

Challenges in Developing Successful Formulated Feed for Culture of Larval Fish and Crustaceans

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ABSTRACT

Expansion and production consistency in commercial farming of aquatic species, particularly species of marine fish, are limited by the lack of successful replacement of live food with formulated feeds. Lack of progress may be due to insurmountable objectives created by the persistence of several biases about what constitutes the proper formulated feed and conditions for larval culture. Poor response to larval diets may have nothing to do with nutrient composition, but rather the result of incompatible culture conditions and unsatisfactory presentation of the diet.

Recognition of the complexity of the ontogeny of nutrient physiology in larvae must be tempered with the need to strive for simplicity in formulation and manufacture. Technical success must be based upon the acceptance of compromise whereby flexibility will ensure progress.

INTRODUCTION

The culture of larvae of many species of fish and crustaceans is precariously dependent upon the availability of live food, whether plant or animal. The newly hatched nauplii of *Artemia* and species of rotifers have generally served as an excellent source of food for larvae of many species of fish and crustaceans. However, live *Artemia* nauplii are obtained through hatching of cysts that are collected from the natural environment and subject to periodic, unpredictable shortages that cannot supply the demand. As a result, prices increase, leading to overall increases in production costs. In addition, another chronic problem is that temporal or spatial differences in cyst collections are reflected in variation in the nutritional quality of hatched *Artemia* nauplii.

That inherent problem has been partially overcome by the use of different methods designed to eliminate presumed nutritional deficiencies associated with both *Artemia* and rotifers (Clawson & Lovell 1992). Efforts often concentrate on the enhancement of the content of two highly unsaturated fatty acids, docosahexaenoic acid and eicosahexaenoic acid. The value of formulated diets that serve as complete replacements for live food is obvious because of both cost and the lack of consistent nutrient quality of live food.

D'Abramo, L.R., 2002. Challenges in developing successful formulated feed for culture of larval fish and crustaceans. In: Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M. G., Simoes, N. (Eds.). *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola*. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

Formulated diets that can achieve consistent and reliable production equivalent to that of live food still do not exist and have definitely been an impediment to the progress of marine fish culture throughout the world. During the past decade significant progress has been achieved but the common use of microdiets, characterized as either microbound, microencapsulated, or microcoated (Tucker, 1998), as exclusive sources of nutrition still eludes us and remains a challenge.

Research that has addressed the development and evaluation of nutritionally complete larval diets has been conducted for over three decades. Most diets, at best, have served as supplements rather than complete substitutions (Kumlu & Jones 1995). The lack of success might in part be due to imposed biases that are partly associated with trying to realize an ideal that may not be attainable. Success may only be achieved through the creation of a diet that possesses less than the ideal characteristics. The highest level of success has been predominantly realized with herbivorous forms of crustacean larvae (Bautista, Millamena & Kanazawa, 1989; Koshio *et al.*, 1989). For these forms, rapid rates of transit time through the gut combined with the highest levels of enzyme activity (Jones, Yule & Holland 1997) provide for the satisfaction of nutrient requirements with diets that do not have to be highly digestible.

Satisfaction of nutrient requirements is achieved through volume of contact per unit of time rather than efficient digestion. In contrast, the comparatively short gut retention time of carnivorous larvae, particularly during early stages of metamorphosis, precludes the use of poorly digestible diets because rapid digestion is critical.

The intent of this paper is to suggest that some of the previous research is based upon biases that need to be relinquished. Successful development of micro diets for larvae in the future may only be achieved through the recognition that a useful diet must be simple to produce and that creation of the ideal diet is not possible due to needs that are often in direct conflict. The goal of developing successful diets depends on a better understanding of nutritional physiology as well as the elimination of biases. My thoughts concerning some of the most important factors and the biases that have been prevalent in the past follow:

FACTORS INFLUENCING THE EFFECTIVENESS OF FORMULATED DIETS

Enzyme activity and digestive capacities At one time, the prevailing explanation for the perennial lack of success in the culture of carnivorous larvae was insufficient enzyme activity. Researchers speculated that the enzyme manufacturing capacity within the gut was far lower than what was needed and that effective digestion was only accomplished through the assistance of exogenous enzymes that originated from the sources of live food (Kolkovski, Tandler & Isquierdo 1997; Kolkovski *et al.*, 1993; Munilla-Moran, Stark & Barbour 1990). However, Lovett & Felder (1990) found that contribution of enzyme activity from *Artemia* prey was very low compared to that measured in larvae of *Penaeus setiferus* larvae. Cahu & Zambonino Infante (1997) found that the lack of good growth in sea bass larvae (15-40 days old) fed a formulated diet was not due to a lack of endogenous enzyme activity. Garcia-Ortega *et al.* (1998) observed a minimal contribution of enzymes

from consumed prey to the larval gut. It appears that although the complement of digestive enzymes available might be qualitatively restricted, particularly during early stages of development, a sufficient quantitative and qualitative array of enzymes do exist to achieve good growth and survival to metamorphosis.

If the complement of enzymes exist, then why do micro diets remain ineffective? The ineffectiveness may reside in a lack of understanding of the specificity of the enzymes that exist at different stages of larval development. The digestive capacities of larvae relative to the ingredients of a formulated feed may be species or stage-specific. Changes in the sequence of qualitative enzyme activity is consistent with the strictly carnivorous feeding activity of early larval stages of the freshwater prawn *Macrobrachium rosenbergii* (Kamarudin *et al.*, 1994) and the ontogenetic change in enzyme activity has also been described for the larvae of the white shrimp *Penaeus setiferus* (Lovett & Felder, 1990). Digestive capacity corresponds to the anatomical development of the digestive system which in turn is related to changes in habitat and diet during metamorphosis (Lovett & Felder 1989). The absence of digestive capacity can be attributed to either physical or chemical characteristics that are not compatible with the enzymatic capacity of very young fish larvae. Digestive capacity may also be based upon the quality of food presented (Zambonino Infante & Cahu 1994a & b). Recent efforts with culture of larvae of *Macrobrachium rosenbergii* using formulated diets that were produced in a variety of ways and contained egg albumin as the primary protein source were unsuccessful. (Ohs *et al.*, 1998). These diets were readily consumed and the guts were full; however, growth and survival were significantly lower than that achieved with live food. This diet was a modification of a similar egg albumin based diet that proved successful for the axenic culture of successive generations of the microcrustacean *Moina macrocopa* (D'Abramo 1979). Recent efforts to feed larvae of *M. rosenbergii* with a diet wherein the primary protein source is egg yolk have proved successful (Kovalenko *et al.*, 2002). This minor change suggests that the egg albumin protein, despite possessing an excellent amino acid profile, is not efficiently utilized.

Although a sufficient quantity of enzymes may be present, they are highly specific and only effective with certain types of protein sources. Therefore, attempts to mimic the ingredient composition of larval diets with that of successful diets for juvenile culture is an approach that may not be possible. One must be aware that the large amount of published literature concerning the nutrition of juveniles may not be applicable to larval forms. Therefore, failure toward the goal of including what would be considered to be an excellent dietary protein source relative to the amino acid composition of the larvae may be due to its indigestible properties for larvae.

Physical form of the diet

The success of any micro diet for larval culture is critically dependent upon physical form. Successful consumption of the diet may be dependent upon the moisture content of the diet, which ultimately affects the physical form of the diet. Ideally, a diet that has low moisture content would be most desirable because it offers advantages relative to storage in a frozen

state for an extended period of time. In contrast, high moisture diets naturally limit the duration of storage and shelf life. In addition, the rate of leaching of water soluble nutrients would presumably be amplified in formulated larval diets containing a high moisture content a dry diet has many appealing benefits but this bias based upon the overriding desire for convenience must not prevail. For dry diets, the method of drying may also influence the performance of a formulated diet. Teshima & Kanazawa (1983) found differences in growth and survival of larvae of *Penaeus japonicus* when fed carrageenan-microbound diets dried under different conditions. High moisture diets may also offer a level of palatability and eventual consumption that cannot be achieved with dry diets.

Shape and size are other important characteristics. The size of formulated diets designed for fish and filter feeding crustacean larvae is generally confined to particles that are small enough to be entirely consumed without the assistance of mouthparts. Larvae of sea bream have been observed to ingest inert particles that are 60 to 80 % of the width of the mouth (Fernandez- Diaz, Pascual & Yufera 1994). However, this need is not always warranted.

Although diets that contain high levels of moisture may not be amenable to the production of particles less than 150 microns, restrictions of particle size imposed by high moisture formulated diets may not be applicable to some species of crustacean larvae that are raptorial feeders. These species possess mouthparts or appendages that permit the grasping and manipulating of particles that are physically modified into sizes that are consumed.

Shape of the particle may also influence consumption of formulated diets. Some species may prefer irregular size particles whereas the consumption of food by other species may be limited to smooth particles. This type of work suggests that videography of the feeding of larva forms will have to become an important part of the evaluation of the consumptive "appeal" of formulated diets. The diet may have the necessary size and nutrient profile but still remain unacceptable. Considerable effort has been devoted to establishing diets that are neutrally buoyant to afford as much access to the diet in the water column. However, achieving this ideal may be unnecessary. Although some diets may have comparatively high rates of sinking, maintenance in the water column can be achieved with appropriate aeration or design of the culture tank. The lack of buoyancy may pose no threat to successful culture of some species that have been observed to feed readily off the bottom.

The lack of ingestion of formulated diets may in fact be the lack of visual stimuli. Some of that stimuli may reside in the movement or color of the live prey, characteristics that are not absent or not normal in formulated diets.

Frequency of feeding

The prevailing thought is that frequent feeding is an important component of successful larval culture. Yet, Rabe & Brown (2000) found that the rate of consumption of live food, *Artemia* and *Brachionus* sp. by larvae of yellowtail flounder was significantly higher when live food was offered 1 X or 2 X per day rather than continuously. These results suggest

that the labor associated with frequent provision of formulated diets may not be necessary for some species of larvae. A more rapid consumption of food and more efficient use of nutrients may actually be achieved through a reduction in the number of feedings per day. A reduction in the frequency of feedings may also reduce the incidence of fouling and reduce the period of time when diets remain unconsumed and subject to leaching of water soluble nutrients. The assumption that frequent feeding is essential for larvae may not be universally applicable and can create undue restrictions on the type of diet that can be used. For example, continuous delivery of moist diets through timed belt feeders may not be possible but such a limitation is non-existent if feeding could be limited to 2-3 X per day without any adverse effects on growth and survival.

Lipid and fatty acid nutrition

Dietary lipid is recognized as an energy source that would seemingly be more critical to achieving satisfactory growth of carnivorous rather than herbivorous larvae. For carnivores, it is assumed that the quantity and quality of carbohydrases produced by carnivorous larvae would be limited based upon the known composition of the natural diet. Lipid in the form of cholesterol, phospholipids, and essential fatty acids has been found to be important for the nutritional physiology of fish and crustacean larvae (Teshima, Ishikawa & Koshio 1993; Jones, Kanazawa & Rahman 1979). Fatty acid composition of the tissue of freshwater versus marine organisms is different and these differences are important considerations in the formulation of larval diets for marine versus freshwater organisms. Recently, the important role of docosahexaenoic acid was demonstrated in feeding experiments with haddock larvae using live and formulated diets (Blair *et al.*, 2002). Certain highly unsaturated fatty acids are found in the tissue at concentrations that are disproportionate to those levels found in the diet, suggesting that these larvae have a tremendous ability to sequester these fatty acids.

Perhaps a threshold dietary level is necessary for the larvae to attain the desired amount in the tissue for normal growth, development, and survival.

The ideal of producing a diet that reflects as much as possible the relative amounts of macronutrients found in the natural diet may be unattainable because establishing the physical integrity of a diet often requires the addition of carbohydrates.

Gut retention time

The rate of passage of food through the gut of a larval fish or crustacean will definitely influence the relative effectiveness of a particular diet. Motility through the gut decreases during ontogeny and average time of food retention accordingly increases as observed in larval lobsters (Kurmalay, Jones & Yule 1990). This information should be an important source of guidance in developing the characteristics of diets for different stages of larval development. If food rapidly passes through the gut, then supply of nutrients over a critical period of time has a better chance of being realized when a diet that has a comparatively low digestibility is used. The lack of high digestibility will be compensated by the

comparatively large volume of food passing through the gut per unit of time. The comparatively long gut retention time of carnivorous fish and crustacean larvae presents a greater challenge because diets must be highly digestible when they pass through the gut. Otherwise, adequate supply of essential nutrients for growth and survival per unit of time cannot be achieved. Achieving good digestibility of formulated diets for carnivorous fish and crustaceans is a difficult challenge because although sufficient enzyme activity is present in the primordial gut, the quality of enzymes is restricted. Ingredient sources of dietary nutrients must be compatible with the existing digestive capacity, particularly during early development of the gut. Larval diets cannot be simply ground pieces of a commercial diet that has proved successful for growth and survival of large specimens.

Bacteria

Previous focus on procedures to limit /remove the presence of bacteria from the culture water of a system where formulated feeds were being tested may, in fact, have been a bias that led to failure. Properly conditioned water with active bacterial populations, rather than "sterile" water, may be one of the more important contributors to successful larval culture using formulated diets. While recognizing the need to follow practices that will not induce abnormally high levels of bacteria, reduction to a level less than that found in the natural environment may reduce the probability of satisfactorily conditioning the gut for digestion of the microdiet. Indigenous bacterial flora may contribute significantly to larval digestion at certain stages of metamorphosis (Pinn, Rogerson & Atkinson, 1997). As gut retention time increases during larval ontogeny, the contributive role of microbiota to digestion may also increase. Microflora may serve as a supplementary source of food and microbial activity in the gut may be a source of vitamins or essential amino acids (Dall & Moriarty, 1983). Also, establishment of the appropriate bacterial flora in the culture water may be conducive to the reduction of the incidence of *Vibrio* and the larval mortality that results. The success with the feeding of some live foods may partly arise from the contribution of associated bacteria that is exposed to the gut epithelium of larval fish and crustaceans. The prevailing thought that bacterial levels must be all but eliminated because of potentially adverse effects on culture needs to be abandoned.

Culture container

The design of the culture container can seemingly exert a marked effect upon the success of food acquisition, specifically for larval forms that are not filter feeding. Consumption of diet may not always be primarily determined by the chemical characteristics, physical properties or attractant value, but rather on the environment into which the formulated diet is introduced. For example, the shape of the container or the magnitude of the movement of water within the container may be important factors contributing to the success of larval culture. Larval forms of the American lobster are successfully grown in a cylindrically tapered tank that maintains suspension of both larvae and food, simulating the planktonic conditions of the natural environment (Chang & Conklin, 1983).

Provision of the proper contrast for larvae to locate food in the milieu of the tank has been assumed to be important for species of raptorial larvae. Provision of a light background color is known to be a very important for some species of larval fish and crustaceans. Creating a color contrast for the efficient removal of uneaten feed from the bottom of a tank, may actually be counterproductive to strengthening food acquisition. Maintaining food in suspension for as long as possible would increase the frequency of encounter for those species of larvae that do not demonstrate a bottom feeding behavior. Special light conditions may also be necessary to optimize food acquisition. Essentially, the culture conditions that afford larvae the best opportunity to encounter/locate and consume the formulated diet cannot be assumed to be similar.

DISCUSSION

Whether formulated diets that will completely substitute for live food can ever be developed remains a question. The complement of desired characteristics for success are often mutually exclusive. For example, creating a water stable particle that offers minimal leaching may be achieved at the expense of the loss of qualities of attraction, palatability or digestion.

However, the leaching of water soluble nutrients does not present a conflicting problem if levels in excess of what is actually required are included to meet the requirements. Other problems may simply have to be confronted with an acceptance that the lack of mouthparts and the presence of a primordial gut in early larval stages of carnivorous species of marine fish require live food.

A nutritionally complete diet that is stable, palatable, and easily digested can be developed with the understanding that the development of the gut in larvae is so dynamic that attempts at specialization will be futile. Future formulation and manufacture of larval diets must avoid becoming stage or species-specific and be based upon a general applicability. A good example is the recently developed egg yolk based diet for larvae of the freshwater shrimp *Macrobrachium rosenbergii* (Kovalenko *et al.*, 2002). Fed exclusively for most of the larval cycle, growth and survival were at least 80 % of that achieved with a live diet of newly hatched nauplii of *Artemia*. This diet is composed of readily available ingredients, is easy to make, and can be produced in a variety of forms, as a high moisture custard diet, dried to produced > 150 micron particles, or even spray-dried.

The ontogeny of larval nutrition is obviously complex and not well understood. Nonetheless, the development of formulated diets must be approached with a consciousness whereby biases and restrictive lofty goals may have to be surrendered and simplicity of thought is the guiding force. That is the challenge.

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