LIVE FEEDS IN AQUACULTURE

Santhosh, B., Ambarish P. Gop, Anuraj, A., Krishna Sukumaran, Muhammed Anzeer and Aneesh K. S.

Vizhinjam Regional Centre of ICAR-Central Marine Fisheries Research Institute, Thiruvananthapuram-695 521, Kerala, India
E-mail: santhoshars@gmail.com

Introduction

Live feeds are the natural food items of fishes and invertebrates. The culture of live-fed organisms for larval nutrition is an important part of the hatchery operation. Successful larval rearing primarily depends on the supply of the right kind of live food organisms in adequate quantity. In general, microalgae and zooplankton form the most important components in the live feed culture. There is a separate chapter for microalgal culture, so we are mainly concentrating culture of zooplankton as live feed in this chapter. The size of feed is an important concern when compared to the mouth size of larvae. Poor vision, improper digestive system and weaker movements of the larvae make it difficult for the larvae to get proper nourishment. Some fish larvae (precautionary type) have good yolk reserve and start feeding at comparatively developed stages while some others (atresial type) start feeding at smaller and lesser developed stages. Many of the freshwater fish larvae, and marine fish larvae like salmon, cobia and clown fish larvae are comparatively larger than grouper and damsel larvae. Larval feed should be smaller, easily digestible, rich in nutrients and allow autolysis. Formulated feeds are generally not suitable for most of the fish larvae may not meet all these requirements and in most 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. Live feed in continuous movement in the water will help the weaker larvae to prey upon these tiny organisms. Copepods, cladocerans, decapod and bivalve larvae, rotifers and ciliates are the important zooplankton organisms which form the food of fish larvae in the wild. The most popular zooplankters used as feed for fish larvae in the fish hatchery are rotifers, cladocerans, artemia and copepods (Lavens and Sorgeloos, 1996).

Rotifers

Rotifers are the most popular larval live feed used in both marine and freshwater hatcheries. Rotifers are very small organisms mostly ranging from 0.1 to 0.5mm belonging to the Phylum Rotifera. Rotifers are naturally abundant in productive freshwater and brackishwater ecosystems. *Brachionus plicatilis B. rotundiformis* and *B. calyciflorus* are the most common rotifers used in hatcheries all over the world (Anitha and Rani Mary George, 2006). Rotifers are small, filter-feeding organisms with high reproduction rates, capable of reproduction through both sexual and asexual methods and can be reared in large densities up to 2000 animals/ml. In general, rotifers can be easily acclimatised and cultured at various salinities. Both *B. plicatilis* and *B. rotundiformis* can be slowly acclimatised to desirable salinity and can cultured at higher densities. *B. calyciflorus* is generally a freshwater form. In general, rotifers are essential in marine finfish hatcheries. Except for the difference in salinities, culture methods are almost the same for all the species. Both *B. plicatilis* and *B. rotundiformis* have three strains developed for hatchery purposes. Large (L) type with lorica ranging from 100-340μ; Small (S) type with a size range of 100-210μ; Super small (SS) type with less than 100μ size.

Ideal water quality parameters for maintaining a successful mass production of *B. plicatilis* and *B. rotundiformis* are -salinity below 35ppt, temperature 20- 28ºC, dissolved oxygen level above 3mg/l, pH above 7.5 and ammonia below 1mg/l. major contaminants in the culture are ciliates and bacteria. Bacterial load especially of *Vibrio* sp. should be below 10^7 CFU/ml. The culture should be free from ciliates like *Uronema* spp. and *Euplotes* spp. In case of severe contamination, continuous 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. Algal feed is ideal for stock culture. Small closed vials are used for maintaining pure stock culture (Dhert, 1996).

Mass culture of rotifers always carries some risks of sudden mortality. Hence it is always ideal to maintain stock culture separately in clean aseptic conditions. Rotifers for initial 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 culture by taking a small sample from a well-maintained stock culture of a hatchery or 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 3000lux using *Chlorella* as feed. It is ideal to add fresh algal culture depending on the density of these tubes. It is better if all the culture tubes are placed on a gentle shaker or a rotating shaft to provide enough oxygen evenly. Ideally, this should be maintained at a density of 2 rotifers/ml up to 200 nos/ml. The stock culture should be periodically recultured and disinfected using mild antibiotics as and when required.

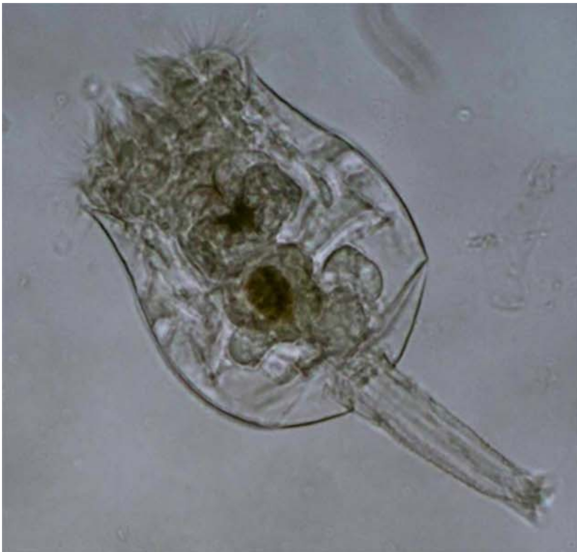
Once the density reaches around 200nos/ml, this can be transferred to Erlenmeyer's flasks of 500ml capacity, with an algal concentration of $1.6x10^6$ cells/ml. Approximately 50 ml of the algae needs to be added daily to maintain the cell density. This can vary depending on the species and culture conditions. The important factors to be monitored are the algal cell density and rotifer density. No aeration is required during this short rearing period. Once the rotifer concentration reaches 200-300 cells/ml (generally within 3 days) the culture is ready for inoculation to 15l bottles. The culture should be passed through the first strainer of 200µ mesh and then strained using 50µ mesh and the filtrate can be transferred to 15l bottles with a density of approximately 50nos/ml for producing starter culture. From this stage onwards we should go for aeration. Fresh algae of a concentration of $1.6x10^6$ cells/ml should be supplied daily ration. Within 7 days the 15l bottle will be full and the culture is now ready for mass culture.

The culture can be maintained using fresh algal culture or commercial algal pastes or with baker's yeast culture and also in formulated diets. Formulated ideal diets for rotifers are now available in the market and several companies are producing rotifer feeds.

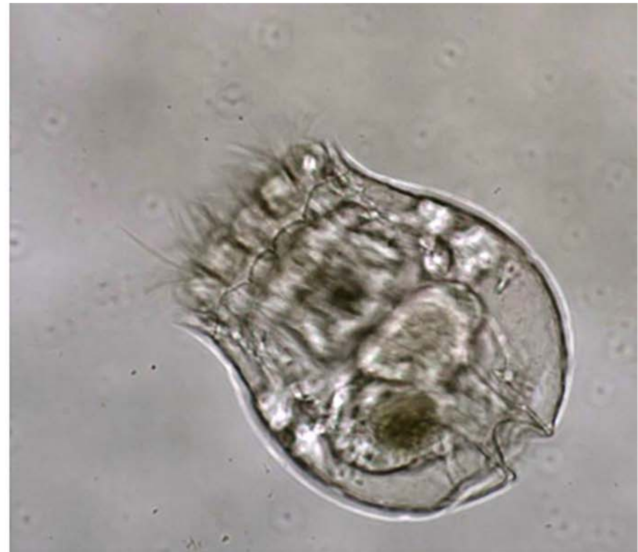
For mass production of rotifers, the hatchery should have the facility for at least any one of the above feeds. Mass culture is generally maintained in large indoor tanks. Continuous harvest can be possible if the rotifer reaches density of 300-500 nos/ml. Daily the rotifers will double their population. Different types of sieves/ strainers can be prepared using micron mesh nets or bolting silk for filtering the mass culture to harvest rotifers. Algal culture needs to be pumped into the culture tank daily and enough aeration should be given to maintain the production.

The nutritional value of rotifers mainly depends on the type of feed used. Rotifer cultured using a mixture of *Tetraselmis*, *Nannochloropsis* or *Isochrysis* or a mixture of these will be higher in DHA and PUFA content than that cultured using *Chlorella*. Rotifers produced using yeasts will be deficient in essential fatty acids and need to be enriched before feeding. Several commercial products are also available for the enrichment

of rotifers. The use of enriched rotifers for feeding the larvae is essential for getting good larval survival. Harvested rotifers can be reared separately in water containing enrichment media. The enrichment time requires the type of media. Simple enrichment can be done by using *Tetraselmis*, *Nannochloropsis* or *Isochrysis* or a mixture of these fed finally for one day to enrich the rotifers naturally. But commercially available enrichment media is also very effective. The media can be added to the harvested rotifers kept in higher concentrations with minimum water and allowed to remain there for a few hours. The enrichment status can be observed by the colour change of the rotifers used. The enriched rotifers can be directly fed to fish larvae.



Brachionus plicatilis (live)



Lorica of *B. plicatilis*

Cladoceran culture

Cladocera or Diplostraca are branchiopod crustaceans commonly known as water fleas. These are more common in freshwaters and are more popular in the culture of freshwater ornamental fishes. *Daphnia* spp. and *Moina* spp. are commonly used in freshwater ornamental fish hatcheries. Cladocerans are comparatively larger when compared to rotifers. These are generally around 1mm in size and are prolific in nature. These are hardy and filter-feeding organisms. Both these species can be cultured easily in small containers. Both these groups can reproduce by sexual and asexual means. When conditions are favourable, cladocerans generally carry brood pouches and release the young ones directly. They will go for sexual reproduction mainly during unfavourable or stressful conditions. Size of popular *Moina* spp. varies between 700-1200µ and for *Daphnia* spp. it varies from 700-4000µ. Generally, these are rich in protein content and need enrichment for increasing PUFA content. Enrichment protocols are the same as in the case of rotifers. *Daphnia* reaches 100-200 nos/ l but *Moina* can reach up to 5000 nos/l (Rottmann et al., 2003). Stock culture can be easily done in small containers and mass culture can be in large containers both in indoor and outdoor conditions. The depth of water should not increase beyond 1m. Outdoor culture needs to be protected from rain.

The containers and water need to be sterilized before culture. Alkaline water with a pH of 7.5 to 8.5 is ideal for the culture. *Moina* is a tropical genus and prefers a temperature range of 24-31ºC and *Daphnia* is a temperate genus and the preference of temperature ranges between 18 to 22 ºC. Both these are nonselective filter feeders that feed on a variety of organisms: bacteria, ciliates, yeast and phytoplankton. Algae can be cultured using inorganic and organic fertilizers. Both batch culture and semi-continuous cultures are possible for these. Regular and partial exchange of water is essential. Cladocerans can be harvested by

passing the culture water through a sieve of 150µ. In general, these are positively phototrophic and can be concentrated using light as in the case of Artemia nauplii. Cladocerans can be stored in inactive condition at 4°C without losing their viability.

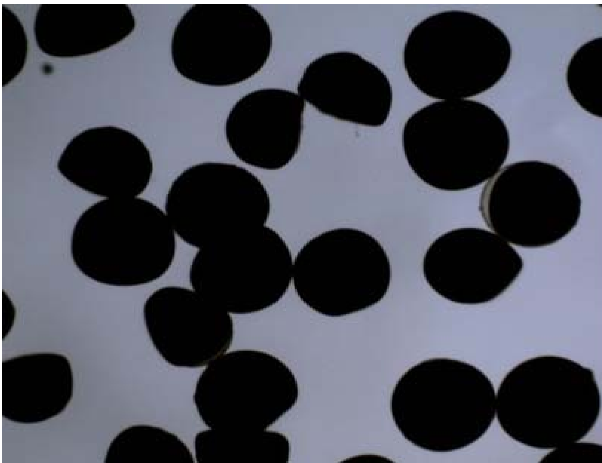
Artemia

Artemia or the brine shrimp which can 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 200tonnes of artemia cyst is marketed annually. Artemia is typically a primitive crustacean belonging to the class Branchiopoda. Total length is about 07-1.2mm and sexes are separate. Artemia is generally produced in hypersaline ponds and can tolerate a wide range of salinity and temperature but the optimum salinity required is 35-38ppt. Artemia can reproduce parthenogenetically but in adverse conditions, it produces dormant eggs (chorion-coated brown eggs) which can be stored in dry conditions without losing their viability for more than 2 years (Stappen, 1996). In dormant condition artemia cyst is round but concave in one or two sides. On hydration this will become spherical and in less than 24 hrs hydration and aeration, this hatches out into the naupliar stage. The freshly hatched out stage is nauplius I with a length of 400-500µ. This stage is popularly used for feeding the larvae. Within 7-8hrs this will change to nauplius II and start feeding minute algae. The larvae again undergo 13 more moults to become adults. Artemia is a filter feeder mainly feeding on microalgae.

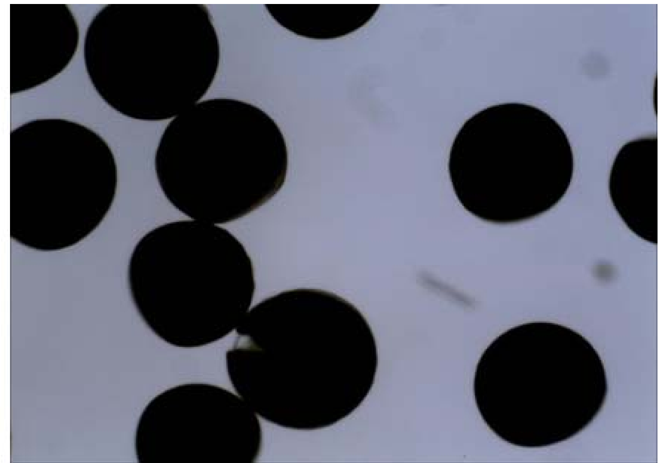
Each gram of artemia cyst contains 200000 to 300000 eggs and almost 50% will hatch within 20-24 hours on proper hydration. The artemia cysts must be properly weighed and kept for hydration in normal sea water of salinity less than 35ppt. The density can be 2g/l approximately and the pH should be above 8 and the temperature should be 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 the larval rearing tank and the species under culture, the requirement of Artemia nauplii should be calculated. Daily measures of the artemia nauplii left over in the tank by examining water in the larval rearing tank and back-calculating the requirement of nauplii/l and the requirement of cysts in g for producing that amount of nauplii. Artemia nauplii if required in large quantities, it is essential to disinfect the cysts before hydration to reduce the bacterial load to increase the quality and quantity of hatching. Soak the cyst in 200 ppm sodium hypochlorite solution for 30 minutes and wash the cyst thoroughly several times with tap water using 125 µ sieve. Cylindroconical tanks are ideal for hatching and aeration should be from the conical tip of the tank. Hydrate the cysts before hatching using tap water for one hour and use filtered sea water for hatching. Remove the aeration before harvesting of nauplii and the nauplii are phototaxic and were easily aggregated using light.

Artemia cysts can be decapsulated and directly used for feeding the fish larvae or they can be stored at 4°C for 1-2 weeks without losing their viability. The decapsulation process is simple but needs constant observation. Sodium hypochlorite solution (0.5g/l) or liquid bleach (5ml/l) are commonly used for decapsulation. The decapsulation process should be monitored properly. Keeping longer duration in bleaching agents will affect the survival seriously. The entire container should be immersed in ice-cold water so that the temperature inside the container should be below 20°C. The time required for the decapsulation process will vary from 5 to 15 minutes. The cysts will turn grey with powder bleach and orange colour with liquid bleach, A few samples should be observed using a stereo microscope and if the cyst wall is dissolved, the cysts should be rinsed using a 125µ sieve several times in water till there is no trace of chlorine. To ensure the removal of chlorine, wash the cysts in 0.1N HCl or 0.1% Sodium thiosulphate

solution ($Na_2S_2O_3$) for one minute. Finally, wash through clean filtered seawater and check the water using chlorine test kits chlorine-free cysts can be directly fed to the fish larvae and can be kept for hatching or they can be sieved and stored in refrigerators at 4°C. Artemia nauplii are nutritionally poor when compared to copepod nauplii and this can be enriched PUFA and DHA using the same method of enrichment as in the case of rotifers. A lot of enrichment media are commercially available now. Artemia biomass can be regularly produced using microalgae in tanks with natural seawater. This can be fed by algal paste or fresh algae. All stages of artemia can be cultivated on a large scale and can be harvested regularly using normal seawater in tropical climatic conditions without much effort.



Artemia cysts before hydration



Artemia cyst after hydration

Artemia nauplius stage I

Artemia nauplius stage II

Copepods

Marine zooplankton especially the copepods are a valuable source of lipids, essential fatty acids, protein, amino acids, easily assimilated carotenoids, minerals and enzymes. Copepods form the major first feed in nature but only a few copepods are reared on such a scale which could be used for hatchery rearing of fish larvae for aquaculture (Rippingale and Payne, 2001, Støttrup and McEvoy, 2003). Many marine fish have small-sized larvae that solely depend on even smaller prey which can be ingested and digested by them. The popular live feeds such as brine shrimp, *Artemia salina*, and the marine rotifers, *Brachionus* spp., rarely get encountered by these early forms of marine fish larvae. Instead, they always prefer copepods especially the calanoid, the abundant crustaceans of the marine environment - which are small in size, nutritionally dominant, and they form first feed not only for the fish larvae but for almost all the vertebrates and invertebrates (Lee *et al.*, 2005). The use of copepods as first feed for larval rearing of many marine

fishes resulted in better growth, nutritional profile and survival compared with the popular, traditional live feeds such as *Artemia* and rotifer (Watanabe *et al*., 1983; Støttrup *et al*., 1986; Kraul *et al*., 1992; Støttrup and Norsker 1997; Schipp *et al*., 1999; Shields *et al*., 1999; Støttrup 2000; Payne *et al*., 2001; Evjemo *et al*., 2004). The better nutritional profile which satisfies the nutritional requirements of the marine fish larvae makes copepods a better feed than *Artemia*. The typical swimming pattern of copepods acts as a stimulus for fish larvae to prey upon them. The ease of use and convenience of having different stages in the life cycle make copepods an appropriate feed from the early stages of fish larvae to the weaning (Lavens and Sorgeloos, 1996). The turbot larvae preferred copepod nauplii over traditional live feed rotifers. The reason might be the attractiveness, visibility and compatible size of the copepod nauplii (van der Meeren, 1991). In addition to that, the exogenous digestive enzymes present in copepods make them important in fish larval digestion (Munilla-Moran *et al*., 1990).

The malpigmentation remains a major problem in hatcheries which is mainly due to insufficient feeding and other traumatic nurturing conditions (McEvoy *et al.,* 1998). When compared with the traditional live feeds or diets, the marine fish larvae fed solely with copepods or fed copepods as a supplement to the popular live feeds *Artemia* or rotifer always reported better growth, survival and normal pigmentation (Kraul 1983; Heath and Moore 1997; McEvoy *et al.,* 1998; Næss and Lie 1998; Nanton and Castell 1999). The use of copepods as live feed will reduce the mortality rate and enhance the production of better-pigmented high-quality fry. This results in higher productivity and economic benefits for the aquaculture industry (Tenaw *et al*., 2015).

The flatfish larvae reared using zooplankton showed normal pigmentation than the larvae fed with *Artemia* nauplii (Seikai *et al.,* 1987, Næss *et al.,* 1995, McEvoy *et al.,* 1998). Better growth and survival is reported in seahorses, *Hippocampus subelongatus* when fed with copepods instead of popular live feed *Artemia* nauplii (Payne & Rippingale 2000b). The administration of copepod along with rotifers resulted in better growth and survival in turbot, *Psetta maxima* (Støttrup & Norsker, 1997), dhufish, *Glaucosoma hebraicum*, and pink snapper, *Pagrus auratus* than one fed with rotifer only (Payne *et al.,* 2001).

Copepod species developed and cultured at Vizhinjam Regional Centre of CMFRI

Larval rearing of many important groups of food fishes such as grouper in the Philipines (Toledo *et al*., 1999, 2005), and Taiwan (Liao *et al*., 2001; Su *et al*., 2005), red snapper in the United States (Ogle *et al*., 2005), flounder in France, cod in Norway and turbot in Norway and Denmark (Støttrup 2003) are successfully done using cultured copepods. *Acartia* sp. has been used as the first feed for the larvae of *Lutjanus johnii*. The use of copepods during larval rearing resulted in a 30% increment in the survival rate after metamorphosis (Fukusho, 1980). The larvae of flame angelfish, *Centropyge loriculus*; crimson jobfish, *Pristipomoides filamentosus*; almaco jack, *Seriola rivoliana* and the peacock hind, *Cephalopholis argus* are successfully reared using semi-intensively produced *Parvocalanus crassirostris* (Toledo *et al*., 1999; Engell-Sørensen *et al*., 2004, Schipp, 2006, Laidley *et al*., 2008).

The successful rearing of fish larvae by using copepods as live feeds includes, Grouper *Epinephelus coioides* (Toledo *et al.,* 1999; 2005, Ranjan *et al*., 2018), Indian Pompano, *Trachinotus mookalee* (Ranjan *et al*., 2018), Herring *Clupea harengus* (Hjelmel *et al.,* 1988), Red seabream *Pagrus major* (Ohno, 1992), Mahi mahi *Coryphaena hippurus* (Kraul, 1993; Schipp, 2006) Flatfish *Scophthalmus maximus* (Bell *et al.,* 2003) Barramundi *Lates calcarifer,* Almaco jack *Seriola rivoliana,* Giant Trevally *Caranx ignobilis* (Schipp, 2006), Florida Pompano *Trachinotus carolinus* (Cassiano *et al.,* 2011) and Atlantic Cod *Gadus morhua* (Karlsen *et al.,* 2015). In successful rearing of all these species, either the copepods alone or a combination with rotifer was used.

The larvae of ornamental fish such as *Dascyllus aruanus* and *D. trimaculatus* failed to consume rotifer during their first feeding (Gopakumar *et al*., 2009b) and the rearing was successful only when they fed with copepod alone as their first feed (Gopakumar *et al*., 2009a). *Pomacentrus caeruleus* (Caerulean damsel), *Chromis viridis* (Blue green damsel), *Neopomacentrus nemurus* (Yellowtail damsel) and *Chrysiptera cyanea* (Sapphire devil damselfish), are also been successfully reared using copepod/in combination with rotifer as live feed (Gopakumar and Santhosi, 2009; Gopakumar *et al*., 2009 a, b). *Neopomacentrus cyanomos* (Regal demoiselle) larvae are reared using copepods and they result good survival and better growth patterns (Rohini Krishna *et al.,* 2016). The use of copepod, *P. crassirostris* as the first feed gave better survival rates than feeding with rotifers in Marcia's anthias, *Pseudanthias marcia* (Anil *et al*., 2018). The larval rearing of *D. carneus* was unsuccessful when tried with rotifer *Brachionus plicatilis* as first feed and they were successfully reared using copepod *Parvocalanus crassirostris* alone from initial feeding to metamorphosis (Anzeer *et al*., 2019). Feeding trials using a combination of copepods *Temora turbinata* and *Pseudodiaptomus serricaudatus* to feed the fry of *Hippocampus kuda* has resulted a better growth when compared to Artemia and rotifer as live feed. The feeding experiments with naupliar stages of *T. turbinata* to feed *Amphiprion frenatus* showed a significant enhancement in colour and higher survival when compared with the larvae fed with popular live feeds *Artemia* and rotifer (Santhosh *et al*., 2018).

Being visual feeders, the presence or absence of light greatly affects the larval feeding and thus the final survival of the larvae. In addition to that the density of quality live feed in the larviculture system also affects the larval survival. During the transition of larvae from one size range to another, the presence of compatible live feeds of both sizes should be provided in the larviculture system (Gopakumar *et al*., 2013). Thus fish will not strive to accept feed of a new size range. The availability of different stages (Nauplii, Copepodite and Adults) having various size ranges within a single species makes the copepod a suitable live feed. They can be used for rearing larvae having a wide range of mouth sizes during their development.

The intensive culture techniques required for year-round production of copepods are not possible in temperate climates where the culture systems will be delimited to extensive or semi-extensive rearing systems in small-scale hatcheries. The year-round intensive production of copepods can be carried out in regions having favourable conditions for feeding larvae of food fish or ornamental fish. Developing a

suitable copepod production system or the development of resting or diapause eggs on a commercial scale will be one of the most important components of the future development of aquaculture (Støttrup and McEvoy, 2003). Details regarding the culture of marine copepods have been summarised and published as a freely downloadable book by CMFRI. More detailed information is already published in this book. Santhosh, B., Anil, M.K., Anzeer, F.M., Aneesh, K.S., Abraham, M.V., Gopakumar, G., Rani, Mary G., Gopalakrishnan, A., Unnikrishnan, C. (Eds.), 2018. Culture Techniques of Marine Copepods. ICAR-Central Marine Fisheries Research Institute, Kochi, Kerala, India. http://eprints.cmfri.org.in/13376/

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