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45 Culture of live feed organisms

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ABSTRACT

In any mariculture practice, availability of seed is one of the prime requisites for sustaining growth, development and to make the activity viable ecologically and economically. As the natural source of seed is often beset with environmental / conservational problems, the dependable source is production through hatchery techniques. Uninterrupted production and supply of live feeds alone can sustain hatchery operations and to feed the emerging larvae or post larvae with different feed requirements at different stages of development / growth. Technologies developed by CMFRI in this line are given in this paper. The method of culturing important live feed organisms such as micro-algae, rotifers, cladocerans and Artemia salina along with their harvesting and preservation are briefly described in this account.

Introduction

In India, the need for carrying out the culture of live feed organisms are increasing day by day since it is playing a significant role in any type of hatchery operations. It is an established fact that the micro-algae, especially the nanoplankters form the basic food of almost all the larval stages of crustaceans, molluscs and fishes. In nature, the larvae feed on whatever organisms present in their surrounding environment. But in a hatchery, the required species of micro-algae and other live feeds have to be supplied in required concentrations to feed the larvae.

The scope of micro-algae as a possible source of protein food was recognized by the researchers in the middle of 20th century. In the past, the main attention has been on single cell protein (SCP) production for human consumption and later many new applications have been evolved including waste

water treatment, nutrient recycling,closed life-support systems, bio-conversion of solar energy and recently in aquaculture. In recent years, there has been renewed interest in producing single cell protein by mass culturing the unicellular micro-algae such as diatoms and nanoplankton flagellates for feeding the larvae of crustaceans, molluscs and fishes. The larvae of prawns and fishes prefer the diatoms as the basic food while the molluscs live on the nanoplankton flagellates, measuring less than 10μ during its larval stages. In the early critical stages of rearing larvae of finfishes and shellfishes, the phytoflagellates(species of *Isochrysis, Pavlova, Dicrateria, Chromulina* and *Tetraselmis*) and other nanoplankters *(Chlorella, Nannochloropsis)* form the basic food. But in the post-larval stages of crustaceans and spat (juvenile stages of molluscs), the diatoms (species of *Chaetoceros, Skeletonema* and *Thalassiosira)* form the primary food. However,most of the post- larval forms of finfishes and crustaceans prefer the animal live feeds such as species of *Brachionus* (rotifer), *Moina* and *Daphnia* (Cladocerans) and *Artemia salina* (Brine shrimp).The general practice in shrimp and fish culture is to use mixed diets consisting of micro-algae feed during the early larval stages followed by the use of zooplankters for the succeeding stages of development.

Research related to the mass culture of live feed organisms presently under way in many research institutions and universities are directed towards the solution of several key problems such as (i)isolation and maintenance of particular species of plankton, both phyto and zooplankton (ii) large scale culture of micro-algae under controlled conditions of light, temperature and aeration and its constant supply in different phases of growth (iii) developing viable methods for the intensive culture of life feeds, its maintenance, harvest and preservation, during its optimum and peak densities and (iv) developing economical feeds which will provide the nutritional needs of the rearing larvae in a hatchery system.

Culture of micro algae

The various aspects of micro-algae culture are: the isolation of required species, identification, preparation of suitable culture media, maintenance of stock culture, mass culture in indoor and outdoor facilities, supply of the culture in required quantities after determining the cell concentration, har-

vest and preservation of the culture for future use.

Isolation: Isolation of suitable species of micro-algae can be done by (i) pipette method, (ii) centrifuge method (iii) agar plating method (iv) by phototactic movement and (v) by serial dilution technique (Gopinathan,1996). After the isolation of the organisms in small culture tubes, it is subcultured again in 50 ml, 100 ml conical flasks for its further purification. Once the culture is purified in small conical flasks, increasing the volume, from 100 ml to 1 litre and later in 3 or 4 litre Hafkin culture flasks, the algae can be kept as stock culture. Filtered and sterilized seawater should be enriched with suitable culture media, not earlier than 3 days prior to the inoculation.

Culture media: For the successful culturing of the micro-algae, various culture media have been used depending on the type of organisms to be cultured and their growth phases. Although most algae are photo - autotrophic and can grow in purely inorganic media, others prefer organic compounds and for many, they are simulatory. Though Schreiber's and Miquel's media were found to be very effective for culturing diatoms and other nanoplankters, several other media have come into existence with the addition of trace metals, vitamins and other organic and inorganic salts. Conway or Walne's medium (Walne, 1974) contains all ingredients and is widely used in the laboratory maintenance and mass culture programmes (Gopinathan,1996). Besides, the laboratory prepared chemicals which serve as nutrients, it is economic to use commercial fertilisers also for the mass production of nanoplankters in open tanks.

Stock culture: Stock culture of different species of micro - algae are to be maintained in a special room adjacent to the mass culture laboratory. The autoclaved or heated seawater after cooling may be poured into the Hafkin culture flasks and the required nutrients added. Walne's medium enriched with vitamins and silicate is the best medium for maintaining the diatoms and without silicate for other micro-algae. About 10 ml of the inoculum in the growing phase of the culture is transferred to the culture flasks and place in front of 2 tube lights (1000 lux) for about 5-6 days at temperature of 25°C. When the maximum growing phase has reached, only one tube light is necessary for further growth of the culture. In the stationary phase of most species of micro-algae under controlled conditions of temperature and light, the stock culture can be kept for nearly 2 months.

Mass culture- Indoor and Outdoor: The containers for the indoor mass culture of micro-algae are: glass carbuoys, polythene bags, transparent perspex tanks, transparent cylindrical FRP tanks and FRP tanks (inside pure white). These containers can be kept on wooden racks and frames providing light and aeration facilities. For the outdoor culture, the containers are 1-5 tonne capacity FRP tanks, 5 - 10 tonne capacity concrete tanks. Fully grown culture from the stock culture room is used as inoculum for the mass culture in these containers. Required quantities of chemical media / fertilizing media may be added. These containers will have the maximum concentration of the cells in the growing phase on 2-4th day and before going to the declining phase, it should be harvested. After estimating the cell concentrations, the culture can be supplied to the hatchery for feeding the rearing larvae.

Growth phases: Increase in cell numbers in a culture follows a characteristic pattern of growth in which the culture may be recognised as (i)lag or induction phase (ii) exponential or growing phase (iii) declining phase (iv) stationary phase and (v) death phase.

Harvest and preservation: The fully grown culture should be harvested during the growing phase of the micro-algae after determining the cell concentration with the help of haemocytometer or Sed-wich Rafter counting cell. If the culture has entered the declining or stationary phase of growth, the metabolites will be very high and the cells may not be in healthy condition. The culture can be preserved either by freezing or drying employing the technique of flocculation (Gopinathan, 1993) to overcome the unfavorable conditions in the hatchery.

The important species of micro-algae used as live feed, procedure for their mass culture and the feeding protocol in various hatcheries are presented in Table 1-4.

Table 1. Micro-algae culture – Important species used as live feed.

Chlorophyceae (Green)	*Chlorella* spp.
	Nannnchloris sp.
	Nannochloropsis spp.
	Tetraselmis spp.
	Dunaliella sp.

Bacillariophyceae (Diatoms)	*Chaetoceros* spp.
	Skeletonema costatum
	Thalassiosira subtilis
Chrysophyceae (Golden Yellow)	*Dicrateria* sp.
	Chryptochrysis sp.
Haptophyceae (Golden brown)	*Isochrysis galbana*
	Pavlova lutheri
	Chromulina freiburgensis
Cyanophyceae (blue - green)	*Spirulina* sp.(freshwater form)
	Synechocystis sp.
Mixed culture	Phytoflagellates
	Nanoplankters
	Centric and pinnate diatoms

Table — 2 Procedure of micro-algae mass culture

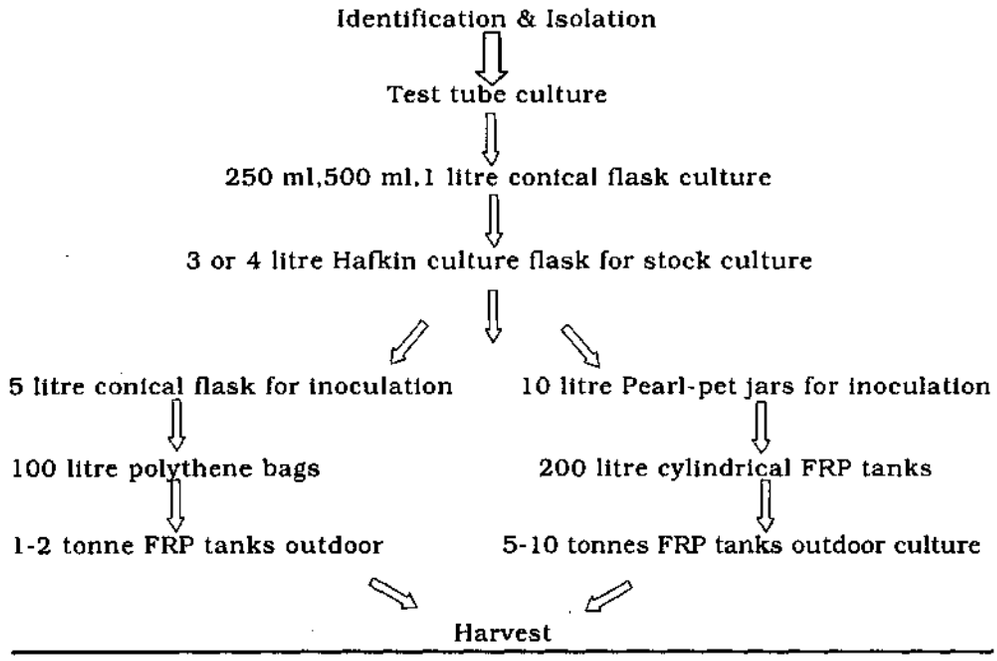


Table 3 Procedure of micro-algae culture:- various stages

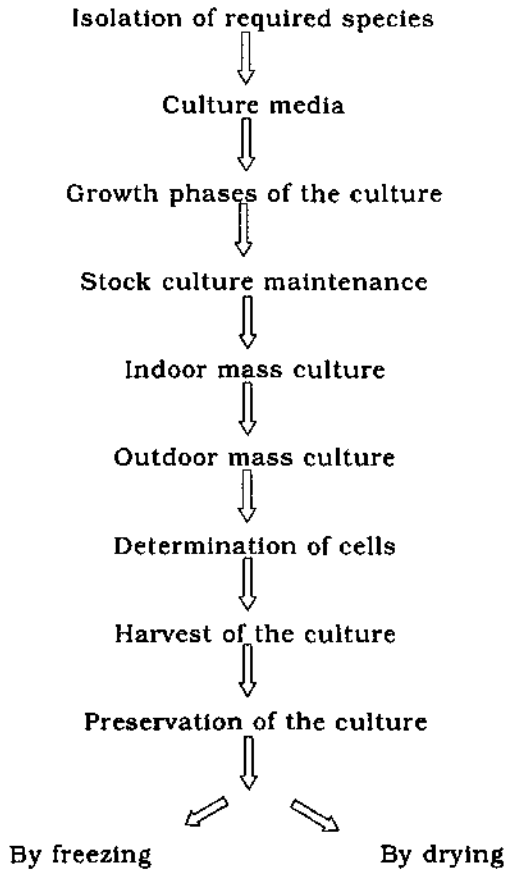


Table 4 Feeding protocol in various hatcheries

A.Prawn Hatchery

Zoea 1 to Mysis 1	Chaetoceros *spp.*
	Skeletonema costatum

Mysis II to Post larvae 5	*Chaetoceros* + *Tetraselmis* + *Artemia* nauplii
PL- 5 to PL-20	Mixed culture + *Artemia* + rotifers + Cladocerans.

B.Fish Hatchery

Larvae to post larvae	Phytoflagellates + Diatoms + *Artemia* nauplii + *Spirulina* (for fresh water forms)

C.Seacucumber Hatchery

Larvae to Auricularia	Phytoflagellates (*Isochrysis* , *Dicrateria*, *Tetraselmis*)
Auricularia to Pentacula	*Chaetoceros* + mixed culture
Pentacula to young one	Algal powder + detritus

D. Molluscan Hatchery

'D' shape to Spat	Isochrysis, *Pavlova* , *Chromulina* and *Dicrateria*
Spat to Juvenile	*Chaetoceros* ,*Skeletonema* , *Thalassiosira* , *Tetraselmis* and other flagellates.
Juvenile to adult	Mixed culture dominated by diatoms,*Chlorella, Tetraselmis* etc.

Problems and prospects: Large scale culture operations with microalgae and other live feeds have been carried out intensively in most of the hatcheries in India. Today, numerous algal waste water treatment systems are in operation in different parts of the world and a few commercial ventures exist that involve the production of high priced protein food derived from microalgae .

For the mass culture of *Nannochloropsis* spp., effluents from different marine grow out systems at Fisheries Harbour Laboratory, Thoppumpady, Cochin were tried on preliminary scale as culture medium in indoors. Based on the encouraging results, a well laid out experiment is in progress to arrive at the best medium for mass culture of *Nannochloropsis* spp. which will in

turn contribute to its sustainable production with economic viability. This will substitute the costly chemical inputs.

It is of significance that the prime goal of all mass culturing ventures is to maximize photosynthetic conversion efficiencies and to optimize the production of plant material. To achieve this on a large scale, it is necessary to take advantage of available solar energy. However, algal mass culture systems when industrialized would pose many bio- engineering problems.

The development of mass culture of micro-algae offers immense scope and should run concurrently with mariculture programmes. Considerable research is needed for elucidating the problems of growth kinetics of different species and also the period of economically viable harvest.

Culture of live feed -zooplankton

Live zooplankters such as rotifers (*Brachionus plicatilis*), cladocerans (*Moina* sp. & *Daphnia* sp.), copepods (*Acartia* sp., *Tigriopus* sp.,etc.), brine shrimps (*Artemia salina*), blood worms (*Chironomus* sp.) and tubifecids (*Tubifex sp.*) play a vital role in the feeding of cultivable species of fishes and crustaceans. Among them the most widely accepted all over the world are rotifers, cladocerans, and the brine shrimp. These are successfully used in hatcheries due to their high nutritive value, short generation time, capacity to grow in dense population and easy to produce in mass scale under controlled conditions. A flow chart of zooplankton culture in general is given in Chart -1.

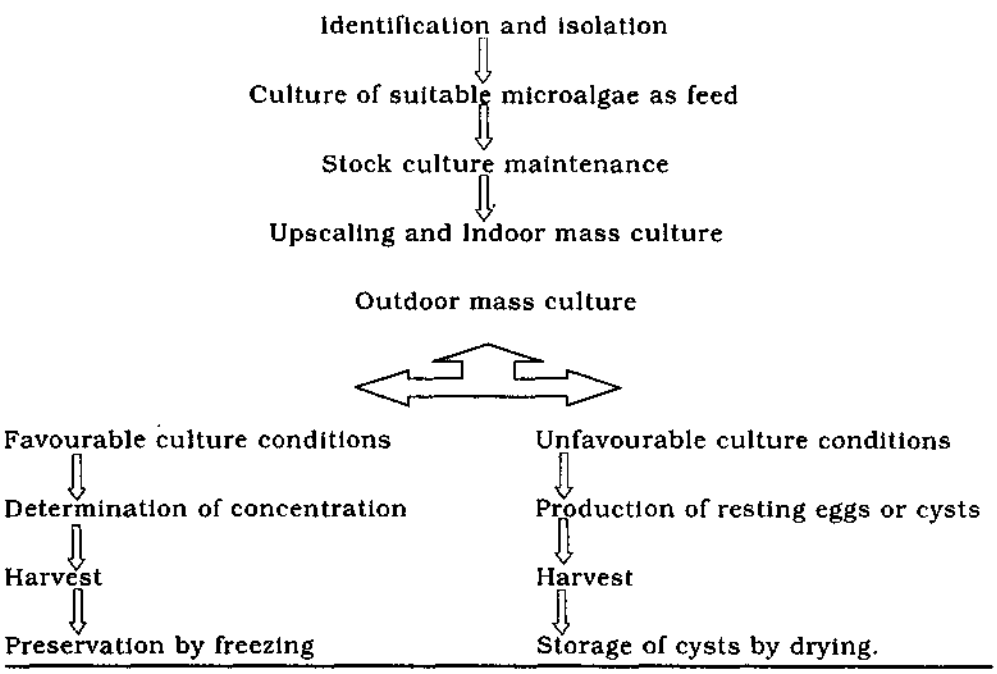
1.Rotifers

Rotifers are the most accepted live feed which is being mass cultured and used in hatcheries world over. Rotifers occur in a variety of aquatic habitats and these were first studied and described by Leeuwenhock as early as 1703. Among rotifers, *Brachionus plicatilis* is considered to be an excellent food source for fish and crustacean larvae. It is euryhaline and distributed in tropical and sub-tropical waters. *B.plicatilis* is a filter feeder and in nature consume particulate food consisting of phytoplankton, detritus and bacteria.

Identification: Lorica flexible, more or less oval, slightly compressed dorsoventrally, anterodorsal margin with six broad-based, acutely pointed, saw-toothed spines, nearly equal in length, foot opening with small sub square aperture dorsally and longer V-shaped aperture ventrally. The life cycle of *B.plicatilis* is by (1) parthenogenesis and (2) sexual.

Chart - 1

Flow chart of zooplankton culture



Isolation and stock culture development: *B.plicatilis* can be isolated with the help of a micropipette, from zooplankton sample kept under a binocular microscope of adequate magnification.The initial step for culture operations is to develop feed for rotifers. Microalgae of suitable size, 2-20µm, such as *Chlorella* spp., *Nannochloropsis* spp., *Isochrysis* spp. and *Tetraselmis* spp. are their preferred food. The methods of stock as well as mass cultures of micro-algae are described in the previous section.To develop a stock culture, transfer a few, live, egg bearing specimens into a small test tube (50 ml capacity) containing algal culture and keep under illumination. The rotifer multiplies very fast by parthenogenesis and a stock culture of required concentration will be developed within 3-4 days.

Mass culture: A technique for mass culture and stable supply is required to feed the early larval stages of fish and crustaceans. To attain this, the initial step is to upscale the stock culture. For this purpose, stock culture

has to be inoculated to culture flasks of 250 ml, 500 ml, 1 litre, 3 litre and 5 litre capacities containing algae. Within a few days concentrated cultures of rotifers will be ready. At this stage, mass culture can be started. Portions of stock cultures can be transferred to bigger containers and provide algal feed. The upscaling in mass culture level can be achieved by inoculating rotifers into larger fibre-glass tanks or concrete tanks. The capacity of tank to be used for culture will depend upon the requirement of rotifers for the hatchery. Daily feeding of rotifers with sufficient algae is essential for better growth.. Continuous aeration and 14-hour illumination per day are known to ensure good growth of rotifers under controlled conditions.

An alternate method of mass culture of rotifer is by culturing algae after fertilizing filtered seawater with groundnut oil cake, urea and superphosphate at the rate of 250, 10 and 5 gms per tonne of water respectively and then inoculating with rotifers.

Rotifer consumes 1.5 to 2 lakh cells of algae per day. Apart from algae, marine yeast, *Candida* sp. and baker's yeast, *Saccharomyces cerevisiae* are also being utilized as feed for rotifers or it can be supplemented with algae in mass culture activities.

The reproductive rate of rotifers in cultures depends on food quality and quantity, salinity, temperature and pH of the medium. They grow very well in salinity ranged from 15-30 ppt and temperature between 26-32°C.

Three methods are being employed for the mass culture of *B.plicatilis* according to the type of harvesting and size of culture tank.

(a) Batch culture:- In this method, the entire culture in a tank is harvested at once and part used as the inoculum for the next culture.

(b) Semi-continuous culture:- In this method, rotifer density is kept constant by periodic harvesting. The portion of rotifers harvested is determined by the amount necessary for the culture density to recover in a day. The size of culture tank can be larger than used in batch culture method.

(c) Continuous culture:- Recently, an automatic continuous culture method is also developed by Yong Fu *et al.*(1997) to improve culture stability as well as to reduce labour and space. The system consists of a filtration unit, a culture unit and a harvest unit. In this system, filtered water and food are

continuously supplied into a rotifer culture tank at a pre-determined rate, and the same amount of culture water is transferred into a harvest tank to obtain rotifers at a significant biomass.

Harvesting and Preservation: Harvesting can be done using appropriate sieves according to the size of rotifer to be supplied as live feed to the larvae. Regular intervals of harvesting will help to maintain the culture in good condition for a longer period. Harvesting should be done when they are with 2-3 eggs attached to the body of rotifers; as the nutritive value of the rotifers will be high in this stage. The harvested rotifers have to be washed in filtered seawater before offering as live feed for the larvae. Otherwise, it can be stored with 10% glycerine in a deep freezer.

Females produce amictic eggs which develop and hatch into amictic females. Under unfavourable conditions such as low oxygen level, high density of population, scarcity of food and wide variations in salinity, the amictic females produce males. Then, by sexual reproduction, the resting eggs or cysts are formed and settled at the bottom of the culture tank. Dry resting eggs can be stored for more than one-year (Philippe Dhert, 1996). It can also be stored in a deep freezer at- 14°C for 2-3 months. The cysts hatch out within 36-48 hours when it is given favourable conditions like hydration in seawater, illumination and aeration. The resting eggs will only hatch into amictic females.

The use of preserved rotifers and resting eggs will help to overcome unforeseen failures of live cultures leading to more efficient use of this live food organism in fish and crustacean hatcheries.

Enrichment: Marine fish larvae have a high requirement for Highly Unsaturated Fatty Acids (HUFA). The amount of these compounds in rotifers has to be increased before feeding the larvae. Rotifers cultured with baker's yeast alone cannot support larval growth as these rotifers lack HUFA. Species high in HUFA such as *Nannochloropsis* spp. are regarded as very good feeds.Enrichment of rotifers with diets such as microalgae, lipid emulsions, fish oils, microcapsules containing lipids, etc. are being used to increase the HUFA content, before offering as live feed for the larvae. Direct and indirect methods of enrichment can be adopted to improve nutritional qualities of rotifers. In the direct method, harvested rotifers are suspended in enrichment medium for about 6-12 hours and in the indirect method the medium is ini-

tially given to the feed and then that feed is given to rotifer.

Problems and prospects: The culture vessels containing the rotifers are likely to be infested with ciliates like *Euplotes* sp. & *Uronema* sp. and other unwanted organisms. Virus and fungi were also reported as infectious agents in mass cultures and cause abnormal mortality of rotifers. Abiotic factors, including water temperature fluctuations, dissolved oxygen; increased ammonia concentrations may lead to decrease in the growth rate of rotifer populations. So, regular monitoring of the culture along with adopting remedial measures is essential in rotifer culture operations.

2.Cladoceran

Under cladocera, *Moina* sp. and *Daphnia* sp. are commonly been used as live food organisms.

***Moina* sp.**

Moina sp. is being used as an ideal live feed in freshwater fish and crustacean hatcheries . It is available naturally in freshwater ponds and pools.

Isolation and development of stock culture: *Moina* sp. can be isolated from zooplankton sample, with the help of a micropipette. *Moina* sp. reproduces parthenogenetically and multiplies very fast, hence a stock culture can be easily developed from a single parthenogenetic female. Usually freshwater *chlorella* sp. is being used as feed for *Moina* sp..

Mass culture: Stock culture has to be upscaled before starting mass culture to ensure that the inoculum is sufficient for the development of mass culture. Fibre glass or concrete tanks of varying capacities can be used for mass production.The capacity of the container will depend upon the daily requirement of *Moina* sp. to the hatchery. Tap water free of chlorine or well water are normally used for the culture activities.

As in the case of rotifer, the initial step for mass culture of *Moina* is the development of feed. The algal feed, particularly freshwater *chlorella* sp. has to be produced in mass scale. Stock culture can be inoculated to mass culture tank containing filtered freshwater. Enough algal culture has to be added daily to these tanks till a dense concentration of *Moina* is attained. An alternate method is to culture the algae using fertilizers like groundnut oil cake(250

gm.), urea(10 gm.) and super phosphate(10 gm.) per tonne of water and when algal concentration attained maximum , stock culture of *Moina* is introduced to the same tank. An advantage of this method is that daily addition of algal feed is not necessary ; thereby labour can be reduced. Vigorous aeration should be provided especially in mass culture tanks for better results.

Females reproduces rapidly and attains maximum concentration within a few days. At this stage the required amount can be harvested using suitable sieves , washed and the concentrated *Moina* can be offered as feed for the larvae. The rest of the quantity remained in the container will act as the inoculum for the next culture. By diluting with filtered freshwater and offering enough algal feed, again *Moina* can be harvested and this method can easily be repeated to have stable supply of this live feed. The excess amount harvested can be stored in deep freezer after mixing with glycerine and this can be utilised in occasions where unforeseen excess demand exists for this live feed.

Under stressful conditions like high density of population,low oxygen level,wide fluctuations in temperature and scarcity of food; the resting eggs will be produced and this can be washed, dried and stored. When the cysts are exposed to favourable culture conditions like hydration, illumination and aeration it will hatch out and give rise to young ones.The production of resting eggs and hatching procedure can be conveniently applied in hatcheries to meet unexpected demand of this live feed.

As it is a freshwater species and because of its low content of essential fatty acids, *Moina* sp. has to be enriched before offering as live feed to fish and crustacean larvae. The same methods of enrichment described in the case of rotifers can be applied for *Moina* sp. also.

***Daphnia* sp.**

Daphnia sp. is a frequently used food source in freshwater larviculture activities. It is available in stagnant water bodies and isolation can be carried out by micropipette method from a zooplankton sample.

Culture method: Development of stock as well as mass culture, harvesting, storing and enrichment of *Daphnia* sp. can be carried out adopting similar procedures as in the case of *Moina* sp..

3.*Artemia salina*

Globally accepted live feed, *Artemia salina* , popularly known as brine shrimp are found in salt pans,lakes or evaporation ponds at salinities from 100 ppt onwards.Different geographical strains are available. Over 300 natural biotopes, spread over the 5 continents have been identified. It is characterized by an elongated body with 2 stalked complex eyes in the head region, 11 pairs of thoracic appendages and an abdomen that ends in a furca covered with spines.

The presence of brine shrimp favour the salt production qualitatively, as it removes algae and impurities from the medium. Also, the salt production will be faster, due to better heat-absorption by the dark red colour developed by the halophilic bacteria which develops in the medium.

Hatching of cysts:*Artemia* nauplii is a widely accepted live feed for the advanced stages of fish and crustacean larvae; and it can be made available by hatching of their cysts. *Artemia* cysts are commercially available in dried form and it can be stored in anaerobic condition for more than a year.When the cysts are transferred to filtered seawater under illumination and vigorous aeration,the biconcave cysts become spherical;after about 24 hours the cyst shell bursts and within a short period of time the free swimming nauplius will be developed. Empty cyst shells and unhatched cysts will be present along with nauplii. From this, nauplii can be separated using its positive phototactic nature. Care should be taken to ensure that no empty shells are added to larval rearing tanks along with nauplii; because, consumption of empty shells may block the gut of the larvae.

Decapsulation of the cysts:A complete separation of *Artemia* nauplii from their empty cyst shells is a difficult task and always it may not be successful also. The presence of empty shells is harmful for the larvae too. A method is available to overcome this situation. The hard shell or chorion of cysts will be removed without affecting the viability of the embryos by short exposure of the hydrated cysts to a hypochlorite solution, a process that is called cyst decapsulation. When the cysts are exposed to hypochlorite solution, the hard shell of the cyst dissolve, and a gradual colour change from dark brown to grey and then to orange can be observed. Since it is an exothermic oxidation reaction, temperature has to be checked at regular intervals and ice has to be added in order to prevent the rise in temperature

above the lethal level of 40°C. The decapsulated cysts can be filtered out, washed, dipped in 0.1N HCl , washed and can be fed to the larvae or can be stored in refrigerator for a few days.

The quality of the *Artemia* nauplii can be enhanced by enrichment diets such as microalgae or oil emulsions. The advantage with *Artemia* nauplii is that it can be made ready as and when required.

Mass culture: Mass production of *Artemia* can be carried out in large fibre glass tanks containing filtered seawater. Introduce *Artemia* nauplii into these tanks, provide with vigorous aeration and add sufficient quantity of algal food.The nauplii moults and passes through different larval stages and feeds on algae, bacteria and detritus which ranges in size from 1 to 40 microns and becomes adult . Both sexual as well as parthenogenetical strains are available. As a result of sexual reproduction, fertilized eggs will be formed and develop into nauplii(ovoviviparous mode of reproduction) and grow; and under stressful conditions cysts (oviparous mode of reproduction) will be formed. Brine shrimp survive for many months, grow from nauplius to adult in less than 2 weeks time. Harvesting can be performed from the water surface , when there is no aeration.

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