Fish Larval Nutrition: A Review on New Developments

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ABSTRACT

Despite considerable progress in recent years, many questions regarding fish larval nutrition remain largely unanswered, and several research avenues remain open. A holistic understanding of the supply line of nutrients is important for developing diets for use in larval culture and for the adaptation of rearing conditions that meet the larval requirements for the optimal presentation of food organisms and/or microdiets. Marine fish larvae fed microdiets have not, at this stage, matched the growth and survival performances demonstrated by larvae fed live feeds such as rotifers and Artemia. This chapter discusses the issues related to the use of microdiets as a sole or partial feed for marine fish larvae. The techniques and methods of manufacturing microdiet particles, chemical and physical properties and the relationship to the ingestion and digestion are described. The aim of the present review is to revise the state of the art and to pinpoint the gaps in knowledge regarding larval nutritional requirements, the nutritional value of live feeds and challenges and opportunities in the development of formulated larval diets.

Keywords: Larval Nutrition, microdiets, Nutritional requirements, Nutritional value.

I. INTRODUCTION

The major objectives of this review are (i) to analyse the current knowledge, research trends and efforts; and based on this analysis (ii) to identify the gaps and bottlenecks that need to be tackled in future research for the advanced and more efficient production of fish larvae. Marine fish larvae are very vulnerable during the first stages of development and have strict requirements for biotic and abiotic conditions to survive, develop and grow properly. There are several recent reviews that cover different aspects of larval nutrition and show the advances in knowledge from different perspectives [1].

In spite of the variety of conditions that a developing larva may face in nature, the current knowledge of nutrition in early stages has been based mainly on laboratory studies carried out following reductionist approaches under artificial conditions based on limited prey types and under relatively constant biotic and biotic conditions. Another aspect to take into account is the variety in ontogeny, feeding physiology and nutritional requirements among species, even within the same family. Consequently, many specific processes cannot directly be extrapolated from findings obtained in model species and require specific studies. Obviously, a good knowledge of the larval nutritional requirements throughout development would contribute to optimize diets and feeding protocols, and thereby improve larval and juvenile quality [2].

Nevertheless, considering the vulnerability of fish larvae, it is always difficult to identify and meet nutritional requirements when several physiological and metabolic constraints are linked and each of them may prevent growth or an appropriate development. An integrated understanding of the different factors and events interacting in the food acquisition and processing is necessary for designing larval diets that meet the larval requirements for optimal ingestion, digestion and absorption of these diets. This review, which covers the gaps in knowledge on fish larval nutritional requirements, should therefore be read together with the review by Ronnestad et al. (in press) that covers the aspects of appetite, feed acquisition and digestive physiology. Considering all these limitations and based on the analysis of the current information available in marine fish nutrition, the present review attempts to identify the most burning gaps to be addressed in future research to achieve a more efficient production of high quality fish larvae [2].

Marine larvae is affected by many external and internal factors [3] [4]. Primarily, the searching, identification and ingestion processes are influenced by physical and chemical factors including colour, shape,
size, movement and olfactory stimuli at a molecular level. Substances secreted by live food organisms that act to stimulate a feeding response belong to a group of chemicals known as ‘feed attractants’, and some have been specifically identified for larvae [3] [5]. Moreover, these physical and chemical factors affect the palatability and influence the ingestion process, which is the precursor to the digestion process. Digestion involves secretion of enzymes, peristaltic movements and, after larval metamorphosis, acid and bile salt secretions. The assimilation and absorption process begins after the food particle is digested and broken down into more simple molecules that can pass across the gut lining. This is further facilitated by the development of brush border and microvilli as well as protein transporters and other transport mechanisms[6] [7].

II. LARVAL NUTRITION

2.1 Larval nutrient requirements

We know very little about the nutritional requirements of marine fish larvae [1]. Both qualitatively and quantitatively they may differ from those of juveniles or adult fish, since fish undergo dramatic morphological and physiological changes, including metamorphosis, during ontogenesis. Moreover, fish larvae grow extremely rapidly, feed continuously and, therefore, the total ingestion of nutrients must be high. In cod larvae, for example, growth rates of up to 30% per day have been measured [8] while some species such as African catfish (Clarias gariepinus) may grow up to 100% per day [9]. The requirement for a particular nutrient can be defined from a physiological point of view as the nutrient intake needed to fulfill a physiological role [10]. However, the design and formulation of diets requires translation of the nutritional requirements into the nutrient content in the diet. Micronutrient requirements, but also requirements for protein / amino acids, fatty acids and so forth, are often given as dietary concentrations / fractions, and, expressed in this way requirements do not always increase under demanding conditions, such as high growth rates and metamorphosis [11]. However, if food intake increases, the absolute intake of each single nutrient will also increase under constant dietary composition. The reason for stressing this argument is to differentiate between requirements for a certain volume of feed and the requirement for a balanced diet, where the different nutrients may be required in different ratios to each other, dependent on the developmental stage and the growth rate of the animal. Nutritional requirements are frequently defined as the ‘requirement for maximal growth and/or survival’ where the relation fish-diet-feeding has an important effect in the determination of the quantitative needs but they can be also defined as a ‘requirement for body maintenance’ as the minimum rate of nutrient expenditure needed to keep the animal alive, ‘requirement for least cost production’ or ‘requirement for fish health’ [12].

2.2 Determination of nutritional requirements for fish larvae

2.2.1 Macronutrients

Direct investigations on the optimum composition of macronutrients for fish larvae are complicated when using live feed due to the feed organism’s own metabolism and nutrient composition. However, [13] used oleic acid (OA) enriched and unenriched Artemia for Senegalese sole (Solea senegalensis) and found that the enriched Artemia gave better growth in one case[13] and a trend of better growth in the other case [13]. This was probably an effect of the higher protein to lipid ratio in the unenriched Artemia, since the non-enrichment was unlikely to change the fatty acid composition in Artemia in any favourable way. The use of experimental microdiets is likewise complicated because of the poor acceptability of most inert diets, and in particular semi-purified ones, by the generalities of species. The deficiencies in some specific nutrients may also mask the results. In spite of this, several attempts to advance this issue have been made. [14] fed microdiets formulated with two protein levels (55% and 62%) to Senegalese sole larvae and found that the larvae fed with the higher protein content grew and survived just slightly better, but exhibited a clear faster rate of eye migration. To our knowledge, real dose–response studies, using more than two levels of variation of macronutrient composition for fish larvae, are lacking. On the other hand, the experimental microdiets offer the possibility of testing different dietary macronutrient contents to explore potential macronutrient preferences. Juvenile and adult fish are able to select the appropriate composition from a variety of diets in relation to their requirement for macronutrients [15].

2.2.1.1 Protein and amino acids

The quality of the dietary protein has a primary relevance. Inclusions of low to medium levels of hydrolysed protein in weaning diets to larval fish have been shown to improve survival and growth. In carp (Cyprinus carpio) and European seabass (Dicentrarchus labrax) larvae, substitution of 60 and 250 g kg$^{-1}$, respectively, of the dietary protein with hydrolysed protein was found to be optimal[16]. In an experiment with cod (Gadus morhua), supplementation of pepsinhydrolysed protein up to 400 g kg$^{-1}$ protein improved survival rates compared with lower levels of supplementation, while a similar experiment with Atlantic halibut (Hippoglossus hippoglossus) did not give improved performance with hydrolysed protein supplementation [17]. Inclusion levels above 500 g kg$^{-1}$ of the protein seem to be detrimental to several fish species.
(gilthead seabream, [18]; carp, [19]; Dicentrarchus labrax, [16]; although noto all (Solea solea, [20]; turbot, Psetta maxima,[21]. The different optima found for different fish larvae may be explained by differences in digestive capacity, but a confounding factor is the high leaching rate of water soluble protein from formulated diets [22]; [23] and differences between fish species in feed ingestion rates.

### 2.2.1.2 Lipid class composition
There is a large body of research on lipid requirements in fish larvae, including both essential fatty acids and phospholipids (PL) to neutral lipids (NL). However, studies aimed at determining the quantitative requirements for these nutrients with dose-response, including at least five dietary levels, are very scarce. Fish larvae fed formulated diets where the lipid was added solely as tri-acyl glycerol (TAG), show poor growth and survival and accumulate lipid droplets in intestinal tissue and in the liver. This is relieved by adding PL to the diet [24]. Dietary PL has been found to be required for the growth and survival of a range of species since the early 1980s [25] [26]. Phospholipids are structural constituents of biomembranes and therefore highly demanded in the fast-growing larvae. Phospholipids are also involved in the digestion, absorption and transport of lipids from the intestine to the rest of the body. There are several indications that fish larvae are unable to synthesize PL in a rate fast enough to cover their high demand and therefore PL need to be included in the diet [26]. Indeed, the first feeding larvae enterocytes are poorly developed and organelles in which PL synthesis occurs in fish, the rough endoplasmic reticulum [27] [28] are scarce.

### 2.2.1.3 Essential fatty acids
There are numerous studies on the effects of essential fatty acids on growth, survival, behaviour and biological functions and processes in marine fish larvae, but few studies quantify the requirements in the different species and in developing larvae. It should be taken into consideration that the relative importance of each fatty acid differs among the species [29]. Dietary n-3 highly unsaturated fatty acids (HUFA) in rotifers, Artemia or microdiets affect larval survival rate and/or growth, as has been found in numerous species including turbot [30], red sea bream (Pagrus major; [31], gilthead seabream [32] 1994 as well as swim bladder inflation in gilthead seabream [32]. They have also been found to increase survival after handling stress (‘activity test’) in several species such as red sea bream [31] or gilthead seabream [33]. They have an effect on swimming, feeding and escaping behaviour [34]; [35] and water reabsorption in red sea bream [31]; [36]; [37] and gilthead seabream larvae [32] on skeleton development [38] and on flatfish pigmentation [39].

### 2.2.1.4 Vitamins
Only a few dose–response studies have been performed to obtain quantitative vitamin requirements in marine fish larvae, the vitamins studied being vitamin A, C, D, E and K. Some of these studies use only two or a few levels of vitamins. Vitamin A is involved in vision, growth, bone development, reproduction and normal maintenance of epithelial tissues. The studies on vitamin A in fish larvae are largely focused on the effects on skeletal development. An increasing number of malformations were found in the caudal region and vertebrae of Japanese flounder [40] and in the vertebrae of turbot [41] fed increasing dietary levels of vitamin A palmitate during metamorphosis. [42] found that 20 mg kg\(^{-1}\) \(\text{Asc}\) was sufficient for normal growth and survival of post-larval turbot and sea bass, when using formulated diets, whilst the Asc requirement for maximum growth in common carp larvae was 45 mg kg\(^{-1}\) [43], both in agreement with [12] requirement assessments for fish. In conclusion, the most studied vitamin in fish larvae is vitamin A, but the focus has often been on toxic effects and not so much on requirements. Nevertheless, the study by [44] indicates a larval requirement for optimal growth and survival is in the range of 10 mg kg\(^{-1}\), which is in line with requirements in juvenile and adult fish [45]; [12]. The maximal non-toxic level of vitamin A for fish larvae is still unknown. Requirements of the other vitamins in marine fish larvae are largely unknown.

### 2.2.1.5 Minerals
Research on mineral requirements in fish larvae only started after 2005 and the number of publications is quite small. [46] enriched Artemia with zinc, manganese or zinc + manganese. Increasing dietary Mn concentration from 12 to approximately 40 mg kg\(^{-1}\) DM gave a significant increase in the growth of red sea bream larvae, from 15 to 30 dph. All Mn, Zn and Zn + Mn enrichment gave a reduction of skeletal deformities, from 53% deformed fish in the control group to 39–41% in the treatment groups.

### III. FOOD IDENTIFICATION AND INGESTION
The first interaction between food particle (live or inert) and larvae occurs in the water column. Following this interaction, the particle can be accepted or rejected. Therefore, it is essential that this interaction (i.e. the feeding process) is maximized and optimized. There are many factors affecting this process including...
particle/organisms concentration, chemical and physical cues and many others. The feeding process includes several steps in the larval process of finding and ingesting food particles (Fig. 11.8, modified from [47]):
1. General and non-specific reaction, initiation of search movements involving chemical and electrical stimuli;
2. Identification of the food particle location involving chemical stimuli;
3. Close identification of the food particle, involving chemical and visual stimuli;
4. Tasting and/or actual feeding requiring chemical stimuli (taste buds).

Various substances, such as free amino acids, nucleotides, nucleosides and ammonium bases, are released from organisms that are prey for fish larvae and are potent inducers of feeding behavior in marine [48] and freshwater fish larvae. Generally planktonic organisms concentrate in ‘patches’ that attract the fish larvae. [49] [50] identified some of the active substances in Artemia rearing water and added these substances to the larvae-rearing tank. The authors then analyzed the effect that individual substances had on ingestion rates by eliminating one substance at a time and observing the differences in feeding activity. When microdiet ingestion rates dropped, the missing substance was regarded as being an active feed attractant. The authors found four amino acids which induced increased feeding activity: glycine, alanine, arginine and ammonium salt – betaine. Furthermore, asynergistic relationship was reported between the amino acids and betaine, which when combined produced a stronger effect than the sum of the individuals. These and other amino acids as well as other substances were also found to be active with other marine species (Table 1).

### Table 1. Amino acids as feed attractant in marine organisms (Source: [51]).

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>Hydrolysate</th>
<th>Free amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainbow trout (Salmo gairdneri)</td>
<td>Mixture of L-amino acids</td>
<td>Adron and Mackie, 1978</td>
</tr>
<tr>
<td>Atlantic salmon (Salmo salar)</td>
<td>Glycine</td>
<td>Hughes, 1990</td>
</tr>
<tr>
<td>Sea bass (Dicentrarchus labrax)</td>
<td>Mixture of L-amino acids</td>
<td>Mackie and Mitchell, 1982</td>
</tr>
<tr>
<td>Pig fish (Oreochromisichthyspilus)</td>
<td>Glycine, betaine</td>
<td>Carr et al., 1977, 1978</td>
</tr>
<tr>
<td>Red sea bream (Chrysophrys major)</td>
<td>Glycine, betaine, alanine, arginine</td>
<td>Gof and Tamura, 1980</td>
</tr>
<tr>
<td>Gilthead sea bream (Sparus aurata)</td>
<td>Glycine, alanine, lysine, Valine, glutamic acid and arginine</td>
<td>Fuket al., 1981, Ina and Matsou, 1980</td>
</tr>
<tr>
<td>Turbot (Scophthalmus maximus)</td>
<td>Inosine and IMP</td>
<td>Mackie and Adron, 1978</td>
</tr>
<tr>
<td>Dover sole (Solea solea) Glycine, betaine</td>
<td>Mackie et al., 1980</td>
<td></td>
</tr>
<tr>
<td>Puffer (Fugu guradus)</td>
<td>Glycine, betaine</td>
<td>Ohsugit et al., 1978</td>
</tr>
<tr>
<td>Japanese eel (Anguilla japonica)</td>
<td>Glycine, arginine, alanine, proline</td>
<td>Yoshi et al., 1979</td>
</tr>
<tr>
<td>Cod (Gadus morhua)</td>
<td>Arginine</td>
<td>Dovinget et al., 1994</td>
</tr>
<tr>
<td>Herring (Clupea harengus)</td>
<td>Glycine, proline</td>
<td>Proline Damsey, 1984</td>
</tr>
<tr>
<td>Glass eel (Anguilla anguilla)</td>
<td>Glycine, arginine, alanine, proline</td>
<td>Mackie and Mitchell, 1983</td>
</tr>
<tr>
<td>Lobster (Homarus americanus)</td>
<td>Glutamate, betaine, taurine, ammonium chloride</td>
<td>Kamsta and Heinsbroek, 1991</td>
</tr>
<tr>
<td>Western Atlantic ghost crab (Ocyepodequadra)</td>
<td>Butanoic acid, carboxylic acid, trehalose, carbohydrates, homarine, asparagine</td>
<td>Trott and Robertson, 1984</td>
</tr>
<tr>
<td>Freshwater prawn (Macrobrachium rosenbergii)</td>
<td>Taurine, glycine, trimethylamine, betaine</td>
<td>Harpaeet al., 1987</td>
</tr>
<tr>
<td>Abalone (Haliotis discus)</td>
<td>Mixture of L-amino acids and lecithin and lecithin</td>
<td>Harada et al., 1987</td>
</tr>
</tbody>
</table>

### IV. ONTOGENY OF DIGESTIVE CAPACITY IN MARINE FISH LARVAE

The development of adequate compound microdiets to replace live foods in the culture of marine fish larvae requires a thorough understanding of the digestion processes occurring during ontogeny [52] [53]. This knowledge is required for overcoming the necessary use of live feeds in the rearing of marine fish larvae. The lack of success in completely replacing live foods with compound microdiets since the onset of first feeding has been historically attributed to the presence of an undeveloped digestive system at the time of hatching and consequent low digestive capacity [54] [55] [56].

### Table 2. The use of marine organism hydrolysates and free amino acids as feed attractants (Source: [57]).

<table>
<thead>
<tr>
<th>Hydrolysate Content</th>
<th>Free amino acids Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digested protein (usually from marine organisms) components such as free amino acids and short peptides</td>
<td>Pure amino acids</td>
</tr>
<tr>
<td>Nutritional value Can be used as partial protein replacement</td>
<td>Can be adjusted and balanced to the AA</td>
</tr>
<tr>
<td>Formulation Unknown and uncontrolled values of AA and peptides as well as other nutrients</td>
<td>Known amounts of AA</td>
</tr>
</tbody>
</table>
Activity | Krill, squid, fish and several crustaceans and molluscs hydrolysates found to be strong attractants. As a ‘general rule’, protein fraction weight between 1000 and 10,000 Dalton is found to have a positive effect on feeding. Only the L-isomers have been found to be active as feed attractants.

Concentrations | Concentrations of extracts and/or hydrolysates made from aquatic animals are harder to quantify than amino acids. However, concentrations that are found to have a positive effect on feeding range from $10^{-2}$ to $10^{-10}$ g/l (when added to the water). In most cases, when incorporated into the diet, the concentration of hydrolysates and extracts released into the water was not determined. Increasing the concentration of amino acids (when added to the water) was found to have positive effects on feeding, range from $10^{-8}$–$10^{-2}$ M.

Synergism | No data available. Synergistic effects were associated with many combinations of amino acids and other substances such as ammonium salts.

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V. DIGESTIVE SYSTEM CAPACITY

Recent research evaluating the effect of specific nutrients on larval digestive physiology and characterizing the metabolic pathways of the assimilated nutrients has revealed an important role of the type (protein vs peptides and amino acids or triglycerides vs phospholipids), quantities (protein or lipid levels), ratios (DHA : EPA : ARA; or essential fatty acids vs other fatty acids for metabolic energy) and availability of dietary nutrients [58] [59]. Given the complexity of the metabolic pathways involved, a more comprehensive approach is needed to further our understanding of the digestive process and nutrient requirements of developing marine fish larvae. Similarly, more molecular research is needed to characterize nutrient transporters in the gut lumen throughout ontogeny, so as to more thoroughly establish the assimilation capacity of developing larvae [60].

VI. DIET MANUFACTURING METHODS

Several microdiet manufacturing methods are currently being used:

1. Microbound diets (MBD) (Fig. 1a);
2. Microcoated diets (MCD) and
3. Micro-encapsulated diets (MED) (Fig. 1b) and
4. Marumerization (MEM) (Fig. 1c).

![Fig. 1.](image_url)

Fig. 1. Microdiets manufactured by different techniques: (a) MBD; (b) MED (photo Manuel Yufera, CICS, Cediz, Spain); (c) MEM (photo Bernard Devresse, BernAqua, Belgium).
6.1 MBD
Currently, the manufacturing process of MBDs is the simplest and most commonly used method of preparation. It consists of dietary components held within a gelled matrix or binder. They do not have a capsule, and it is suggested that this facilitates greater digestibility and increased attraction through greater nutrient leaching [61] [62]. Some commercial microdiets are manufactured using extrusion and then crushed and sieved to the required particle sizes.

6.2 MCD
The MCD method is based on coating or binding small MBD particles to reduce leaching [63] [64] [65]. The coating layer is usually lipids or lipoproteins. This method is not often used in commercial processes.

6.3 MED
MED particles are made using several different techniques. The particle usually has a membrane or capsule wall, which separates dietary materials from the surrounding medium (Fig. 11.14b,c). The capsule wall helps maintain the integrity of the food particle until it is consumed preventing leaching and degradation of the nutritional ingredients in the water. However, this attribute may restrict leaching of water-soluble dietary components and therefore reduce the larvae’s attraction to the food particles [61] [66]. The capsule wall is also thought to impair digestion of the food particle [67] (Fig. 11.14b).

6.4 MEM
Mechanical encapsulation involves processes such as spray drying, fluidized bed drying, cold micro extrusion marumerization (MEM) and particle-assisted rotational agglomeration. The last two techniques have gained attention in the past few years with commercially available diets produced using these methods. Initially developed for pharmaceutical processes, these methods involve purpose-built machines. MEM is a two-step process of cold extrusion followed by marumerization (spheronization).

VII. GAPS AND BOTTLENECKS IN OBTAINING KNOWLEDGE ON NUTRITIONAL REQUIREMENTS OF MARINE FISH LARVAE
The most studied topic in marine fish larval nutrition is polyunsaturated fatty acid metabolism and requirement, and even within this topic quantitative requirements still have to be determined in most European fish larvae. For all other nutrients, requirement studies using dose–response designs and at least five dietary levels are largely lacking. Moreover, the few existing studies have typically been performed in the later larval stages, and requirements in early life are likely to be somewhat different.

The main reason for this scenario is a lack of appropriate diets that can be used for running requirement studies. Nutrient concentrations in live feed may be difficult to control, due to the organisms’ own metabolism and formulated feeds have technical limitations, such as high leaching rates and low digestibility. Lately, there has been an improvement in formulated diets and increased knowledge on how to control the nutrient composition of live feed. Therefore, we are now in a better position to do these studies. However, the knowledge on larval diets needs to be improved further in order to increase the quality of nutrient requirement studies.

We also do not know enough about the behaviour of marine fish larvae in relation to feed intake and the consequences this may have for nutrient digestion and absorption, for example the bioavailability of the different nutrients. Studies on topics such as the effects of feeding regimes, feeding intensity, diurnal rhythms and so forth, on gut passage time and the bioavailability of nutrients are needed to build a good framework for how to design and run requirement studies.

When nutrient requirement studies are designed in the future it is important to measure the relevant biological responses in addition to growth and survival, because the requirement for growth can be different from, for example the requirement for optimal innate immune response, normal pigmentation and muscle-, skeleton and neural system development. Another aspect that should be taken into account is the interaction of nutrients with other nutrients and with environmental conditions.

VIII. FUTURE PROSPECTS
It is clear that complete replacement of rotifers and Artemia, as finfish larvae first food items, with microdiet has not been achieved commercially without reduced growth and survival performances. As described in the chapter, the reasons for this lack of success can be related to several factors and disciplines that need to be addressed using an integrative approach. First and foremost, the particles need to be attractive to the larvae. Therefore, feed attractants need to be incorporated or coated onto the particles. This should involve diet manufacture techniques that will limit leaching, particularly of amino acids.
To date, larval nutritional requirements are only partially identified and much is still unknown. With the introducing of better microdiets with higher attractability and better digestibility, the nutritional requirements of marine fish larvae can be defined more easily. Minerals, vitamins, specific proteins and amino acid balance should be looked at combined with FCR, both calculated and actual. This research will lead to better feeding strategies and will enable the use of nutritional tools, as is the case with fish nutrition. Finally, a better uniformity in the design and execution of nutritional trials will enable the comparison of data from different systems and different trials.

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[56.] G. J. Holt, Feeding behavior of North Sea turbot (Scophthalmus maximus) and Dover sole (Solea solea) larvae elicited by chemical stimuli, Mar Biol, 113, 1992, 543–558.


