

A Comprehensive Review of Live Feed(artemia) Enrichment for Fish Larvae

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Abstract

Live prey such as *Artemia* nauplii and rotifers are widely used as starter feeds in marine fish larviculture. However, their inherent nutritional deficiencies necessitate enrichment protocols to enhance their dietary value. Bioencapsulation with essential nutrients, including n-3 highly unsaturated fatty acids (HUFAs), amino acids (e.g., methionine), vitamins (A and C), selenium, and iodine, has been developed to improve larval growth, survival, and reduce malformations such as incomplete eye migration in flatfish and skeletal deformities. Enrichment with n-3 HUFAs, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), is critical for larval development, while methionine supplementation addresses amino acid deficiencies in *Artemia* compared to natural copepod prey. Vitamins A and C play vital roles in pigmentation, vision, collagen synthesis, and stress resistance, yet their incorporation into lipid-based enrichment emulsions remains challenging. Selenium, in both inorganic (sodium selenite) and organic (selenoyeast) forms, enhances oxidative stress resistance and immune response, while iodine is essential for thyroid hormone synthesis and successful flatfish metamorphosis. Advances in enrichment techniques, including liposome encapsulation, offer promising solutions for delivering both hydrophilic and lipophilic nutrients. Optimizing these protocols is crucial to meet species-specific larval requirements and improve hatchery production efficiency.

KEYWORDS: *Artemia* Enrichment, HUFA-Highly Unsaturated Fatty Acids, Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Methionine, selenium, vitamins and minerals.

INTRODUCTION:

Nutrients can be incorporated into live preys through “bioencapsulation” or “enrichment” protocols (Sorgeloos et al., 2001), *Artemia* nauplii and rotifers are commonly used as the start feed for fish larvae worldwide. Although feeding *Artemia* provides reasonable growth during the initial feeding stages in marine fish species in culture, diets based solely on *Artemia* throughout the entire live food period have resulted in a high degree of abnormalities such as malpigmentation and incomplete eye migration in cultured species of flatfish Seikai, 1985, Næss et al., 1995, Næss and Lie, 1998. The best feeds for *Artemia* are live microalgae such as *Nannochloropsis*, *Tetraselmis*, *Isochrysis*, and *Pavlova*. Moreover, a

combination of live phytoplankton fed to Artemia cultures have demonstrated superior enrichment characteristics (i.e., increased HUFAs) over feeding single phytoplankton species. In addition to live algae, Artemia cultures can be enriched by feeding a wide variety of processed foods, including yeasts, fish meal, soybean powder, egg yolk, and micronized rice bran. Like rotifers, the inherent nutritional value of Artemia is low, resulting in similar enrichment requirements. Artemia are enriched with commercially available enrichment medium such as DC DHA Selco, almagamag. Etc. Once enriched, Artemia are rinsed, concentrated, enumerated, and then fed to larvae.

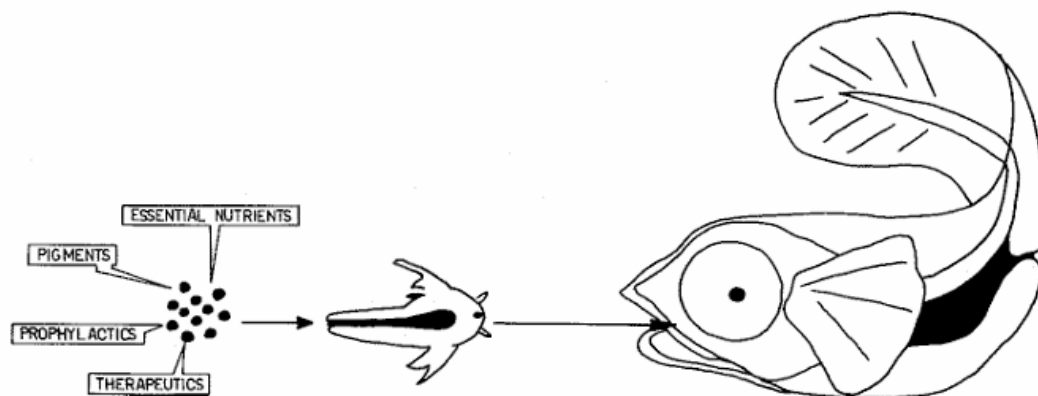
ARTEMIA: Artemia (Brine shrimp) is an important live feed, most commonly used at completion of the rotifer stage and prior to conversion of the larva to an inert feed. It represents the transitional feed for the larvae, after which artificial feeds can be used. Brine shrimp is typically a primitive crustacean belonging to the class Branchiopoda with a total length of about 7-12 mm. Artemia nauplii are the most and widely used food item mainly due to its convenience and availability. The



unique property of Artemia is the formation of dormant embryos, called cysts. Cysts are available year-round in large quantities along the shorelines of hypersaline lakes and coastal lagoons, which can be collected, processed and stored or commercially made available.

FEEDING ENRICHMENT OF ARTEMIA: Artemia are non-selective filter feeders and therefore will ingest a wide range of foods. The main criteria for food selection are particle size, digestibility, and nutrient levels. The best feeds for Artemia are live microalgae such as Nannochloropsis, Tetraselmis, Isochrysis, and Pavlova. Moreover, a combination of live phytoplankton fed to Artemia cultures have demonstrated superior enrichment characteristics (i.e., increased HUFAs) over feeding single phytoplankton species. But some unicellular algae are not appropriate for sustaining Artemia growth. For example, Chlorella, and Stichococcus have a thick cell wall that cannot be digested by Artemia. In addition to live algae, Artemia cultures can be enriched by feeding a wide variety of processed foods, including yeasts, fish meal, soybean powder, egg yolk, and micronized rice bran. Like rotifers, the inherent nutritional value of Artemia is low, resulting in similar enrichment requirements. Artemia are enriched with commercially available enrichment medium such as DC DHA Selco, almagamag

etc. Once enriched, Artemia are rinsed, concentrated, enumerated, and then fed to larvae.



ENRICHMENT WITH $n-3$ HUFA: The dietary requirements of $n-3$ poly-unsaturated fatty acids (PUFA), particularly docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have been documented for various species of marine fish (Izquierdo et al., 1992, Sargent et al., 1999, Watanabe, 1993). DHA, as well as its ratio to EPA, appears to be critical during the early larval stages as it affects growth and survival of marine fish (Furuita et al., 1996, Reitan et al., 1994, Sargent et al., 1997, Watanabe, 1993). Because of its role as a precursor of the eicosanoids, arachidonic acid was later added to the list of dietary essential fatty acids for marine fish (Castell et al., 1994a, Castell et al., 1994b, Estevez et al., 1999, Sargent et al., 1999). Various enrichment emulsions have been formulated differing in the fatty acid composition of their triglycerides. All these changes in the formulation of the enrichment diets offer more possibilities to satisfy the needs of different species and help to reduce problems related to diseases, stress resistance, malformation, and pigmentation in numerous fish species (Sorgeloos et al., 2001). Enrichment of fatty acids in *A. urmiana* cysts in order to improve its quality and fish food value by increasing significantly the levels of EPA, DHA, and EPA/DHA ratio using fish oil can be considered as a cheap and easily adoptable method for commercial hatchery operations.

ENRICHMENT WITH METHIONINE: *Artemia* nauplii can successfully be enriched with free methionine, the high retention of free methionine in the *Artemia* nauplii following transfer to fish tanks shows that it is possible to offer fish larvae a feed with a high level of FAA, based on enrichment of *Artemia* nauplii. The need for dietary FAA of marine fish larvae is also suggested by the large pool of FAA found in marine invertebrates such as copepods, which are the natural food for the larvae in the sea Yancey et al., 1982, Fyhn et al., 1993. In contrast, *Artemia* nauplii, and especially nauplii of *Artemia franciscana*, which is the common strain used in aquaculture, contain markedly lower levels of FAA compared to wild copepods

Fyhn et al., 1993, Helland, 1995. This is especially true for the essential amino acid methionine, which in *Artemia* nauplii exists at trace or low levels only Fyhn et al., 1993, Helland, 1995. This suggests that methionine is a limiting amino acid for fish larvae when fed *Artemia* nauplii (Conceição et al., 1997). Based on these findings, it would be pertinent to study the possibility of enriching *Artemia* with FAA in order to improve their nutritional value for marine fish larvae. Liposomes are spherical lipid vesicles (10 nm to 20 µm) containing an aqueous volume enclosed by a phospholipid membrane. These vesicles are potent delivery vectors for hydrophilic (dissolved in the aqueous volume) as well as hydrophobic (embedded in the lipid membrane) nutrients and are potential carriers for FAA. By encapsulating water-soluble substances into suitably sized liposomes, these vesicles may be readily ingested by *Artemia* nauplii similarly to the non-selective filter feeding of micelles in lipid emulsion enrichment.

ENRICHMENT WITH VITAMINS: Vitamins are a heterogeneous group of compounds with a wide range of biological activities. Vitamins can be classified according to their solubility, as either lipid or water-soluble compounds (Gouillou-Coustans and Guil laume, 2001). Among liposoluble vitamins, vitamin A represents an essential nutrient for fish since they cannot synthesize this compound de novo. Vitamin A has been widely studied in fish because it is involved in vision and most of the farmed species are visual feeders (Hunter, 1981). Consequently, dietary deficiencies in vitamin A can alter normal function of vision in larvae, thereby reducing their hunting ability. Moreover, dietary vitamin A deficiencies have been related to the occurrence of pseudoalbinism in flatfish such as halibut (*Hippoglossus hippoglossus*), turbot (*Psetta maxima*) and Japanese flounder (*Paralichthys olivaceus*). This syndrome is characterized by the lack of pigmentation in the body skin of the individual (Seikai et al., 1987; Estévez and Kanazawa, 1995). Furthermore, low vitamin A content in the live food such as *Artemia* can lead to an incomplete migration of the eye during flat fish metamorphosis (Saele et al., 2003). In contrast, hypervitaminosis of vitamin A can cause skeletal deformities in the jaw (Haga et al., 2003) and has been related to the backbone disease in Japanese flounder (Dedi et al., 1995, 1997). In regard to hydrosoluble vitamins, vitamin C or ascorbic acid is essential in the synthesis of collagen and cartilage in vertebrates (Halver, 2002). Fish lack the enzyme gulonolactone oxidase required in its synthesis (Dabrowski, 1990), and have to satisfy vitamin C requirements through the diet. Additionally, vitamin C also offers antioxidant (Hwang and Lin, 2002) and immunostimulant (Cuesta et al., 2002) properties, and protection against stress (Henrique et al., 2002). For these reasons, most diets used in aquaculture contain mega doses of vitamin C as a strategy to prevent diseases in cultured fish. Despite the fact that

Artemia nauplii contain vitamin A, vitamin C and free amino acids (Merchie et al., 1997; Takeuchi et al., 1998; Helland et al., 2000), enrichment products normally include these compounds to enhance the natural content of nauplii. However, inclusion of these compounds in the enrichment diet is somewhat difficult because of their different polarity. Fish oil emulsions are the basis of many of the commercial products used in *Artemia* enrichment worldwide. The lipid micelles formed when the product is dispersed in water possess a lipophilic core which impedes the incorporation of hydrosoluble molecules. In the case of vitamin C, this handicap has been overcome using lipophilic derivatives such as ascorbyl palmitate which is stable in the emulsion micelles (Merchie et al., 1995). On the other hand, amino acids are water-soluble compounds and consequently their inclusion in lipid emulsions is problematic. Liposomes represent a potential alternative for the enrichment of live preys in both hydrophilic and lipophilic nutrients (Hontoria et al., 1994).

ENRICHMENT WITH MINERAL: Iodine is an essential part of the (TH) thyroid hormones but the sources of iodine in larval flatfish nutrition has received little attention despite that Solbakken et al. (2002) reported a 700-fold higher level of iodine in copepods compared with *Artemia*. Furthermore, they found that the larvae-fed copepods contained significantly higher levels of iodine compared with larvae-fed *Artemia*. Iodine is found in the seawater as iodate and iodide, in organic components and in most organisms (Wong 1991). Flatfish metamorphosis is initiated by the actions of thyroid hormones (TH) and iodine is an essential part of these hormones. Hence, an iodine deficiency may lead to insufficient levels of TH and incomplete metamorphosis.

ENRICHMENT WITH SELENIUM: Despite progress made with live feed (i.e. rotifers, *artemia*), enrichments in essential fatty acids for marine fish larvae, little is known on the micronutrient requirements such as selenium (Se). Selenium is a critical component of several enzymes maintaining important biological functions such as cellular oxidation, and therefore plays a key role in oxidative and stress status of marine larvae. The levels of Se found in the larvae's natural diet (i.e. copepods) is generally higher than those of the enriched live preys used in hatcheries. *Artemia nauplii* can be enriched with Se using inorganic (sodium selenite) and organic (selenoyeast). Se has also been shown to play a protective role by reducing oxidative stress caused by heavy metals such as copper (Cu), resulting in enhanced immune response in fish. Bioavailability of Se, as per other minerals (NRC, 2011), is greatly dependent upon its form when accumulated in the diet (Pacitti et al., 2015). One of the most common forms of dietary Se is sodium selenite (Na_2SeO_3 , hereafter referred to as Na-Se), a highly water-soluble inorganic compound. An alternative Se source is selenoyeast (Se-yeast), an

organic source of Se produced by exposing yeast (*Saccharomyces cerevisiae*) to Na–Se (Suhajda et al., 2000), which results in an accumulation of selenomethionine (Se-Met). The latter (organic) form of Se is regarded to be more bioavailable to organisms, thus explaining why it is broadly used as a livestock feed additive (Wang and Lovell, 1997; Rayman, 2004; Thiry et al., 2012). Nevertheless, inappropriate dietary Se levels can induce toxicity effects.

Likewise, supplementation with minerals such as **zinc** and **manganese** contributes to better skeletal development and reduces deformities in fish larvae. These interventions not only correct deficiencies but also strengthen the animals' ability to withstand the challenging conditions of intensive production.

CONCLUSION

The enrichment of live prey such as *Artemia* nauplii and rotifers with essential nutrients including n-3 HUFAs (DHA and EPA), methionine, vitamins (A and C), selenium, and iodine play a crucial role in enhancing larval fish growth, survival, and overall health in aquaculture. These enrichment protocols help bridge the nutritional gap between cultured live feeds and natural prey like copepods, mitigating common issues such as malpigmentation, skeletal deformities, and incomplete metamorphosis in marine fish larvae. Advances in bioencapsulation techniques, particularly the use of liposomes for delivering both hydrophilic and hydrophobic nutrients, offer promising solutions for optimizing nutrient delivery. However, challenges remain in balancing enrichment formulations to avoid toxicity while ensuring bioavailability. Future research should focus on species-specific enrichment strategies and the development of cost-effective, stable enrichment products to further improve hatchery production efficiency and larval quality. By refining these protocols, aquaculture can better replicate the nutritional benefits of natural prey, ensuring healthier and more robust fish larvae for sustainable production.

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