Utilizing egg fatty acid consumption during embryogenesis to develop and improve broodstock and larval diets

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Abstract

Aquaculture is considered to be an alternative to the harvesting of wild populations. However, there remain several constraints in larviculture of marine species: the quality of newly hatched larvae is highly variable; larvae are vulnerable to fluctuating environmental conditions; many larvae have reduced energy reserves and have underdeveloped digestive systems; inability to provide diets of appropriate nutritional profile to broodstock and larvae. Recent research has emphasized enhancement of broodstock and larval nutrition via fatty acid (FA) analysis of the eggs. Yolk reserves are the source of energy and nutrients during embryonic development and the pre-feeding larval period, therefore, the FA composition of eggs can be as a good indicator for determining the nutritional profile of the larval diet. We hypothesize that the FA consumed during embryonic development will be the ones required during larval development. Available data suggests that the greater the consumption of docosahexaenoic acid during embryonic development, the greater the need for a DHA-rich larval diet. Fatty acid composition of broodstock diets might also be improved by adding the essential fatty acids that are most consumed during embryonic development. FA trophic markers can elucidate the feeding ecology of potential aquaculture species and promote the development of diets.

**Keywords:** fatty acid; egg; larval nutrition; broodstock diet; trophic markers

1. Major constraints of marine larviculture

The culture of marine species is relatively recent and has been performed commercially for the past 40 years. During the 20th century advanced techniques were developed to successfully culture marine species. The discovery of the *Artemia* and rotifer lifecycle, the primary prey items used to feed marine larvae, permitted several breakthroughs in the culture of marine fish species such as cobia, flounder, sole, cod, seabream, sea bass, barramundi and moi and some crustaceans, particularly shrimp such as *Penaeus kerathurus*, *P. japonicus* and *P. monodon* (FAO 2006). Aquaculture accounts for almost 50% of the world’s food fish and is perceived as having the greatest potential to meet the growing demand for food. World aquaculture has grown tremendously during the last 50 years from an annual production of less than a million tons in the early 1950’s to 59.4 million tons in
2004. In 2004, 336 species belonging to 115 families including fish, aquatic plants, crustaceans and molluscs were cultured (FAO 2006). Despite the advancement of aquaculture techniques there remain several bottlenecks in the larviculture of marine species.

 Marine species are much more difficult to rear than their fresh and brackish water counterparts for various reasons. Of the numerous causes, variable larval quality, maintenance of appropriate environmental parameters and the nutritional profiles of current prey items and broodstock diets have received much attention.

Larval quality

Consistent larval quality remains problematic for aquaculture (Lavens & Sorgeloos 1991). The ability of broodstock to produce competent larvae often declines in captivity (Harrison 1990; Palacios, Ibarram, Ramirez, Portillo & Racotta 1998). While stress and environmental factors may contribute to this decline, the ability of an animal to sequester adequate nutrient reserves prior to and during oocyte development can also be a major impediment (Teshima & Kanazawa 1983; Teshima, Kanazawa, Koshio & Horinouchi 1989; Palacios et al. 1998).

The embryo and pre-feeding larvae of most crustaceans and marine fish are lecithotrophic, as their nutrition is solely supplied by egg yolk reserves (Harrison 1990) that must contain all nutrients required for maintenance and development (Anger 1998; Rosa Calado, Narciso & Nunes 2007). Parental condition and quality of maternal nutrition have been shown to affect reproductive performance and the viability of resultant embryos (Cahu, Guillaume, Stéphan & Chim 1994; McCormick 1999; Kokita & Nakasono 2000; Green & McCormick 2005). This may affect the physiological condition of newly hatched larvae and subsequent development, survival and growth, particularly during the period when the larvae are dependent upon endogenous energy reserves (Rainuzzo, Reitan & Olsen 1997; Anger 2001). The quality and quantity of nutrients in egg yolk is dependent on maternal body reserves, capacity for biosynthesis, and dietary intake during maturation (Harrison 1990). Maternal nutrition must be augmented to provide sufficient energy and appropriate nutrients to meet the metabolic cost of biosynthesis and of mobilization of nutrients for the manufacture of gonads, oocytes and egg yolk (Smith, Ritar, Johnston & Dunstan 2004). Broodstock dietary intake must also provide and replace all essential nutrients lost to the eggs in support of embryogenesis and early larval development (Harrison 1990). An improvement in broodstock nutrition and feeding has been shown to: promote (or induce) maturation;
enhance fertility, sperm quality and promote mating; and increase fecundity by improving egg quality, egg quantity, hatching rates, viability of offspring and aquaculture productivity (Izquierdo, Fernández-Palacios & Tacon 2001; Lochmann, Goodwin, Lochmann, Stone & Clemment 2007).

Commercial feeds formulated and marketed for broodstock became available in the mid-1980’s and are based on general production diets fortified with several nutrients, especially n-3 polyunsaturated fatty acids (PUFA), presumed important for gonad maturation. However, formulation of broodstock diets requires enhanced knowledge of the lipid, fatty acid and amino acid requirements of the broodstock. Recent research has emphasized determining the nutritional requirements, particularly fatty acids (FA), of broodstock (to increase egg and larval quality) and larvae (to increase growth, survival, and productivity) that are essential to various biological processes.

**Maintenance of environmental conditions**

In nature, successful reproduction is partially dependent upon the release of larvae during periods when both abiotic and biotic factors, such as temperature, predation and food availability are optimal for survival (Thorson 1950; McConaugha 1985; Anger 1987). In captivity, natural optimal conditions must be reproduced to enhance the survival and development of larvae. Available culture data and optimization models have shown that as conditions, such as temperature, salinity, prey and/or stocking density, deviate from optimal values, survival, growth and metabolism are reduced (Agar 1999; Figueiredo & Narciso 2006; Penha-Lopes, Figueiredo & Narciso 2007).

**Larval diet**

Most newly hatched larvae, particularly marine fish larvae, have an underdeveloped digestive system (Blaxter 1986) lacking some proteases such as trypsin, a proteolitic enzyme responsible for 40-60% of digestion. Without the necessary amount of trypsin, marine larvae require live prey containing this enzyme to facilitate digestion (Kumlu & Jones 1995a, b). Two live prey items that predominate in larviculture are the rotifer, *Brachionus* spp., and the anostracan, *Artemia* sp., due to established culture techniques and small size (Dhert, Rombaut, Suantika & Sorgeloos 2001; Hagiwara, Gallardo, Assavaaree, Kotani & de Araujo 2001). According to Watanabe, Kitajima & Fujita (1983), without the mass culture of
rotifers, larval rearing of marine fishes would be virtually impossible. The discovery that newly hatched Artemia nauplii were an excellent food source for several larval decapod species (Sorgeloos, Dhert & Candreva 2001) and ease of obtaining Artemia cysts for culture in captivity (McConaugha 1985) were important breakthroughs in the culture of several marine species. While heavily utilized, these prey items lack many of the essential fatty acids (EFA) required of marine larvae. Rotifers and Artemia can be deficient in eicosapentaenoic acid (EPA, 20:5n-3), although most common Artemia strains used have a relatively high content of EPA (Sargent, McEvoy & Bell 1997; Dhert et al. 2001; Sorgeloos et al. 2001). Rotifers and Artemia nauplii naturally lack 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) (Fernández-Reiriz, Labarta & Ferreiro 1993; Narciso & Morais 2001) which will also suppress conversion of EPA to DHA (see section 2).

Since failure to provide correct EFA is a primary cause of unsuccessful culture (Rainuzzo et al. 1997), it is common practice to enrich rotifers and Artemia with EFA (Fernández-Reiriz et al. 1993; Han, Geurden & Sorgeloos 2000; Dhert et al., 2001; Monroig Navarro, Amat, González, Bermejo & Hontoria 2006). Enrichments include microalgae, lipid emulsions, fish oils and protists (spray dried single celled heterotrophic marine protist Schizochytrium sp.)(Sargent, McEvoy, Estevez, Bell, Bell, Henderson & Tocher 1999a). However, all dietary oils rich in DHA (particularly marine fish oils) contain substantial but variable amounts of EPA and minor and variable amounts of ARA. Additionally, rotifers and Artemia nauplii supplemented with enrichment products preferentially catabolize DHA relative to EPA (Navarro, Henderson, McEvoy, Bell & Amat 1999), thus, the final DHA:EPA ratio of the enriched prey is substantially less than the starting fish oil (McEvoy, Navarro, Amat & Sargent 1995; Rainuzzo et al. 1997). Therefore, once the prey is removed from the enrichment solution and added to the larval tanks, catabolization of the enrichment product causes alterations to the nutritional profile (Estévez, McEvoy, Bell & Sargent 1998). Prey items need replacement with adequate frequency to ensure the available prey is always of proper nutritional quality, which is costly and time consuming.

Recent research has focused on alternative prey that could overcome the nutritional deficiencies of current prey items (Bamstedt, Wild & Martinussen 2001; Chen, Sheng, Lin, Gao & Lv 2006). Several prey items such as ciliates, barnacle nauplii, nematodes and copepods were examined, with copepods being considered the most promising (Shields, Bell, Luizi, Gara, Bromage, & Sargent 1999; Bell, McEvoy, Estevez, Shields & Sargent 2003;
Chen et al. 2006). The advantages of copepods as a live feed are a preponderance of phospholipid levels and ratios of DHA: EPA: ARA more closely resembling a larva’s natural diet (Shields et al. 1999; Payne & Rippingale 2000; Bell et al. 2003; Peck & Holste 2006; Rajkumar & Kumaraguru, 2006; Jepsen, Andersen, Holm, Jørgensen, Højgaard & Hansen 2007; Sørensen, Drillet, Engell-Sørensen, Hansen & Ramløv 2007); however, this ratio is dependent on the copepod’s algal consumption and can vary between life stages (nauplii, copepodites and adults) (Bergé & Barnathan 2005). The advantages of developing protocols for feeding copepods to marine fish larvae are great (Chen et al. 2006), but may be partially offset by logistic or economic disadvantages as copepod culture is still unreliable and cultures cannot be maintained at sufficient densities to support an aquaculture industry.

2. Importance of Fatty Acids

The lipids are very important in growth, development and survival of marine species (Harrison 1990; Anger 1998): 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 triglycerides and wax esters, which are the major components of fats and oils (Bergé & Barnathan 2005). A variety of fatty acids (FA) occur in marine organisms, such as saturated, branched, monounsaturated and polyunsaturated FA.

Polyunsaturated fatty acids (PUFA) are essential components in higher eukaryotes that confer fluidity, flexibility and selective permeability to cellular membranes, affecting many cellular and physiological processes, including cold adaptation and survival, modulation of ion channels, endocytosis/exocytosis, pathogen defense, and activities of membrane-associated enzymes that are sensitive to the biophysical properties of lipid membranes (Izquierdo et al. 2001; Bergé & Barnathan 2005). The uptake of n-3 PUFA by broodstock and larvae has been correlated with various aspects of reproductive performance and embryo and larval viability (Primavera, Lim & Borlongan 1979; Harrison 1990; Abi-ayad, Melard & Kestemont 1995; D’Abramo 1997; Anger 1998). Bruce, Oyen, Bell, Asturiano, Farndale, Carrillo, Zanuy, Ramos, Ramos & Bromage (1999) showed that different broodstock diets resulted in varying levels of arachidonic acid (ARA), EPA and DHA in the eggs of the European sea bass *Dicentrarchus labrax*. Fernandez-Palacios,
Izquierdo, Robaina, Valencia, Salhi & Vergara (1995) reported an optimum level of n-3 PUFA in the diet of adult gilthead seabream, *Sparus aurata*, resulted in improved fecundity, hatching success and larval survival. The nutritional quality of broodstock diet, particularly its content in EPA and DHA, were shown to influence spawning and egg quality in red sea bream (*Pagrus major*) (Watanabe, Itoh, Murakami, Tsukashima, Kitajima & Fujita 1984; Watanabe 1985). Cahu *et al.* (1994) showed that the concentration of phospholipid and highly unsaturated fatty acids significantly influenced spawning rate and egg and tissue composition in the shrimp, *Penaeus vannamei*.

For larvae, EPA is effective in promoting 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. (Mourente, Rodriguez, Tocher & Sargent 1993; Bell, Batty, Dick, Fretwell, Navarro & Sargent 1995; Jones, Yule & Holland 1997; Sulkin & McKeen 1999). Arachidonic acid promotes growth, survival and improves resistance to acute stress in marine larvae and postlarvae (Bell & Sargent 2003). Several authors have suggested that both the concentration and ratio of all three EFA (DHA: EPA: ARA) are nutritionally important to larvae of marine species (Bell *et al.* 1995; Rainuzzo *et al.* 1997; Sargent, Bell, McEvoy, Tocher, Estevez 1999b; Bell & Sargent 2003). While the optimum ratio would be species specific, a ratio of ca. 10 DHA: 5 EPA: 1 ARA has been suggested (Sargent *et al.* 1999b; Castell, Blair, Neil, Howes, Mercer, Reid, Young-Lai, Gullison, Dhert & Sorgeloos 2001). However, an imbalance in the ratio of these EFA has been found to promote deleterious effects. For example, high amounts of EPA in relation to DHA may create an imbalance in the structural composition of the phospholipids, which could affect the normal growth and the quality of the larvae (Rainuzzo *et al.* 1997). A relative excess of EPA also competitively inhibits the production of eicosanoids from ARA, and vice versa (Sargent *et al.* 1999b; Bell & Sargent 2003).

Fatty acid requirements of freshwater and marine species are known to vary both qualitatively and quantitatively (Sargent, Henderson & Tocher 1989; Bell & Sargent 2003). In freshwater species, due to greater yolk reserves and higher capacity for synthesizing highly unsaturated fatty acids from C18 precursors, FA requirements can be met by supplying linolenic acid (18:3n-3) and/or linoleic acid (18:2n-6), although better growth performance can be achieved by supplying the “bioactive” forms of the n-3 PUFA, particularly EPA and DHA (Kanazawa 1985). Marine species can convert EPA to DHA, albeit at low rates not likely to fully meet the high demand for DHA during larval growth (Sargent *et al.* 1997).
Ecosapentanoic acid (20:5n-3) is chain elongated to 22:5n-3 and hence to 24:5n-3. The latter is then converted by a Δ-6 desaturase to 24:6n-3, which is finally chain shortened by peroxisomal β-oxidation to DHA (22:6n-3) (Voss, Reinhart, Sankarappa & Sprecher 1991; Teshima, Kanazawa & Koshio 1992; Sargent et al. 1997) (Figure 1). However, Δ-6 desaturase also actively converts 18:3n-3 to 18:4n-3, so that 18:3n-3 and 24:5n-3 are substrates for the same enzyme. The result is that dietary 18:3n-3 (in higher amounts than 24:5n-3) will competitively suppress the conversion of EPA (20:5n-3) to DHA (22:6n-3) (Buzzi, Henderson & Sargent 1996; Sargent et al. 1997) (Figure 1). Also, marine species are not capable of converting 18:3n-3 to EPA (20:5n-3) and 18:2n-6 to ARA (20:4n-6) due to the fact that they have low to negligible Δ-5 fatty acid desaturase activity (Ghioni, Tocher, Bell, Dick & Sargent 1999; Tocher & Ghioni, 1999; Bell & Sargent 2003) (Figure 1). Since the long chain PUFA DHA, EPA and ARA cannot be sufficiently synthesized de novo by marine species, they are considered essential dietary constituents for marine species and must be included in the diet (Sargent et al. 1989; Sargent et al. 1999b; Bell & Sargent 2003; Anger 2001). Due to the competition between 18:3n-3 and 24:5n-3 for the enzyme Δ-6 desaturase, it is essential not to add 18:3n-3 to the diets to minimize this competition and increase the conversion of EPA to DHA.

**Figure 1** – Polyunsaturated fatty acid aerobic pathway in heterotrophic eukaryotic marine organisms
Studies utilizing varying levels of EFA in both broodstock and larval diets have previously been used to determine optimal fatty acid requirements. However, according to Sargent *et al.* (1999a), the definition of the optimal levels of EFA for a particular species can be readily obtained by direct lipid analysis of the eggs. The FA profile of recently spawned eggs and their dynamics through embryogenesis has the potential to provide information of the FA profile (quantity, quality and ratio of EFA such as EPA, DHA and ARA) of the broodstock and larval diet. The most utilized/consumed FA during embryonic development might be those required in higher concentrations.

3. Use of egg fatty acid consumption during embryogenesis to determine larvae fatty acid requirements

Larval requirements differ between species (Sargent *et al.* 1989) as a reflection of their dietary and metabolic adaptation to different habitats (Sargent *et al.* 1999b; Morais, Narciso, Calado, Nunes & Rosa 2002; Rosa *et al.* 2007). In culture settings, the nutritional requirement of larvae after the transition from endogenous to exogenous feeding must be known to provide a nutritious diet for first-feeding larvae (Rainuzzo *et al.* 1997). Heming & Buddington (1988) hypothesized the optimal formulation 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 (Subramonian 1991; Rosa *et al.* 2007). Thus a larval diet with the same nutritional profile may enhance survival and growth during first feeding.

However, besides yolk composition of recently spawned eggs, the differential FA consumption within eggs during embryonic development may be a more useful determinant of the nutritional requirements of marine larvae. We hypothesize the fatty acids which are most consumed during embryogenesis are those which should be present in the larval diet while those which are not consumed might not need to be provided. Available data supports this hypothesis. We used DHA as a case-study since it is an essential fatty acid (i.e., it cannot be synthesized *de novo*) and it known to be absent in newly hatched *Artemia* nauplii; *Artemia* nauplii can however be enriched with DHA, so that larval culture results obtained with an unenriched and enriched *Artemia* nauplii can be compared. Data available suggests a relationship between the consumption of DHA during embryonic development of crustaceans...
and fish and the need to provide a prey with DHA reserves (Table 1). For example, in crustaceans, recently spawned eggs of *Armases cinereum* had 2.64 µg.mg dw⁻¹ of DHA; however, the embryos did not significantly consume DHA during embryonic development (Figueiredo, Penha-Lopes, Anto, Narciso & Lin 2008a). Staton & Sulkin (1991) reported survivorship of larvae was 86.3% (15.2 days) when fed newly hatched *Artémia* nauplii (which lacks DHA). Recently spawned eggs of *Lysmata seticaudata* contained 9.59-12.86 µg.mg dw⁻¹ of DHA, and during embryonic development, 1.43-4.65 µg.mg dw⁻¹ were consumed (Calado, Rosa, Nunes & Narciso 2005a); the resultant larvae survived and grew well when fed with newly hatched *Artémia* nauplii (75.41%) but the survival to juvenile was higher if fed DHA-enriched *Artémia* nauplii after zoea V (83.4%) (Calado, Figueiredo, Rosa, Nunes & Narciso 2005b; Figueiredo & Narciso 2006). Recently spawned eggs of *Macrobrachium rosenbergii* had 0.67 µg.egg⁻¹ of DHA, and during embryonic development, 0.42 µg.egg⁻¹ were consumed (Clarke, Brown & Holmes 1990). The resultant larvae survived and grew well when fed newly hatched *Artémia* nauplii but survival to juvenile was greater if the diet was supplemented with DHA (Devresse, Romdhane, Buzzi, Rasowo, Léger, Brown & Sorgeloos 1990; Alam, Ang & Cheah 1993; Alam, Ang & Begum 1995). Recently spawned eggs of *Palaemon serratus* had 14.84 µg.mg dw⁻¹ of DHA and embryos consumed 10.37 µg.mg dw⁻¹ during embryonic development. The resultant larvae were unable to successfully develop when fed newly hatched *Artémia* nauplii (which lacks DHA), while when fed DHA-enriched *Artémia* nauplii and *Isochrysis galbana*, improved survival and growth was observed (Wickins 1972; Narciso & Morais 2001). In *Maja brachydactyla*, 4.97 of the initial 25.29 µg.mg dw⁻¹ DHA was consumed during embryonic development (Figueiredo & Narciso 2008). Larvae fed newly hatched *Artémia* nauplii had lower survival to juvenile (18%), longer larval duration (24 days) and more aberrant forms than individuals fed DHA-enriched *Artémia* nauplii (42.2-46% survival, 22 days, no aberrant forms and larger juveniles) (Urcera, Arnaiz, Rua & Coo 1993; Andrés, Estévez & Rotllant 2007). However, others crustacean species known to consume greater amounts of DHA during embryonic development have been impossible to successfully culture to the juvenile stage without a DHA rich diet. Recently spawned eggs of *Nephrops norvegicus* had 42.10 µg.mg dw⁻¹ of DHA, but during embryonic development embryos consumed 28.29 µg.mg dw⁻¹ (Rosa, Morais, Calado, Narciso & Nunes 2003). The resultant larvae did not achieve the juvenile stage when fed unenriched *Artémia* nauplii, however, when fed DHA-Selco 24h enriched *Artémia* nauplii, some larvae reached the juvenile stage (0.6%, 51.7 days) (Figueiredo & Vilela 1972; Rotllant, Charmantier-Daures, Charmantier, Anger & Sardà 2001); since the
species consumes a very high amount of DHA during embryogenesis, we suggest the larvae require a prey with a much greater DHA content than a DHA-enriched *Artemia* nauplii.


<table>
<thead>
<tr>
<th>Species</th>
<th>Newly spawned egg’s DHA content</th>
<th>DHA consumed during embryogenesis</th>
<th>Larval culture with newly hatched <em>Artemia</em> nauplii (no DHA)</th>
<th>Larval culture with <em>Artemia</em> nauplii enriched or supplemented with DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Armases cinereum</em></td>
<td>2.64 µg.mg dw(^{-1})</td>
<td>Not consumed</td>
<td>86.3% survival (15.2 days)</td>
<td>No data available</td>
</tr>
<tr>
<td><em>Lysmata seticaudata</em></td>
<td>9.59-12.86 µg.mg dw(^{-1})</td>
<td>1.43-4.65 µg.mg dw(^{-1})</td>
<td>75.41% survival (19 days)</td>
<td>83.4% survival (19 days)</td>
</tr>
<tr>
<td><em>Maja brachydactyla</em></td>
<td>25.29 µg.mg dw(^{-1})</td>
<td>4.97 µg.mg dw(^{-1})</td>
<td>18% survival (24 days) presence of aberrant forms, smaller juveniles</td>
<td>42.2-46% survival (22 days) no aberrant forms, larger juveniles</td>
</tr>
<tr>
<td><em>Palaemon serratus</em></td>
<td>14.84 µg.mg dw(^{-1})</td>
<td>10.37 µg.mg dw(^{-1})</td>
<td>0-20% survival</td>
<td>73-81% survival</td>
</tr>
<tr>
<td><em>Nephrops norvegicus</em></td>
<td>42.10 µg.mg dw(^{-1})</td>
<td>28.29 µg.mg dw(^{-1})</td>
<td>0% survival</td>
<td>0.6% survival (51.7 days)</td>
</tr>
<tr>
<td><em>Dicentrarchus labrax</em></td>
<td>2 µg.egg(^{-1}) (neutral)</td>
<td>Not consumed</td>
<td>80-98.5% survival</td>
<td>84-92% survival</td>
</tr>
<tr>
<td><em>Sparus aurata</em></td>
<td>0.6 µg.egg(^{-1}) (neutral)</td>
<td></td>
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<tr>
<td></td>
<td>0.7 µg.egg(^{-1}) (phospholipid)</td>
<td></td>
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</tr>
<tr>
<td><em>Macrobrachium rosenbergii</em></td>
<td>0.67 µg.egg(^{-1})</td>
<td></td>
<td>44% survival</td>
<td>56% survival</td>
</tr>
</tbody>
</table>
Like crustacean larvae, marine fish larvae exhibit a similar pattern of DHA consumption during embryogenesis and requirement of a DHA rich larval diet. Sea bream, *Sparus aurata*, eggs 40h post fertilization had 0.6 µg.egg\(^{-1}\) and 0.7 µg.egg\(^{-1}\) of DHA in neutral and phospholipid fatty acids, respectively. At 10 hours post hatch, larvae had consumed 0.15 µg.egg\(^{-1}\) and 0.2 µg.egg\(^{-1}\) of DHA in neutral and phospholipid fatty acids, respectively (Rønnestad, Koven, Tandler, Harel & Fyhn 1994). Previous experiments have shown sea bream larvae require DHA-enriched prey for proper growth and development (Robin & Vincent 2003; Robin & Peron 2004; Monroig *et al.* 2006). In contrast, European sea bass, *Dicentrarchus labrax*, eggs 15 h post fertilization had 2 and 1.2 µg.egg\(^{-1}\) of DHA in neutral and phospholipid fatty acids, respectively, but did not significantly consume either forms of DHA during embryonic development (Rønnestad, Koven, Tandler, Harel & Fyhn 1998). Experiments have shown that sea bass larvae can be successfully raised with newly hatched *Artemia* nauplii (80-98.5% survival) (Navarro Hontoria, Varo & Amat 1988) and display similar survival with DHA-enriched *Artemia* nauplii (84-92% survival) (Navarro, McEvoy, Amat & Sargent 1995).

Available data suggests that there is no relationship between the DHA content in newly hatched egg and the need for a prey rich in DHA (Table 1). Data confirms that the higher the consumption of DHA (µg.egg\(^{-1}\) or µg.mg dw\(^{-1}\)) during embryonic development of crustaceans and fish, the greater the requirement of a DHA rich larval diet (Table 1). If no significant consumption of DHA occurs during embryogenesis, larval survival and growth should not be significantly affected when fed a DHA deficient prey (Table 1). However, available data is still insufficient to validate this hypothesis. While the observed trend seems to be coherent and to support the hypothesis made (Table 1), 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). Therefore, we suggest a set of standardized and controlled experiments across a range of species be conducted to validate this hypothesis.

According to Rønnestad (1995), developmental changes in egg or larval composition are often erroneously reported as proportions; proportional numbers are related to a component that also changes over time, such as dry or wet matter, total fatty acids and total lipids, e.g. *Alpheus saxidomus* (Wehrtmann & Graeve 1998), *Palaemonetes schmitti* (Wehrtmann & Graeve 1998), *Lepidophthalmus louisianensis* (Nates & McKenney 2000) and *Anguilla japonica* (Furuita, Unuma, Nomura, Tanaka, Okuzawa & Yamamoto 2006).
Comparisons of data based upon relationships with such constituents, particularly regarding synthesis and catabolism, may be misleading and in some cases contradict conclusions drawn from data based on absolute individual measurements. Despite the fact that the egg fatty acid profile of several important commercial crustacean and fish species has been analyzed, authors presented the fatty acid profile of a mixture of eggs at different stages of development or only reported the fatty acid profile of one egg stage (e.g. *Diplodus sargus* (Cejas, Almansa, Jérez, Bolaños, Felipe, Lorenzo 2004) and *Anguilla japonica* (Furuita *et al.* 2006)), preventing the determination of FA consumption or synthesis. For other species, the egg fatty acid dynamics during embryogenesis was analyzed, but to best of our knowledge, there is no available data on the effect of prey containing different EFA concentrations/ratios on larval survival and growth (e.g. *Nautilcaris magellanica* (Wehrtmann & Kattner 1998), *Plesionika martia* (Morais *et al.* 2002), *Macropius tuberculatus* (Rosa *et al.* 2007), *Chlorotocus crassicornis* (Rosa *et al.* 2007), and *Uca rapax* (Figueiredo, Penha-Lopes, Anto, Narciso & Lin 2008b)). Due to a paucity of available data presented as absolute values, besides absolute individual measurements, data reporting proportions of dry matter (µg.mg dw⁻¹) were considered. This approach was considered since, during embryonic development, egg volume and wet weight increases, but this increase is primarily due to water uptake and therefore egg dry matter may not significantly change, and possible errors are minimized.

4. Use of egg fatty acid profile and consumption through embryogenesis to improve broodstock nutrition and conditions in captivity

Understanding nutrition-reproduction interactions and determining the nutrient requirements for successful maturation and spawning are needed to enable year-round, large volume hatchery production (Harrison 1990; Watanabe 1995). The egg fatty acid profile (particularly newly laid eggs) has already elucidated information about species life history, feeding ecology and habitats (Cahu, Fauvel & Aquacop 1986; Cahu *et al.* 1994; Narciso 1999; Rosa *et al.* 2007). A high EPA/DHA ratio indicates the species occupies a high trophic level as DHA is highly conserved throughout the food chain; a low EPA/DHA ratio indicates the species occupies a low trophic level. A high percentage of C18 C20 PUFA, such as linoleic acid (18:2n-6), linolenic acid (18:3n-3) and EPA indicates they are consuming primary producers (herbivory) (Narciso & Morais 2001; Dalsgaard, St. John, Kattner, Müller-Navarra & Hagen 2003). A low 18:1n-7/18:1n-9 ratio and high percentage of 18:1n-9 indicates
carnivory (Auel, Harjes, da Rocha, Stübing & Hagen 2002; Scott, Kwasniewski, Falk-Petersen & Sargent 2002; Dalsgaard et al. 2003). A high proportion of odd-numbered FA (particularly 15:0 and 17:0, which are biosynthesized by marine heterotrophic bacteria abundant in sediments) indicates the existence of scavenger or detritivorous behaviour (Volkman, Barrett, Blackburn, Mansour, Sikes & Gelin 1998).

For candidate aquaculture species, utilizing these trophic markers from fatty acid analysis of eggs has the potential to elucidate information regarding broodstock nutrition as one may develop a broodstock diet similar to the natural diet, particularly in lipid and EFA content, to enhance egg and larval quality. This could be particularly important for species with a relatively unknown ecology, as it can also be used to verify the exactness of the existing descriptions of well-known species feeding ecology; Figueiredo & Narciso (2008) reported that the egg fatty acid profile of the spider crab *Maja brachydactyla* revealed they are more herbivorous than previous studies suggested. However, determination of not only the optimal levels of inclusion, but also the appropriate proportion of dietary fatty acids of the n-3 and n-6 families would still be required. Also, trophic markers may be used to infer a species preferred habitat (e.g. bottom feeding detritivore versus open water carnivore), which could aid in determining broodstock tank size/shape and need for substrate.

The differential FA consumption within eggs through embryonic development may also be a useful determinant of the nutritional requirements of the broodstock diet, as constituents acquired from the diet are incorporated into the oocytes and egg yolk. However, despite being plausible, to our knowledge no available data exists regarding this hypothesis. The egg fatty acid consumption during embryogenesis should be studied in wild stocks (eggs spawned and matured in nature) and assessed with standardized and controlled experiments across a range of species to validate this hypothesis.

5. Conclusion

Despite progress in the aquaculture industry, there still remain several bottlenecks in the larviculture of marine species including: variable larval quality; vulnerability of larvae to fluctuating environmental conditions; larval characteristics such as reduced energy reserves at hatch and an underdeveloped digestive system; ability to provide prey items/diets of appropriate nutritional profile to broodstock and larvae. We acknowledge each variable and
their interactions represent significant problems to larviculture; however, we hypothesize utilizing information obtained through analysis of egg FA consumption through embryogenesis to improve and develop the nutritional profile of larval and broodstock diets. The FA profile of recently spawned eggs may provide information about the FA profile (quantity, quality and ratio of EFA such as EPA, DHA and ARA) of broodstock and larval diets, however, this may be insufficient to determine the optimal FA composition of the larval diet as certain FA are preferentially consumed during embryogenesis. We hypothesize larval diets could be developed utilizing the consumption of FA by comparing the recently spawned egg and the pre-hatching egg FA composition. The most utilized/consumed FA during embryonic development may be the FA that should constitute a greater percentage of the larval diet. Available data seems to support this hypothesis (DHA case-study): the higher the consumption of DHA (µg.egg⁻¹ or µg.mg dw⁻¹) during embryonic development of crustaceans and fish, the greater the requirement of a DHA rich larval diet. However, if no significant consumption of DHA occurs during embryogenesis, larval survival and growth should not be significantly affected when fed a DHA deficient prey. Additionally, we also hypothesize the differential FA consumption within eggs during embryonic development may be a useful determinant of the nutritional requirements of the broodstock diet, as constituents acquired from the diet are incorporated into the oocytes and egg yolk. However, further research is required to determine if information obtained from analysis of the egg FA consumption could be used as an indicator of the nutritional profile of the larval and broodstock diet. Standardized and controlled experiments across a significant range of species are still required to validate these hypotheses.

For potential aquaculture species with a relatively unknown ecology, trophic markers obtained via egg FA analysis can provide valuable information since the DHA: EPA ratio, and proportions of 18:1n-9 and odd-saturated FA reflect adult feeding habits (e.g. herbivore versus carnivore) and habitat (e.g. pelagic versus demersal) (Auél et al. 2002; Scott et al. 2002; Dalsgaard et al. 2003; Volkman et al. 1998; Rosa et al. 2007; Figueiredo & Narciso 2008; Figueiredo et al. 2008a, b). Utilizing FA trophic markers, one may develop a broodstock diet similar to the natural diet, particularly in lipid and EFA content, to enhance egg and larval quality.
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