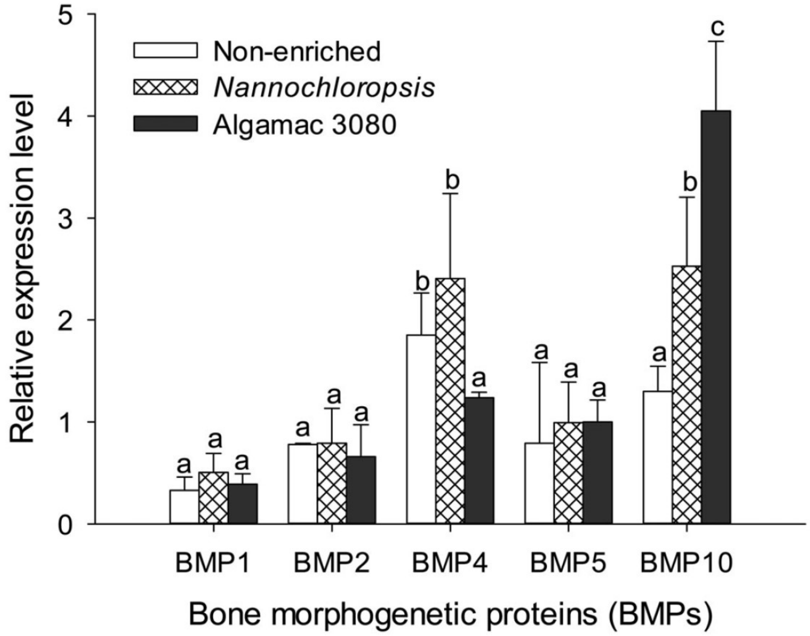

Fig. 1. Relative expression levels of BMPs in golden pompano larvae fed enriched and non-enriched *Artemia* nauplii.

Previous studies have indicated that poly unsaturated fatty acids play an important role in bone formation of fish (Izquierdo et al. 2010), and dietary fatty acids can alter the composition of bone and cartilage (Liu et al. 2004). Abnormal development of fish larvae may be caused by insufficient dietary n-3 highly unsaturated fatty acids (HUFA) in live food (Hamre et al. 2002). A 50% reduction of deformed fish was observed when fish larvae were fed with higher levels of dietary DHA (Izquierdo et al. 2010). In the present study, fish fed *Artemia* nauplii enriched with Algamac 3080 showed twofold lower jaw malformation than those fed non-enriched *Artemia* nauplii or *Artemia* nauplii enriched with *Nannochloropsis*. Skeletal malformation was reduced in fish fed *Artemia* enriched with Algamac 3080, which is coincident with the high DHA content in the feed. This indicates that a dietary DHA level of 2.56% may be suitable for skeletal development in golden pompano larvae.

BMP2 and BMP4 are closely related proteins involved in key embryonic processes. In the present study, the expressions of BMP1 and BMP2 were not significantly affected by the nutrient enrichment by 28 DPH, suggesting that the expression of BMP1 and BMP2 in golden pompano may be less sensitive to nutrient enrichment after the bone structure is formed and mineralized.

BMP4 plays diverse roles during vertebrate development. Thus, it has been used to evaluate the effect of micronutrients on the skeletal development of marine fish larvae (Villeneuve et al. 2006). In the present study, the expression levels of BMP4 in fish fed non-enriched *Artemia* nauplii and *Nannochloropsis* enriched *Artemia* nauplii were significantly higher than fish fed Algamac 3080 enriched *Artemia* nauplii. Furthermore, jaw malformation in the treatment of non-enriched and *Nannochloropsis* was significantly higher than in the treatment of Algamac 3080. These results are consistent with the finding reported by Villeneuve et al. (2006), in which jaw malformation increases when the expression of BMP4 is up-regulated.

Unlike other BMPs, BMP10 is expressed predominantly in the adult heart and to a lesser extent in the liver and lung (Neuhaus et al. 1999). In the present study, nutrient enhancement altered the expression of BMP10 in golden pompano larvae, and the expression of BMP10 was corresponding to jaw malformation.

In summary, nutrient enhancement can affect jaw malformation in fish larvae during the *Artemia* nauplii feeding phase. Feeding golden pompano larvae with enriched *Artemia* nauplii significantly reduced the jaw malformation rate, but also decreased survival at the same time. Reduction of jaw malformation may be due to the mortality of fish larvae during test time. However, this may need further verification. Nutritional manipulations can significantly affect the expression levels of

BMP4 and BMP10, and the concentration of dietary DHA can significantly affect the expression of BMP10. The expressions of BMP4 and BMP10 varied between different dietary treatments, and the expressions of BMP4 and BMP10 correspond to jaw malformation in golden pompano larvae during the *Artemia* nauplii feeding phase. This study suggests that measures of BMP4 and BMP10 in golden pompano may serve as a suitable indicator for jaw malformation in the field and aquaculture facility, leading to rapid assessment of nutrient status affecting fish jaw malformation.

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From Zhenhua Ma, Jing Hu, and Gang Yu, South China Sea Fisheries Research Institute, and Jian G. Qin, Flinders University Adelaide. Gene expression of bone morphogenetic proteins and jaw malformation in golden pompano Trachinotus ovatus larvae in different feeding regimes. Journal of Applied Animal Research, 46:1. Under CC license 4.0

ΩHF

New research developments in aquatic animal larval feeding and nutrition—recent literature

Abstract excerpts compiled by Meredith Brooks, Hatcheryfeed

Growth performance and intestinal morphology of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae fed on live and dry feeds

Researchers investigated the effects of both live and dry feeds on the growth and intestinal morphology of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae. They developed five diets, including two diets containing a combination of live and dry feeds, and tested them on larvae from 2 days post hatch to 6 and 11 days post hatch. After the feeding trial, both final weight and specific growth rate were significantly higher in larvae fed nauplii combined with dry feed. The best FCR rates were achieved with the diet containing only nauplii and the diet containing both nauplii and dry feed.

Results found that different starter feeds affect intestinal morphological development and growth of African catfish larvae. Specifically, a gradual reduction of nauplii combined with an increase in dry feed stimulated morphological development, resulting in improved growth performance.

Onura, C.N., Van den Broeck, W., Nevejan, N., Muendo, P., and Van Stappen, G. Growth performance and intestinal morphology of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae fed on live and dry feeds. Aquaculture, March 2018; 489: 70-79.

Full paper: https://doi.org/10.1016/j.aquaculture.2018.01.046

Combined diet of yeast, fermented soybean meal, and microparticulate as larval feed in extensive rearing systems for seed production of the oriental river prawn *Macrobrachium nipponense*

This study aimed to evaluate the effects of different types of larval feed on seed production of the oriental river prawn *Macrobrachium nipponense.*

Researchers developed four diet groups A through D, each containing equal levels of total protein content, and tested them against one no-feeding control. Higher amounts of oriental river prawn larvae and postlarvae were achieved from diet C containing yeast and fermented soybean meal and diet D containing yeast, fermented soybean meal and a microparticulate diet. The trials also found that the increase of larvae can result in an increase in phytoplankton in the water, helping to improve water quality.

Final results demonstrated that diet D was the most effective at increasing per unit larval yield and has a low cost of production, which can assist in the expansion of oriental river prawn aquaculture.

Yao, Z.L., Zhao, Y., Ma, H.Y., Liu, H.J., Wang, H., and Ji, X.S. Combined diet of yeast, fermented soybean meal, and microparticulate as larval feed in extensive rearing systems for seed production of the oriental river prawn *Macrobrachium nipponense*. Aquacult Int (2018) 26: 757.

Full paper: https://doi.org/10.1007/s10499-018-0240-8

Growth, Survival, and Whole-body Proximate and Fatty Acid Composition of Haddock, *Melanogrammus aeglefinus L.*, Postlarvae Fed a Practical Microparticulate Weaning Diet

In an effort to further the development of high quality hatchery feeds for haddock (*Melanogrammus aeglefinus L.*) larvae, Researchers in Canada developed a practical microparticulate diet (PMD). The diet was compared with a high-quality imported feed through a series of feed and weaning trials.

The PMD formulation was demonstrated to be a highly suitable diet for haddock larvae. In addition to a high rate of feed acceptance and survival, the fish fed the PMD had higher final fork lengths, wet weights, and weight gains. This indicates the diet has good potential for future use in hatcheries and research studies.

The study was the first to demonstrate that a high rate of survival (>88%) of haddock can be achieved through the weaning stage from days 54 to 61 post-hatch; previous studies achieved between 35% and 2-5%. Researchers compared the co-feeding periods for all studies and suggested that earlier co-feeding periods may result in lower survival rates.

Lall, S. P., Lewis-McCrea, L. M. and Tibbetts, S. M. (2018), Growth, Survival, and Whole-body Proximate and Fatty Acid Composition of Haddock, *Melanogrammus aeglefinus L.*, Postlarvae Fed a Practical Microparticulate Weaning Diet. J World Aquacult Soc, 49: 83-95. doi:10.1111/jwas.12462

Full paper: https://onlinelibrary.wiley.com/doi/full/10.1111/jwas.12462

A web-based combined nutritional model to precisely predict growth, feed requirement and waste output of gibel carp (*Carassius auratus gibelio*) in aquaculture operations

A new combined nutritional model has been constructed to predict growth, feed requirement, and waste output of gibel carp (*Carassius auratus gibelio).* The web-based model was developed using data from several fish farms and published studies, and is able to predict nutritional needs for fish during various stages of life. Researchers classified carp into three growth stanzas using the thermal-unit growth coefficient (TGC) model; stanza 1 is first feeding from 1 to 13.5g, followed by 13.3 to 175.5g, and >175.5g.

An online platform was developed for farmers and managers to use the system, and the design and implementation of the model is described in the published study.

Liu, X., Sha, Z., Wang, C., Li, D., and Bureau, D.P. A web-based combined nutritional model to precisely predict growth, feed requirement and waste output of gibel carp (*Carassius auratus gibelio*) in aquaculture operations. Aquaculture, July 2018; 492: 335-348.

Full paper: https://doi.org/10.1016/j.aquaculture.2018.04.019

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