Zooplankton for marine fish larval feed

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Introduction

Larval nutrition is an important part of the hatchery operation. Successful larval rearing primarily depends on the live feed and zooplankton forms the most important component in the live feed. Larval feed should be smaller, easily digestible, rich in nutrients and allow autolysis. Formulated feed may not meet all these requirements and in most of the cases this will reduce the larval survival rate than live food. Moreover, the fish larvae have poor vision and less developed olfactory and digestive organs. The size of feed is important when the mouth size of larvae is concerned. Poor vision, improper digestive system and weaker movements make it difficult for the larvae to get proper nourishment. Some fish larvae (precautionary type) are with good yolk reserve and they start feeding at a comparatively developed stage while some others (atresial type) start feeding at smaller size and lesser developed stage. Salmon, cobia and clown fish larvae are comparatively larger than grouper and damsel larvae. Live feed in continuous movement in the water will help the weaker larvae to prey upon these tiny organisms. Copepods, cladocerans, decapod larvae, rotifers and ciliates are the important zooplankton organisms which form the food of fish larvae in the wild. The most popular zooplankters used for fish larvae are rotifers, artemia and copepods.

Rotifers

Rotifers are larval live feeds used in both marine and freshwater hatcheries. Rotifers are very small organisms mostly ranging from 0.1to 0.5 mm and belong to the Phylum Rotifera. Brachionus plicatilis and B. rotundiformis are the common species used in hatcheries all over the world. Rotifers are filter feeding organisms with high reproduction rate, capable of both sexual and asexual reproduction and can be reared in large densities upto 2000 animals/ml. B. plicatilis and B. rotundiformis have three strains developed for hatchery purpose; i) L type with lorica ranging from 100-340*µ*; ii) S type with a size range of 100-210*µ* and iii) SS type with less than 100*µ* size.

Ideal water quality parameters for maintaining successful mass production of B. plicatilis are -salinity below 35 ppt, temperature 20- 28ºC, dissolved oxygen above 3 mg/l, pH above 7.5 and ammonia below 1 mg/l. Major contaminants in the culture are ciliates and bacteria. Bacterial load especially of Vibrio sp. should be below 107 CFU/ml. The culture should be free from ciliates like Uronema sp and Euplotes sp. In case of severe contamination, washing through a flow through system with 50*µ* mesh plankton net can regain the pure culture. Intensive indoor culture is mainly by batch-culture using microalgae as feed.

Mass cultures of rotifers always carry some risks of sudden mortality. Hence it is ideal to maintain stock culture separately under aseptic conditions. Rotifers for starter culture can be collected from the wild and isolated through a series of antibiotic treatments and purified culture without any contamination can be prepared. It is always easy to start the culture by taking a small sample from a well maintained stock culture in a hatchery or from a laboratory. All the culture tubes and filters should be properly sterilised before going for stock culture. The stock should be maintained at 28ºC with proper illumination of approximately 3000 lux using Chlorella as feed. It is ideal to add fresh algal culture on daily basis to these tubes. It is better if all the culture tubes are placed on a gentle shaker or a rotating shaft for providing sufficient oxygen. Ideally this should be maintained at a density of 2 rotifers/ml upto 200 nos/ml. The stock culture should be periodically re-cultured and disinfected using mild antibiotic as and when it requires. Once the density reaches around 200 nos/ml, this can be transferred to Erlenmeyer's flasks of 500 ml capacity with an algal concentration of 1.6 x 106 cells/ml. Approximately 50 ml of the algae should be added daily and no aeration is required during this short rearing period. The concentration will reach 200-300 cells/ ml within 3 days period and now the culture is ready for inoculation to 15 l bottles. The culture should be passed through first strainer of 200*µ* mesh and then strained using 50*µ* mesh and the filtrate can

be transferred to 15 l bottles with 2 l water and a density of approximately 50 nos/ml for producing starter culture. This stage onwards we should go for aeration. Fresh algae of concentration of 1.6 x 106 cells/ml should be supplied as daily ration. Within 7 days the 15 l bottle will be full and the culture is now ready for mass culture.

The culture can be maintained using fresh algal culture/ commercial algal pastes/ or with baker's yeast/ formulated diets. Ideal formulated diets for rotifers are now available in market and Selco is one such company producing rotifer feeds. For mass production of rotifers, the hatchery should have facility for providing at least any one of the above feeds. Mass culture is generally maintained in large indoor tanks. Continuous harvest is possible if the rotifer reaches a density of 300-500 nos/ml. The rotifers will double its population daily. Different types of sieves/ strainers prepared using 50μ mesh net can be used for filtering the mass culture during harvest. Algal culture should be pumped in the culture tank on a daily basis and enough aeration should be given to maintain the production.

Nutritional value of rotifers mainly depends on the type of feed used. Rotifer cultured using Tetraselmis, Nannochloropsis or Isochrysis or a mixture of these will be higher in DHA and PUFA content than that cultured using Chlorella. Several commercial products are also available for enrichment of rotifers. Use of enriched rotifer for feeding larvae is essential for better larval survival. Harvested rotifers can be reared separately in water containing enrichment media. Simple enrichment can be done using Tetraselmis, Nannochloropsis or Isochrysis or a mixture of these fed finally for one day to the rotifers. Commercially available enrichment media can be added to the harvested rotifers kept in higher concentrations with minimum water for few hours. The enrichment status can be observed by the colour change of the rotifers used or by biochemical estimation of PUFA levels using a gas vchromatograph. The enriched rotifers can be directly fed to fish larvae.

Brachionus plicatilis (live)

Lorica of B. plicatilis

Artemia

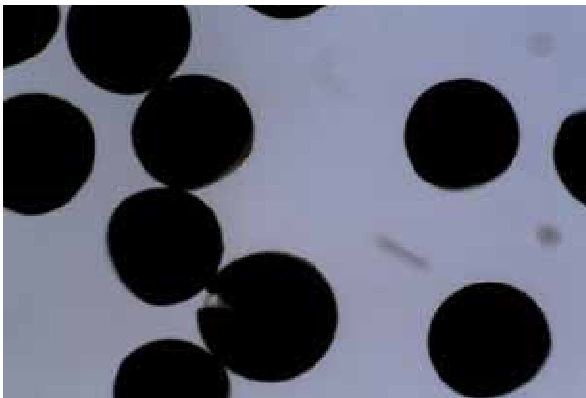
Artemia or the brine shrimp which has the ability to make dormant eggs called cysts is the world's most popular and widely used live feed. The artemia cysts can be stored in dry condition for a longer period and over 200 tonnes of artemia cyst is marketed annually all over the world. Artemia is typically a primitive crustacean belonging to the class Branchiopoda with a total length of about 07-1.2 mm and sexes separate. Artemia are produced in hyper saline ponds and can tolerate wide range of salinity and temperature but with an optimum requirement of 35-38 ppt. Artemia can reproduce parthenogenetically and in adverse conditions, it produce dormant cysts (chorion coated) which can be stored in dry condition without losing its viability for more than 2 years.

In dormant condition artemia cyst is round but concave at one or two sides. On hydration this will become spherical and in less than 24 h hydration and aeration, this hatches out into nauplii. Freshly hatched nauplii is Instar I with a length of 400-500μ and is popularly used for feeding the larvae. Within 7-8 h this will change to Instar II and start feeding on minute algae. The larvae

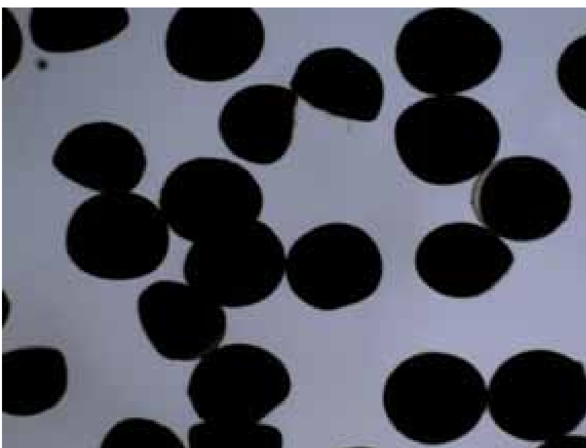
again undergo 13 more moults to become adult artemia. Artemia is a filter feeder mainly feeding on microalgae.

Each gram contains 200000 to 300000 of artemia cysts and almost 50% will hatch within 20-24 h on proper hydration. The artemia cysts must be properly weighed and kept for hydration in normal seawater of salinity not less than 35 ppt. The density can be 2 g/l and the pH should be above 8 and the temperature around 28°C. Strong aeration and illumination (above 2000 lux which can be achieved using fluorescent tubes) are essential for ensuring maximum hatching. Depending on the volume of larval rearing tank and the species under culture, requirement of artemia nauplii should be calculated. Daily measure the artemia nauplii left over in the tank by examining water in the larval rearing tank and back calculate the requirement of nauplii/l and requirement of cysts in g for producing that amount of nauplii.

Artemia nauplii if required in large quantities, it is essential to decapsulate the cysts before hydra-



Artemia cysts before hydration



Artemia cyst after hydration

Artemia Instar I

Artemia Instar II

tion to increase the quality and quantity of hatching. Soak the cyst in 200 ppm sodium hypochlorite solution for 30 minutes and wash thoroughly with tap water using 125μ sieve. Cylindroconical tanks are ideal for hatching and aeration should be from the conical tip of the tank. Remove aeration prior to harvesting of nauplii and since they are phototactic, can be easily aggregated using light.

Artemia cysts can be decapsulated and directly used for feeding the fish larvae or this can be stored at 4oC for 1-2 weeks without losing its viability. Decapsulation process is simple but need constant observation. Sodium hypochlorite solution (0.5g/l) or liquid bleach (5ml/l) are commonly used for decapsulation. Decapsulation process should be monitored properly. Keeping longer duration in bleaching agents will affect the survival. The entire container should be immersed in ice cold water so that temperature inside the container should be below 20oC. The time required for decapsulation process will vary from 5 to 15

minutes. The cysts will turn grey with powder bleach and orange colour with liquid bleach, few samples should be observed using a stereo microscope and if the cyst wall is dissolved, the cysts should be rinsed using 125μ sieve several times in water till there is no trace of chlorine. In order to ensure the removal of chlorine, wash the cysts in 0.1N HCl or 0.1% Sodium thiosulphate solution (Na2S2O3) for one minute. Finally wash through clean filtered seawater and check the water using chlorine test kits and chlorine free cysts can be directly fed to the fish larvae or can be kept for hatching or this can be sieved and stored in refrigerator at 4oC.

Artemia nauplii are nutritionally poor when compared to copepod nauplii and this can be enriched using PUFA and DHA by the same method of enrichment of rotifers. Artemia biomass can be regularly produced using microalgae in tanks with natural sea water. This can be fed by algal paste or fresh algae. All stages of artemia can be cultivated in large scale and can be harvested regularly using normal seawater without much effort.

Copepods

One of the major problems in culture of marine fishes is lacunas in development of complete larval feed. In hatcheries, rotifers and brine shrimps are used as live feed for marine fin fishes during the early life stages. Apart from these organisms, due to the presence of desirable characters such as size and nutritional value, copepods also play a role in larval rearing. Copepods are tiny planktonic crustaceans with more than 10000 species living in a variety of ecological niches. They are a good source of proteins, amino acids, fatty acids, vitamins and minerals and the nauplii are successfully used as first feed for fish larvae in cases where rotifers were inadequate in their nutritional value. Copepods contain the essential unsaturated fatty acids (HUFA) which makes them more appropriate food for fish larvae. Most of the early fish larvae are evolutionarily adapted for feeding on copepods than on other animals.

Copepods are successfully cultured in finfish hatcheries of many countries especially for feeding atresial larvae of certain fishes like groupers. Cultured species includes calanoid as well as harpacticoid copepods. In a study conducted on the wild-caught fish larvae, it was revealed that calanoid copepod nauplii were an essential item in the early feed of many fish species. Calanoids of the genera Acartia and Gladioferens have been proved as important live feed for improving survival of some fish species. Promising species are found in the genera Centropages, Acartia, Labidocera and Eurytemora. On the other hand, Harpacticoid copepods also are a good source of larval and juvenile fish feed in aquaculture. Alone or as a supplement, in many cases harpacticoids have been proved to improve primary growth than rotifers and brine shrimps. The morphological minuteness of harpacticoids enables their feeding by gap-limited fish larvae such as the groupers and snappers. The presence of rich natural fatty acids in it increases the value of copepod in aquaculture. At commercial scale, only a few copepods have been successively reared. Due to several reasons, most of the copepod rearing trials is done in small scale lasting for few weeks or months only. Modern technologies by means of pond or bag culture ; with an input of large quantities of sea water or by the placement of bags of various mesh sizes in open sea, copepod culture can be successfully enhanced.

Due to their compatible size and suitability to culture technologies, harpacticoids seems to be the most suitable one for culture. In natural environment, harpacticoids exhibit a detritivorous behavior and they are adaptable to both formulated artificial feeds and algal cultures and can be grown upto a density of 1,15,000 individuals L-1. Calanoid species such as Acartia tonsa, Calanus hamatus, Eurytemora affinis and Gladioferens imparipes have already being used as larval fish feed. Along with these, symbiotic copepods also have a role in the marine finfish rearing. Copepods of the family Mycolidae like Pseudomyicola spinosus indicates that, this species has a potential for development into a live feed for finfish larvae due to its easy availability, planktonic naupliar larvae, suitability to culture and its property of having maximum fecundity rate. Thus, the naupliar larvae of P. spinosus is largely cultivated and released in a mussel bed to improve its population. Considering their importance mass culture of several copepods are being taken up which are beneficial for aquaculture.

Species popularly cultured

Of the planktonic copepods in estuarine and coastal habitats, calanoids are the most abundant

taxa of pelagic realm forming an extreme connecting link between phytoplankton and fishes in the inshore ecosystem. Among calanoids, the easier one to cultivate is the Acartia spp. than Calanus spp. and Temora spp. Most of the species present in Calanoida are of ≈ 1.0 mm total length, with a size range of 0.4-10.0 mm. Acartia clausi and Calanus finmarchicus are the most widely studied calanoids followed by Temora longicornis, Paracalanus parvis, Calanus helgolandicus, Pseudocalanus elongates, Acartia tonsa, Centropages hamatus, Centropages typicus and Temora stylifera.

Based on studies conducted by the Japanese planktologists and aquaculture scientists on the improvement of copepodal massculture, 13 species were recommended for mass cultivation. These includes Acartia clausi (A. hudsonica or A. omorii), A.longiremis, Eurytemora pacifica, Euterpina acutifrons, Microsetella norvegica, Oithona brevicornis (O. davisae), O. nana, O.similis, Pseudodiaptomus inopinus, P. marinus and Tigriopus japonicas. Among these, T.japonicas is the only one which is produced on a large scale and used in marine fish farming. Maintenance of cultivation has a lot of difficulties including frequent replacement of water, high demand for algal diet as well as low and unstable population growth.

Pseudodiaptomus serricaudatus

Culture methods- Harpacticoids & Calanoids

Copepod culture can be done by continuous as well as batch culture. Normally coastal species with shorter life span and with a wider tolerance to salinity and temperature alterations are preferred for aquaculture. In the late 90's, Taiwan was the first country to start copepod culture. Without water exchange, copepods can be grown in ponds with low salinity. Some culturists are of the opinion that the ponds with clay are more suitable for these planktonic blooms as the beneficial nutrients are available. Even sandy ponds are found suitable as culture field. In routine culture, rotifers can be cultured to a high density of 2000 nos/ml. Many copepods can be cultured commercially at a density of 20-90 nos/ml. These include Paracalanoid spp. Bestolina simili, Parvocalanus crassirostris and a harpacticoid, Eupertina acutifrons. The culture technique of each copepod is different.

More than 60 copepod species have been raised in laboratory. For promoting the culture and improving cost-effectiveness of marine copepods in aquaculture industry, the development of appropriate culture techniques is essential. Copepods can be cultured extensively, intensively and semi-intensively. Copepods can be extensively developed in tanks, outdoor ponds, lagoons or enclosed fjords. By using appropriate mesh sizes these cultured copepods can be made available to fish larvae. Planktonic copepods including Acartia, Centropages and Temora can be cultured in such systems. In extensive systems, culture is done normally on the basis of microalgal booms induced by agricultural fertilizers. Occurrence of parasitic infections on most species is the major problem in extensive copepod production. Due to risk of parasitic transmission harvesting copepods from natural environments is not desirable. As copepods are also natural prey for fish in aquaculture ponds, they can be used semi-intensively on an industrial scale. In intensive culture methods, we can get reliable and sustained production. Long generation-time of most species is the major problem in intensive production of copepods. For intensive mass production, Harpacticoids are recommended as the suitable ones than Calanoids. Harpacticoids are promising species for intensive cultivation due to their tolerance to temperature and salinity, ability to feed on a large amount of live and inert diets and high fecundity rate with relatively short life cycle (5-29 days). They can also be cultured in high densities (exceeding 100,000 individuals per litre) as they possess planktonic nupliar stages and have capability to utilize wastes. But calanoid culture is more beneficial than harpacticoids because of their pelagic

behavior and as they are the most readily available prey to fish larvae. By providing appropriate temperature, sufficient live feed (algae) as well as frequent exchange of seawater with the use of advanced mesh of varying measurements , continuous and a reliable supply of large scale copepods can be got.

Temora turbinata

Temora turbinata, a common calanoid copepod is an important food for many fish species. This species has been reported as an important candidate species for mass culture. T. turbinata has capacity to tolerate wide ranges of environmental conditions. This is a slow moving non-myelinated calanoid copepod species which shows very low escape reflex. Mass culture of T. turbinata has been standardised by CMFRI. The culture started with a few isolated specimens from the plankton sample in a beaker, then to buckets and now in sintex tanks of 1000 liter capacity. Such tanks are maintained in the hatchery. The culture is fed with a mixture of Nannochloropsis and Isochrysis. This species has several advantages over several other species tried here. It has no brood pouch or egg sacs. Eggs are scattered in the culture medium and can be collected easily by collecting the bottom samples. Cannibalism is very rare in all stages of their life. This species is very hardy and can even tolerate ciliate contamination up to certain extent.

T. turbinata takes almost 17-18 days to mature. It has six naupliar stages and 5 copepodite stages. It is difficult to distinguish the sexes. Males have slightly different antennules and caudal ramus, cephalothorax is slightly slender and the fifth leg of male is totally modified. Females are more common in collections.

Collection and Isolation

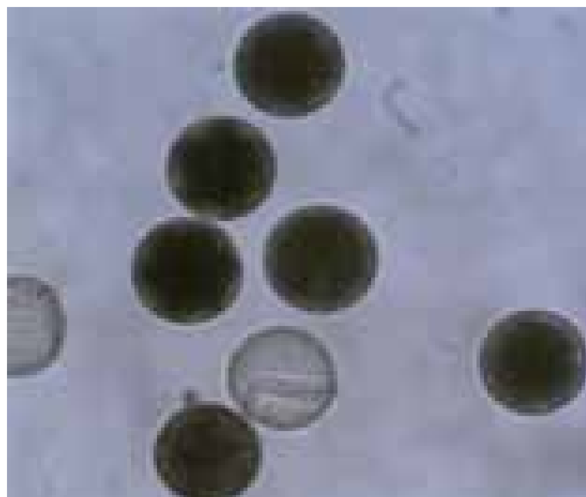
Collection was done using plankton net during early hours and the efficiency of collection procedure mainly depends on the mode, time and location of collection. The marine copepod T. turbinata was collected from plankton collected using 330μm mesh plankton net. At that time, the water temperature was around 24-26c and salinity range was 34-35 ppt. The generation was enhanced by continuous culture maintained in the hatchery for many months with a nutritional supply of I. galbana and N. oculata. From the planktons collected, copepods with similar features were picked up by using a fine dropper under a stereo dissecting microscope. Different species were sorted out and maintained in monoculture for evaluating their adaptability for mass culture. The Calanoid copepod, T. turbinata has satisfied all the sufficient characters for mass culture; with a size ranging from 60 to 440μm, which can be used as a feed for many species of marine fish. This can be cultured up to 4-5nos ml–1 in a temperature range between 27 □C to 32 □C and a salinity of 30-32 ppt.

T. turbinata (male)

T. turbinata (female)

Culture

Culture was started in 500 ml beakers and later transferred to 1 liter beakers. Under different culture conditions copepods can be raised. Mass culture of T. turbinate was done in five 500 liter tanks. Tanks were filled using chlorine treated and de-chlorinated water filtered through a 5 micron filter bag from an outdoor tank of 200 m3. To the contamination-free water the resident population of T .turbinata has to be introduced. The tanks should be placed in 60% shade. Using a refractometer, the salinity of the resident water should always be maintained at 30 ppt (+/-2 ppt). The optimum temperature should be maintained at 27 - 30°C. A mixture of I. galbana and N. oculata has to be supplied regularly. This helps in preventing contamination of the culture system by ciliates upto a certain extent. Mild aeration is also required in these tanks. Siphoning off of the sediment from the tanks on alternate days are required to prevent ciliate growth, thereby enhancing population. By passing through a series of filtering mesh of 20μ, 110μ and 330μ animals with different ages, ovigerous females and mating pairs were sorted out and brought for further culture from the sediments collected.



T. turbinata eggs

Naupliar stages of T. turbinata

Feeding

Supply of an optimal diet is an important factor for culturing copepods. By means of gut analysis and faecal examination the feeding of copepod can be determined. While selecting food for a species the particle size as well as the digestibility of the feed has be taken into consideration. The chemical composition of the algal feed may also be considered as it may also have some effects on survival. In the case of T. turbinata the particle size should not be more than 10μ. The ideal algal feed for T. turbinata was I. galbana and N. oculata ($\approx$2-5 μ). For feeding, stock and mass cultures are prepared. Using a compound microscope, supply of unicellular algae can be quantified. The amount of food required is directly proportional to the copepod biomass present in the culture media. Feed of 2 x 104 cells/ml/day is sufficient for young nauplii. Then, it can be gradually increased upto 1 x 105 cells/ml/day till they mature. Daily assessment of population density is essential. If the water appears cloudy, the feed rate should

be decreased. The supply of sufficient amount of feed will successfully facilitate the peak production.

Cleaning

The major threat occurring to the copepod culture is ciliate infection. Total removal of ciliates is an impossible task. So, by means of proper cleaning, it can be avoided. Daily removal of accumulated fecal debris, wastes and uneaten food materials can be done by siphoning. The siphoned water has to be collected in separate buckets. The buckets should be contamination free. Later the supernatant portion of the filtrate should be filtered through a 100 micron filter to recover adults if any. The bottom filtrate can be stored in buckets with mild aeration for 3-4 days. Every day the developed copepod nauplii in the buckets were filtered using a 150μ and 20 μ mesh, washed thoroughly in freshwater for 30 sec and also in seawater. The adults if any collected in 150μ filter can be deposited into the main tank itself. The young nauplii collected through 20μ filter can be washed with seawater and put in another fresh bucket for further growth or it can be used for feeding. Renewal of resident seawater should be done in every 2 weeks and a replacement of tank to be done in every 4 weeks for preventing ciliate development. Although, the volume to be removed is not critical, the exchange of 10% of water is most effective at the time of removing debris from the bottom.

Harvesting

It may take about one month for a tank to reach a density of minimum 1000 copepods/l and only then it becomes ready for a harvest. In a harvesting, there may be eggs, nauplii and adult copepods. In a continuous culture, 5-10% can be regularly harvested. The harvesting can be done by siphoning out the water. Production can be enhanced by proper cleaning and adequate supply of feed.

Problems

The main problem in T. turbinata culture is the ciliate infestation in tanks. Overfeeding, fecal contamination and accumulated debris results in emergence of ciliates. Ciliate growth can be assessed by the cloudy nature at the bottom of the tanks. The deficiency of feed is another problem in the decline of culture population. The feed provided has to be proportional to the biomass present. Adults feeding on eggs and early larvae will result in total collapse of the population if sufficient feed is not provided. The feed provided should be contamination free, especially of ciliates. If a mesh of 20 μ is used for filtering the feed, it will help in preventing ciliates to an extent. Matured algae should be fed to attain a successful growth. Immature or collapsed algal feed shall lead to a decline in population. The settled debris and accumulated wastes in resident sea water is also a substrate for development of ciliates and other dangerous organisms. So renewal of sea water in resident tanks is essential to create a healthy environment for the cultured species.