



# Bio Enriched Feeds: A Promising Feed for Hatchery

Siddhnath<sup>1</sup>, Shiv Mohan Singh<sup>1\*</sup>, Ravikant Bharti<sup>1</sup>, Abdul Aziz<sup>2</sup>, Subir Pradhan<sup>2</sup>, Bhagchand Chhaba<sup>3</sup>

<sup>1</sup>Dept. of Fish Processing Technology, Faculty of Fishery Sciences, WBUAFS Kolkata

<sup>2</sup>Dept. of Fisheries Economics and Statistics, Faculty of Fishery Sciences, WBUAFS Kolkata

<sup>3</sup>Dept. of Aquatic Environment Management, Faculty of Fishery Sciences, WBUAFS Kolkata

\*Email: [shivmohan.singh98@gmail.com](mailto:shivmohan.singh98@gmail.com)

**Abstract:** *The culture of shellfish larvae under controlled hatchery conditions requires not only the development of specific culture techniques, but in most cases also the production and use of Bio enriched feeds as feed for the developing larvae in hatchery. The present study describes the preparation techniques currently employed for the culture of the Bio enriched feeds commonly used in larvae culture, as well as their application potential in terms of their nutritional properties and feeding methods. The article is divided into different sections according to the Bio enriched feeds used in hatchery, namely technique, preparations of bio enriched feed and nutritional value.*

**Keywords –** Hatchery, Bio enriched feed, Shrimp larvae.

## Introduction

Information on nutrition of aquatic animals is scanty. The traditional feed of prawn larvae consists of zooplankton, chopped fish and mussel meat. Most preferred larval food is *Artemia* nauplii. Steamed chicken egg custard is used for indoor larval rearing. Rotifers, Cyclops, copepods and insect larvae can also be fed to prawn larvae along with *Artemia* in prawn hatchery. *Acetes* spp. (fresh/frozen) are used for feeding shrimp larvae. Many hatcheries continue to prepare their own larval feed because of high cost of commercial feeds, and live foods remain an essential feeding component of hatchery operation. Bio enrichment is the process involved in improving the nutritional status of live feed organisms either by feeding or incorporating within them various kinds of materials such as micro diets, microencapsulated diets, genetically engineered baker's yeast and emulsified lipids rich in w3HUFA (Highly Unsaturated Fatty Acid) together with fat soluble vitamins. (Lavens *et al.*, 2000)



### Factors to be considered prior to bio enrichment

- **Selection of the carrier or biofeed:** This is a very important aspect taking into account the acceptability of the organism and its size. Commonly used carriers (Imelda 2003) and their size ranges are listed as under :
  - a. Microalgae: 2 - 20  $\mu$
  - b. Moina: 400 - 1000  $\mu$
  - c. Rotifers: 50 - 200  $\mu$
  - d. Daphnia: 200- 400  $\mu$
  - e. Artemia: 200 - 400  $\mu$
- **Nutritional quality, digestibility and acceptability before and after Enrichment:** This requires extensive studies on all commercial species. This study will form a baseline to conclude upon whether to go in for bio enrichment or not.
- **Fixing up the level of the enriching media to be incorporated into the carrier organism:** This depends on the nutritional quality of the carrier before incorporation and is also based on the feeding trials conducted in the laboratory.
- **Economic feasibility of enrichment.**
- **Purity of the culture of the carrier organism.**
- **other criteria**
  - I. It should be easily procurable.
  - II. Culture should be economically viable.
  - III. Catchability of the carrier by the target species.
  - IV. It should be easily reproducible.

### Techniques of bio enrichment:

There are essentially two methods which are widely used for bio enrichment, - the direct method, and the indirect method.

**1. The indirect method:** It is based on the fact that baker's Yeast contains a fairly high amount of monoethylenic fatty acids and no w3HUFA, and that the fatty acid composition of rotifers is readily affected by the fatty acids of the culture organisms. A new type of yeast has been developed as a



culture organism for rotifers in order to improve upon the nutritional value for fish larvae of rotifers cultured on baker's yeast (Imada *et al.*, 1979). This new type of yeast designed as co-yeast, was produced by adding fish oil or cuttle fish liver oil as a supplement to the culture medium of baker's yeast, resulting in higher levels of lipids and w-3 HUFA, the essential fatty acid (EFA) for both marine and freshwater finfish and shellfish larvae. In a similar manner *Artemia* nauplii and *Moina* are also enriched with w-3HUFA. This method is so called because live feeds are enriched with w-3 HUFA together with the lipid.

**2. The direct method:** This method was first developed by Watanabe *et al.*, (1983). Where is in a homogenate prepared by an emulsion of lipids containing w-3HUFA. Raw egg yolk and water is directly fed to the carrier organisms to enrich them directly. The use of both the methods, direct and indirect will significantly improve the dietary value of live feeds by allowing them to take up from the culture medium not only w-3 HUFA, but also fat soluble vitamins together with lipids (Watanabe *et al.*, 1983). Temperature and density of the carriers more dictate the incorporation.

### **Preparation of enrichment media:**

For the preparation of emulsified lipids the w-3 HUFA concentration in the lipid source should be very high. In an ordinary preparation about 5 gm. of the fish oil is homogenized for 2-3 minutes in a homogenizer or mixer or by vigorous shaking. Proper emulsification is ensured by observing the emulsion under a microscope. The preparation may be stored under refrigeration until use. Emulsifiers may be added to maintain the emulsion. If not, a violent shaking prior to use reforms the emulsion. The enrichment media may be supplemented with water and fat soluble vitamins like A, D, E and K prior to homogenization. Enrichment of *Artemia* nauplii and rotifers with w-3 HUFA is dictated by two factors - lipid content in the emulsion, and type of lipid source. The amount of lipid source depends on the population density of the carriers, their feeding activity and the water temperature. The nauplii or rotifers are harvested using plankton net of 60 u mesh size washed with clean sea water or freshwater and fed to the larvae of finfish or shell fish in adequate numbers.



## Live food enrichment

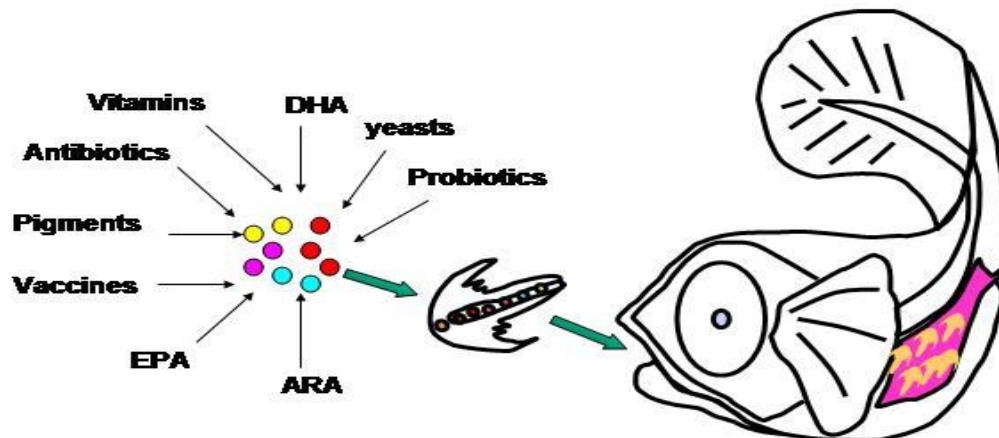


Figure 1- Source: Merchie, G. (1996). Use of nauplii and meta-nauplii. *Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper.*

In many cases, determination of nutritional requirements of fatty acids and other nutritional components is done by comparing commercially available enrichments differing in their EFA or other nutrients (Cutts *et al.*, 2006). The disadvantage of this approach is that these commercial products usually differ from each other in more than one component. Today, there is large variety of commercially available enrichments with different levels and ratios of EFA, anti-bacterial agents (Eichelburg 1978), vitamins (Merchie *et al.*, 1997) etc. However, in some cases, especially with new species or susceptible larvae, different levels and/or other ingredients are needed in the enrichments. For example, immune-stimulants and ‘mega’ doses of vitamins E and C are used to increase stress resistance and reduce deformities. In public aquaria and ornamental fish hatcheries, stress resistance and coloration enhancement is needed. These can be achieved with high levels of carotenoids. Currently, there are no commercially - available ‘tailor-made’ enrichments aimed at research institutes as well as commercial hatcheries and public aquaria.

Supplementing nutritional components, such as vitamins or calcium, into live brine shrimp has been practiced by aquaculture hatcheries for around 10 years. This bio-enrichment or bioencapsulation of brine shrimp nauplii (instar 2 or adults) began using emulsified fish oils containing high HUFA's or highly unsaturated fatty acids for marine finfish and crustacean larvae (Anantharaja 2007). This 'breakthrough' enabled the culture of many other new marine species to be developed (flounder, sea



bass, tuna, ornamental marine sp.). Today, there are at least a dozen different Artemia enrichment formulas on the market.

Although we do not market any enrichment formula, we do enrich and freeze live adult Artemia with a HUFA formula and Spirulina algae for the aquaculture and aquarium markets (Vigani *et al.*, 2015). However, almost all of the sales for these two enriched products go to the aquaculture market due to the "unawareness" of the benefits of bio enrichment in the aquarium trade.

Live food enrichments play an important role in delivering nutrients to marine fish larvae. Today, there are many enrichment products with different levels and ratios of EFA, anti-bacterial agents and vitamins. Species-specific enrichments are, however, lacking. Sometimes, especially with new species, it is desirable to have a product with a targeted nutritional profile.

### **Enrichment with nutrients**

As mentioned previously, an important factor affecting the nutritional value of Artemia as a food source for marine larval organisms is the content of essential fatty acids, eicosapentaenoic acid (EPA: 20:5n-3) and even more importantly docosahexaenoic acid (DHA: 22:6n-3). In contrast to freshwater species, most marine organisms do not have the capacity to biosynthesize these EFA from lower chain unsaturated fatty acids, such as linolenic acid (18:3n-3). In view of the fatty acid deficiency of Artemia, research has been conducted to improve its lipid composition by prefeeding with (n-3) highly unsaturated fatty acid (HUFA)-rich diets. It is fortunate in this respect that Artemia, because of its primitive feeding characteristics, allows a very convenient way to manipulate its biochemical composition. Thus, since Artemia on molting to the second larval stage (i.e. about 8 h following hatching), is non-selective in taking up particulate matter, simple methods have been developed to incorporate lipid products into the brine shrimp nauplii prior to offering them as a prey to the predator larvae. This method of bioencapsulation, also called Artemia enrichment or boosting, is widely applied at marine fish and crustacean hatcheries all over the world for enhancing the nutritional value of Artemia with essential fatty acids (Van Stappen 1996).



For enrichment the freshly-hatched nauplii are transferred to an enrichment tank at a density of 100 (for enrichment periods that may exceed 24 h) to 300 nauplii.ml<sup>-1</sup> (maximum 24-h enrichment period); the enrichment medium consisting of disinfected seawater maintained at 25°C. The enrichment emulsion is usually added in consecutive doses of 300 mg.l<sup>-1</sup> every 12 h with a strong aeration (using air stones) being required so as to maintain dissolved oxygen levels above 4 mg.l<sup>-1</sup> (the latter being necessary to avoid mortalities). The enriched nauplii are harvested after 24 h (sometimes even after 48 h), thoroughly rinsed and then fed directly or stored at below 10°C so as to minimize the metabolism of HUFA prior to administration, i.e. HUFA levels being reduced by 0-30% after 24 h at 10°C. By using these enrichment techniques very high incorporation levels of EFA can be attained that are well above the maximal concentrations found in natural strains. These very high enrichment levels are the result not only of an optimal product composition and presentation, but also of proper enrichment procedures.

Apart from EFA, other nutrients such as vitamins and pigments can be incorporated in *Artemia*. Fat soluble vitamins (especially vitamin A and vitamin E) were reported to accumulate in *Artemia* over a short-term (9 h) enrichment period with vitamin A levels increasing from below 1 IU.g<sup>-1</sup> (Wet weight basis) to over 16 IU.g<sup>-1</sup> and vitamin E levels increasing from below 20 Ig.g<sup>-1</sup> to about 250 Ig.g<sup>-1</sup>. Recently tests have also been conducted to incorporate ascorbic acid into live food. Using the standard enrichment procedure and experimental self emulsifying concentrates containing 10, 20 and 30% (on a Dry weight basis) of ascorbyl palmitate (AP) in addition to the triglycerides, high levels of free ascorbic acid (AA) can be incorporated into brine shrimp nauplii. For example, a 10%-AP inclusion in the emulsion enhances AA levels within freshly-hatched nauplii by 50% from natural levels (500 Ig g<sup>-1</sup> Dry weight). By contrast, however, a 20 or 30% addition increases AA levels in *Artemia* 3-fold and 6-fold respectively after 24 h enrichment at 27°C; with (n-3) HUFA levels remaining equal compared to normal enrichment procedures. Moreover, these AA concentrations do not decrease when the enriched nauplii are stored for 24 h in seawater (Van Stappen, 1996).



## Conclusion

The Bio enriched applied as feed for shrimp larvae in hatchery contain nutrients that enhanced survival, growth promotion, the appetizer to increase consumption and anti stress characteristics and, will therefore be of immense use in the culture of shrimps. Such techniques would have important application in the shrimp hatchery.

## References

1. Lavens, P., Thongrod, S., & Sorgeloos, P. (2000). Larval prawn feeds and the dietary importance of Artemia. *Freshwater prawn culture: the farming of Macrobrachium rosenbergii*, 91-111.
2. Imada, O., Kageyama, Y., Watanabe, T., Kitajima, C., Fujita, S., & Yone, Y. (1979). Development of a new yeast as a culture medium for living feeds used in the production of fish seed. *Bulletin of the Japanese Society of Scientific Fisheries*.
3. Watanabe, T., Kitajima, C., & Fujita, S. (1983). Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture*, 34(1-2), 115-143.
4. Merchie, G. (1996). Use of nauplii and meta-nauplii. *Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper*, 361, 137-163.
5. Cutts, C. J., Sawanboonchun, J., Mazorra de Quero, C., & Bell, J. G. (2006). Diet-induced differences in the essential fatty acid (EFA) compositions of larval Atlantic cod (*Gadus morhua* L.) with reference to possible effects of dietary EFAs on larval performance. *ICES Journal of Marine Science*, 63(2), 302-310.
6. Anantharaja, K. (2007). *Effect of bio-enriched live feeds on the growth, survival and maturity of Macrobrachium rosenbergii (De Man)* (Doctoral dissertation, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu Fisheries University).
7. Van Stappen, G. (1996). Use of cysts. In *Manual on the production and use of live food for aquaculture* (Vol. 361, pp. 107-136). FAO.
8. Vigani, M., Parisi, C., Rodríguez-Cerezo, E., Barbosa, M. J., Sijtsma, L., Ploeg, M., & Enzing, C. (2015). Food and feed products from micro-algae: market opportunities and challenges for the EU. *Trends in Food Science & Technology*, 42(1), 81-92.
9. Eichelburg, R. J. (1978). *U.S. Patent No. 4,118,512*. Washington, DC: U.S. Patent and Trademark Office.
10. Merchie, G., Lavens, P., & Sorgeloos, P. (1997). Optimization of dietary vitamin C in fish and crustacean larvae: a review. *Aquaculture*, 155(1-4), 165-181.
11. Imelda, J. (2003). Bioencapsulation of live feeds 1-6.