Culture and Maintenance of Marine Copepods as the Live Feed

Santhosh B., Aneesh K.S. and Muhammed Anzeer

Vizhinjam RC of ICAR-Central Marine Fisheries Research Institute
santhoshars@gmail.com

Introduction

Copepods are the most common planktonic crustaceans that occur in almost all kinds of water bodies on the earth's surface. There are more than 210 families, 2400 genera and 24,000 species identified in this group. Planktonic copepods are considered to be the most abundant metazoans on earth. From the Lower Cretaceous period onwards, these groups were diversified and adapted to almost all kinds of aquatic habitats and successfully colonized everywhere. In the marine environment, copepods are present in all types of water bodies from pelagic to deep sea and from sea shore to deep hydrothermal vents. Some of them are adapted to live inside the body cavity of many animals. These form the important secondary producers/primary consumers and ultimately contribute significantly to the food chain in large ecosystems. Almost all types of marine organisms, directly or indirectly depend on these small organisms for their food. Copepods form important food for many marine fishes and invertebrates. Certain fishes and fish larvae are evolutionarily adapted for feeding on copepods. Copepods are nutritionally superior to almost all live feeds. Many fishes, especially those with weak fish larvae, totally depend on copepod nauplii for their survival at least for the initial few days. Due to their smaller naupliar stages and nutritional superiority, copepod cultures became an integral component in marine finfish hatcheries.

Copepods are much superior in nutrition than most of the popular and common live feeds in the hatchery. Feeding marine fish larvae with copepods increases their survival and growth rates, reduces deformities and enhances pigmentation and stress tolerance. Due to these properties, copepods are much more suitable for larval rearing than almost all other popular live feeds.

Among the planktonic copepods, three major groups are very important in terms of live feeds- calanoids, cyclopoids, and harpacticoids. All these three groups are being utilized as live feeds in the hatcheries. There is always a limitation in the high-density cultivation of copepods. Rotifers can be cultured to a high density of 2000/ml. But in the case of most of the copepod cultures, the density rarely exceeds 2-3/ml for adults and 10/ml for nauplii. Calanoids are comparatively more difficult to culture than cyclopoids and harpacticoids in small quantities. But large-scale cultures are easier for calanoids. Calanoids have smaller pelagic larvae and can be more easily produced on a large scale in hatcheries. Copepods that scatter their eggs are ideal for large-scale cultivation in hatcheries.

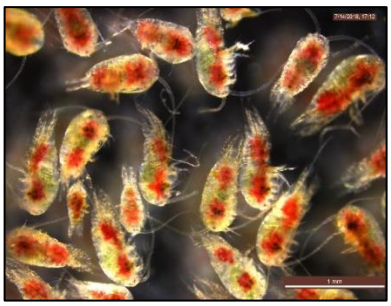
Species and culture

More than 60 species of copepods have been raised in laboratories, but very few are popular for large-scale production. Copepods can be cultured extensively, intensively, and semi-intensively. The extensive cultures are mainly in tanks, outdoor ponds, lagoons or enclosed fjords. By using sieves of appropriate mesh sizes, these cultured copepods can be made available to fish larvae. In extensive systems, culture is done normally by producing microalgal blooms using ordinary agricultural fertilizers. Agriculture fertilizers, both organic and inorganic, were used with or without the combination of fishmeal, rice bran, wheat bran and fish feeds as inputs for nutrients. But here the main disadvantage is the unpredictable nature of production. Semi-intensive culture is generally carried out in indoor tanks with a regular supply of microalgae in combination with other inert feeds. Here regular harvest is possible and yields a mixed culture of different species of copepods. Here also the culture may not be stable for longer periods. CMFRI recommends intensive culture for large-scale production. Intensive culture is developed generally by maintaining a selected isolated pure culture of copepod species with desired qualities. Basically, there are small stock culture units, large mass culture units, and modified culture tanks fitted with structures for harvesting naupliar stages on regular basis. Specialized nauplii collection units can be attached to mass culture units also. All water quality parameters need to be regularly monitored and adjusted.

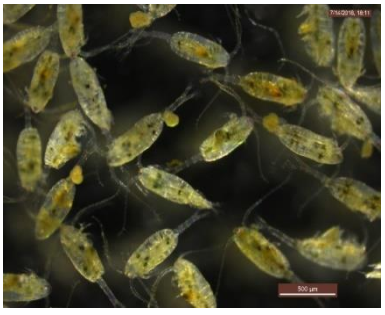
Though intensive culture may not be economical, most of the hatcheries prefer this because of the assured production of copepods and nauplii of desired size and species. This will help larvae to thrive well during critical periods of larval rearing. Once the critical period is crossed, the larvae can be fed with enriched rotifer and *Artemia*.

Copepod species developed for use as the live feed

Pure stock and mass cultures of 12 species of copepods that have been identified as suitable for larval rearing are maintained at Vizhinjam Regional Centre of CMFRI. Calanoid copepods (*Temora turbinata, Pseudodiaptomus serricaudatus, Acartia spinicauda, A. bilobata, A. southwelli, A. tropica, Parvocalanus crassirostris, Bestiolina. coreana, B. similis*), Cyclopoid copepods (*Oithona brevicornis* and *Dioithona oculata*) and Harpacticoid copepod (*Euterpina acutifrons*) are being produced in CMFRI. *Apocyclops cmfri* is a promising new species identified from Karwar waters and cultured here.



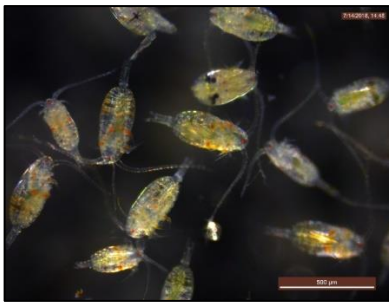
Temora turbinata



Pseudodiaptomus

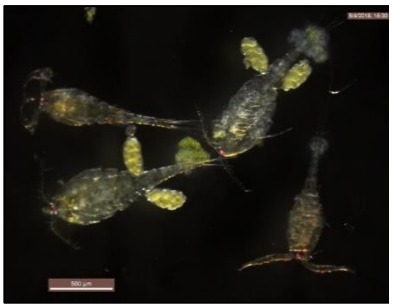
Acartia southwelli

serricaudatus

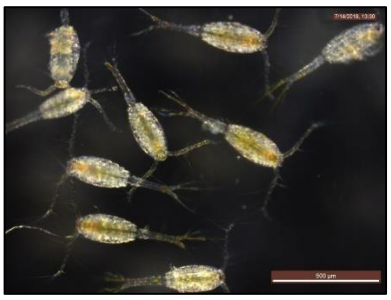


Parvocalanus crassirostris

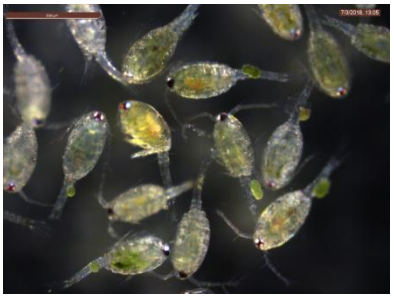
Bestiolina similis



Apocyclops cmfri



Oithona brevicornis



Dioithona oculata

Euterpina acutifrons

Copepods developed by CMFRI

Stock culture

The stock culture of microalgae is a prerequisite for copepod culture. Microalgae need to be contamination free. The algal requirement is comparatively less when compared to other live feeds but the purity of the culture is very important. Contaminated algal culture can collapse the stock of copepods. For each species of copepods algae or a combination of algae in the recommended doses are essential.

Stock culture of copepods can be done in tanks of 50-500 litre capacity or even in bigger tanks. Tanks of plastic, HDPE or fibre are ideal. Cement tanks are not ideal. White colour or light colours are desirable because they support visibility. It is easy to assess the population and health of copepods on white background. Cleaning is also easy on white background. In stock culture, the health status of the copepod is more important than its population status.

A few hundred copepods are sufficient to inoculate in stock culture tanks. Tanks attain maximum density within 10-20 days depending on the species cultured. Since production is totally dependent on population, regular harvest is possible even from the stock culture without affecting the total population. Normally the stock culture can continue for 2-3 months with proper maintenance. There are basically no differences in the maintenance of stock and mass culture.

Maintenance of stock culture

On alternate days, the sediments need to be siphoned off from the culture tanks to reduce the ciliate growth and also to maintain good growth of copepods in the culture. The siphoned sediment and water should be kept in 20-litre buckets with mild aeration for a few hours for the settling of debris. Live copepods, eggs, and larval forms accumulated in the clear surface of the buckets can be carefully filtered out by passing through a filter of the sieves of the desired mesh size. Copepods collected can be washed and reintroduced in the culture tanks. The sediment can be diluted again and this process can be repeated several times so that all healthy and live copepods are collected and introduced back into the culture. This process is essential for egg broadcasting species because all the eggs will be settled in the bottom with faecal pellets and wastes. In this way, ciliates and dead organisms can be regularly removed from the tanks. In the case of egg-producing copepods, the filtrate needs to be diluted with clear filtered sea water and kept for one or two days with mild aeration. The freshly hatched nauplii can be sieved out regularly using a 30µm sieve and can be introduced back into the culture system. The water level in the culture tank should be brought back to the original level by adding clean, dechlorinated or ozonized and filtered seawater. The tank needs to be washed and reused completely after one month of culture.

Another method of keeping stock culture is without siphoning the bottom for 7 days. Keep on adding feed and maintain water quality parameters without disturbing the culture. After 7 days, remove the bottom debris and transfer the entire culture into another fresh tank. The old tank can be washed, sterilized, and reused.

Mass culture

For the mass culture of 1000-5000 litres, 50-100 litres of inoculum is required. Inoculum needs to be cultured in 1000-litre tanks for inoculating 5 tonne or 10 tonne tanks. Up to 75% of the stock can be used for culture. The inoculum will be ready again within 8-10 days and the tanks will be ready for harvest within 10-25 days period. Thus, a series of tanks starting from 100 litre, 500 litre, 1000 litre and 5 tonnes/ 10 tonnes are necessary for establishing a large-scale production system. Care should be taken to increase the volume of water slowly with an increase in population after inoculation, especially in large systems. All tanks should never be filled beyond 75-80% of their capacity. Flat base round drainable tanks are ideal and the water depth less than 1m. Complete indoor tanks also can be used with normal lighting conditions. All tanks should be placed in a bit elevated position so as to assist easy siphoning of bottom samples. Mild aeration is essential in all tanks.

Mass culture can be done as batch culture and continuous culture. For batch culture, the entire tank content can be harvested. For continuous culture, daily harvest is possible from the larger tanks. Most of the species can be cultured in both ways but continuous culture is more successful in egg-broadcasting copepods of the genera *Parvocalanus, Bestiolina, Temora* and *Acartia*.

Maintenance of mass culture

Mass culture tanks of any capacity can be used for large-scale production. Mild aeration needs to be provided accordingly the entire area of the tanks needs to be aerated but there should not be any turbulence or strong flow of water. The inoculum needs to be introduced into 4-5 times higher volume and slowly increase the water level to optimum tank size. In large volumes of water, unused algae may settle down as debris and can create the development of unwanted organisms. If conditions are favourable within one week, copepods can reach the maximum capacity of production. In most of the species, the maximum volume can be attained within 7-15 days. Depending on the level of sediments, bottom debris needs to be siphoned off from the culture tanks to reduce the ciliate growth and to maintain good growth of copepods in the culture. The siphoned sediment and water should be kept in buckets with mild aeration for a few hours for the settling of debris. If a large volume of water needs to be filtered, use one or more sieves to collect the sediments. In such cases, an open wide flat tray can be used to reduce the pressure of outflowing water. Live copepods, eggs and larval forms accumulated in the clear surface of the buckets can be carefully filtered out by passing through a filter of the desired mesh size. Copepods collected can be washed and reintroduced in the culture tanks. The sediment can be diluted again, and this process can be repeated several times so that all healthy and live copepods are collected and introduced back into the culture. This process is essential for egg broadcasting species because all the eggs will be settled in the bottom with faecal pellets and wastes. In this way, ciliates and dead organisms can be regularly removed from the tanks. In the case of egg-producing copepods, the filtrate should be diluted with clear filtered sea water and kept for one or two days with mild aeration. The freshly hatched nauplii can be sieved out regularly using 30µm sieve and can be introduced back into the culture. The water level in the culture tank should be brought back to the original level by adding clean, dechlorinated or ozonized and filtered seawater.

Sieves of different mesh sizes made from plastic pipe connectors and bolting silk

Problems in the culture

The main problem in culture is ciliate infections in tanks. Overfeeding, faecal contamination, and accumulated debris results in the emergence of ciliates in culture. Ciliates growth can be assessed by the cloudy nature at the bottom of the resident tanks. If care is not given, it will result in a total decline in the population. The prevalence and mean intensity of

ciliates in the culture tanks and epibionts on the copepods should be evaluated at regular intervals. *Euplotes* sp. is the most common ciliate in the culture system and *Voticella* sp. is the most common epibiont on the copepods.

The culture can withstand the ciliates up to a certain extent. If ciliates exceed, the culture can be siphoned out and washed through dechlorinated filter water using sieves of 70-8µ and fresh culture should be initiated. The threshold level of ciliates in the bottom water sample of culture tanks is estimated as 7-8 nos/ml and if the level exceeds more than 10 nos/ml, there can be a sharp decline in the population of copepods in the culture tanks.

The deficiency of feed is another reason for the decline of the culture population. The feed provided should be proportional to the biomass present. If the sufficient feed is not provided, it will result in cannibalism by adults of some species, especially *Acartia* spp. In that stressed situation, adults start feeding on eggs and early larvae which may lead to the reduction or total collapse of the population.

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 some extent. Pure and mature algae need to be fed to maintain long-term culture. Immature or collapsed algal feeds shall lead to a decline in population. The settled debris and accumulated wastes in resident sea water is also a substrate for the development of ciliates and other dangerous organisms. So, the regular renewal of seawater in resident tanks is essential to create a healthy environment for the culture.

Cleaning

A major threat to the copepod population is ciliate infection. The total removal of ciliates is an impossible task. So, by means of proper cleaning, ciliates can be reduced to a large extent. The daily removal of accumulated faecal debris and waste food materials can be done using separate siphoning tubes. In mass culture tanks siphoning can be done on alternate days as described in earlier. 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 as mentioned in the earlier section. The very young nauplii collected through a 20µ filter can be washed thoroughly with dechlorinated sea water and can be used for feeding the larvae. Every week, the sides of the culture tanks and bottom should be slowly and carefully wiped using the appropriate brush without disturbing the water. Aeration should be stopped for at least one hour, and all the sediments should be allowed to settle down at the bottom. The sediments can be carefully siphoned off and treated in a manner similar to that discussed earlier. As far as possible use all items like sieves, pipes, buckets etc separately for each tank. Enough care should be taken to avoid cross-contamination. Care should be taken for siphoning out the sediment or eggs or copepods. The filter should be placed in a trough and allow the water to overflow through the trough in such a way that the disturbance of the water inside the filter should be minimum.

****