15. Development of artificial diets for fish larvae

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Introduction

A generalised feeding protocol for marine finfish larvae begins with the provision of rotifers at first feeding followed by *Artemia* nauplii and larger Artemia as larvae increase in size, to a point where artificial diets are introduced and larvae are weaned from live feed organisms. In addition to rotifers and *Artemia,* microalgae are usually cultured in mariculture hatcheries to feed them. Therefore, most mariculture hatcheries culture three different live foods (microalgae, rotifers and *Artemia*) to provide food for the larvae of a single target species.

Artificial diets

Not surprisingly, efforts have been made to develop artificial diets to replace live foods for marine fish larvae. The major factors influencing this development are outlined in Table 15.1. Although cost is the major impetus for research into development of artificial diets, from a nutritional standpoint, live foods (rotifers and/or *Artemia*) are far from ideal. Artificial diets offer the opportunity to develop diets with better nutritional compositions.

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Table 15.1. Problems Associated with the Use of Live Foods.

- **Expense**
  Live food organisms may contribute up to 50% of hatchery operating costs. Most of this cost is associated with labour.
- **Facilities**
  Live food production requires substantial commitment of space and infrastructure.
- **Nutritional inconsistency and/or deficiency**
  Live foods vary in their nutritional composition according to source, age and culture techniques. *Artemia* and rotifers lack some essential nutrients and must be enriched prior to use.
- **Availability**
  Australian hatcheries rely on a continuing supply of adequate quantities of imported *Artemia* cysts. There are also quarantine issues.
- **Disease and/or crashes**
  The introduction of disease, and live food culture 'crashes' can be major problems for mariculture hatcheries.

Artificial diets for finfish larvae must be attractive and readily ingested. They must also be stable in aqueous suspension and maintain integrity. They should be digestible, their nutrients readily assimilated by fish larvae, and of appropriate nutritional composition. One of the major potential advantages of artificial diets is the ability to adjust their nutritional composition to suit the exact requirements of target larvae. This is not possible with live foods. Two types of micro-particle have been used to present artificial diets to fish larvae; these are (a) micro-encapsulated diets (MED) and (b) micro-bound diets (MBD). Both have been used extensively in nutritional studies with finfish larvae. The major difference between the two is that MED have a membrane or capsule wall which separates dietary materials from the surrounding medium. The capsule wall may restrict leaching of water soluble dietary components and therefore reduce the attractiveness of the food particles. The capsule wall is also thought to impair digestion of the food particle and, as such, MED are considered to be of limited use for marine fish larvae. MBD, on the other hand, do not have a capsule wall; this facilitates greater digestibility and increased attractability through greater nutrient leaching. Both MED and MBD are generally dried prior to use and this may hinder their digestion.

Many studies have been conducted to assess the nutritional value of micro-particulate artificial diets for marine finfish larvae. Generally, they result in lower survival and poorer growth of larvae compared to those fed live foods. The major problems in developing artificial diets for marine finfish larvae are (a) diet attractiveness and (b) poor development of the digestive system in young fish larvae. Artificial diets need to be attractive to marine fish larvae and to be ingested at a similar rate to live prey. One of the major problems in this regard is that carnivorous fish larvae rely heavily on the visual stimulus of moving prey to initiate a capture response. The lack of movement of artificial diets in aqueous suspension is a major factor influencing their low ingestion rate. Most marine finfish larvae are poorly developed at hatching; the digestive tract in the larvae of most species is a straight tube that, with time, becomes segmented into the different sections of the gut. The digestive tract is fully developed only after 'metamorphosis', when the stomach with gastric glands and pyloric caeca are developed. At first feeding, the digestive tract in most fish species contains the enzymes related to metabolism (digestion, absorption and assimilation) of molecules such as proteins, lipids and glycogen. Enzyme activity has been observed to be relatively low compared with that of adult fish. Each enzyme develops independently during ontogenesis, with variations related to fish species and temperature. Secretion of acid and pepsin to aid digestion occurs only after metamorphosis is completed and a functional stomach is present.

Live food organisms consumed by the larvae are thought to assist digestion by 'donating' their digestive enzymes, either by autolysis or as zymogens that activate larval endogenous digestive enzymes. However, other evidence has led to contradictory views regarding the role of the live food contribution in the digestion process of fish larvae. Live food organisms contain a 'package' of enzymes, gut neuropeptides and nutritional 'growth' factors that enhance digestion. These substances are frequently omitted in formulated diets. Moreover, particulate diets for larvae contain proteins and other ingredients that are difficult to digest (especially since formulated diets contain 60–90% dry matter while zooplankton has only 10%).

Inclusion of digestive enzymes, especially proteases, in the diets for fish larvae has been reported to significantly improve nutrient utilisation and performance of larvae, but still not as much as larvae fed on live food. The inclusion of pre-hydrolysed proteins in artificial diets has given mixed results depending on percentage of hydrolysate and larval age. It remains unclear whether a unique combination of hydrolysates coupled with nutrient absorption transporters can be comparable to live zooplankton. The effect of including digestive system neuropeptides to formulated diets has also been investigated in recent years. The results suggest that including bombesin may increase assimilation of diets and larval growth. However, other trials with juvenile fish have shown no effect of these additions of the neuropeptide.

There is little doubt that the development of suitable artificial diets for marine finfish larvae would increase the profitability of larval production by reducing or eliminating the requirement for live feed organisms. However, research into artificial diets for finfish has not received as much attention as that for crustacean larvae for which successful artificial diets have been developed and are now commercially available. Total replacement of live prey with artificial food particles is still not possible for most marine fish larvae and more research is required in this field. However, the profitability of larval production may also be increased by reducing the requirement for live feed organisms through partial replacement of live foods. While not totally replacing live foods, this option can result in considerable cost savings. More research is required to establish the degree to which live feeds can be replaced with existing artificial diets and the degree to which weaning onto artificial diets can be advanced. For example, weaning of European seabass (*D. labrax*) larvae 15 days earlier has enabled savings in *Artemia* production of up to 18%. Development of more suitable artificial diets for marine finfish larvae will require research into the following key areas.

(1) **Improved ingestion**

Artificial diets are ingested at a lower rate than life foods and are negatively buoyant. This may lead to overfeeding and water quality problems. Better ingestion of artificial diets requires more attractive diets and important factors to be addressed include buoyancy, colour, odour and movement. Many feed attractants have been identified, but we still don't understand completely their mode of action on feeding behaviour and the digestive tract.

(2) **Improved digestion**

Once ingested, artificial diets need to be efficiently digested. Improved digestibility may be possible through more selective use of binders, by incorporating digestive enzymes into artificial food particles and by developing soft food particles. We now know more about pancreatic hydrolazes in larval fish but understand less about mechanisms responsible for their developmental patterns.

(3) **Nutritional requirements**

Little is known about the nutritional requirements of marine finfish larvae; research is needed to establish more specific requirements. This knowledge may be acquired by developing more attractive and digestible artificial food particles whose nutritional composition can be manipulated in nutritional studies.

(4) **Culture system design**

The settling of artificial food particles can reduce water quality and the availability of food particles to fish larvae. Appropriate system design (e.g. tank shape and aeration) can be used to maximise the availability of artificial food particles within the water column and to reduce settling and resulting water quality problems.

The development of successful artificial diets for fish larvae will require a multidisciplinary approach addressing all of these factors.