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Fish Larval Nutrition: A Review on New Developments

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----------ABSTRACT----------
Despite considerable progress in recent years, many questions regarding fishlarval 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.*

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I. INTRODUCTION

The major objectives of this review are (i) to analyse thecurrent knowledge, research trends and efforts; and basedon 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 abiotic 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, identificationand ingestion processes are influenced by physical and chemical factorsincluding colour, shape,

size, movement and olfactory stimuli at a molecularlevel.Substances secreted by live food organisms that act to stimulate a feedingresponse belong to a group of chemicals known as 'feed attractants', andsome have been specifically identified for larvae [3] [5]. Moreover, these physical and chemical factors affect the palate andinfluence the ingestion process, which is the precursor to the digestionprocess. Digestion involves secretion of enzymes, peristaltic movementsand, after larvae metamorphosis, acid and bile salt secretions. The assimilationand absorption process begins after the food particle is digested andbroken down into more simple molecules that can pass across the gutlining. This is further facilitated by the development of brush border andmicrovilli 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 ofmarine fish larvae [1]. Both qualitatively andquantitatively 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 (Clariasgariepinus) 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 fulfil 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

2.2.1Macronutrients

Direct investigations on the optimum composition ofmacronutrients for fish larvae are complicated when usinglive feed due to the feed organism's own metabolism andnutrient composition. However, [13]used oleic acid (OA) enriched and unenrichedArtemiafor Senegalese sole (Soleasenegalensis) and found that theunenrichedArtemia gave better growth in one case[13] and a trend of better growth in theother case [13]. This was probably aneffect of the higher protein to lipid ratio in the unenrichedArtemia, since the non-enrichment was unlikely tochange the fatty acid composition in Artemia in anyfavourable way.The use of experimental microdiets is likewise complicatedbecause of the poor acceptability of most inertdiets, and in particular semi-purified ones, by the generalityof species. The deficiencies in some specific nutrientsmay also mask the results. In spite of this, severalattempts to advance this issue have been made. [14] fed microdiets formulated with two protein levels (55% and 62%) to Senegalese sole larvae and foundthat the larvae fed with the higher protein content grewand survived just slightly better, but exhibited a clear fasterrate of eye migration. To our knowledge, real dose–response studies, using more than two levels of variationof macronutrient composition for fish larvae, are lacking.On the other hand, the experimental microdiets offerthe possibility of testing different dietary macronutrientcontents to explore potential macronutrient preferences.Juvenile and adult fish are able to select the appropriatecomposition from a variety of diets in relation to theirrequirement for macronutrients [15].

2.2.1.1Protein and amino acids

The quality of the dietary protein has a primary relevance.Inclusions of low to medium levels of hydrolysedprotein in weaning diets to larval fish have been shown toimprove survival and growth. In carp (Cyprinuscarpio)and European seabass (Dicentrarchuslabrax) larvae, substitutionof 60 and 250 g kg)1, respectively, of the dietaryprotein with hydrolysed protein was found to be optimal[16]. In an experimentwith cod (Gadusmorhua), supplementation of pepsinhydrolysed protein up to 400 g kg)1 protein improvedsurvival rates compared with lower levels of supplementation,while a similar experiment with Atlantic halibut(Hippoglossushippoglossus) did not give improved performancewith hydrolysed protein supplementation [17]. Inclusion levels above 500 g kg)1 of the proteinseem to be detrimental to several fish species

(giltheadseabream, [18]; carp, [19]; Dicentrarchuslabrax, [16]; although notto all (Soleasolea, [20]; turbot, Psetta maxima,[21]. The different optima found fordifferent fish larvae may be explained by differences indigestive capacity, but a confounding factor is the highleaching rate of water soluble protein from formulateddiets [22]; [23] and differencesbetween fish species in feed ingestion rates.

2.2.1.2Lipid class composition

There is a large body of research on lipid requirements infish larvae, including both essential fatty acids and theratio of phospholipids (PL) to neutral lipids (NL). However,studies aimed at determining the quantitativerequirements for these nutrients with dose-response,including at least five dietary levels, are very scarce.Fish larvae fed formulated diets where the lipid isadded solely as tri-acyl glycerol (TAG), show poor growthand survival and accumulate lipid droplets in intestinaltissue and in the liver. This is relieved by adding PL tothe diet [24]. Dietary PL has beenfound to be required for the growth and survival of arange of species since the early 1980s [25] [26]. Phospholipids are structuralconstituents of biomembranes and therefore highlydemanded in the fast growing larvae. Phospholipids arealso involved in the digestion, absorption and transportof lipids from the intestine to the rest of the body. Thereare several indications that fish larvae are unableefficiently to synthesize PL in a rate fast enough to cover their high demand and therefore PL need to be includedin the diet [26]. Indeed, the firstfeeding larvae enterocytes are poorly developed andorganelles in which PL synthesis occurs in fish, the roughand smooth endoplasmic reticulum [27] [28] are scarce.

2.2.1.3Essential 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] 1994as 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 behavior [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].

2.2.1.4Vitamins

Only a few dose–response studies have been performed toobtain quantitative vitamin requirements in marine fishlarvae, the vitamins studied being vitamin A, C, D, E andK. Some of these studies use only two or a few levels ofvitamins.Vitamin A is involved in vision, growth, bone development,reproduction and normal maintenance of epithelialtissues. The studies on vitamin A in fish larvae are largelyfocused on the effects on skeletal development. Anincreasing number of malformations were found in thecaudal region and vertebrae of Japanese flounder [40] and in the vertebrae of turbot [41] fed increasing dietary levels of vitamin Apalmitate during metamorphosis.[42] found that 20 mg kg)1 Asc was sufficient for normal growth and survival of post-larvalturbot and sea bass, when using formulated diets, whilethe Asc requirement for maximum growth in commoncarp larvae was 45 mg kg)1[43], both in agreement with [12] requirement assessments for fish.

In conclusion, the most studied vitamin in fish larvaeis vitamin A, but the focus has often been on toxic effectsand not so much on requirements. Nevertheless, thestudy by [44] indicates a larval requirementfor optimal growth and survival is in the range of1–10 mg kg)1, which is in line with requirements in juvenileand adult fish [45]; [12]. Themaximal non-toxic level of vitamin A for fish larvae isstill unknown. Requirements of the other vitamins inmarine fish larvae are largely unknown.

2.2.1.5Minerals

Research on mineral requirements in fish larvae onlystarted after 2005 and the number of publications is quitesmall. [46] enriched Artemia with zinc,manganese or zinc + manganese. Increasing dietary Mnconcentration from 12 to approximately 40 mg kg)1 DMgave a significant increase in the growth of red sea breamlarvae, from 15 to 30 dph. All Mn, Zn and Zn + Mnenrichment gave a reduction of skeletal deformities, from53% deformed fish in the control group to 39–41% inthe treatment groups.

III. FOOD IDENTIFICATION AND INGESTION

The first interaction between food particle (live or inert) and larvae occursin the water column. Following this interaction, the particle can be acceptedor rejected. Therefore, it is essential that this interaction (i.e the feedingprocess) is maximized and optimized. There are many factors affecting thisprocess including

particle/organisms concentration, chemical and physicalcues and many others.The feeding process includes several steps in the larval process of findingand ingesting food particles (Fig. 11.8, modified from [47]:

1. General and non-specific reaction, initiation of search movements involvingchemical and electrical stimuli;
2. Identification of the food particle location involving chemical stimuli;
3. Close identification of the food particle, involving chemical and visualstimuli;
4. Tasting and/or actual feeding requiring chemical stimuli (taste buds).

Various substances, such as free amino acids, nucleotides, nucleosidesand ammonium bases, are released from organisms that are prey for fishlarvae and are potent inducers of feeding behavior in marine [48] and freshwater fish larvae. Generallyplanktonic 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-rearingtank. The authors then analyzed the effect that individual substances hadon ingestion rates by eliminating one substance at a time and observing thedifferences in feeding activity. When microdiet ingestion rates dropped,the missing substance was regarded as being an active feed attractant. Theauthors 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 alsofound to be active with other marine species (Table 1).

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

Rainbow trout (*Salmogairdineri*)	Mixture of L-amino acids	Adron and Mackie, 1978
Atlantic salmon (*Salmosalar*)	Glycine	Hughes, 1990
Sea bass (*Dicentrarchuslabrax*)	Mixture of L-amino acids	Mackie and Mitchell, 1982
Pig fish (*Orthopristischrysopterus*)	Glycine, betaine	Carr *et al*., 1977, 1978
Red sea bream (*Chrysophrys major*)	Glycine, betaine Glycine, alanine, lysine Valine, glutamic acid and arginine	Goh and Tamura, 1980 Fuke*et al*., 1981 Ina and Matsui, 1980
Gilthead sea bream (*Sparusaurata*)	Glycine, betaine, alanine, arginine	Kolkovski*et al*., 1997
Turbot (*Scophthalmusmaximus*)	Inosine and IMP	Mackie and Adron, 1978
Dover sole (*Soleasolea*) Glycine, betaine	Glycine, inosine, betaine	Mackie *et al*., 1980 Metaille*et al*., 1983
Puffer (*Fugupardalis*)	Glycine,betaine	Ohsugi*et al*., 1978
Japanese eel (*Anguilla japonica*)	Glycine, arginine, alanine, proline	Yoshii *et al*., 1979
Cod (*Gadusmorhua*)	Arginine	Doving*et al*., 1994
Herring (*Clupeaharengus*)	Glycine,	prolineDamsey, 1984
Glass eel (*Anguilla anguilla*)	Glycine, arginine, alanine, proline Alanine, glycine, histidine, proline	Mackie and Mitchell, 1983 Kamstra and Heinsbroek, 1991
Lobster (*Homarusamericanus*)	Glutamate, betaine, taurine, ammonium chloride	Corotto*et al*., 1992
Western Atlantic ghost crab (*Ocypodequadrata*)	Butanoic acid, carboxylic acid, trehalose, carbohydrates, homarine, asparagine	Trott and Robertson, 1984
Freshwater prawn (*Macrobrachiumrosenbergii*)	Taurine, glycine, trimethylamine, betaine	Harpaz*et al*., 1987
Abalone (*Haliotisdiscus*)	Mixture of L-amino acids and lecithin and lecithin	Harada *et al*., 1987

IV. ONTOGENY OF DIGESTIVE CAPACITY IN MARINE FISH LARVAE

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

Table 2. The use of marine organismshydrolysates and free amino acids asfeed attractants (Source: [57]).

	Hydrolysate	Free amino acids
Content	Digested protein (usually from marine organisms) components such as free amino acids and short peptides	Pure amino acids
Nutritional value	Can be used as partial protein replacement	Can be adjust and balanced to the AA
Formulation	Unknown and uncontrolled values of AA and peptides as well as other nutrients	Known amounts of AA

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 ofamino 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 asammonium salts

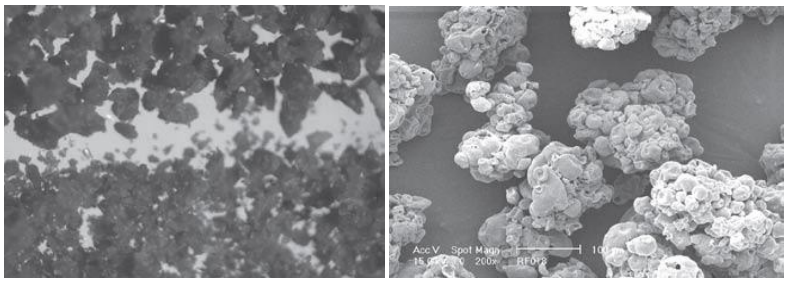
V. DIGESTIVE SYSTEM CAPACITY

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

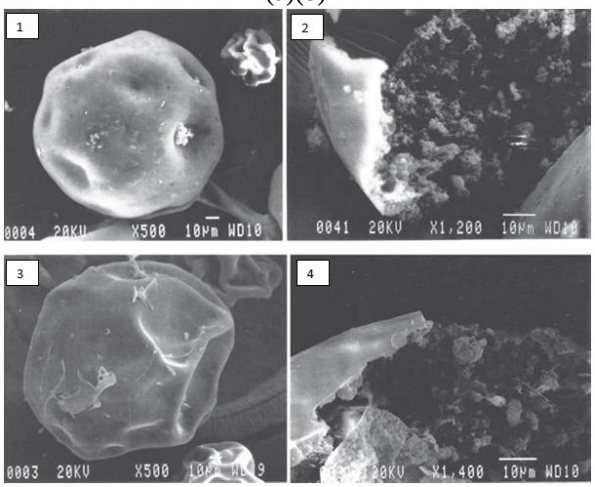
VI. DIET MANUFACTURING METHODS

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).



(a)(b)



(c)

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 particleassisted 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 finfishlarvae 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 andmuch 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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