COPEPOD CULTURE TECHNIQUES FOR MARINE FINFISH LARVAL REARING

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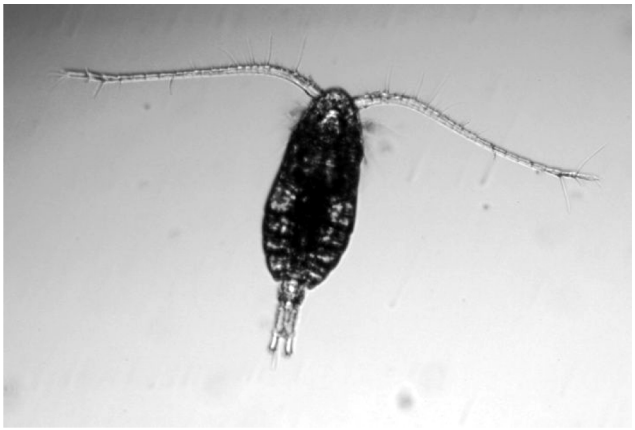
Copepods are the most numerous metazoans on earth and they represent about 80 % of the zooplankton in the ocean. In nature, they constitute a vital link in the marine food chain from primary producers to higher trophic levels i.e. fish. They are the natural choice as food for many marine finfish larvae. Various studies have demonstrated that copepod may have a higher nutritional value than *Artemia* sp., as the nutritional profile of copepods appears to be better suited to the nutritional requirements of marine fish larvae. In addition, they can be administered in different forms, either as nauplii as a starter feed or as copepodites/full grown copepods until weaning. This makes them a suitable group as live feed in aquaculture.

Biology

The name copepod is derived from the Greek word '*Kope*' means 'oar' and '*podos*' means 'foot' and refers to the flat, paddle-like swimming legs. Around 200 families with 1650 genera and 11,500 species of copepod were described by 1993. Free-living copepods inhabit a variety of habitats, ranging from the planktonic copepods that inhabit the world's ocean, through benthic species that live on the surface of microalgae or inhabit microscopic spaces in marine sediments, to subterranean species living in groundwater or in deep sea hydrothermal vents. Almost one-third of marine copepod species are parasites or live in symbiotic relationship with other organisms. The most commonly used species in mariculture are free-living copepods belonging to orders Calanoida, Harpacticoida and Cyclopoida.

i. Calanoida

The calanoid copepods are predominantly pelagic; however , they are available at all depths, with some near –bottom and benthic species. They are selective feeders; feeding mainly on small phytoplankton cells by filtration, or preying upon a variety of animal prey including copepod eggs. They are distinguished by their long antennules, as long as the body itself or even longer, with upto 27 segments and biramous antennae used as accessory locomotory appendages.



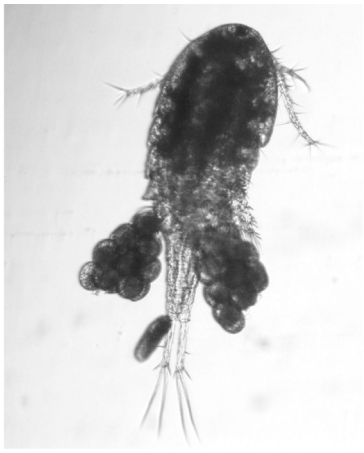
ii. Harpacticoida

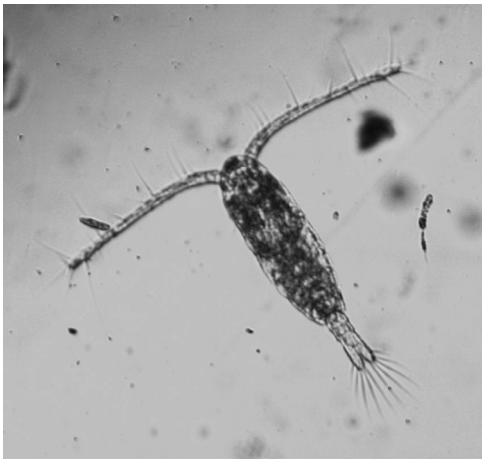
The harpacticoids include more than 50 % of copepod species, are primarily marine, free living, benthic organisms. They are distinguished by their short antennules, fewer than 10 segments and biramous antennae.

iii. Cyclopoida

The cyclopids include pelagic, epibenthic, benthic and parasitic species, which inhabit both freshwater and marine environments, although they are far more abundant in freshwater. In the marine environment, cyclopoida belonging to the family Cyclopinidae are predominantly benthic and those of the Oithonidae are

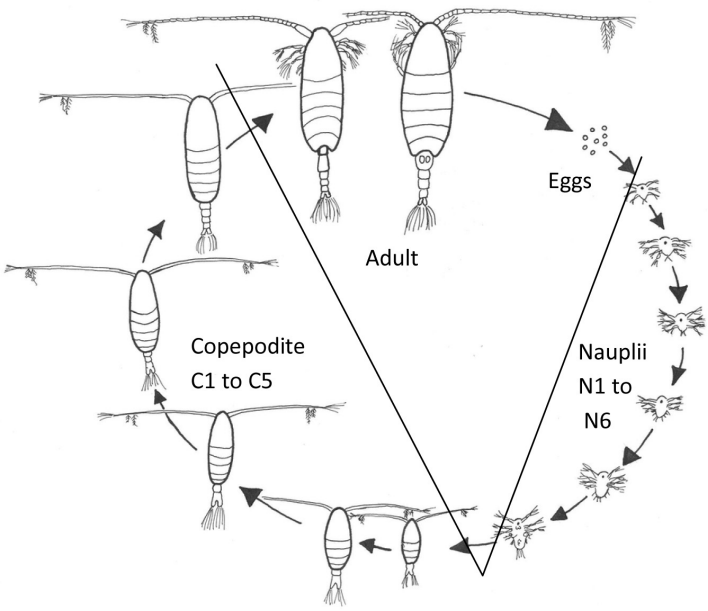
planktonic. Cyclopoids have uniramous antennae used to help catching food. The antennules in cyclopoids are shorter than in calanoids, rearly reaching beyond the cephalothorax.





Life cycle

Generally, the body of most copepods is cylindriconical in shape, with a wider anterior part. The whole body consists of two distinct parts, the cephalothorax (the head being fused with the first of the six thoracic segments) and the abdomen,



Life cycle of copepod modified from http://www.st.nmfs.noaa.gov

which is narrower than the cephalothorax. Males are generally smaller than females counterpart. During copulation the male grasps the female with his first antennae, and deposits the spermatophores into seminal receptacle openings, where they are glued by means of special cement. Generally eggs remain attached to females as an ovisac or they get released directly in the water column. The eggs hatch as nauplii and after five to six naupliar stages, the nauplii turn to copepodites. Generally five copepodite stages are passed before the adult stage is reached. Each stage moults for developing into the next advanced stage. The entire development phase may take from less than one week to as long as one year.

Species used in Mariculture

The different copepod species used in mariculture as live prey for marine finfish larvae are listed in following table

Copepod species	Marine finfish
Calanoida	
Acartia longiremis	Wolf fish, *Anarhichas lupus*
A. pacifica, A. plumose	Asian sea bass, *Lates calcarifer*; grouper, *Epinephelus fuscoguttatus*
A. sinjiensis	Red snapper, *Lutjanus argentimaculatus*
A. tonsa	*Fundulus* spp.; *Elops saurus*; Turbot, *Scophthalmus maximus*
Acartia spp.	Golden snapper, *Lutjanus Johnii*; Red snapper, *L. argentimaculatus*
Eurytemora affinis	Striped bass, *Morone saxatilis*
Gladioferens imparipes	Dolphin fish, *Coryphaena hippurus*; Sea horse, *Hippocampus angustus*; West Australian dhufish, *Glaucosoma herbraicum*; pink snapper, *Pagrus aurata*
Harpacticoida	
Euterpina acutifrons	Grey mullet, *Mugil cephalus*; Mahimahi, *Coryphaena hippurus*

Tigriopus japonicas	Black sea bream, *Mylio macrocephalus*; Sand borer, *Silago sihama*, Nassau grouper, *Epinephelus striatus*
Tisbe holothuriae	Turbot, *Scophthalmus maximus*; Dover sole, *Solea solea*
Tigriopus japonicas	Yellow-fin sea bream, *Acanthopagrus latus*
Cyclopoida	
Apocyclops borneoensis	*Acanthopagrus cuvieri*
A. royi	Grouper, *Epinephelus* spp.
Oithona sp.	Striped patio, *Eugerres brasilianus*

Selection of copepod species for mariculture

The following criteria are used for selecting a species for use in mariculture.

i. Natural occurrence

ii. Size of copepod and nauplii

iii. Type of spawning – free spawner/egg carrying

iv. Daily production

v. Highly fecund

vi. Short generation time

vii. Wide thermal and salinity tolerance

vii. Easy adaptation to laboratory conditions

Collection and isolation

Copepods can be collected from sea, coastal water and estuarine area by using zooplankton net of 160 µm during early morning. The collected sample should be carried to the lab in sufficient water and screened through 500 µm to remove bigger particles. The sample should be washed thoroughly and sieved with a 150 µm mesh to remove debris and unwanted material. Then the sample can be divided according to the size wise. Finally a single species of copepod can be selected with the help of dissection and streozoom microscope. This selected copepod can be cultured for one week before final isolation of a single species.

Stock culture

Stock culture of a single copepod species can be maintained in 5-20 L containers with continuous aeration. They need to be fed with single or mixed species of phytoplankton according to their requirement. The copepods should not be overfed since they are filter feeders and if they are overfed they may die due to choking of filtering apparatus. The whole culture needs to be sub cultured every month to avoid contamination. For this, each time the whole water content should be filtered through 150-200 µm and the retained adult copepods need to be cultured with fresh seawater and phytoplankton.

Culture techniques

The basic culture techniques of various copepod species is either Indoor or Outdoor culture.

I. Extensive and outdoor cultures

i. Harvest of wild zooplankton

Copepods have been used for larval rearing of marine species for several decades. They are either collected directly from nature, often near to mangrove area where natural densities are high, and used directly as live prey, or inoculated into outdoor tanks or ponds in land based systems to produce live zooplankton for fish larval rearing. They are either used live or harvested, frozen, dried or freeze-dried for later use as an inert diet. Several types of filtering devices have been developed for collecting wild copepods such as floating propeller-induced device that directs the water current through elongated plankton nets. Different sizes of copepods can be segregated by increasing the mesh size of the larger gauze depending upon the requirement of copepod size.

ii. Production in outdoor ponds or large tanks

Copepod culture is carried out in 350-5000 m^3 ponds and tanks in Europe and Asia for feeding larvae of different marine finfish such as cod, grouper, flatfish and turbot. Generally filtered sea water is used in these systems. Sea water is passed through 20-40 µm mesh to fill pond or tank, so that natural phytoplankton can be transferred to the ponds without accompanying zooplankton or potential predators. The phytoplankton can be monitored and additional nutrients, generally commercial fertilizers in small quantities can be added, as and when required.

Generally, in low nitrate concentrations, small flagellates will develop faster, but when the nitrate levels are non-limiting (>5 µM) and oxygen concentrations are high, large diatoms develop faster, which supports prolific copepod production. Silicate is sometimes added which encourages development of diatoms, leading to the better copepod production. Generally, nutrient levels in the water intake and the type of phytoplankton found in the rearing tanks are closely monitored so it can be manipulated at any time to enhance the production of phytoplankton for copepod proliferation. The copepods are filtered from the wild by using zooplankton net to inoculate the rearing tanks. In Asia a mesh size of 400-600 µm was used to inoculate outdoor tanks with copepodite and adult stages for grouper lraval rearing. The copepod cultured in this system are primarily calanoid species namely, *Eurytemra affinis, Temora longicornis, Centropages hamatus, Acartia* spp.*, Calanus finmarchicus, Paracalanus parvus* and *Pseudocalanus elongatus*, but other copepods such as *Oithona similis and Tisbe sp*. also have been cultured.

In this type of production system, a bloom of rotifers will appear initially followed by a bloom of copepod nauplii and other zooplankton which replaces the rotifers. An advantage of outdoor ponds culture system over the extensive systems is that to rely on the local production of zooplankton over one generation before using them as food. Another advantage of this system is avoidance of any parasites collected with wild zooplankton. Trematodes and cestodes, which infect marine fish, have been identified in copepods. Thus, feeding wild zooplankton directly to the fish increase the risk of infection. Since many of these parasites use copepods as intermediate hosts between compulsory hosts, the use of the first generation nauplii in this system reduces the risk of parasite transfer to fish larval system.

In some places, copepod cultured tanks are emptied and dried during the winter at the end of the season, however, in some cases half the water is left in the tank or pond which helps in producing resting eggs copepods, which survive in the sediment until the following year. These resting eggs are used as starter zooplankton culture in the following year when the larval rearing season starts again.

II. Intensive or *Indoor culture*

Several attempts were undertaken to intensively culture copepods in indoor conditions since the early 1990s. This is in response to shortage of *Artemia* eggs and diversification of marine finfish culture into new species with very small mouth

gape larvae such as grouper, snapper and other marine ornamental fish. These small gape mouth larvae are difficult to rear on traditional live prey, such as rotifers and *Artemia* nauplii.

Generally, a single species of Calanoid or Harpacticoid copepod dominates in a group of copepod species when cultured in a tank. This might be due to one copepod species taking advantage of some environmental parameter to outcompete other species. The mechanisms for competition have not been fully explored, although production of toxic metabolites and genetic adaptation to particular environmental conditions (niches) has been proposed. These dominant species of copepods are ideal candidate species for intensive rearing.

Several attempts to mass-culture copepods in intensive systems have been undertaken with varying success and have resulted in the development of different systems for particular species of copepods. Rearing in larger volumes (>10 l for Calanoids and >2 l for Harpacticoids) may be more representative of the conditions required for mass rearing.

Calanoids

The most frequently cultured calanoid species belong to genera found in coastal waters, namely *Acartia, Centropages, Eurytemora, Parvocalanus* and *Temora*. These copepods are small, with relatively short generation time, a wide thermal and salinity tolerance, and easy adaptation to laboratory conditions. When reared in outdoor multispecies cultures, they tend to dominate over time.

The calanoid copepods are filter feeders which feed mainly on phytoplankton by filtering water. In culture systems too, their main diet is phytoplankton although *Acartia tonsa* has been cultured on rice bran also. In many cases, copepods are reared on monoalgal diets, which may not comply with all the requirements for maximum egg production and nauplii development. The rate of egg production in copepods is dependent on the size, quantity and quality of the algae provided. This is not similar for every species. Each species requirement is different in terms of type and quantity of phytoplankton. As a general rule, to reach food saturation, high ingestion rates and high egg production rates, cell concentrations of around 10^3 cells ml^{-1} would be sufficient using larger cells such as *Thalassiosira weissflogii* and *Ditylum brightwellii* (>12 µm in mean diameter), around 10^4 cells ml^{-1} using smaller cells such as *Rhodomonas baltica* (around 5-12 µm) and 10^5 cells ml^{-1} using yet

smaller cells such as *Isochrysis galbana* or *Pavalova lutherii*. Larger calanoid species may be less efficient in feeding on the smaller algal sizes. Generally feed is provided on daily basis depending upon the consumption of the feed since high concentration of feed sometimes lead to crash of copepod culture. Feed level can be monitored either by counting the algal cells to ensure specific densities or be regulated according to the water turbidity in the culture tanks.

A combination of at least two algal species with high n-3 polyunsaturated lipid content, and of different sizes can be utilized by the nauplii, copepodite and adult stages, probably will be an adequate diet for copepod culture. Since the fatty acid distribution in adults and their non-feeding offspring reflects that of the adult diet, this would also ensure a suitable fatty acid distribution in the copepods used as live feed. A combination of *I. galabana*, high in DHA (DHA: EPA=11.68), and *Nannochloropsis oculata or Chaetoceros calcitrans*, high in EPA (DHA: EPA=0.05), can be used for the culture of *Parvocalanus* sp. *and Acartia spinicauda* (3:1 by cell numbers). The food ration should be adjusted according to water turbidity, ranging from 1×10^4 to 1×10^5 cells ml^{-1} day^{-1}.

The basic culture technique of different calanoid copepod species is same, given below is description of *Acartia spinicauda* and *Parvocalanus* sp. culture. Copepod is being cultured in 100 L to 10 t tank in indoor systems. Generally, the containers used for culture are cleaned, washed and dried for 2 days before stocking with copepods to avoid contamination. The tank is filled with water up to one-tenth level of tank height. The thoroughly washed adult copepod collected with 150-200 µm filter can be stocked in the tank @ 10-20 no per liter. The different phytoplankton species such as *Nannochloropsis oculata, Isochrysis galbana*, *Chaetoceros calcitrans* are used as copepod feed. The feeding should be carried out in such a way that the feed should finish overnight. The copepod *Acartia spinicauda* and *Parvocalanus* sp. should be fed with mix culture of *Nannochloropsis oculata* and *Isochrysis galbana*, in 1:3 ratio @ 10^4-10^5 cell per ml. The bottom of the tank can be siphoned once in 4 days to avoid the accumulation of debris in the bottom otherwise it will deteriorate the water quality. The water quality is to be maintained in such a way that ammonia nitrite nitrite should not go beyond 0.5 ppm and 0.1 ppm. The dissolved oxygen level should always be more than 3.0 ppm. Fresh sea water is added every day to the tune of one tenth of the culture container. The adult copepods as well as nauplii can be harvested from 10^{th} day to

the 30th day in continuous culture, where as in batch culture the copepods can be harvested all at a time and the container can be re-inoculated with adult copepod for starting fresh culture.

Nauplii of *Acartia spinicauda*

In this system, even the eggs sedimented to the bottom can be collected daily by siphoning the bottom, simultaneously siphoning out debris, faecal matter and associated ciliates. During siphoning, the eggs can be concentrated on a 50-60 µm sieve, allowing most of the debris and ciliates to pass through and be removed from the culture.

Harpacticoids

Harpacticoids have been cultured in batch and continuous systems to provide food for marine fish larvae. Improvements in growth and survival were observed when it was used either as the only food source or as a supplement to traditional feeds. There are several advantages in using harpacticoids in culture:

- High tolerance to a wide range of environmental conditions
- Ability to feed on a wide range of live or inert diets
- High reproductive capacity
- Relatively short life cycles
- Ability to be cultured in high densities

- Requirement for surface area rather than volume
- Planktonic naupliar stages
- Can be used as tank cleaners in rotifer cultures, other copepod cultures or larval tanks

The culture conditions for harpacticoids are simpler than those for calanoids. Filtered, artificial or non-treated seawater may be used, and a whole range of inert food is acceptable to many harpacticoid species. This simplifies the culture method and eliminates the need for culture of phytoplankton. A mixture of two algal species would be the preferred choice for feeding herpecticoid copepods. Algae, which quickly sediments, are appropriate for benthic copepods, possibly because bacteria colonise these cells, and the mixture of algae and bacteria may be a superior dietary combination for harpacticoids. Algae such as *Skeletonema costatum*, *Chetocerous calcitrans* and *Tetraselmis suecica* quickly sediment, whereas species such as *Isochrysis galbana* remain in suspension and may be less available to the harpacticoids.

Harpacticoids can be also reared on a variety of inert feed. However, as discussed earlier, food quality affects development and fecundity, and should be considered carefully. The use of inert feed may cause hygiene problems in the culture tank. A mixed diet may provide the best nutritional value. The protein content of the diet affects both the development time and fecundity. This may not be valid for all harpacticoids, but it may be appropriate to ensure a diet containing around 50% protein for most harpacticoids.

Cyclopoids

Very few cyclopoid species have been reared in the laboratory. *Oithona* spp. or *Apocyclops* spp. appear to be the best candidates and they are relatively easy to culture over several generations in the laboratory. *Oithona* spp. is an ideal supplement to the traditional live feed for orange spotted grouper. The basic culture techniques of *Oithona* spp are similar to the calanoid copepod *Acartia spinicauda* and *Parvocalanus* sp.

Culture tank size and shape

Many Calanoids and Cyclopoids copepod require large volumes for their culture and the adult density rarely exceeds 100 per litre. However, higher densities have

been achieved in *Acartia spinicorda*, *Paraclanus parvus, Acartia clausi*, *Eurytemora long icorrils* and E. *affinis*. Among the Cyclopoids, *Oithona* spp. appears to be the best candidate and they are relatively easy to culture at higher densities. Different shape and size of the tank has been used in calanoids and Cyclopoids copepod culture such as cylindrical, rectangular, round. The bottom should be flat to enable easy siphoning of eggs from the bottom. A higher tank height to tank diameter is considered advantageous in reducing the surface area to be siphoned and reduce loss of copepods. Furthermore, a central air-stone may be sufficient to ensure proper circulation within the tank column without too vigorous aeration. A low tank height to bottom surface area may result in high numbers of adult copepods being siphoned together with bottom debris.

The mass culture of benthic harpacticoid is dependent on the available surface area rather than culture volume. Addition of small balls or other material in culture tanks helps in increase in surface area which will provide more space for the culture of harpacticoids copepod.

Temperature and salinity

Temperature plays a fundamental role in the life of copepods, but their ability to adapt to temperatures even beyond their natural range is remarkable. Geographical populations are genetically adapted to the conditions of their natural habitat. Estuarine species are more tolerant to lower salinities. Coastal species have wider thermal and salinity tolerances than oceanic species. In culture, it is preferable to choose species with similar thermal, salinity optima to those present in the rearing facility. Generally, *Acartia spinicauda*, *Parvocalanus* sp. and *Oithona* spp were able to grow in salinity regime of 16-34 ppt and temperature regime of 24-32 ^{o}C.

Contamination

Generally in culture systems where culture medium is not exchanged daily, waste products such as fecal matter, moulting shells etc and superfluous feed may accumulate and generate problems with ciliates and other contaminants, which may lead to collapse of the culture.

The main contaminants which pose problems in copepod culture are as follows

i. Bacteria

ii. Ciliates

iii. Rotifers

iv. Other copepods

The following steps should be carried out as precautionary approach to avoid contamination of copepod cultures.

i. Use of the same siphon pipe for the entire copepod tank should be avoided. Each tank should have a separate siphon pipe.

ii. Other items such as filters, mugs, and buckets etc should also be separate for each tank.

ii. Generally, in commercial facilities, contamination by rotifers is the most likely cause of the crash of a copepod culture, since the rotifers with their higher reproductive rate would quickly outcompete the copepods. It is therefore important to keep these cultures strictly apart.

iv. The presence of other copepods may pose a problem, so care should be taken while adding fresh seawater and feed to the culture tank. The water used for the phytoplankton culture as well as copepod culture should be filtered with 10 µm filter bag.

v. Generally ciliates proliferation is more in over fed copepod culture tanks. Sometimes these ciliates act as a feed for these copepods during the periods of low phytoplankton concentrations. However ciliates also compete for the same feed with the copepod, thus care should be taken to avoid contamination with ciliates. It is advisable to empty the culture tank using 60 - 80 µm mesh size gauze if ciliate contamination is more, which retains the adult copepods, but allows the ciliates to be washed out. Then, culture can be started afresh.

vi. Generally, Bacteria constitute a part of the diet of copepods. Sometimes, cultures may succumb to uncontrolled proliferation of bacteria. Some bacteria, such as *Vibrio* sp., are known to infect copepods in eutrophic coastal waters, resulting in lower survival rates with further contamination of the fish larval rearing system which leads to mass mortality of the fish larvae also.

Conclusion

More than 20 species of copepods were used as feed in larval rearing of marine finfish. Most of them have been used in temperate climate. Though the mass culture technique of these copepods has been developed, it has not transferred as technology in hatcheries. In India, the Research Centre of CMFRI, Vizhinjam has isolated three species namely *Acartia spinicauda*, *Parvocalanus* sp. and *Oithona* sp. Their mass culture has been successfully carried out and given as first feed for larvae of orange spotted grouper, *Epinephelus coioides* at Regional center of CMFRI, Visakhapatnam to enhance survival rate. Thus, they can be considered as promising copepod species for mass culture and marine finfish larval rearing in tropical conditions.

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