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The Consumption of DHA during Embryogenesis as an Indicative of the Need to Supply DHA during Early Larval Development: A Review

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Abstract

The establishment of an adequate larval diet for crustacean and fish often involves a series of time-consuming and expensive trial and errors. Despite being nutritionally poor, rotifers and *Artemia* are the most commonly used preys in larviculture. Whether (and to what extent) the prey needs to be enriched with essential fatty acids differs from species to species. We hypothesized that the DHA content of a newly spawned eggs and its consumption through embryogenesis can be a good indicator of the need to enrich the prey with DHA. In order to assess this hypothesis, we performed a search in the scientific literature and compared DHA consumption through embryogenesis with larval culture success with unenriched and DHA-enriched *Artemia nauplii*, respectively a prey poor and rich in DHA of fish and crustacean. Data available from previously published studies suggests that, higher the consumption of DHA during embryonic development, greater the requirement of a diet rich in DHA during early larval development; and when, although present, DHA is not consumed during embryogenesis, larvae seem to be able to successfully develop with diet poor in DHA (i.e. using solely their reserves). Further studies will be necessary to better validate this hypothesis, but if confirmed, it may allow a reduction of time and costs associated with the establishment of an adequate larval diet.

Keywords: DHA; Embryogenesis; Enrichment; Fatty acid consumption; Larval diet; Profitability

Introduction

The development of an aquaculture industry relies on the profitability of its culture protocols. The methods utilized to raise a species not only have to be reliable and highly productive, as they have to be relatively inexpensive [1]. This imposes several challenges to producers since a culture protocol that guarantees higher survival is not necessarily the most productive or profitable [2]. For instance, higher stocking densities may generate lower survival, but be more productive than lower stocking densities [2]. One of the greatest expenses for an aquaculture facility is the feeds, particularly the ones destined to the larvae.

The Need for Live Prey

Most newly hatched marine larvae, particularly marine fish larvae, have an underdeveloped digestive system [3] lacking some proteases such as trypsin, a proteolytic enzyme responsible for 40-60% of digestion. Without the necessary amount of trypsin and other proteases, marine larvae require live prey containing these enzymes to facilitate their digestion [4-6]. Additionally, the movement of live preys in the water column is an attractant to larvae, and this is difficult to imitate in artificial diets. For these reasons, artificial diets generally can only be introduced to totally or partially substitute live prey later in development [6]. The rotifer *Brachionus* spp. and the anostracan brine shrimp *Artemia* sp are the predominant live prey items in larviculture. Their popularity advents from their small size and established culture techniques [7,8]. While heavily utilized, these prey items lack many of the essential fatty acids (lipids) required by marine larvae.

The Role of Lipids and Fatty Acids

Lipids are fundamentally important for the growth, development and survival of marine species [9,10]: phospholipids are structurally bound in membranes where they fulfill crucial physiological functions;

triacylglycerides constitute a major energy reserve that can be rapidly mobilized during periods of nutritional, thermal or osmotic stress; sterols are precursors of hormones. Fatty acids constitute the essential part of triacylglycerides and wax esters, which are the major components of fats and oils [11].

Fatty acid requirements of marine species are known to vary both qualitatively and quantitatively [12,13]. Marine species can convert Eicosapentaenoic Acid (EPA) to Docosahexaenoic Acid (DHA), albeit at low rates not likely to fully meet the high demand for DHA during larval growth [14]. Eicosapentaenoic acid (20:5n-3) is chain elongated to 22:5n-3 and hence to 24:5n-3 (Figure 1). The latter is then converted by the enzyme Δ -6 desaturase to 24:6n-3, which is finally chain shortened by peroxysomal β -oxidation to DHA (22:6n-3) [14-16] (Figure 1). However, Δ -6 desaturase also actively converts linolenic acid (18:3n-3) to 18:4n-3. Thus, both 18:3n-3 and 24:5n-3 are substrates for the same enzyme. Since 18:3n-3 is in higher amounts than 24:5n-3, it will competitively suppress the conversion of EPA (20:5n-3) to DHA (22:6n-3) [14,17] (Figure 1). Also, marine species are not capable of converting 18:3n-3 to EPA (20:5n-3) and 18:2n-6 to arachidonic acid (ARA, 20:4n-6); this is due to the fact that marine animals generally have low to negligible enzyme Δ -5 fatty acid desaturase activity [13,18,19] (Figure 1). Since the long chain polyunsaturated fatty acids DHA, EPA and ARA cannot be sufficiently synthesized *de novo* by marine species,

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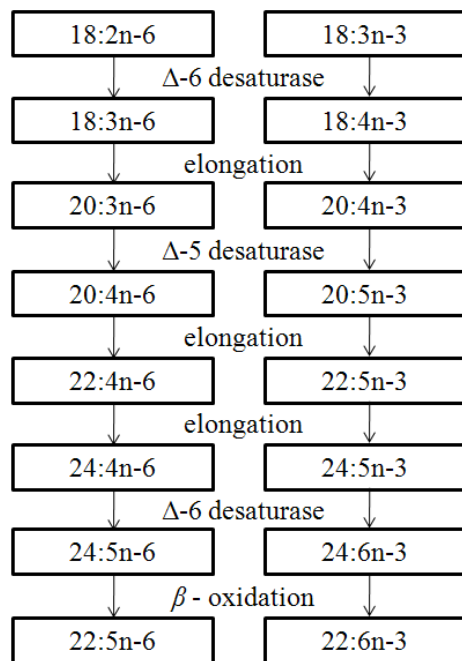


Figure 1: Polyunsaturated fatty acid aerobic pathway in heterotrophic eukaryotic marine organisms.

they are designated by essential fatty acids and must be included in all marine animals' diet [12,13,20,21]. Essential fatty acids have important functions on larval development. EPA is effective in promoting larval survival while DHA appears to be particularly important for promoting larval growth (hastening larval duration) and development of neural tissues such as the brain and retina [22-25]. ARA promotes growth, survival and improves resistance to acute stress in marine larvae and post larvae [13].

Advantages and Disadvantages of Different Live Preys

Rotifers and *Artemia nauplii* have a small size (generally 0.1-0.5 mm and less than 0.5 mm, respectively), and therefore are adequate as preys in the larval culture of several fish and crustaceans. However, they can be deficient in Eicosapentaenoic Acid (EPA, 20:5n-3), although most common *Artemia* strains used have a relatively high content of EPA [7,14,26]. Both rotifers and *Artemia nauplii* naturally lack, or have insignificant quantities of, Docosahexaenoic Acid (DHA, 22:6n-3) and are rich in linolenic acid (18:3n-3) and, to a lesser extent, linoleic acid (18:2n-6) [27,28]. Furthermore, the presence of high amounts of 18:3n-3 suppresses the conversion of EPA to DHA. Since failure to provide correct essential fatty acids is a primary cause of unsuccessful culture [29], it is common practice to enrich rotifers and *Artemia* with essential fatty acids [7,27,30,31]. Enrichment consists in feeding an enrichment product rich in the desired essential fatty acids to filter-feeding *Artemia* and rotifers. This way, one can encapsulate the required essential fatty acids that the prey naturally lacks and create a nutritionally adequate prey. Enrichment products include microalgae, lipid emulsions, fish oils and protists (spray dried single celled heterotrophic marine protist *Schizochytrium* sp.) [32].

Recent research has focused on the efficacy of using alternative preys, such as ciliates, *Barnacle nauplii*, nematodes and copepods (alone or in conjunction with traditional prey items), to overcome

dietary nutritional deficiencies [33-35]. Copepods are considered the most promising alternative live prey to feed marine fish larvae, due to a preponderance of phospholipids and ratios of DHA: EPA: ARA [36,37] that closely resemble the natural diet of the larvae. Despite the continuous effort to develop protocols to rear copepods in captivity [38-41], their culture in high densities is still unreliable and therefore incapable to support an aquaculture industry.

Larval Nutritional Requirements during Early Larval Development

Larval nutritional requirements differ between species [12] as a reflection of their dietary and metabolic adaptation to different habitats [20,42,43]. Ideally, in culture settings, the nutritional requirement of larvae after the transition from endogenous to exogenous feeding would be known. Unfortunately, this is often not the case. Heming and Buddington [44] hypothesized that the optimal formulation to provide a nutritious diet for first-feeding larvae should replicate the yolk composition of recently spawned eggs. In oviparous organisms, endogenous yolk reserves are responsible for providing nutrients and energy for proper development of the embryo and larvae during the lecithotrophic phase [43,45]. Thus, according to Heming and Buddington [44], a larval diet with the same nutritional profile of the recently spawned egg should enhance survival and growth during early larval development. However, it is known that fatty acids are not equally consumed. Furthermore, some fatty acids are consumed, while others can be conserved or even produced during embryogenesis (Figure 1).

Enrichment of prey items with fatty acids to feed the larvae is an expensive procedure in larval culture due to the elevated cost of enrichment products and labor. Most enrichment products contain essential fatty acids, particularly DHA. DHA cannot be synthesized *de novo* by marine animals and thus it can only be conserved or consumed during embryogenesis. The quantity of DHA necessary to supply to each larval species is usually determined by expensive and laborious trial and errors. Therefore, the development of a fast technique that could indicate the need to supply DHA during early larval development could be quite beneficial for the aquaculture industry.

DHA Consumption During Embryogenesis as an Indicator for DHA-Supply to Larvae

We hypothesize that the quantity of DHA consumed during embryogenesis is an indicative of the need to supply DHA during early larval development. Specifically, the more DHA consumed during embryogenesis, the greater is the need to supply DHA during early larval development (e.g., through DHA enrichment of nutritionally poor preys or the used of an additional prey with adequate DHA content). Conversely, if the newly spawned eggs are rich in DHA, but this fatty acid is not consumed during embryogenesis, the larval diet might not need to be supplemented with DHA; larvae may be able to successfully develop using solely the DHA conserved through embryogenesis. Note that we are not hypothesizing that this fatty acid is not required; we solely hypothesize that we might not need to supply it in the diet during early larval development. Since DHA is fundamental for the successful development of larvae, the need to supply DHA can be evaluated by comparing larval development success with diets poor and rich in DHA.

Methods for Bibliographic Review

We focused the literature review on fish and crustaceans species

Species	Newly spawned egg's DHA content (µg.mg dw ⁻¹)	DHA consumed during embryogenesis (µg.mg dw ⁻¹)	Larval culture with newly hatched <i>Artemia nauplii</i> (no DHA)	Larval culture with <i>Artemia nauplii</i> enriched or supplemented with DHA
<i>Armasces cinereum</i>	[47] 2.64	[47] NS	[48] 86.3% survival (15.2 days)	ND
<i>Lysmata seticaudata</i>	[55] 9.59-12.86	[55] 1.43-4.65	[56,2] 75.41% survival (19 days)	[56,2] 83.4% survival (19 days)
<i>Palaemon elegans</i>	[42] 11.8	[42] 2.7	[57,4,58] 88.5-92% survival (12 days)	ND
<i>Solea senegalensis</i>	[59] 30.7	[59] 2.8	[60,61,62] 29-87% (11-19 days)	ND
<i>Maja brachydactyla</i>	[63] 25.29	[63] 4.97	[64] 18% survival (24 days), presence of aberrant forms, smaller juveniles	[64] 42.2-46% survival (22 days), no aberrant forms, larger juveniles
<i>Palaemon serratus</i>	[42,28] 14.84-15.6	[42,28] 8.1-10.37	[28,65] 0-20% survival	[28,65] 73-81% survival and growth
<i>Nephrops norvegicus</i>	[66] 42.10	[66] 28.29	[66] 0% survival	[67,68] 0.6% survival (51.7 days)

Table 1: Newly spawned egg's DHA content, DHA consumed during embryogenesis (µg.mg dw⁻¹), and larval culture success with a prey poor or lacking DHA and with a prey enriched with DHA of crustacean and fish species (NS – no significant consumption; ND – no data available; Figueiredo et al. [47]; Staton and Sulkin [48]; Calado et al. [55]; Calado et al. [56]; Figueiredo and Narciso [2]; Morais et al. [42]; Rochanaburanon and Williamson [57]; Kumlu and Jones [4]; Sanders et al. [58]; Mourente and Vazquez [59]; Canavate and Fernandez-Diaz [60]; Dinis et al. [61]; Yufera et al. [62]; Figueiredo and Narciso [63]; Urcera et al. [64]; Narciso and Morais [28]; Wickins [65]; Rosa et al. [66]; Figueiredo and Vilela [67]; Rotllant et al. [68]).

which fatty acid content through embryogenesis and larval culture have been previously studied. Specifically we searched for published data on DHA content in newly spawned eggs, DHA consumption during embryogenesis (obtained as the difference between the DHA in newly spawned eggs and pre-hatching eggs), and larval culture success with a prey rich in DHA and with a prey lacking DHA. In order to avoid possible misleading information regarding synthesis and catabolism of fatty acids, data based upon relationships with variable constituents, specifically percent data, were not considered. Only studies in which DHA content was presented as an absolute individual measurement or as a proportion of dry matter (µg.mg dw⁻¹) were considered. Additionally, in order to standardize the data as much as possible, only studies of larval culture using *Artemia* were considered. *Artemia* is considered as relatively complete diet, except for the fact that, regardless of its origin or strain, it is poor in DHA (naturally lacks or has a reduced quantity of DHA), but can be easily enriched [26,46]. Therefore, studies of survival and growth of larvae fed with (unenriched) *Artemia nauplii* and DHA-enriched *Artemia nauplii* can be used to compare the effect of a diet poor in DHA and a diet rich in DHA.

Data Available

A review to the existent data on DHA consumption during embryogenesis, and larval culture success of crustacean and fish species with *Artemia nauplii* (poor in DHA) and enriched *Artemia nauplii* (rich in DHA) (summarized on Tables 1 and 2), suggests that the consumption of the essential fatty acid DHA during embryonic development might be a good indicator of the necessity to provide DHA to marine larvae. Overall, existent data shows that greater the consumption of DHA during embryogenesis, the more DHA a larvae requires in its diet to successfully develop (Tables 1 and 2). On the other hand, when DHA is present in newly spawned eggs, but is not significantly consumed through embryogenesis, larvae seem to be equally able to successfully develop with a diet poor in DHA (Tables 1 and 2). However, due to the reduced number of species studied (ten species of crustaceans and three species of fish with data on DHA consumption through embryogenesis and larval culture with *Artemia nauplii*, unenriched and enriched with DHA, was simultaneously available), further studies are required to confirm these indications.

The newly spawned eggs of all crustacean species contained DHA, but its quantity and consumption through embryogenesis differed between species. The wharf crab *Armasces cinereum* did not significantly consume DHA during embryonic development [47] and its larvae developed successfully with newly hatched *Artemia nauplii* (poor in DHA) [48]. All the other crustaceans studied (Monaco shrimp *Lysmata seticaudata*, Rockpool prawn *Palaemon elegans*, Giant river prawn *Macrobrachium rosenbergii*, Common prawn *Palaemon serratus*, Spider crab *Maja brachydactyla*, Norway lobster *Nephrops norvegicus* and Shrimp *Nauticaris magellanica*) consumed DHA during embryogenesis. Overall, as the consumption of DHA during embryogenesis increased, the success of rearing the larvae with a prey poor in DHA decreased. Greater the consumption of DHA during embryogenesis, the greater

Species	Newly spawned egg's DHA content (µg.egg ⁻¹)	DHA consumed during embryogenesis (µg.egg ⁻¹)	Larval culture with newly hatched <i>Artemia nauplii</i> (no DHA)	Larval culture with <i>Artemia nauplii</i> enriched or supplemented with DHA
<i>Dicentrarchus labrax</i>	[69] 2 (neutral) 1.2 (phospho-lipid)	[69] NS	[70] 80-98.5% survival	[71] 84-92% survival
<i>Nauticaris magellanica</i>	[72] 0.29-0.37	[72] 0.15-0.16	ND	[73,74] 85.7% until zoea IX, but strong mortality from zoea X to decapodid stage
<i>Sparus aurata</i>	[75] 0.6 (neutral) 0.7 (phospho-lipid)	[75] 0.15 (neutral) 0.20 (phospho-lipid)	[76,77] 15% survival	[76,77,31] 9-28% survival
<i>Macrobrachium rosenbergii</i>	[78] 0.67	[78] 0.42	[79,80,81] 44% survival	[79,80,81] 56% survival

Table 2: Newly spawned egg's DHA content, DHA consumed during embryogenesis (µg.egg⁻¹), and larval culture success with a prey poor or lacking DHA and with a prey enriched with DHA of crustacean and fish species (NS – no significant consumption; ND - no data available; Rønnestad et al. [69]; Navarro et al. [70]; Navarro et al. [71]; Wehrtmann and Kattner [72]; Wehrtmann and Alborno [73]; Wehrtmann and Alborno [74]; Rønnestad et al. [75]; Robin and Vincent [76]; Robin and Peron [77]; Monroig et al. [31]; Clarke et al. [78]; Devresse et al. [79]; Alam et al. [80]; Alam et al. [81]).

was the relative success of feeding larvae with a prey rich in DHA. It is important to notice that the Norway lobster (or scampi) *Nephrops norvegicus* consume a very high amount of DHA during embryogenesis, thus we suggest the larvae require a prey with a much greater DHA content than the one provided in the existent study. The larval culture failure of the shrimp *Nauticaris magellanica* past zoea IX is probably due to the increase or change in nutritional requirements during late development.

The larvae of the three fish species studied (sea bream *Sparus aurata*, sole *Solea senegalensis* and European sea bass *Dicentrarchus labrax*) exhibit a similar pattern of DHA consumption during embryogenesis and requirement of DHA during early larval development.

General Conclusions

Available data suggests that the more a species consumes DHA (μg . egg⁻¹ or μg .mg dw⁻¹) during the embryonic development the more it will require diet rich in DHA during early larval development (Tables 1 and 2). If the newly spawned egg has DHA, but no significant consumption of DHA during embryogenesis occurs, larval survival and growth do not seem to be greatly affected when larvae are fed with a DHA-poor prey (Tables 1 and 2).

Species which recently spawned eggs contain DHA, but that do not consume DHA during embryogenesis, probably do not need to be fed with a DHA-rich prey during early larval development because they still have DHA reserves. Later in development, when their DHA reserves are exhausted, they will likely require a diet rich in DHA; but, by that time, since they are more developed, this essential fatty acid may be provided, for example, as a pellet food. As the consumption of DHA during embryogenesis increases, the greater may have to be the quantity of DHA provided through the diet to the larvae. The DHA consumption during embryogenesis can provide the producers with an indication for DHA requirements during early larval development. Since enrichment and enrichment products are expensive, a better knowledge of the quantity of DHA required for larvae to successfully develop can avoid unnecessary expenses, and contribute to the establishment of a profitable methodology.

Future Studies and its Possible Implications

Despite our hypothesis being supported by all published studies, existing data is still insufficient to draw a definite conclusion, and more studies are needed to confirm it. Moreover, larval culture success is due to a combination of multiple aspects, such as larval culture conditions and larval diet composition, particularly EFA (and their relative proportions, DHA:EPA:ARA) [3,20,23,29]; but, since DHA is an essential fatty acid and we only compared studies that used the same prey (*Artemia nauplii*), which composition is well known, our conclusions will likely stand to further studies. We suggest that a set of standardized and controlled experiments across a significant range of species are still required to test the hypothesis that information on DHA consumption during embryogenesis could be used to develop and/or improve the larval diets and reduce productions costs. Further studies should: 1) determine the DHA consumption through embryogenesis (i.e., determine DHA in newly spawned and pre-hatching eggs of wild individuals), 2) culture larvae fed the same prey enriched with different known quantities of DHA, while controlling for DHA:EPA:ARA ratios, and larval survival, growth and presence of abnormalities registered (long-term effects of feeding a DHA-poor diet should also be determined), and 3) the results for multiple species should be compared to determine if the DHA consumption during embryogenesis could

be an indicator of the DHA content required in the larval diet. If this hypothesis was validated, a relatively simple procedure of determining fatty acid consumption during embryogenesis could: (1) avoid expensive and laborious trial and errors to find the most nutritionally suitable diet for larvae, (2) lead to a faster development of profitable larval aquaculture feeds for some species, and (3) potentially minimize enrichment (and its cost) to the strictly necessary.

For now, the consumption of DHA during embryogenesis can be used as a “rule of thumb” to decide whether, and to which extent, the larvae may need DHA in their diet, and thus possibly contribute to a faster development of culture protocols for novel species of interest

Species	Newly spawned egg's DHA content (μg .mg dw ⁻¹)	DHA consumed during embryogenesis (μg .mg dw ⁻¹)
<i>Uca rapax</i>	[49] 2.6	[49] NS
<i>Uca annulipes</i>	[50,51] 0.77-2.74	[50,51] NS
<i>Uca inversa</i>	[50] 4.1	[50] NS
<i>Uca urvillei</i>	[50] 2.0	[50] NS
<i>Uca chlorophthalmus</i>	[50] 2.4	[50] NS
<i>Uca vocans</i>	[50] 3.9	[50] NS
<i>Palaemon concinnus</i>	[82] 8.6-15.7	[82] NS-7.7
<i>Chlorotocus crassicornis</i>	[43] 15.1	[43] 1.4-4.6
<i>Macropipus tuberculatus</i>	[43] 17.2	[43] 2.9
<i>Plesionika narval</i>	[83] 10.7	[83] 4.5
<i>Plesionika martia martia</i>	[43,42] 20.5-24.5	[43,42] 5.3-6.7
<i>Sarda sarda</i>	[84] 32.7	[84] 5.7
<i>Hippocampus guttulatus</i>	[85] 17.3	[85] 11.7
<i>Polybius henslowii</i>	[43] 34.4	[43] 13.9
<i>Dentex dentex</i>	[86] 43.3	[86] 16.6
<i>Homarus gammarus</i>	[87] 29.9	[87] 20.4

Table 3: Newly spawned egg's DHA content and DHA consumed during embryogenesis (μg .mg dw⁻¹) (NS – no significant consumption; Figueiredo et al. [49]; Torres et al. [50]; Penha-Lopes et al. [51]; Penha-Lopes et al. [82]; Rosa et al. [43]; Guerao and Abello [83]; Morais et al. [42]; Ortega and Mourente [84]; Faleiro and Narciso [85]; Mourente et al. [86]; Rosa et al. [87]).

to aquaculture. For example, there are several species of fish and crustacean which DHA consumption through embryogenesis has been studied (summarized in Table 3) and for which we can make predictions about the DHA requirements of the larvae. Provided the newly hatched larvae are able to readily feed on *Artemia nauplii*, we expect that Uca larvae will not require *Artemia nauplii* to be enriched with DHA to successfully develop [49-51] (Table 3). Newly spawned *Artemia nauplii* have been used in the past to rear the larvae of Uca sp. [52], but survival and growth data was never reported. The species that consume a significant amount of DHA during embryogenesis, such as shrimp *Plesionika narval* and *P. martia*, Atlantic bonito *Sarda sarda*, Seahorse *Hippocampus guttulatus*, Henslow's swimming crab *Polydora henslowii*, will probably require a diet rich in DHA to complete development. Common dentex *Dentex dentex* and the European lobster *Homarus gammarus* consume a very significant amount of DHA during embryogenesis (similar to *Nephrops norvegicus*), therefore a DHA-enriched *Artemia* might still be an insufficient source of DHA. Rueda and Martinez [53] reported that *Dentex dentex* displays high mortality and presents malformations when fed rotifers and *Artemia* and requires a diet more rich in DHA, which is coherent with our hypothesis. When *Homarus gammarus* larvae are fed newly hatched *Artemia nauplii* and mysids, they do not complete larval development [54].

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