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# IMPORTANT LIVE FOOD ORGANISMS AND THEIR ROLE IN AQUACULTURE

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## INTRODUCTION

Live food organisms include all plants (phytoplankton) and animal (zooplankton) lives grazed upon by economically important fishes. Phytoplanktons are generally eaten by zooplankton. Thus, phytoplankton forms the basis of the food chain. Live foods are able to swim in water column and are constantly available to fish and shellfish larvae are likely to stimulate larval feeding response (David, 2003). In an aquatic ecosystem, these live food organisms constitute the most valuable resource for aquaculture. Most of the fish and shellfish larvae in nature feed on small phytoplanktonic and zooplanktonic organisms. However, natural fish food organisms are usually not abundant in clear pond water, but are abundant in ponds having greenish water. The green colour indicates the presence of phytoplankton and other natural food organisms. In the natural food web, zooplankton constitutes a major part of the diet for marine fish larvae and it is generally believed that copepods can meet the nutritional requirements of fish larvae (Evjemo *et al.*, 2003).

Artificial larval feeds are no match to live food organisms in terms of acceptance, nutritional and other factors. Feeding habit of fishes in natural water bodies is different among the species but all the fishes require protein rich live food for their better growth, efficient breeding and survival (Mandal *et al.*, 2009). Advances in live food enrichment technique have helped to boost the importance and potential of live food organisms in the raising of larval aquatic species. The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food for feeding fish larvae, fry and fingerlings (Lim *et al.*, 2003). The availability of large quantities of live foods organisms such as marine rotifer (*Brachionus plicatilis* and *Brachionus rotundiformis*) and *Artemia* nauplii to meet the different

stages of fry production has contributed to the successful fry production of at least 60 marine finfish species and 18 species of crustaceans (Dhert, 1996). A common procedure during the culture of both larvae of fish and prawns is to add microalgae (i.e. “green water”) to intensive culture systems together with the zooplankton prey (Tamaru *et al.*, 1994), has become popular practice these days.

Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1998) and hence are commonly known as “living capsules of nutrition”. Providing appropriate live food at proper time play a major role in achieving maximum growth and survival of the young ones of finfish and shellfish. To achieve maximum production and profitability, the nutritional components of natural foods must be identified and quantified. Nutritional status of live food organisms can improve through various techniques of enrichment and bioencapsulation. It is obviously agreed that the production of live food organisms continues to be a very important first step in intensification of aquaculture, both horizontally as well as vertically.

## Importance of live food organisms in aquaculture

Success of aquaculture depends on healthy cultured stock. A disease free healthy stock can be maintained by feeding live food to the cultured stock along with supplemented artificial feed. Supplemented artificial feed can not meet all the elements required for the growth of fish. So, fish and shellfish must be fed with live food. For getting good return from rearing of larvae of fish and shellfish they should be fed with nutrient rich diet. Larval rearing is one of the riskiest phases of aquaculture, but it could be one of the most profitable venture. Special planning and strategies are required to overcome the risk of high mortality during this phase of culture. Zooplankton is required as a first food for many cultured fish; for others it contributes to faster growth and higher survival. Larvae of fish and shellfish cannot feed artificial supplemented feed. They require small size live foods for their nutrition. Live foods are easily digestible protein rich diet for fish and shellfish. These live foods can be purchased from the market, which is costly and may not be available as and when required. It will also increase the production cost. But these live foods can be cultured easily and economically. Live foods include both phytoplanktonic as well as zooplanktonic organisms. Phytoplankton consist of chlorophyll bearing organisms e.g. *Microcystis*, *Volvox*, *Eudorina*, *Oscillatoria*, etc. and non photosynthetic plants or saproplankton e.g. bacteria and fungi, where as zooplankton comprise plankters of animal origin. In the tropical areas it mainly comprises protozoans (e.g. *Arcella* sp., *Diffflugia* sp., *Actinophrys* sp., *Vorticella* sp. etc.), rotifers (e.g. *Brachionus* spp., *Keratella* sp., *Asplanchna brightwelli*, *Polyarthra vulgaris*, *Filinia opoliensis* etc.) and the planktonic forms of crustaceans (*Artemia* spp.), cladoceran (*Moina* spp., *Daphnia* spp., *Ceriodaphnia* sp. etc.), ostracoda (*Cypris*, *Stenocypris*, *Eucypris* etc.) and copepods (*Mesocyclops leuckarti*, *M. hyalinus*, *Microcyclops varicans*, *Heliodiaptomus viduus*, etc.) and their larvae.

Microalgae are utilized as live feed for all growth stages of bivalve molluscs, for the larval/early juvenile stages of abalone, crustacean, some fish species and zooplankton in aquaculture food chains. Over the last four decades, several hundred microalgae species have been tested as food, but probably less than twenty have gained widespread use in aquaculture. Microalgae must possess a number of key attributes to be useful as aquaculture species (Kawamura *et al.*, 1998). Microalgae have an important role in aquaculture as a means of enriching zooplankton for feeding fish and other larvae. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments and sterols, which are transferred through the food chain. In nature, zooplankton is one of the primary foods of fish larvae. Two of the dominant zooplankton groups are Rotifera (rotifers) and Copepoda (copepods). These two groups are the preferred prey for shrimp and fish and are they are the most widely used live feeds by aquaculturists. The intensive larval culture of most marine fish depends on a large supply of zooplankton.

## Important live feeds

Among the different types of live feed available for use in aquaculture, some important ones are discussed below:

### Microbes

Yeast can be directly used as a primary food source for many larvae but it is mainly used as a feed for zooplankton which is grown for use in larviculture. It is an important ingredient in artificial larval diets. Yeast has also been evaluated as supplement or replacement for algae in the feeding of post larval penaeid shrimps.

In contrast to the use in terrestrial animals, the use of bacteria as probiotics in case of fishes is no longer restricted to feeds only. Their use as a prophylactic measure is gaining momentum day by day in live food production to stabilize and promote larval rearing through a microbiologically balanced system. Probiotic materials have been used as food supplement in fish culture. The fish fed with such probiotics grew well without requiring drugs added (Hirata *et al.*, 1998). Today commercial preparations of useful bacteria like *Bacillus subtilis*, *B. polyrixa*, *B. negaterium* etc. are available in ready to use packs. Most probiotics used in aquaculture belong to the LCB, of the genus *Bacillus*, to the photosynthetic bacteria or to the yeast, although other genera or species have also been mentioned (Vazquez *et al.*, 2005; Wang, 2007). These bacteria are consumed by several groups of zooplankton like protozoans, rotifers and copepods. *B. plicatilis* can grow by feeding on microorganisms such as yeast and bacteria (Yamasaki and Hirata, 1990).

Generally, bacterial cells have good nutritional value as they contain essential amino acids, protein and polysaccharides. Being a rich source of exogenous enzymes, bacteria

also helps in digestion and absorption process in the gut of larvae or food organisms by breaking down the larger particles into smaller ones. After proper selection, the bacteria can be established in the gut of larvae through food or through live food enrichment. In larviculture, probiotic treatment results in better growth rate, survival and disease resistance in larvae.

## Micro algae

Algae are chlorophyll bearing unicellular or multi-cellular plants. When multi-cellular, they may be colonial or filamentous. Most of them are aquatic. Besides chlorophyll, they also show various carotenoid pigments which impart different colours to them. According to the nature of photosynthetic pigments, algae are further classified into three divisions such as Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). Brown and red algae are mostly marine forms while green algae i.e. Chlorophyta is mostly freshwater and free floating type. Brown algae contain iodine and algin. Some red algae are the source of agar jelly, used in the preparation of ice creams and culture media. Chlorophyta (green algae) serve as initial food producers and the first link in the aquatic food chain, both in freshwater and marine ecosystems.

The use of micro algae as a possible source of protein food was recognized by the researchers in mid 20<sup>th</sup> century. In the beginning, the main attention had been given on the production of single cell protein for human consumption. Later on, however, many new applications came to be recognized viz. wastewater treatment, nutrient recycling, bio-conservation of solar energy, etc. In recent years, mass culture of unicellular algae such as diatoms (viz. *Chaetoceros* and *Skeletonema*) and small phytoplankters (viz. *Isochrysis*, *Tetraselmis* and *Chlorella*) is becoming quite popular for feeding larvae of fishes, prawns, shrimps and molluscs in aqua hatcheries.

Importance of micro algae in aqua hatcheries not only owes its nutritional attributes but more so for its small size ranging from 5 to 25 microns meeting the feed size requirements ideally well for early stages of various aquatic animals. Today, micro algae is used as an essential food source for rearing all stages of marine bivalve molluscs (clams, oysters, scallops), gastropods (abalone, conch), larvae of fishes (cod, halibut, tilapia) and shrimps (*Penaeus* sp). Micro algae also constitute an important source of food for live food organisms (rotifers, copepods, cladocerans, brine shrimp etc.) used in aqua hatcheries. Micro algae are frequently supplied together with rotifers during first feeding of marine larvae. This technique has normally enhanced survival as well as growth. Two types of live feeds have been used for rotifer production; live algal cells such as *Nannochloropsis oculata*, *Tetraselmis tetrathele* and *Chlorella vulgaris*, and a supplementary food such as baker's yeast (Maruyama *et al.*, 1997). Importance of micro-algae as larval food is also because it stimulates enzymatic synthesis and on-set of feeding in young larvae. Besides, it also acts as water conditioner by stripping off the nitrogenous substances.

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Microalgae grown to late-logarithmic growth phase typically contain 30 to 40% protein, 10 to 20% lipid and 5 to 15% carbohydrate (Brown *et al.*, 1997). When cultured through to stationary phase, the proximate composition of microalgae can change significantly (Harrison, 1990). PUFAs derived from microalgae, i.e. docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) are known to be essential for various larvae (Sargeant *et al.*, 1997). The content of vitamins can vary between microalgae. Ascorbic acid shows the greatest variation, i.e. 1 to 16 mg g<sup>-1</sup> dry weight (Brown and Miller, 1992).

## Infusoria

Infusoria refers to microscopic single celled animalcules belonging to the class - Ciliata of phylum - Protozoa. Besides being small in size, they are soft bodied and nutritionally very rich and therefore, serve ideally as starter diet for early stages of fish larvae. In the early development stages of fish larvae, *infusoria* or small live organisms are indispensable (Zableckis, 1998). *Paramoecium* and *Stylonychia* are the most common forms of freshwater infusoria while *Fabrea* and *Euplotes* are of marine ones.

## Culture of freshwater Infusoria

Generally, for *infusoria* culture, lettuce, cabbage and banana peels are commonly used as media as they stimulated growth of organisms. Take a glass jar or aquarium, filled with de-chlorinated filtered water and add 2 to 3 banana peelings. Cover the container with a cloth to prevent the entry of mosquitoes and flies. Keep the container in a cool place where natural diffused light is available. After 2 to 3 days the water will turn milky and also emit foul smell. This is due to the multiplication of a large number of bacteria causing decay of banana peelings. A film of slime will be formed on the water surface. In about 4 to 5 days, the water will turn clear, becoming transparent with light yellowish colour. This is because of the floating spores of *infusoria* in the air which have settled on the water surface break up and disintegrate. The culture is now ready for feeding the early stages of fish larvae. Once the culture reaches the peak density, it must be harvested, if not, the density will suddenly fall due to lack of space and oxygen depletion. After 50 % harvesting, add 2 to 3 drops of milk and again fill the glass jar with fresh water. The culture will sustain for a week. *Infusoria* can also be cultured by above method by putting bruised lettuce leaves or take some dry paddy husk and boiled it in water and take the solution for culture. *Infusoria* can be collected using 0.12 mm fine mesh cloth from foul smelling water bodies.

## Rotifers

Rotifers are popularly called as wheel animalcules. They are an important group of live food organisms for use in aqua hatcheries. *Brachionus*, which is the most known form

of all rotifers, serve as an ideal starter diet for early larval stages of many fish and prawn species in marine as well as freshwater. Species of the genus *Brachionus* (Brachionidae: Rotifera) are well represented in different water bodies worldwide (Pejler, 1977). Depending on the mouth size of the cultured organisms, small (50 to 110 micron length) or large (100 to 200 micron length) rotifers are used. There are about 2,500 species of rotifers have been known from global freshwater, brackish water, and seawater. *B. plicatilis* is the species used most commonly to feed fish larvae in hatcheries around the world. It is a euryhaline species, small and slow swimming, with good nutritional value. It is well suited to mass culture because it is prolific and tolerates a wide variety of environmental conditions. The rotifer, *B. plicatilis* and *B. rotundiformis*, have been indispensable as a live food for mass larval rearing of many aquatic organisms (Maruyama *et al.*, 1997). By way of significant developments in larval rearing technology of fishes, demand for the rotifer is further increasing.

The nutritional value of rotifers for larval fish depends on the rotifers' food source. Highly unsaturated fatty acids (HUFA) are essential for the survival and growth of fish larvae (Whyte and Nagata, 1990). Rotifer feeds containing DHA (docosahexaenoic acid, 22:6n-3), and EPA (eicosapentaenoic acid, 20:5n-3) can be valuable for marine fish larvae. Depending upon their food source, rotifers are composed of about 52 to 59% protein, up to 13% fat and 3.1% n-3 HUFA (Awais, 1992; Oie and Olsen, 1997). High nutritional value of rotifers is of major importance for survival and growth of the fish larvae, and several cultivation techniques, including feeding with different algae, baker's yeast and artificial diets, are used to improve their nutritional quality.

Maintaining large cultures of rotifers and their production on a predictable basis is a major problem. The food of rotifers appears to be the key element in their mass production. Presently, fresh baker's yeast is mostly used as the main diet ingredient for rotifers. However, its freshness, a criterion that is difficult to evaluate, can greatly influence the dietary value of the yeast for the rotifers and as a consequence, determine success of rotifer culture. Several measures are taken to deal with the problem such as supplementation of baker's yeast with micro algae, improving the nutritional quality of rotifers through vitamin C supplementation, treatment with antibiotics to prevent bacterial contamination and use of probiotics, i.e. the addition of beneficial bacteria in rotifer culture.

## Culture of Rotifers

To get pure culture of *B. plicatilis* stock culture is need to be developed. In order to start stock culture collect *B. plicatilis* from the stagnant salt water/brackish water bodies with the help of a scoop net having 50 to 100 micron mesh. After collection, place the content in a plastic bucket and bring to the laboratory. Dilute the sample by adding fresh clear water having similar salinity as that of field sample water containing *B. plicatilis*. Examine the sample under microscope and pick up *B. plicatilis* with the help of fine dropper whenever observed and inoculate in a 10 ml glass tube containing 5 ml of water. Feed

the *B. plicatilis* with yeast @ 200 ppm or *Chlorella* at a cell density of  $10 \times 10^6$  cells per ml. Serially dilute the test tube cultures daily through several large test tubes of 20 ml containing 10 ml of water. Gradually increase the volume to 50 to 10 ml capacity beakers and then to 1 to 2 litre jar or beakers. This way obtained stock cultures containing 100 to 150 individuals per ml. These cultures are used as inoculum for mass culture.

Normally, *B. plicatilis* is mass cultured in 10 to 15 ppt saline water, where maximum reproduction occurs. Among the several methods, continuous culture method is extensively used. In this method, after fertilization of the culture tank with ammonium sulphate (100 gm/1000l), single super phosphate (10g/1000l) and urea (10g/1000l), the tank is inoculated with *Chlorella*. As *Chlorella* reaches its peak density ( $10 \times 10^6$  to  $20 \times 10^6$  cells/ml), tank is re-fertilized with the same medium as was initially used. When *Chlorella* cells are consumed, Baker's yeast is introduced at the rate of 1.0 gm/ million *B. plicatilis*/day. When *B. plicatilis* population reaches 100 to 150 individuals per 1 ml, about 25 % of culture is harvested and transferred to the another tank. This procedure helps in uninterrupted supply of *B. plicatilis* for aqua hatcheries. In order to maintain long term culture in the same tank, about 25 % water is replaced from tank bottom with fresh filled water at an interval of 5 days.

## Artemia

*Artemia* commonly known as brine shrimp are zooplankton, like copepods and *Daphnia*, which are used as live food in the aquarium trade and for marine finfish and crustacean larval culture. There are more than 50 geographical strains of *Artemia* has been identified. Many commercial harvesters and distributors sell brands of various qualities. Approximately 90 % of the world's commercial harvest of brine shrimp cysts (the dormant stage) comes from the Great Salt Lake in Utah. Normally 2,00,000 to 3,00,000 nauplii are hatched from each gram of high quality cysts (Treece, 2000).

Of the live food used in aqua hatcheries, *Artemia*, constitute the most widely used organism. It is an organism closely related to shrimp belonging to the order - Anostraca of the class - Crustacea and phylum - Arthropoda. The biggest advantage of using *Artemia* is that one can produce live food on demand from dry and storable powder i.e. dormant *Artemia* cysts which upon immersion in seawater regain their metabolic activity and within 24 hours, release free swimming larvae (nauplii) of about 0.4 mm length.

*Artemia* has high nutritive value and high conversion efficiency. All the life stages of *Artemia*, i.e. cysts (after decapsulation), nauplii, juveniles, sub-adults are used as feed. Today, in majority of the commercial aqua hatcheries, *Artemia* nauplii is virtually used as a sole diet. Frozen adult *Artemia*, are widely used by aquarists, fish breeders and aquaculturists. *Artemia* biomass is also used as food additive for domestic livestock or extraction of pharmaceutical products as also in making protein rich food products. It is even used for human consumption in some countries. Owing to its great utility, *Artemia* trading is a growing business in several parts of the world.

An important characteristic that influences the suitability of *Artemia* in aqua hatcheries is the size of nauplii, which can vary greatly from one geographical source to another. This is one of the reasons why the local strains of *Artemia* in India are not performing so well in aqua hatcheries and, therefore, hatchery operators have to depend on imported *Artemia* cysts.

In comparison to *Moina*, the *Artemia* nauplii provides better growth and survival performance to fry and adults of guppy. With combined feeding using *B. calyciflorus* and *Artemia* nauplii, feeding of discus larvae becomes less tedious and more practical for use in commercial breeding of discus, and this would also eliminate the risk of larvae being eaten up and shorten the breeding interval, thereby leading to higher yield of fry (Lim *et al.*, 2003).

## Production of artemia nauplii

*Artemia* cysts are hatched into nauplii following the standard technique (Van Stappen, 1996) involving the different steps i.e. hydration of cysts, decapsulation of cysts and hatching of decapsulated cysts.

Decapsulation of hydrated cysts of *Artemia* is accompanied by treating them with chemicals which dissolve the shells. The hydrated cysts are kept in 5% sodium hypochlorite solution @15ml for every one gram cyst. The oxidation process starts immediately as a result of which heat is produced and temperature rises beyond 40°C. In order to prevent damage of embryo from the rising temperature, the containers containing the hydrated cyst are placed inside a trough containing cool water. During this period, the cysts are constantly stirred using a glass rod to facilitate uniform cooling. As the chorion dissolves, a gradual change in the colour of cysts can be noticed from dark brown to white. In about 5 to 10 minutes, the chorion gets dissolved and the decapsulated cysts are filtered on a 100 micron mesh. The decapsulated are then thoroughly washed in freshwater to remove the traces of toxic chlorine. In order to ensure complete removal of chlorine, the decapsulated cysts are given a dip in 0.1 % sodium thiosulphate solution.

Though *Artemia* cysts can be hatched directly into nauplii, but the decapsulation process is considered desirable because it helps in improving the hatching percentage and also in eliminating the chance of disease contamination in hatcheries. The decapsulated cysts are stocked @ 0.5 to 1.0 gm/litre of sea water. The cysts are kept in suspension with vigorous aeration. The cysts hatch into nauplii in about 12 to 24 hrs, depending on the strain of *Artemia*, quality of cysts and water temperature. The optimum environmental condition required for proper hatching are: temperature - 27 to 30 °C, salinity - 25 to 30 ppt, pH-7.5 to 8.5, light intensity-1000 lux and dissolved oxygen - up to saturation point.

## Copepods

Copepods are common zooplankton of freshwater and brackishwater. They are natural feeds for larvae and juveniles of many finfish and crustaceans and it is generally believed

that copepods can meet the nutritional requirements of fish larvae (Evjem *et al.*, 2003). In the wild, most marine fish larvae feed on copepod eggs and nauplii during the first few weeks of life. Because some species of copepods have very small size larvae (a necessity for some species of fish larvae) and can have very high levels of HUFAs and other essential nutrients, they are an excellent food source for first-feeding larvae. In fact, a number of marine larval fish cannot be reared using rotifers as the first feed but have been reared on either laboratory reared or wild caught copepod nauplii. Research with several species, such as the turbot and red snapper, has shown that when offered mixed plankton diets, young larvae consume more copepod nauplii than rotifers and prefer copepod nauplii because of the differences in size and swimming patterns of the two prey types. Consequently, there is considerable interest in the use of copepods as feed sources for small marine larval fish. Copepods are cylindrical with a trunk comprised of 10 segments, consisting of head, thorax and abdomen. Adult copepods size range from 0.5 to 5.0 mm. The larval stages consist of six naupliar and six copepodite stages. Main suborders of copepods found in brackishwater are calanoids (*Acartia*, *Calanus* and *Pseudocalanus* spp.), harpacticoids (*Tisbe* and *Tigriopus* spp.), and cyclopoids. Herbivorous copepods are primarily filter feeders and typically feed on very small particles. But they can feed on larger particles, which give them an advantage over the rotifers. Copepods can also eat detritus. They differ from *Artemia* and rotifers in that they do not reproduce asexually. Copepods mate after maturing and female produces 250 to 750 fertilized eggs (rotifers produce 15 to 25 per female). The copepod lifespan is 40 to 50 days (5 to 12 days for rotifers), and it has a longer generation time (1 to 3 days for the rotifer and 7 to 12 days for the copepod).

In contrast to rotifers, copepods are more difficult to culture on a commercial basis. Only a few species of copepods, such as *Tigriopus japonicus*, have been mass cultured successfully. Even this technique employs the combination of rotifer culture and the use of baker's yeast or omega-3 yeast as feed. Unfortunately, the amount of yeast used to produce the copepod and rotifer combination outdoors is fairly high. There are outdoor production systems that can produce large numbers of copepods; however, these systems are very inefficient in terms of number of copepods per litre of culture water. Considerable work needs to be done on culture and harvest techniques before copepods become as widely used as rotifers. One interesting advantage of copepods is that under appropriate conditions some species will produce a resting egg similar to that of *Artemia*. So once commercial techniques are developed, copepod eggs could be collected in large numbers and stored for months, like *Artemia* (brine shrimp) and rotifer cysts. Photoperiod and temperature largely determine the production of copepod resting eggs. Laboratory production of these eggs is possible, but has not yet proved to be economically feasible. It is hoped that using copepods as a food source can improve the culture of a variety of species, such as the red drum, by reducing the size variability and mortality. The use of copepods, especially the harpacticoids, is well documented in marine fish culture. The Japanese have routinely cultured the copepods *Tigriopus* and *Acartia* for rearing fish larvae approximately 7mm in length. The growth and biochemical composition of *Coryphaena hippurus* larvae

that were fed with copepod (*Euterpina acutifrons*) survived well under stressful conditions. A system for the mass culture of a benthic marine harpacticoid copepod, described by Sun and Fleeger (1995), should be useful for aquaculture. Other species of copepods considered to be promising for mass culture are *Acartia clausi*, *A. longiremis*, *Eurytemora pacifica*, *Euterpina acutifrons*, *Oithona brevicornis*, *O. similis*, *Pseudodiaptomus inopinus*, *P. marinus*, *Microsetella norvegica* and *Sinocalanus tenellus*.

**Evjem et al. (2003) reported that** copepodid and adult stages of the marine copepods *Temora longicornis* and *Eurytemora* sp. had a total lipid content varying between 7% and 14% of dry weight (DW). The predominant fatty acids of all copepods were docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and the saturated fatty acid 16:0. In *T. longicornis* and *Eurytemora* sp., DHA constituted 26-42%, EPA 15-24% and 16:0 8-12% of total fatty acids. In *C. finmarchicus*, the DHA content ranged between 21% and 32.5%, whereas the content of EPA and 16:0 was 15-21% and 9-15% of total fatty acids, respectively. The sum of n-3 HUFA was highest in *T. longicornis* and *Eurytemora* sp. (55-62% of total fatty acids) and lowest in *C. finmarchicus* (38-47%). The protein content of various copepods varied between 52.4% and 57.6% of dry weight (DW), and was significantly higher than in *A. franciscana* (41% in newly hatched nauplii and 34% after 24-h enrichment).

## Cladocerans

Cladocerans are generally called ‘water fleas’. Cladocera is an order of sub-class - Branchiopoda and class - Crustacea of the phylum - Arthropoda. Two cladocerans, namely *Daphnia* and *Moina* are important as live food. *Daphnia* is found in freshwater ponds, tanks and lakes, all over the world. It swims by rapid jerks of the two large antennules. *Daphnia* contains a broad spectrum of digestive enzymes such as proteases, peptidases, amylase, lipase and even cellulase which serve as exoenzymes in the gut of fish and prawns. Being larger in size than *Moina*, it serves as live food for advanced stages of fishes. *Moina* are primarily inhabitants of temporary ponds or ditches. It is smaller in size (0.5 to 2 mm) than *Daphnia* containing 70% more protein and therefore, goes well as a replacement for *Artemia* in aqua hatcheries. *Moina* has also been extensively utilized as live food in many hatcheries and in the maintenance and culture of aquarium fishes of commercial importance (Martin et al., 2006).

Cladocerans have the advantage of high reproduction rates, wide temperature tolerance and the ability to thrive on phytoplankton and organic wastes. They are mainly freshwater zooplankters; most do not tolerate salinities higher than 3 ppt., and are generally not found in brackishwater, except *Diaphanosoma celebensis*. In Asia, there is a growing use of this species. This is a saline-tolerant (1 to 42 ppt) water flea in the 400 to 800 micrometer range that has been successfully cultured in backyard hatcheries. The organism must be enriched before it is fed. This enrichment is accomplished with a source of DHA, but usually not one with an oil emulsion base because of gill and water fouling problems. Other cladocerans considered as promising species are *Evandne tergestina*, *Penilia*

*avirostris* and *Podon polyphemoides*. The cladoceran *Moina macrocopa* has been used in Southeast Asia as feed for sea bass fry immediately after weaning from *Artemia* and prior to feeding minced fish flesh. A related cladocera, *Moina salina*, has been used in finfish culture.

The nutritional content of *Moina* varies considerably depending on their age and the type of food they are receiving. The protein content of *Moina* usually averages 50% of the dry weight. Adults normally have a higher fat content than juveniles. The total amount of fat per dry weight is 20-27% for adult females and 4-6% for juveniles. In ornamental fish culture, *Moina* used to be the most common live food organism for feeding young fish larvae.

## Culture of Moina

For pure culture of *Moina*, stock culture need to be developed. In order to start stock culture, collection of *Moina* is done from freshwater ponds and tanks with the help of scoop net having 250 to 500 micron mesh. After collection the content of the net is placed in a plastic bucket and brought to the laboratory. The sample is then diluted by adding clear freshwater and examine under a microscope. Pickup *Moina* with the help of a fine dropper. Each *Moina* so picked up are inoculated in 20 ml glass tube containing 10 ml of filtered water. Feeding of *Moina* is done with yeast @ 200 ppm or *Chlorella* at a cell density of  $10 \times 10^6$  cells/ml. Each gravid *Moina* produces 8 to 10 offsprings in about 24 hours. Dilution of the test tube culture is done daily through several 100 ml beakers. The volume is increased to 1 to 2 litre beakers or jars. Feeding is continued in similar manner as in the test tube culture. After 4 to 5 days, these cultured *Moina* are used as inoculums in mass culture tanks.

In mass culture methods, the culture tanks are treated with groundnut oil cake (75 ppm), single super phosphate (20 ppm) and urea (8 ppm). After fertilization, the tank is inoculated with *Chlorella* or mixed phytoplankton. When algal blooms are developed within 3 to 4 days, *Moina* is inoculated @ 40 to 50 nos./litre depending on the availability of stock culture. *Moina* multiples rapidly, feeding on phytoplankton blooms, bacteria and small particles of groundnut oilcake. It attains a peak density of 20,000 to 25,000 nos./litre in 5 to 7 days after inoculation. After attaining peak density, it is regularly harvested to feed the larval stages. As a result of *Moina* multiplication and reduction of nutritional status of water, *Chlorella* concentration declines. In order to maintain optimum *Chlorella* concentration, partial water exchange from the tank bottom and re-fertilization with groundnut oil cake (75 ppm) is done at an interval of 4 to 5 days after commencement of first harvesting of *Moina*.

## Tubifex

*Tubifex* is a type of worm of class - Oligochaeta under the phylum - Annelida. Clusters of these worms are commonly seen in sewage drains. When disturbed, they jerk into the

mud. Though not for larval and post-larval stages, but *Tubifex* make an ideally suited diet for brooders of various ornamental fishes. Among the natural food organisms, red worm (*Tubifex tubifex*) is one of the best candidates owing its short generation time, occurrence in a vast range of habitats and tolerance to a wide spectrum of environmental variables (Kaster, 1980).

Mahmut *et al.* (2003) reported the percentage crude protein, lipid, ash and moisture content of *T. tubifex* were  $11.02\pm 0.58$ ,  $2.14\pm 0.06$ ,  $1.83\pm 0.16$  and  $18.78\pm 0.83$  respectively. Total fatty acid content was 7.28 mg/100 mg dry weight and  $\omega$ -3 (C18:3n-3 and C20:5n-3) and  $\omega$ -6 (C18:2n-6c and C20:4n-6) fatty acids composed 18%, 22% of the total, respectively. The most abundant amino acids (amino acid g/100 g protein) were lysine ( $6.54\pm 0.12$ ), leucine ( $6.52\pm 0.13$ ) followed by arginine ( $5.39\pm 0.04$ ), valine ( $4.92\pm 0.09$ ), treonine ( $4.81\pm 0.09$ ), phenylalanine ( $4.36\pm 0.09$ ), isoleucine ( $4.31\pm 0.08$ ), tyrosine ( $2.74\pm 0.07$ ), histidine ( $2.67\pm 0.03$ ) and methionine ( $1.82\pm 0.04$ ). Total carotenoid level present in *Tubifex* is  $15.02\pm 0.80$  mg/kg.

## Culture of Tubifex

*Tubifex* can be easily cultured on mass scale in container with 50 to 75 mm thick pond mud at the bottom, blended with decaying vegetable matter and masses of bran and bread. Continuous mild water flow is to be maintained in the container, with a suitable drainage system. After the arrangement of the system, the container is inoculated with *Tubifex* worms, which can be obtained from nearby muddy canals or sewage canals. Within 15 days, clusters of *Tubifex* worms developed. This can be removed with mud in masses by means of a spade and kept in large wide mouth plastic container. When worms will come to the surface due to lack of oxygen, they are collected and washed under brisk stream of water to remove residual mud attached to the body. Since their gut still may contain mud which they have eaten. Therefore, they should be kept long enough under stream of water for the mud to be evacuated from the intestine. After proper cleaning only the worms are fed to the fishes.

## Chironomid Larvae

Chironomids (non-biting midges) are one of the most ubiquitous, diverse, and ecologically important groups of aquatic macro invertebrates (Coffman and Ferrington, 1984). *Chironomid* is an insect belonging to the order - Diptera of the class - Insecta of phylum - Arthropoda. These are commonly called as blood worms due to the presence of haemoglobin in their body fluid (Madlen, 2005). Larvae initially live in soft tubes made from organic matter. Fully grown larvae are dark red in colour.

The *chironomid* larvae are recognized as an important food for many fishes and cultured invertebrate (Habib *et al.*, 1992; Tidwell *et al.*, 1997). These live foods are very popular in aquarium fish trade. Chironomid larvae are excellent source of protein (De La Noue and Choubert, 1985), lipid, vitamins and minerals (Mclarney *et al.*, 1974). The relatively high protein content (56%), the high digestibility (73.6%) as mentioned by De

La Noue and Choubert (1985), and the apparent function in small quantities as a growth promoter in fish and crustacean diets (Tidwell *et al.*, 1997) make chironomid larvae a rich food for many organisms. It was suggested by Armitage (1995) that the preference of bottom feeder organisms for chironomid larvae and pupae as food source is related to their high energy content (with a utilization energy of 4.1 to 6.1K Cal g<sup>-1</sup>).

## Culture of chironomid larvae

They represent an abundant group of benthos insects in freshwater ecosystem. They have high reproductive capacity, each female lay about 2300 eggs in one batch which hatch in about three days at temperature (18-22°C). The larvae attain a size suitable for feeding purposes in 16-20 days (Madlen, 2005). Flat trays filled with water are added with soil and compost manure or organic matter or decaying vegetable matter to attract chironomid flies to deposit eggs. Some time the *Chironomid* flies even lay eggs in clear stagnant waters also. The *Chironomid* larvae are herbivorous in feeding habits and feeds on algae, detritus, decaying organic matter etc (Habib *et al.*, 1992). Initially the larvae live in soft tubes made up of organic matter which can be clearly seen at bottom of the track. After 2 to 3 days, they come out of the tubes and freely swim in water vertically. The larvae are harvested with scoop net and washed thoroughly before feeding. It constitutes one of the staple food items in the ration of nearly all carnivorous young fishes.

## Enrichment of live food organisms

The most important factor governing the nutritional quality of live feeds for aquaculture practices is the essential fatty acid content, particularly eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), commonly called as highly unsaturated fatty acids (HUFA). In recent years, much emphasis has been given to enhance the nutritional status of live food organisms through various techniques of enrichment and bioencapsulation. The nutrient which are lacking or present insufficiently in food organisms can be made available by allowing them to grow for a defined period in a medium containing appropriate quantities of the required nutrients. Today, various kinds of micro particulate and emulsified formulations are used for boosting these live foods with essential fatty acids and other crucial components, including pigment and vitamins.

The filter feeding nature of *Artemia* allows a very convenient way to manipulate biochemical composition. To improve the lipid composition of both *Artemia* nauplii and juveniles, researchers (Sorgeloos *et al.*, 2001) have developed several enrichment products like unicellular algae, compound diets, micro particulate diets, etc. The use of liposomes as enrichment products provides different advantages and possibilities (Tonheim *et al.*, 2000). This outstanding achievement in larviculture has resulted in increased survival, higher growth rate and greater resistance to stress and disease. The enrichment technique is becoming more prevalent for intensification of culture practices and in the future, it will help initiate commercial culture of new marine fish species.

Live feed enrichment techniques may also be an excellent tool for prophylactic and therapeutic measures of fish and shrimp larviculture to administer drugs and vaccines to culture organisms. This will help to minimize the loss of expensive drugs into the environment which otherwise may prove detrimental to animal and human health. The use of ascorbyl palmitate as an ideal source of vitamin C supplementation in live food can provide an important tool to build up stress and disease resistance during larval rearing in hatcheries.

## Major constrains in live feed culture

Considering several factors, live feed remains the most practical solution for larval rearing for aquaculture species. However, it is not easy to maintain a steady supply of adequate quantities of live feed at appropriate times in intensive culture systems. Among the constraints of micro algae production the primary one is its cost of production, especially in smaller hatcheries. Difficulties in getting pure strain, lack of infrastructure facility like controlled environmental laboratory for culture maintenance etc. are some other prime areas of concern. Live feed also act as a carrier of diseases to the larvae of fish and shellfish, therefore, maintenance of hygiene is very important during their production. The new technology of enrichment process is a costly affair for poor and medium level farmers. Similarly, the high infrastructure and labour requirement along with the variable cost for live feed production illustrates the need to develop suitable modified culture technology. Although several strains of artemia are available in India, selection and suitability of the available strains are of major concern and hence the aqua hatcheries are more dependent on imported cysts. Nutritional status of the live feed organisms needs to be summarised for feeding different larval stages of fish and shellfish. Therefore, more research thrust should be given on suitability of many of the available live food organisms.

## Future prospects

A good selection of micro algal species is available to support the aquaculture industry. However for some particular applications for industry sectors, new species with improved nutritional quality or growth characteristics could improve hatchery efficiency. The use of microalgae either as a full or partial enrichment should be considered for improving the nutritional quality of zooplankton.

Finfish producers are concerned with improving the quality, quantity and cost effectiveness of their live feed production facilities. Many of them now supplement cultures with omega yeast, vitamins (E, D, C and B<sub>12</sub>), marine oils or other HUFA sources, and vitamin B<sub>12</sub> producing bacteria to improve feed quality. Today, live feeds for fish larvae are being improved by adjusting their biochemistry through controlling their diet and supplementing the cultures with microencapsulated feeds or emulsified oils. While algae and rotifers are the most widely used live food items, their use is not without problems and limitations. Rotifer and copepod cultures are subject to collapse or “crash”.

Producers are finding new species of live food organisms better suited for specific culture situations. For many commercial species, the co-feeding of live and artificial feeds during the larval stages is recommended. Microencapsulated diets do have one very positive attribute - they are an alternative way to administer vaccines and therapeutic agents to larvae. Even though the large-scale, intensive production of microalgae and rotifers is expensive and often unreliable, the production of live food organisms continues to be a very important first step in aquaculture.

Ciliates are consumed by larval fish and crustaceans in the wild and are considered promising candidates for mass production. Besides being the microscopic ciliates are soft bodied and nutritionally rich. They reduced the organic load for the culture system by consuming the fine organic particles. Further, understanding of its nutritional potential needs to be explored.

Earthworms are excellent food for cultured fish species; it can be used alone and in combination with other foods. Earthworm has been found to be a good source of protein (Hilton, 1983). Earthworm accelerates growth, improves sexual performance, stimulates the appetite, makes feeds more attractive, so the animals come to feed better and waste is avoided.

Fairy shrimp, the freshwater relatives of the more popular *Artemia*, offer interesting possibilities as live food in larval culture (Prasath *et al.*, 1994). They are probably more appropriate for freshwater fish and crustacean cultures that depend on live foods. Their high carotenoid content makes them a candidate for colour enhancement in ornamental fish culture. They are also amenable to enrichment and bio-encapsulation making them an ideal candidate for the delivery of valuable nutrients and other molecules to the young larvae. The nutritional value of freshwater fairy shrimps has been documented by Munuswamy *et al.* (1992). The cysts of fairy shrimps contain 45-50% protein; and 5-6% of lipids. Both larvae and adult live fairy shrimps can be used as live food to a variety of aquaculture organisms. There is a wide scope of further research for perfection and standardization of different culture techniques of live food organisms.

## CONCLUSION

The high cost of *Artemia* cysts has increased fish production costs and cheaper alternative diets with similar nutritional quality needed to be maintain the cost competitiveness of the fish in the global market. The industrial development of aquaculture has been hampered by the lack of suitable live feeds for feeding the fish at their various production stages. Here an attempt has been made to make aware about the recent developments in the applications of several live food organisms in intensive culture of fish and shellfish. The availability of on-grown live food would not only offer farmers and exporters a better alternative option for feeding to their fish, but more importantly, the possibility of enhancing the fish performance and quality through bioencapsulation.

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