Live feed culture and larval rearing of marine finfishes

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Introduction

The marine fish larvae are generally classified into altricial and precocial types. The altrial type of larvae are having very less yolk reserves at hatching and hence, the larvae are in an undeveloped stage when the yolk sac is completely exhausted. The development of digestive system is also very primitive in these types of larvae. On the contrary larvae of precocial species hatch in an advanced stage of development. Many of our suitable species of marine fin fishes for aquaculture are characterized by having the altrial type of larvae which poses challenges in their larviculture. When the yolk reserves are fully exhausted, the larval size and mouth gape are very small and the perceptive powers for searching and taking external feed is also very less. The period when the yolk reserves are fully exhausted and larvae need to resort to exogenous feeding is a critical period in the larviculture of most marine fin fishes. Unless proper live feeds of required size are provided in sufficient densities in the larviculture media and its nutritional requirements especially in terms of PUFA are met, large scale mortality is bound to happen at this stage. Hence it is evident that the larviculture of marine finfish having altrial larvae is really challenging and proper management of live feed is the most vital pre-requisite for the success in terms of survival and growth of the larvae.

Live feed culture

Live feeds are the main items in the diet of cultured fish larvae and they are of particular importance when rearing marine fish larvae of the altrial type. The low digestive capacity of the altrial larvae might not be the only aspect responsible for them requiring live feeds. Live preys are able to swim in the water column and are thus constantly available to the larvae. Most formulated diets tend to aggregate on the water surface or sink within a few minutes to the bottom and are thus normally less available to the larvae than live feeds. In addition, since larvae are visual feeders adapted to attack moving prey in nature, the movement of live feed in the water is likely to stimulate larval feeding responses. Live prey with a thin exoskeleton and high water content may be more palatable to the larvae once taken into mouth, compared with the hard, dry formulated diet. The availability of appropriate live feed is the prime requisite for the successful larviculture of marine finfishes. The chief live feeds employed are (a) rotifers (b) copepods (c) Artemia nauplii. Microalgae form the basic requirement for live feed culture and hence microalgal culture is the first step in live feed production.

Microalgae

Microalgae constitute the first link in the oceanic food chain. Nearly 16 genera of microalgae are commonly employed for aquaculture purposes. They are generally free living, pelagic and in the nannoplankton range (2-20μm). In aquaculture, microalgae are produced as a direct food source for various filter feeding larval stages of organisms. They are also used as a direct food source in the production of rotifers, Artemia and copepods which in turn are used as food for the carnivorous larvae of many of the marine fish species. For rearing marine fish larvae according to the 'green water technique' microalgae are used directly in the larval tanks. This technique is nowadays a normal procedure in marine larviculture and is reported to improve fish larval growth, survival and feed ingestion. The role of microalgae in the rearing water is attributed to (i) providing of nutrients directly to the larvae (ii) contributing to the preservation of live prey nutritional quality (iii) promoting changes in the visual contrast of the medium and its chemical composition and (iv) playing an important role in the microflora diversification of larval gut.

Growth dynamics

A basic understanding of the algal growth dynamics is necessary to carry out their culture. An algal culture goes through the following phases

Lag or induction phase in which there is no increase in cell numbers

Exponential phase in which cell multiplication is rapid.

Declining Phase in which the growth and multiplication of cells will be arrested and slowly the cells show the symptom of decline.

Stationary Phase in which the culture will be stationary without any further cell division for a few days. In the stationary phase if the cells get a new environment, they may start further growth and reproduction.

Death Phase in which the cells will lose its viability and start dying. At this stage the culture will become useless either for re-culturing or for feeding.

Culture methods

The following are the steps involved in micro algal culture

i. Preparation of culture media

ii. Identification and isolation of the required species

iii. Stock and working culture maintenance

iv. Mass culture

Preparation of Media: Culture media mostly consists of nitrates and phosphates in the ratio 10 : 1 (N : P) besides trace metals and vitamins. Silicate is essential for culturing diatoms, as they have siliceous cell walls. The composition of the two commonly used media viz. Miquel's medium and Conway or Walne's medium is given below:

Miquel's Medium

A. Potassium nitrate - 20.2 g

Distilled water - 100ml

B. Sodium orthophosphate - 4 g

Calcium chloride - 2 g

Ferric chloride - 2 g

Hydrochloric acid - 2 ml

Distilled water - 100 ml

0.55 ml of A and 0.50ml of B are added to one litre of filtered and sterilized seawater.

Conway or Walne's Medium

A. Potassium nitrate - 100 g

Sodium orthophosphate - 20g

EDTA (Na) - 45 g

Boric acid - 33.4 g

Ferric Chloride - 1.3 g

Manganese chloride - 0.36 g

Distilled water - 1 litre

B. Zinc chloride - 4.2 g

Cobalt chloride - 4 g

Copper sulphate - 4 g

Ammonium molybdate - 1.8 g

Distilled water - 1 litre

C. Vitamin B1 (Thiamin) - 200 mg in 100 ml distilled water

Vitamin B12 (Cyanocobalamine) - 10 mg in 100 ml distilled water

Prepare A , B and C in different reagent bottles. Add 1ml of A , 0.5ml of B and 0.1ml of C to one litre of filtered and sterilized seawater.

Equipments and Glasswares: For identification of microalgae as well as for the determination of cell concentration of the culture, a powerful microscope is necessary. For stock culture maintenance the glasswares required are micropipettes, droppers, reagent bottles, culture tubes, conical flasks, Haufkin culture flasks, haemocytometer etc. For mass culture 10 litre polythene bags, 20 litre glass carboys, 100 litre Perspex tanks and 250 litre cylindrical transparent FRP tanks are used for the indoor culture while 250 litre , 500 litre and one tonne fiberglass tanks and 5 tonne concrete tanks are used for the outdoor culture of micro algae.

Isolation of algal species: Twenty litres of water is collected from the water body and enriched with nutrients and left under light until algal bloom occurs. The nutrient added for enrichment should be appropriate to the species required to be isolated. The isolation of a single algal cell from the bloom can be accomplished by any one of the following methods:

1.Simple capillary pipette isolation Method: The mixed plankton sample is kept in a petridish under a binocular microscope. The desired species is isolated using a capillary pipette and transferred to culture tubes having suitable sterile culture medium.

2. Centrifuging method: By repeated centrifuging the water samples and then by inoculating the deposits, we can isolate several microalgae.

3. Serial dilution Method: This method is used mainly for the isolation of phytoflagellates(i.e. motile species). This involves systematic dilution of the inoculaum in five stages (1, 10-1, 10-2, 10-3, 10-4 or 4 steps 0.001, 0.01, 0.1 and 1ml) so that the subject species is well separated from any contaminant. The species thus isolated is transferred to the culture tubes.

4. Agar plating Method: Agar medium is prepared by adding 1.5 gm of agar to one litre of suitable culture medium. This agar medium is sterilized in an autoclave for fifteen minutes under 120 lbs pressure and 100ºC temperature. Now the medium is poured in sterilized 15 cm petri dishes and kept for 24 hrs. The required species can be picked by platinum needle or loop under microscope and streaked on the surface of agar plate. After inoculation, these petridishes are placed in an incubation chamber for 7-8 days providing light (1000 lux) and constant temperature (25ºC). Within this time , the required species , if it has grown into a colony is removed by platinum loop under microscope and transferred to culture tubes. Further from the culture tubes to small conical flasks and larger flasks, the algae can be grown on a mass scale.

Stock culture Maintenance: The pure culture (0.1ml) isolated from the mixed culture is inoculated into 20 ml culture tubes or 50 ml culture flasks filled with enriched water and incubated in light intensity of 1000 lux (2 tube lights) with photoperiod of 12,hours to produce one million cells/ml. This forms the stock or starter culture for mass culture and thus can be maintained for 15 days. The above procedure should be repeated every 15 days in order to maintain the vigour of the culture.

Working culture maintenance: Some of the 50 ml flasks containing the starter culture are used for inoculating 250 ml flasks. After two days, culture in 250 ml flasks are transferred to 2 litre flasks with enriched water and incubated in light (1000 lux) with aeration for two days to get a density of three million cells/ml. This again is inoculated into 20 litre carboys with enriched water to get three million cells/ml density.

Mass culture: Large scale outdoor culture of microalgae required for hatcheries can be carried out economically by enriching with the following ingredients

Ground nut oil cake -250 gm/tonne
Urea - 10 gm/tonne
Superphospahate - 5 gm/tone

Soak the groundnut oil cake in water, then thoroughly smash the same to obtain a milky suspension which can be filtered through a cloth to remove larger sediments. The milky filtered suspension along with the inorganic nutrients (urea and superphospahate) is added to enrich the water. The required inoculum for mass culture is added and kept under sunlight. The two methods of mass culture commonly employed are – batch culture and semicontinuous culture.

Batch culture: In this method the entire culture is harvested when the cell density reaches the desired level. Then the culture tank is filled with enriched water and the required inoculum is added. When the cell density reaches the desired level the entire culture is harvested. Batch culture method is the most reliable method, but it is labour intensive.

Semicontinuous culture: Here the microalgae are allowed to grow until a certain cell density is reached. Then it is partially harvested and fresh medium is added. The growth and harvest procedures are repeated several times before the water quality mandates that the tank be drained and cleaned. It involves less labour but is a less reliable method.

Counting of Micro algal cell density: The apparatus used for counting cells is a haemocytometer with an improved Neubauer ruling. Before counting, both the cover slip and chamber must be rinsed clean and dried. The face of the counting chamber is composed of two gridded surfaces separated by canals. The cover slip is placed on the support bars along the canals and a drop of homogeneously mixed algae suspension is delivered from a Pasteur pipette by touching the pipette tip to the edge of the cover slip where it hangs over the V-shaped loading port. Slight pressure will cause the algal suspension to flow evenly across the surface, but not into the canals or on top of the cover slip.

A small drop of 5 to 10% formalin mixed into

the sample is sufficient to immobilize cells for counting. Each half of the haemocytometer contains nine large grids. Only those algal cells which fall within the four large corner grids are counted. Each large corner grid is further subdivided into 16 small squares. Moving systematically back and forth across the squares, a minimum of 200 algal cells are counted in as many grids as necessary . To determine the algal cell density (number of algal cells per milli litre) in the suspension, the number of algal cells counted is divided by the large corner grid area covered, multiplied by 10,000. For example, if 300 algal cells were counted in 1.5 large corner grids (or 24 small squares), the cell density is 300 algal cells/1.5 corner grids x 10,000 = 2 x 10 6 cells per ml.

Whenever microalgae are used as a direct food source or as an indirect food source in the production of rotifers, Artemia or copepods, growth of the animals is usually superior when a mixture of several microalgal species is used. This probably occurs as different species compensate one another for eventual deficiencies in given nutrients. Special care is needed when selecting microalgae for on growing live feeds for marine fish larvae, in order to avoid the nutritional deficiencies of the latter especially in terms of n-3 highly unsaturated fatty acids. Deficiencies in the n-3 PUFA contents of microalgae may cause severe mortalities and quality problems in marine fish larvae. Such deficiencies may also cause reduced fecundity of rotifer and copepod cultures. Microalgae like Chlorella sp., Nannochloropsis sp., Tetraselmis sp., Dunaliella sp., and Pavolova sp., Isochrysis sp. can be used as algal diet for growing the rotifers. The size, nutritive value, proliferation rate and digestibility of the algae are the critical factors for selecting the algae for the use in marine hatchery.

Recently commercial microalgal products are developed which can also be effectively employed for larviculture. These include microalgae concentrates, frozen and freeze dried microalgae and microalgal pastes. Results of these products are generally good.

Precautions for maintaining culture asepsis and to prevent contamination are very much needed. All fluids and surface that come into contact with the culture must be sterilized. Natural water used for the culture should be free of pollution and stable in quality. A sand filter reduces the sizes of suspended particles to 10-20 μm, thereby eliminating most of the zooplankton. Additional filtration by cartridges or sieves reduces particle size to 1 μm. Chemical sterilization such as chlorination – dechlorination and by activated carbon can also be practiced. Natural filtered water is enriched by the addition of mineral salts required for photosynthesis, i.e. metabolisable nitrogen, phosphorus and trace elements including iron and silicon for diatoms. A chelating agent EDTA is often added to prevent precipitation of ferric hydroxide. Vitamins such as thiamine or biotin should be added with due caution because of their rapid degradation due to heat. These salts constitute the enrichment media, the most commonly used being f/2 medium and the medium of Conway .Temperature is often controlled between 18 and 25ºC, but this should be fine tuned to each species.

Batch cultures are generally run according to production cycles of 3-7 days. Once illuminated tanks have been cleaned and filled with filtered sterilized water, enrichment medium is added and aeration is provided, and an inoculum is introduced. The algal strains are provided in few milliliters of culture in a test tube. Starting from this sample successive volumes of increasing size are inoculated in order to prepare the biomass required to reach inoculum concentration in the production tanks. The cultures obtained in hatcheries seldom exceed a density of 6 x 106 cells ml-1 at the end of 5 days. The costs of producing microalgae in hatcheries include labour (90%) amortisation (6%), energy (3%) and miscellaneous expenses (1%). In Industrial facilities specialized in the production of microalgae and exploiting production system in controlled conditions such as photobioreactrors, the cost of production can be reduced considerably.

When phytoplankton was included in larval rearing tanks, the survival, growth and food conversion index of many marine finfish species were better than in clear water condition. The green water technique (larviculture in an endogenous bloom of phytoplankton and rotifers) and the 'pseudo green water technique' (larviculture in a tank supplemented daily with exogenous phytoplankton and rotifers) have much commercial application in marine finfish larviculture. Micro algae can also influence live feed and larval microbiology. It has been found that exudates of some algal species can either enhance or inhibit

the feeding activity of copepods in cultures .These substances are also involved in the settlement of micro flora required in the gut of fish larvae to prevent intestinal opportunistic bacteria from causing disease. Bacteria associated with live feed can be transmitted to larval fish during feeding. As live prey actively ingest bacteria, it is possible to introduce favourable bacteria as probiotic. In the 'green water technique' of larviculture micro algae contribute to maintaining the nutritional quality of live food and also positively influence on the settlement of a healthy intestinal micro flora in fish larvae. Micro algae can also possibly influence the endotrophic stages (egg and pre–larvae) and early exotrophic stages. Micro algal background has an important effect on the timing and intensity of first zooplanktonic feeding. Micro algae also play a role in intestinal transit and gut repletion. Improvement in the survival at first feeding is the main result of larviculture with micro algae. Improvement in growth efficiency during rotifer period is another result of micro algal background in larval tanks. Early enhancement of digestive and assimilative functions improves the survival and growth of fish larvae and favours the transition to exotrophy. The use of micro algae in tanks increases the production of pancreatic and intestinal digestive enzymes and improves the quality of gut flora. Even after the endo-exotrophic phase, micro algae have a positive effect on larviculture and may increase the resistance of larvae to further stressing or adaptive conditions. The indirect effects of micro algae on larvae are mainly related to water quality, luminosity, the bacteriology of water and the quality and accessibility of rotifers. It is thus evident that strategic use of micro algae in hatcheries during the very early life of marine fish improves the success of first feeding, a prerequisite for efficient survival, growth and quality in fish larviculture.

Rotifers

Rotifers have been used as live feed for cultured marine fish, since four decades. It is well known that a continuous, stable and reliable supply of nutritionally adequate rotifers is the key to the larviculture of marine finfish. Rotifers of the species Brachiounus rotundiformis and B. plicatilis are almost indispensable for larval rearing of most marine finfish .

The success of rotifer cultivation is dependent on selecting the most suitable rotifer species or strain for local culture conditions, maintaining water quality in culture tanks and choosing the most appropriate culture technique. Size, the type of reproduction and reproductive rates are species or strain specific. Culture temperatures, salinities, type of food and its quality - all influence the type of reproduction and its rates. Mass production of rotifers is achieved by encouraging rotifers to reproduce asexually, since sexual reproduction results in males and resting eggs. The amount of food that has to be supplied daily to each tank depends on the reproductive rate of rotifers. Usually 1-4 g of baker's yeast is supplied per million rotifers per day.

The optimal range of pH for culturing rotifers is 7.5 – 8.5 and the pH affects the percentage of unionized ammonia in the water. The pH of cultures play an important role since the toxicity of NH3-N is a function of pH, temperature and salinity. The optimal level for ammonia is <1 mg l-1 and the acceptable level of ammonia and nitrite levels is 6-10 mg l-1. Rotifer cultures require aeration and the dissolved oxygen level should be maintained above 4 ppm . Surplus food is one of the major factors for the deterioration of water quality. This can be avoided by dividing the daily food ration into four to six meals a day or by continuous feeding using a peristaltic pump.

Stock cultures of rotifers are maintained for long periods which facilitate their availability to mass culture wherever they are needed. Natural seawater should be filtered through a 1.0 μm bag filter and heat sterilized at 1200C at 15psi atmospheric pressure for 30 minutes to avoid fermentation of insoluble precipitates. The cool sterile seawater can be employed for stock culture. Erlenmeyer flasks (100 ml in volume) or 50 ml sterile disposable tubes can be used for culture. Each heat sterilized flask is filled with 10 – 20 ml sterile sea water and 40 – 60 rotifers are added. Usually, a salinity of 30ppt is suitable for most strains. A drop of concentrated algae is added to each culture and the flasks or tubes are incubated at temperatures ranging from 20 – 350c. The cultures are fed ad libitum every 2 days with concentrated algae. Cultures are renewed every 7-10 days. Culture of Nannochloropsis sp was found to be the most convenient source of food for rotifer cultures. As in the case of microalgae, mass cultures are done by batch, semi continuous and

continuous culture methods.

Evaluating the physiological state of rotifer culture is very important in hatcheries since larval production depends on a predictable and reliable supply of rotifers. Six parameters viz egg ratio, swimming velocity, ingestion rate, viscosity, enzyme activity and diseases are employed for assessing the state of health of rotifer cultures.

The nutritional quality of rotifers is improved by enrichment, in which rotifers are collected or harvested from culture tanks into containers where they are kept at very high densities and incubated for 8 – 20 hours with enrichment dietary components like HUFA. In addition to nutritional enrichment, rotifers can be enriched with antibiotics or with probiotic bacteria. The nutritional value of rotifers depends on their dry weight, caloric value and chemical composition .The number of rotifers consumed by the larvae determines the quantity of food reaching their gut. In red sea bream, the number of rotifers consumed daily increases with size or age of the larva, 55 – 72 rotifers per 3.9 mm length larvae to 4700 per 11.4mm length larva .

Various methods of storing rotifers have been studied. Frozen rotifers are not usually adequate as feed because of leaching of nutrients. Live B .plicatilis can be stored at 40C at relatively high densities for at least one month . Rotifers can be kept at -10 C without feeding or water exchange for about 2 weeks .B. rotundiformis strains are less tolerant to 40 C than B .plicatilis rotifer strains and the strains known as SS type are most susceptible and showed lowest survival. Amictic eggs of rotifers can be preserved by cryopreservation in liquid nitrogen after they have been impregnated with cryoprotective agents like dimethyl sulfoxide (DMSO). This method ensures full preservation of genetic traits of importance to aquaculture. Cryopreservation is not a suitable method for preservation of large numbers of rotifers for direct use as feed.

Artificially produced rotifer eggs have been tried as an alternative to daily production of rotifers. The production of these eggs can be manipulated by environmental factors, such as salinity, food quality and quantity, rotifer culture density, exchange of culture media and temperature and varies between B. plicatilis and B. rotundiformis. The cost of producing resting eggs is very high and therefore not yet been extensively adapted in hatcheries.

It is evident that rotifer cultures will continue to be indispensable in marine finfish hatcheries. Current methodologies of producing and enriching rotifers are meeting the requirements of the industry. The current need to have very small sized rotifers is difficult to achieve, although several super small strains have been found and cultured. Improved methods for predicting the health of cultured rotifers may be useful in preventing culture crashes). Using preserved rotifers may eliminate the dependence on daily production of rotifers. Cheaper methods of resting egg production are another field which requires research attention in future.

Copepods

Copepods are a major component of the natural diet of marine fish larvae. The advantages of copepods over rotifers are that copepods have wide range of body sizes both within and between species. The early stage nauplii and copepodites can be extremely useful as initial prey for species that have very small larvae with small mouth gape at first feeding.

In extensive methods copepods are collected from nature and inoculated into outdoor tanks to mass produce for fish larval rearing. The larvae are then transferred at densities of 0.01 to 0.321-1. Additional prey may be added during the larval rearing when necessary to maintain prey densities in the range of 200 – 500 l-1. By this method from 1986 to1994 a total of around 2 million juvenile cod was produced .Disadvantages of this system include the inability to control production and thus food levels and predators. Lack of food results in differential growth in fish larvae.
Outdoor production of copepods in ponds or large tanks of 350 – 5000m3 is carried out in Europe and Asia for cod, grouper and flatfish. Filtered seawater by using filters of around 20 – 40 μm is generally used in these systems. Phytoplankton bloom can be induced by application of commercial fertilizers. Filtering devices that allow for selective sieving are used to collect primarily nauplii (80 – 250 μm) and copepodite stages (80 – 600 μm) to inoculate the rearing tanks. A mesh size of 400 – 600 μm was used to inoculate outdoor tanks for grouper rearing with copepodites and adult stages 3 days before stocking the

newly hatched fish larvae at densities of 5 m-3. In this system, using wild harvested copepods, an average survival of 3.4% at harvest correspond to an average production of 0.17 grouper Epinephelus coioides juveniles m-3. Regular monitoring of densities of live prey in these outdoor systems is important for the successful rearing of marine fish larvae. An advantage of outdoor ponds over the extensive systems that rely on the local production of zooplankton is the possibility of culturing the zooplankton over one generation before using them as food. Moreover, feeding wild plankton directly to the fish increases the risk of infections. Several attempts to mass culture copepods in intensive systems have been undertaken with varying success. Species with relatively short generation at ambient temperatures are best suited for aquaculture purposes. Species inhabiting in coastal environments are normally more tolerant to variations in salinity and temperature and have a wider thermal and salinity tolerance. The most frequently cultured calanoid species belong to the genera found in coastal waters, such as those of genera Acartia, Centropages, Eurytemora and Temora. These copepods are small, with relatively short generation time and a wide thermal and salinity tolerance and are easily adaptable to laboratory conditions. Aeration is required to maintain phytoplankton in suspension and to create small turbulence which helps to distribute copepods within the culture tanks. Most calanoids require large volumes and the adult density rarely exceeds 100 per litre. Successful hatch culture of the calanoid Acartia sp was achieved in 1000 litre polyethylene tanks, 1.3m in diameter with a conical base. The tanks are emptied after the 8 day hatch cycle and cleaned and a new batch culture was started . Contamination of copepod culture by bacterial blooms, ciliate infection, other copepods or rotifers may pose a problem. In commercial facilities, contamination by rotifers is most likely to cause the collapse of copepod culture, since the rotifers with their higher reproductive rate would quickly out compete the copepods. Hence these cultures should be strictly kept apart.

Ciliates are utilized by copepods and in periods of low phytoplankton concentration constitute the major dietary source. Ciliates are often an indication of overfeeding and if ciliates are noted in cultures it is advisable to empty the culture using a 60 or 80 μm mesh, which retains the adult copepods, but allows the ciliates to be washed out. Harpacticoid copepods have several advantages for culturing .They include (i) High tolerance to a wide range of environmental conditions. (ii) Ability to feed on a wide range of live or inert diets. (iii) High reproduction capacity. (iv) Relatively short life cycles (v) Ability to be cultured in high densities. (vi) Requirement for surface area rather than volume (vii) Planktonic naupliar stages (viii) Can be used as tank cleaners in rotifer cultures, other copepod culture or larval tanks.

Filtered seawater can be used for harpacticoid culture and most feeds are acceptable to many harpacticoid species. Algae which quickly sediment are also good feed because bacteria colonise these cells, and the mixture of algae and bacteria form a good dietary combination for harpacticoids. Photoperiod influence offspring production and sex ratio. A photoperiod of 12 L / 12 D was shown to be most favourable for offspring production. Many harpacticoids have wide thermal and salinity tolerances. Ciliates and rotifers in the culture tanks compete for food and may lead to crash of copepod culture.

Improved growth, survival and / or rates of normal pigmentation have been documented for several marine fish species fed copepods alone or as a supplement to other traditional live feeds. The improvements in larval growth, survival and normal pigmentation are generally attributed to the levels of DHA, EPA and / or arachidonic acid (ARA) in the diet and in particular to the DHA: EPA ratio in the diet. Copepods which constitute the major diet for marine fish larvae in nature contain high levels of DHA and other PUFA. DHA levels in wild copepods can be more than 10 times higher than in enriched Artemia.

The interest in copepod culture as live feed is gaining momentum in recent years for the rearing of altricial larvae. A few of the culture methods developed to date can be adapted in commercial hatcheries. However there is a need to evolve intensive culture methods for copepods in future. It is felt that the future expansion of mariculture especially of marine finfish depends largely on the development production of resting eggs of copepods on commercial scale.

Artemia

Artemia is widely used in the mass culture of

different sea bream species, sea bass species, wolf fish, cod, turbot, halibut, flounder species, milk fish, surgeon and many shrimps, prawn, crabs and lobsters. Nauplii in instar I and II stages are the most widely used forms of Artemia in aquaculture. They are the earliest and easiest live feed, being obtained directly from the cysts.

Several factors are critical for the successful hatching of Artemia cysts. Optimal hatching conditions are constant temperature, 15-35 ppt salinity, pH around 8.0. Minimum oxygen levels of 2 mgl-1, preferably 5 mg l -1, maximum cyst densities of 2 gl-1 , and strong illumination (2000 lux). Best hatching results are achieved in containers with conical bottom, aerated from the bottom. Transparent or translucent containers will facilitate inspection of hatching, especially when harvesting.

Strong illumination (above 2000 lux at the water surface) is essential, at least during the few hours after complete hydration, to trigger the start of embryonic development. It is advisable to keep the hatching tanks indoors and to provide artificial illumination, so as to ensure good standardisation of the hatching process. When hatching large quantity of cyst, bacterial load rapidly develops. Reducing bacterial development during hatching will improve the hygienic status of nauplii and may result in better hatching. It can be achieved through simple disinfection of the cyst using the liquid bleach solution, through decapsulation. Attention should be paid to the selection of Artemia cyst batches with good hatching synchrony (less than 7h between hatching of first and last nauplii) and high hatching efficiency (more than 2 lakhs nauplii per gram).

After hatching and before feeding to fish larvae, the nauplii should be separated from the hatching wastes (empty cyst shells, unhatched cyst, debris, microorganism and hatching metabolites). Decapsulation of cysts results in disinfection of the cysts and also eliminates the introduction of cyst shells to culture tanks.

Most marine fish larvae cannot synthesise DHA, EPA or Arachidonic acid from shorter chain precursors and they must be provided in the larval diet, hence Artemia is enriched for enhancing the nutritional value for using as a live feed. Although Artemia is often an inferior food source for fish larvae compared with wild zooplankton, the ability to produce any amount of biomass within 24 hrs, and the constant improvement of enrichment products ensure its continued use in marine fish larviculture. It is quite possible that Artemia will gradually be replaced by formulated diets; it is obvious that the use of nauplii will continue in hatcheries at least for a few more years.

Larval feeding behavior

After egg hatching, fish larvae go through important changes to reach the juvenile stage, the most evident being a dramatic biomass increase. Feeding success in fish larvae is critical for obtaining the nutrients and the energy necessary for healthy growth and development that allows them to survive to the end of the larval period. Feeding behavior is the result of interaction of complex processes viz. searching, detection, attack, capture, ingestion, digestion and evacuation. Each of them has a specific pattern that changes throughout development. The feeding strategy is related to the specific characteristics of each species. Availability of suitable prey is one of the most determinant biotic factors, but feeding mode and amount of food intake are also influenced by prevailing environmental conditions.

Searching and detecting food

Searching for prey and detecting them depend on the appropriate functioning of some organs and tissues that become progressively available throughout development. From hatching, larvae are progressively aware of different external stimuli that indicate the presence of potential food items. Searching depends basically on swimming capacity, while detection depends largely on sensory organs. Food detection occurs by means of visual, chemical and mechanical stimuli. Olfaction allows detection of distant stimuli, sight allows the identification of objects at medium and relatively short distance , while touch and gestation need very close or direct contact with the source of stimulus. Most marine fish hatch with immature anatomical features. The sensory organs develop quickly during the first days after hatching. Sight allows the larvae to perceive objects that are relatively close. Altritial marine teleosts hatch with undeveloped eyes although the pigment in the retina appears in a few hours or days. This early retina has only one type of photoreceptor that allows vision only under bright light. Double and mosaic cone structures and rod photore-

ceptors appear later and enable vision at low light intensity. Olfaction allows for more remote detection of a stimulus. The olfactory organ appears early during embryonic development. Olfactory placodes and pits are already present at the onset of feeding and develop further by the late larval stage. The intra and extra oral taste buds develop or proliferate some days or weeks after the first feeding. Mechanical stimuli such as touching or water movements are detected by neuromasts and the lateral line system. In larval fish some few free neuromasts are already present at hatching and progressively proliferate during their growth and development. The progressive development and completion of all these sensory organs increase the capacity for detection and recognition of potential prey.

Locomotor capacity

Basically fish larvae exhibit alternating periods of swimming ability and inactivity. Swimming speed, pause duration, reactive distance, perception angles and duration of predation cycle define the changes in behavior during searching and attack throughout development. At first feeding, even the smallest larvae have some primordial hunting habits , but the efficacy increases with development and growth, changing from passive feeding to an active prey searching capacity.

Capture and ingestion

Capture success relies not only on development stage and concomitant hunting capacity but also on the availability and accessibility of prey. Once the prey is perceived, the foraging has three possible results: unsuccessful attacks, aborted attacks and successful attacks. After mouth opening, fish larvae need to learn hunting and have to do it quickly. High prey availability and accessibility are crucial for successfully initiating feeding. Prey size and swimming ability are primary factors determining the efficacy with which the prey is caught. The ability to start feeding after mouth opening is typically affected by prey size. During the very early stages with low swimming capacity, encounter opportunity depends on prey density. Mouth gape limits the dimensions of the prey that can be ingested. Prey/gape ratio determined in different species usually ranges between 25 and 60%. Searching for appropriate prey of adequate size has been a priority for rearing fish larvae. The established prey sequencing is based on rotifers of different sizes and Artemia nauplii and meta nauplii. However, there is a need to search for live feeds below 100 micron size for rearing of very small marine fish larvae. Eventhough copepod nauplii can be employed for this purpose, mass scale production of copepod nauplii for large scale larval rearing is a major constraint. Overall , the current commonly used live feeds, Brachionus spp. and Artemia spp. meet well the feeding behavior of larvae except very small larvae at mouth opening.

Factors affecting larviculture

Since most of the larvae are visual feeders providing the required light affect the larval survival. During the critical period, the density of the live feed and its nutritional qualities determine the percentage of the survival of the larvae. The density of the larvae of the concerned species should also be regulated in the larviculture tanks for getting good survival. When changing from smaller size live feed to larger size, co-feeding with both sizes of live feeds is needed for a few days. Weaning to formulated feed has to be done with great care. First feeding of the day can be done with appropriate size formulated feed. Feeding with live feed can be continued till all the larvae are weaned to formulated feed. Different sizes of formulated feeds need to be used as per the mouth size of the larvae. The marine fish larvae exhibit highly differential growth even from very early stages (in the case of cobia, starting from the first week) and hence grading from an early stage is also very much needed for increasing the survival. In addition, variety of other factors such as tank colour, size of the tank, water temperature, water quality, etc., affect the larval survival and growth. From the foregoing, it is clear that the larviculture of marine finfish is highly complicated, unless each and every factor is taken care of, the survival and growth of the larvae will be very meager.