Marine larval fish production: a nutritional perspective.

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Introduction

Many of the marine species that are currently being produced on a commercial basis are members of the group of fishes known as pelagic spawners. The reproductive strategy employed by these fishes involves the production of large numbers of small, buoyant eggs which, after being released into the water and fertilized, drift freely in surface currents with no further parental attention. Newly hatched larvae typically carry an elliptical yolk sac containing an oil globule. Although, the yolk and oil globule function as nutrient stores for further development of the newly hatched larvae, these stores tend to be small and influenced by a variety of factors. Upon initiation of feeding the larvae often have minimal nutrient reserves and a small mouth gape which limits the type of prey that they can consume. As a result, larvae do not do well on foods of sub-optimal nutritional value.

Survival through the larval stages is quite tenuous and is generally considered the most critical period affecting marine fish recruitment as well as aquaculture development. Finding prey items of appropriate size and nutritional characteristics is a difficult challenge in the wild and one of the primary concerns in the culture of larvae. Successful feeding is dependent on larvae locating appropriate food items, ingesting the items and assimilating nutrients from the food source. This process is complicated by a number of factors which include: 1) limited and variable energy reserves giving a relatively small window of opportunity prior to starvation, 2) small size of the larvae reducing the effective distance the larvae can travel, as well as the size of the particle that can be consumed, 3) extremely fast growth rates and minimal nutritional reserves, and 4) undeveloped

digestive system, which may not digest and assimilate some food sources as efficiently as a fully developed fish.

In addition to nutritional requirements, the tolerance of various environmental and culture conditions varies from species to species. Such factors can provide considerable challenges towards the development of production protocols for each species. To facilitate a broader understanding of nutritional challenges of larval culture, this paper will review general nutritional concepts with regard to the production of marine fish larvae.

Broodstock management

In the wild, fish respond to environmental ques that trigger the onset of maturation and spawning. This is generally a slow process that allows both tissue reserves and extrinsic food sources to be incorporated into gonadal tissue. After suitable stimulation final oocyte maturation, ovulation and spawning will occur. In an aquaculture situation, broodstock are often held in unnatural conditions and spawning is often induced or manipulated in some way. Quite often the food source (prepared feeds), timing and duration of spawning (hormone induced or temperature/photo-period manipulation) is artificially manipulated. For many species these manipulations have a direct impact on egg and larval quality.

Although, there are a number of factors influencing egg quality (husbandry condition, genetic make up, stage of oocyte development) we often overlook the fact that the nutritional reserves of the egg and early larval stages originate from the female. Hence, if oocyte growth and release are altered adequate nutrient reserves may not be transferred to the egg and larvae. It has been well established that broodstock nutrition, as well as the timing of oocyte release, will influence the nutritional reserves of the egg and larvae (Bromage and Roberts 1995). With respect to the management of brood stock, one of the most critical factors influencing egg quality is the nutritional quality of the feed. Diets containing inappropriate balances and/or deficiencies of nutrients have been shown to negatively impact spawning frequency as well as egg and larval quality. Although research in this area is limited, there are clear examples in the literature and numerous examples under practical conditions of larval rearing problems originating from broodstock nutrition. In species such as the red sea bream, *Pagrus major,* (Watanabe and Kiron, 1995) and gilthead sea bream, *Sparus aurata,*

(Zohar et al., 1995) problems with egg and larval quality have been traced back to low levels of polyunsaturated fatty acids (PUFA), phospholipids, astaxanthin and caretenoids in the diet.

Lipids critical to broodstock nutrition include essential PUFA, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are critical for the development of neural tissues and compounds important in stress response and adaptation. Multiple spawning species such as the red drum, *Sciaenops ocellatus,* red sea bream and gilthead sea bream generally rely on exogenous (dietary) sources of lipids to derive the lipids deposited in the egg. It has been demonstrated that species such as the red and gilthead sea bream respond rapidly (within 15 days) to changes in dietary lipid composition (Higgs and Dong, 2000). Based on work with a variety of species it is clear that HUFA's, vitamins C, E and the carotenoid astaxanthin (a widely occurring animal pigment especially common in crustaceans), play important nutritional roles in determining egg quality. This is why it is common to either feed fresh feeds (e.g. fish, shrimp and squid) supplemented with vitamin and lipids or a high quality prepared diets (50-55% protein, 10-15% lipids) in conjunction with fresh feeds.

Broodstock nutrition will directly influence the nutritional reserves of the egg and newly hatched larvae. When considering the nutritional status of larvae and potential problems seen in larval development (e.g. early mortality, shock syndrom) consideration must be given to not only the nutritional quality of the larval feed but the nutritional status of the broodstock. Although, responses are species specific this is particularly important for species that are reared under artificial conditions and the spawning period is controlled or artificially extended (e.g. through temperature photo-period manipulation). Consequently, it is becoming more common to enhance the quality of brood stock diets, minimize the length of time brood stock are spawned before they are allowed to reabsorb and regenerate there gametes and conduct regular measures of egg and larval quality. Common measures of egg quality include: fertilization rate, egg weight, size and shape of the oil globule. For a review on brood stock management and seed quality the reader is referred to Bromage and Roberts (1995).

Larval feeding

To understand larval nutrition, one must also have a good understanding of the steps in larval development, particularly with respect to the digestive system. The general development and

functionality of the digestive systems of larvae been reviewed by Tanaka (1973) and Govoni et al. (1986). Upon hatching, most marine fish are fairly undeveloped and do not have functional eyes or digestive systems. Using endogenous nutrients, the larvae continues to develop to a first feeding stage. Upon initiation of feeding the eyes are generally pigmented, the jaw and mouth are functional. At this point the digestive system includes a fairly undifferentiated tube like alimentary canal, liver, pancreas and gallbalder. Digestion of food occurs in the midgut and hindgut regions. As the larvae progresses toward becoming a juvenile, the digestive system will continue to differentiate with the formation of pyloric cecae and differentiation of the stomach.

Because a number of studies attempting to rear larvae on artificial diets have meet with limited success many authors concluded that exogenous enzymes may be required for proper digestion. It has been suggested by a variety of authors, that larvae rely on exogenous enzymes from the prey to aid in digestion and activate zymogens (inactive enzymes) released by the larvae; thus increasing digestion and growth rates. As exemplified by the comprehensive work of Lazo (1999), it was concluded that red drum, *Sciaenops ocellatus*, larvae have a highly functional digestive system at first feeding. Lazo, concluded that growth and survival is lower in red drum larvae fed a microparticulate diet alone compared to those fed zooplankton or a combination of zooplankton and microparticulate diet. However, no dietary-induced differences in activity of pancreatic enzymes (trypsin, lipase and amylase) were detected among the larvae subjected to these dietary regimes. Additionally, Lazo reported that exogenous enzymes played a minor role in the digestive process of the larvae. A similar conclusion was reached by Garcia-Ortega et al., (2000), who reported that heat treatment of decapsulated *Artemia* cysts reduced the nutritive value and enzyme activity of decapsulated cysts fed to the African catfish, *Clarias gariepinus*. However, no changes in enzyme activity of the larvae were detected for any treatments, indicating that decreased growth was due to heat damage of the food source and not the level of exogenous enzymes. Hence, low growth and survival of larvae offered micro-particulate diets can not be attributed to low digestive enzyme production. More likely the prepared diets failed to stimulate ingestion, provide sufficient nutrients in a digestible form and suitable levels for proper development.

In many instances, live or natural food sources will continue to be the methods of choice. When provided at suitable densities for proper ingestion rates, larvae do not have problems digesting

live prey. However, the offered prey items must be compatible with the larvae's gape width (mouth size) and swimming speed. If the prey is too large, larvae will not be able to capture it, conversely, if the prey is to small the energy invested in capturing numerous small items may be greater than that obtained from the food. When dealing with live prey, it is also critical that they are maintained at a suitable density as this will influence both encounter rate and capture efficiency. If the density is too low, the encounter rate will be infrequent and to much energy will be expended searching for prey. If the density is too high the larvae will ineffectively capture and digest the prey. In both instances, proper development of the larvae will be inhibited.

The most common live feeds include: *Artemia*, rotifers and copepods. Other less used zooplankton include cladocerans (water fleas), copepods, and tintinnid ciliates. Although live foods are often good sources of nutrition, for many species of fish larvae these traditional food sources have been found to be inadequate to support larval development. Even in instances where these prey items are considered sufficient the nutritional quality of live prey items can vary considerably possibly resulting in increased variability in the quality of the larvae. Because the nutritional content of prey items, such as rotifers, is a reflection of it's food source, the nutritional quality of the rotifers as a larval food source will vary with culture conditions used to rear the rotifer. The proximate composition of rotifers consists of 52-59 % protein, up to 13 % fat, and 3.1 % n-3 highly unsaturated fatty acids (HUFA). However, this composition will vary considerably depending on the food source the rotifers were reared on (or enriched with) as well as the amount of time the rotifer is deprived of food. It has been well established that one of the limiting factors in larval fish feeds is the level of omega-3 or n-3 HUFA. Hence certain feeds containing HUFAs (especially, DHA, 22:6n-3, docosahexaenoic acid and EPA, 20:5n-3, eicosapentaenoic acid) can be valuable as food sources for rotifer and/or enrichments . Although, we can manipulate, within a certain range, the nutritional quality of live feeds, we must also remember that they are live. Consequently, if live feeds are left without food for an extended period (this may be as little as 2-4 hrs) their nutritional quality will decline and may not be suitable to support proper development of the larvae. Consequently, when dealing with live feeds, you must ensure that they are either collected and immediately fed or properly stored (chilled down and/or held with feed). Additionally, one must consider that if the prey is left in the culture system for an extended period of time it's nutritional quality will change hence

either food for the prey must be provided or the prey should be flushed from the system on a regular basis.

For many species and production situations, the shift to prepared feeds is critical to the continued development of the industry. Prepared feeds currently do not work for all species; however, there are a number of species that co-feeding of prepared and live foods has been demonstrated to enhance production. Examples include: seabass, *Dicentrarchus labrax;* gilthead seabream, turbot ,*Scophthalmus maximus*; Atlantic halibut, *Hipoglossus hippoglossus* (Rosenlund *et al* 1997) and the red drum (Lazo 1999). The primary advantage of micro-particulate feeds is that their nutritional content can be controlled and manipulated, hence, it is a stable source of nutrients.

Because of the inherent differences between feeding a live and formulated diet, the rearing protocols developed with live feeds can result in disastrous results (Barrows and Rust, 2000). Quite often, when prepared feeds do not work it is not due to inadequacies of the feed but inadequacies of the culture conditions. Live foods have a number of advantages, they can be fed less often, they have less impact on water quality and they stay suspended in the water column. Conversely, prepared feeds do not stay in the water column. Quite often changing the tank design (conical bottom vs flat bottom) and circulation patterns (e.g. increased aeration or using an up-welling system) are required to resuspend inert feeds. Additionally, water exchange rates or biological filtration is required to maintain adequate water quality. If the culture protocols are not adjusted to resuspend the food and maintain water quality the use of prepared feed will not be successful. In most species for which intensive larval rearing techniques have been established the use of larval feeds as a co-feeding, and in some cases as the primary food source, have been demonstrated and provide a variety of advantages over traditional live food systems.

Larval production systems

In general, marine larval fish production can be characterized as a continuum of techniques ranging from extensive to intensive systems. The most common is extensive outdoor production in which primary production is stimulated by pre-fertilizing outdoor ponds and a natural ecosystem is allowed to develop. In this situation, other than manipulations of fertilization regimes and pre-stocking period. There is little or no control over the food source (wild plankton such as Rotifera

"rotifers" and a sub-class of the Crustacea, Copepoda "copepods"). A typical production schedule includes filling the ponds with filtered sea water. Two days after filling, the pond is often fertilized with inorganic fertilizers (such as ammonia nitrate and phosphoric acid) and then two days later with organic fertilizers (cottonseed meal). Seven to ten days after fertilization the pond should have adequate zooplankton blooms to support larval production. For red drum the initial target for zooplankton (copepods, copepod nauplii and rotifers) counts is 200 /liter. Once the targeted plankton bloom has been reached, the ponds are stocked with 3 day old larvae at a density of 400,000 larvae/acre. The eggs and newly hatched larvae are held in indoor tanks until they are ready to be stocked into ponds. This allows for the evaluation of hatching rates, larval quality and assures that the larvae are maintained in a healthy environment until they are ready to feed.

In operations for which the fish are to be released into the wild for restocking purposes, the fish are harvested as juveniles (25-30 days post hatch). Whereas fish being used for commercial grow out are offered a swim-up feed (generally trout feed) that is broadcast into the pond starting at day 14-21 depending on temperature, availability of natural food sources and size. This method works very well producing consistent production of 10-25% survival (from egg to young juvenile) depending on weather conditions and management strategies. Although, this is by far the most common strategy for the production of marine fish, it has two primary drawbacks: 1) a number of species will not tolerate pond conditions 2) production is dependent on environmental conditions that vary seasonally and from year to year.

To maintain better control on the availability of food sources and environmental conditions many production systems rely on semi-intensive methods. Such systems rely on natural phytoplankton blooms similar to those developed in ponds to supply the base of the food web. However, unlike pond systems since the volumes are smaller and the larvae reared at higher densities, food sources are often collected either from the laboratory or from outdoor productions systems concentrated and then added to the larval rearing tank to enhance natural production. Hence, this system uses techniques from both extensive and intensive systems. This is a common system in Asia and has been successfully applied to a number of species. Work by Watanabe et al. (1998) describes initial success of mass production of *L. analis* juveniles in a 30,000 L (7,926 gal) outdoor larval rearing tank. The culture tank was prepared by filling with unfiltered seawater, fertilizing with

inorganic fertilizer and inoculating the culture water with *Nannochloropsis oculata* followed by ss-type rotifers, *Brachionus plicatilis*. Eggs were then stocked into the system at a density of 10.5 eggs/L (39.7 eggs/gal). Both rotifers and algae were supplemented to the culture tanks from batch cultures. Newly hatched *Artemia* nauplii, as well as lipid enriched nauplii, were fed at 1/L (3.8/ gal) from day 7 to 35 post hatch with artificial feeds introduced at day 24 post-hatch. On day 38 post-hatch, the fish averaged 0.3g and had a survival from day 2 post-hatch of 14.3%.

Because food items (phytoplankton and zooplankton) are added to the culture systems, semi-intensive systems have more control over food density and the quality or type of live prey items. Such system are quite common and range from a very basic systems where all food items are collected and concentrated by hand to completely automated systems that collect the zooplankton and pump it into the various larval rearing systems. As this system is further intensified, we reach intensive production systems in which the larvae are reared at higher densities often times in clear water systems, in indoor controlled conditions for which all food items are provided at metered levels.

Commercial production of sea bream (*Sparus aurata*) is an excellent example of intensive larval rearing. Rearing conditions are usual under intensive conditions at a density of 100-150 larvae/L, temperature 19-21ºC and salinity 30- 38‰. Larval rearing generally takes 50-60 days until metamorphoses. Rearing tanks are circular or cylinder conical with a central drain and capacity ranges from $2m^3$ to 5 m^3. Husbandry during the first 15 days of larval rearing is very important. Water is generally filtered through 1µ cartridge and a green water technique is used (i.e. algae is placed in the culture system as opposed to a clear water system without algae). The use of some algae in the culture system has a variety of advantages but primary serve to enhance water quality and provide food for live prey while they are in the larval tanks, but also improves appetite, initial growth rate, survival and viability of fry (Reitan et al., 1993; Oie et al., 1997). Water flow in the tanks range from 0.8 – 2.8 L/min (2-4 exchanges per day) and is designed to produce a uniform larvae distribution in the tank. At 3 days after hatching (DAH), the larvae will begin feeding (mouth is open, eyes pigmented and pectoral fins present). The first feeding prey is enriched rotifers, *Brachionus plicatilis* maintained at a constant concentration in the tanks (10 rotifers/ml) As a physoclist species the conditions to provide the swim bladder formation are very important, because

the initial inflation through the pneumatic duct occurs only during a limited period of the larval life. This period is short, lasts from 5 until 12 DAH at 20ºC. The swim bladder first appears as a small reflective drop in larvae around 5 DAH, and it is located between vertebral column and the digestive tract (Soares et al, 1994). Typically it develops in two stages, the primary inflation in which the bladder became ellipsoidal and a highly reflective bubble and the expansion when a second drop appears followed by a fusion with the primary bubble causing a posterior increase in size. During the first stage the pneumatic duct is opened to the digestive tract, and the larvae need to gulp air to inflate the bladder. If this process failed, the duct closes and a functional bladder will not form. The absence of a functional swim bladder has been identified as a cause of skeletal malformations such as lordosis and scoliosis (Kitajima *et al.*, 1981, Chatain, 1994) and increased mortality for this and other species (Chatain and Ounais-Gouschemann, 1990).

Consequently, during the first two weeks of larval rearing it is important to remove the oily layer at the water surface in the tanks, which usually results from the enrichment techniques use for the rotifers and/or the use of micro-particulate diets. Air gulping is indispensable for initial bladder inflation in the larvae, requiring larval access to the water surface which that film avoid. More recently, the use of a blower associated with a floating trap improved the inflation rate of the swim bladder (Chatain, 1990). Once the fish reach 17-20 DAH, *Artemia* nauplii are introduced in a co-feeding method at a concentration of 2-5-nauplii/ ml. After 25 days larvae are only fed on Artemia till 30 DAH, when the weaning onto inert diets starts. The weaning is always given in co-feeding with Artemia using different size pellets. Although, current protocols for sea bream are primarily based on live foods, recent work has demonstrated that the best growth is supported by co-feeding micro-bound diets with live foods (Kissil et al., 2000).

Summary

Culture techniques for the mass production of marine larval fish has made considerable strides particularly with reference to controlled spawning and intensive larval rearing. Although these fields have matured considerably, we are still heavily dependent on live and fresh foods to provide essential nutrients to both broodstock and larvae. As our understanding of the nutritional requirements for maturation, reproduction and larval rearing expands it is becoming more common

to replace fresh or live feeds with compounded diets. Although, fresh feeds (live or frozen) have a number of advantages they are often costly, difficult to produce or obtain on a year round basis and they are also potential sources of pathogens. As our understanding of the nutritional requirements of species expand so does our use of prepared feeds in both maturation and larval rearing. It is now common practice for a number of marine species to use prepared feeds in both maturation and larval rearing. The co-feeding of larvae with both live and prepared feeds can result in not only a reduction in the need for live prey items but increases in larval quality. It should be noted the advances we are seeing with regard to the use of micro-particulate larval feeds in a synergistic advance in culture techniques, system design, nutrition, feed processing and a better understanding of larval physiology. Consequently, if prepared feeds are to be successfully integrated into a given production system one also has to make corresponding changes to culture techniques.

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