

Zooplankton Culture for Mariculture

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Introduction

Plankton is vast and diverse group of organism incapable of free swimming. They drift in the mercy of water current. Plankton is described as the smallest group of plants and animals in the aquatic ecosystem. The term 'plankton' was coined by the German founder of Quantitative Plankton and Fishery Research, Victor Hensen in 1887. It is derived from a Greek word 'Plano' meaning 'to wander' and it has the same etymological root as 'planet'. They are seen in both marine and freshwater ecosystems. Planktons are broadly classified into two major groups 'Phytoplankton and Zooplankton'. Phytoplanktons are microscopic plant community in the aquatic environment. They synthesize organic carbon from inorganic material by the process of photosynthesis. They are considered as the primary producers in the aquatic ecosystem. Major phytoplankton groups are diatoms, dinoflagellates, blue green algae, *etc.* Zooplankton is the small animal community in the aquatic environment considered as the primary consumers or the secondary producers of aquatic ecosystem. The zooplankton occurrence and distribution influence the pelagic fishery. Major zooplankton group present in the aquatic environment are Copepoda, Decapoda, Amphipoda, Ostracoda, *etc.*

Zooplankton

Zooplankton (fig: 1) is the diverse delicate and very useful group of animals that drift in water current in the aquatic ecosystems. It plays a very important role in shaping the aquatic ecosystem. They are distinguished from phytoplankton on the basis of morphology or the mode of nutrition. They are the heterotrophic group of the

plankton community. They can feed on phytoplankton, other zooplankton and detritus. They range in size from single celled protozoa to large jelly fishes. Generally zooplankton is distributed according to the availability of the phytoplankton and suitable environment.

Classification

Zooplankton can be categorized into different groups according to their mode of life, size and feeding habit.

Based on the life pattern

- Holoplankton: -Spending their whole life as plankton. E g: Amphipod, copepod, ostracods, etc.
- Meroplankton: -Plankton forms that do not spend their entire life as plankton.
E g: -Cephalopods, Fish, Scyphozoan, etc.

Based on the size of the plankton

- Micro zooplankton:-Size ranges from 20 μm to 200 μm and it is the main source of production in the sea
- Macro zooplankton :-Size ranges from 200 μm to 2mm
- Mega zooplankton: -Size >2mm

Based on the food preference

- Herbivores:-they consumes phytoplankton
- Carnivores: -they consumes small animals
- Omnivores:-they consumes mixed diet of plant and animal material
- Detritivores:-they consumes dead organic material

Zooplankton Culture

Successful hatchery production of fishes and other organisms in aquaculture depends on the availability of the planktonic organisms of appropriate size for feeding their larvae. Freshly hatched *Artemia nauplii* have been the popular larval feed used by the aquaculturists for a long time. But the high cost of *Artemia* cyst has led to the aquaculturists to search for other suitable

zooplankton which could be easily cultured on a large scale. Different varieties of rotifer sp., copepods and cladoceran are used for the culture. All these organisms which have high reproductive rate, short generation time, and the ability to live and grow in crowded culture conditions have been found to be useful as live feed organism for larval rearing of cultivable species of fishes. Among them *Brachionus plicatilis*, *B. rotundiformis*, *Pseudodiaptomus annandeli*, *P. serricaudatus* and *Moina* sp. have been the most successfully cultured in small and large scale in the mariculture hatchery of the Central Marine Fisheries Research Institute, Kochi, and given as feed for fish larvae.

Importance

Zooplanktons play an important role in the pelagic food web by controlling phytoplankton production and shaping of pelagic ecosystem. It plays critical role as food source for larval and juvenile fishes. The zooplankton population dynamics, reproduction and survival rate etc. are important factors for recruitment to fish stock. Zooplankton occupies a key position in the pelagic food web as it transfers organic energy produced by unicellular algae through photosynthesis to higher trophic levels. It is regarded as the most important environmental factor controlling the year class strength of a large number of commercial fish stock. The ecological significance of zooplankton communities depends on the diversity, behavior and interaction of their species, which play the main role in channeling energy up the food web and exercising top-down control through grazing or predation. Natural (variation in current) and man-made factors can strongly affect zooplankton density and distribution. Zooplankton contributes to the removal of surplus anthropogenic carbon dioxide from the atmosphere through sedimentation and burial of organic and inorganic carbon compounds.

Sample collection

For culture zooplankton samples are collected during early morning hours from the coastal waters of Kochi, Kerala (N 10° 01.764'; E 076° 12.955'). Sampling is done using 60- μ m-mesh plankton net. Thus collected contents are transferred to 1L sample bottle containing pre-filtered water from the collection site itself. Almost immediately, the samples are transported to laboratory for processing. On arrival, the salinity of the sample water is checked using Hand Refractometer/Salinometer and the salinity of filtered seawater is adjusted accordingly. The samples are then rinsed thoroughly using filtered seawater of salinity 25-35 ppt. for removal of contaminants.

The zooplanktons are segregated based on their size using sieves of different mesh size in order to obtain adult planktons and later stage planktons. Initially, a 500- μ m-mesh sieve was used to remove fish and prawn larvae and later a series of sieves 300- μ m-mesh size to 60- μ m-mesh for screen to eliminate smaller zooplanktons such as rotifers, copepod nauplii and barnacle nauplii, etc. The selected zooplanktons for culture are then rinsed vigorously several times with filtered seawater (25-35ppt) to prevent contamination. Rinsing is also done periodically to reduce the rotifer numbers in copepods and other planktonic cultures. After rinsing, the remaining adult zooplanktons are maintained in 1L to 3L culture flasks and fed with phytoplankton/algal culture.

Algal Culture as phytoplankton feed

Algal culture was separately maintained for feeding the zooplanktons. The algae we maintained for feeding the zooplanktons are *Tetraselamis gracilis*, *Nanochloropsis* sp., *Chlorella* sp., *Chaetoceros calcitrans* and *Isochrysis galbana*. The cultures of these species are maintained in controlled conditions in the laboratory. Stock cultures are maintained in 500 ml conical flasks and 4000 ml Haffkine flasks containing filtered seawater at 23 °C temperature,

with 32ppt. salinity and 2100 W tube lamps are used to provide artificial illumination and fertilized with Wallness medium.



Figure 1 stock and mass culture of algae

Meanwhile, the collected zooplanktons are identified under light microscope (4 x magnification) using the key given by Kasthurirangan (1963). Based on diagnostic morphological characters, the zooplanktons are sorted as individuals of single discrete culture in each culture flask and labeled accordingly.

Copepod culture

Copepods constitute the first vital link in the marine food chain leading from primary producer to fish. Copepods are mostly marine and many of the species occupy freshwater or estuarine habitats. Almost one-third of marine copepod species are parasites or live in symbiotic relationship with other organisms. The most commonly used species in aquaculture are free-living copepods belonging to three of the ten orders of copepods (reviewed by Huys & Boxshall 1991): Calanoida, Harpacticoida and Cyclopoida.

Axénisation

Prior to start of stock culture of copepod, the axénisation of copepods has to be done by antibiotic treatment method. The antibiotics Benzyl Pencillin Sulphate, Streptomycin Sulphate and Chloramphenicol are mixed in different proportions (400mg+80mg+10mg) respectively and stored as “dry mix”. 50ml

sterile sea water is used for the mixing the antibiotic dry mix. 6 sterile test tubes are taken and the first one was kept empty added 6 ml of the sterile sea water to the second tube and continued up to the sixth tube. To the empty first tube added 6 ml of sea water and 6 ml of the antibiotic mix. The tubes are shaken well and the 6 ml solution from the first tube was added to the second tubes which already contain 6 ml sea water. Later took 6 ml from the second tube and transferred to the third tube. Likewise all the tubes are mixed with antibiotics and 30-60 numbers of zooplanktons are added to all the tubes. A few drops of sterility test medium are added to all the tubes. The cultures are then incubated at 37 °C for 24 hrs in dark. The bacterial growths were checked by streak plate method. After axenisation culture is sub cultured and maintained (Droop1967).

Production of copepods

Batch culture of copepods is relatively straight forward once proper environmental and nutritional conditions are met. The culture flasks are stocked with adult copepods (10-25 individuals/ml). The cultures are maintained in 1L conical flask containing filtered sea water (25-35 ppt.) and fed with live algae in the ratio 1:25 (v/v). The algal feed was given on alternate days. The cultures are rinsed with filtered sea water and the eggs, nauplii, and adult from the detritus are separated into fresh culture flasks every week either manually or through filtration. The adult would begin producing eggs, sperms in 9-12 days; thereafter egg production would initially raise, and then reaches the peak and finally it falls.

Stock culture

Culturing large volumes of copepods on algae and baker's yeast diets always involve some risks of sudden mortality of the population. Technical or human failures and also contaminations with pathogens or competitive filter feeders are the main causes for lower reproduction which can eventually result in a complete crash of the population. Relying only on mass cultures of copepods for

inoculating new tanks is too risky an approach. In order to minimize this risk, small stock cultures are generally kept in closed vials in an isolated room to prevent contamination with bacteria and/or ciliates. These stock cultures which need to generate large populations of copepods as fast as possible are generally maintained on algae.



Figure 2 Stock culture of copepod

Stock cultures of calanoid, cyclopoid and harpacticoid copepod are maintained in 1 to 3L flask with culture media. The culture media was chlorinated sea water, salinity of the water 25-30ppt as measured by hand held refractometer. Feed is added every day depending on the size of the container and care must be taken to avoid over feeding, which is indicated by cloudy media or excess precipitation food. No aeration is provided for stock culture upto 3 L. Culture water is renewed once a week, by passing the culture through a series of mesh size 500 μm , 300 μm , 200 μm , 100 μm and 60 μm sieves. These sieves of varying mesh sizes are used in order to collect the copepods at different stages of their lifecycle. The cultures were maintained at 23-33°C (Rhodes, 2003).

Mass culture

The cultures are maintained in 50 L square tank, 3000 L cylindro-conical tank and 1 tone round outdoor culture tanks with continuous aeration (Merrylal James and Martin Thompson 1986).



Figure 3 mass culture of copepod

Rotifer culture

Rotifers have been used as food organism for cultured marine fish larvae. A continuous and stable supply of nutritionally adequate rotifers is the key to the flourishing culture of marine finfish in various parts of the world. They also used as a food for culturing penaeid shrimp (Samocha et al. 1989) and crabs (Keenan & Blackshaw). It serve as a 'living capsule', providing the nutrients required by the cultured marine fish larvae for proper development. Large number of rotifers may be required per day for raising marine fish larvae in commercial hatcheries (Lubzens et al. 1997, 2001). The range of 20,000 to 100,000 rotifers per fish larvae during the 20 to 30 days of culture (Kafuku& Ikenoue1983; Lubzens et al. 2001). The methods developed for providing an adequate supply for rotifers to a variety of small mouthed fish larvae rely on extensive studies into their biology, feeding, reproductive strategies, genetics, physiology and biochemistry. Most rotifers are free-crawling or swimming and colonial forms are also known

(Ruttner-Kolisko 1974; Pontin 1978; Wallace & Snell 1991; Nogrady et al. 1993). About 20000 species populate in freshwater and several species are known from the brackish water and marine water.



Figure 4 *Brachionus plicatilis* (Rotifer)

Isolation

The brachionidae family comprises a large number of species but most of them are in fresh water, only few species are in marine form. Two of these (*Brachionus plicatilis* and *B. rotundiformis*) are being used extensively in mass cultures and serve as early developmental stages of marine fish larvae. Rotifers grow naturally in brackish water ponds and can be easily isolated under a binocular microscope with a fine pipette. As the rotifer is parthenogenetic, it is easy to isolate a few egg-bearing female in a petri dish containing filtered brackish water and by feeding them with a suitable algal food to build up a stock culture within a few days.

Stock culture

Selected species of rotifers such as *Brachionus plicatilis* and *B. rotundiformis* are isolate and take 10 to 20 numbers of egg-bearing rotifers are put into 5 numbers of 250 ml flask contain 200 ml of filtered brackish water/sea water (15 to 25 ppt.) and add phytoplankton such as *Nanochloropsis* sp. and *chlorella* sp. are given as feed . Daily monitoring of the culture, water exchange and routine filtering of the water are necessary to prevent contamination and also to maintain the water quality.

Mass culture

Three methods are used today for obtaining large number of rotifers: batch culture, semi-continuous culture and continuous culture.

Batch culture

Rotifers are introduced at low density into 'green water' produced in fertilized tanks or ponds. Rotifers are harvested after all the algae have been consumed and used as food for fish larvae. A small number is used to inoculate newly prepared 'green water' ponds or tanks. A recent adaptation (Lubzens et al. 1997) for batch culture relies on a series of tanks. The culture system depends on short production cycles (1 week) with a cleaning interval of tanks and aeration tubing. The culture started in each tank (500L to 1 tone tank) filled with sea water salinity of 25ppt, in a one fifth of its volume sterilize with hypochlorite solution. Introduce rotifers with a density of 200-300per ml. rotifers are mainly fed with Baker's yeast, daily ration is divided into 3 to 5 meals. Daily increase the volume of the water, to increase the rate of rotifers in the tank. At the end of the culture period density may reach to 300-500numbers per ml. The final volume of the tank reaches after 4-5 culture days. The rotifers are then sieved and concentrated, and the culture tank and its accessories are sterilised in hypochlorite solution (10ppm of commercial grade bleach) for 24h, followed by through rinsing with sea water before reuse. The concentrated rotifers are immersed in a brief period in fresh water, for remove the contaminants. Most of the concentrated rotifers are enriched with essential fatty acids and proteins before being to feed the fish and a small fraction is used for new culture.

Semi continuous

This is a periodic harvesting of rotifers by removal of part of the culture medium and replacing it with new sea water (Hirata 1980). This method has been termed the 'thinning method' (Fukusho 1989b). The volume removed depends on the reproductive rate of

the rotifer and harvesting removes only the number of rotifers gained by reproduction from the previous harvesting period. 6-7% of biomass can be removed daily in rotifer culture maintained in Baker's yeast (Hirano 1987). Culture continues for several days.

Continuous culture

This is based on the chemostat or turbidostat models of microorganisms and is fully controlled and highly dependable (temperature, pH, oxygen supply and density of cultured organism) (Walz 1993; Walz et al 1997). They offer easy manipulation of rotifer physiological and nutritional quality. Log-phase produced rotifers can be harvested and their nutritional quality is maintained by providing adequate food organisms (James et al. 1983; James and Abu-Razeq 1989a,b,1990).

Artemia Nauplii Production

All Brine shrimp eggs need to be stored in a tightly sealed container, free from moisture and in a cool environment at or below 28°C (Refrigeration is need for short term storage, for a long term storage eggs are best kept at below freezing).



Figure 5 The cyst pack, cyst and the nauplii of Artemia (Brine shrimp)

Hatching environment

For hatching the cyst was done in a conical container. The salinity of the water is 30 ppt and the pH is 8. The optimum temperature may be 26 to 28°C, maintaining a light source during the entire

incubation period is recommended to obtain optimum hatch result and for temperature control. Constant aeration is necessary to keep cyst in suspension and to provide sufficient oxygen levels for the cysts to hatch. Strong aeration should not damage or hurt the brine shrimp cyst or nauplii. The stocking density of the cyst is 1gram per liter. Cone or 'V' bottomed containers are best to ensure that cyst remain in suspension during hatching. Be sure to thoroughly wash the hatching cone with a light chlorine solution, rinse and allow to air dry between uses. Avoid soap, soap will leave a slight residue which will foam from aeration during hatching and leave cysts stranded above the water level. Generally the optimum incubation time is 24 hours. Egg which has been properly stored for more than 2-3 months may require additional incubation time up to 30-36 hours. Oftentimes eggs will hatch in as few as 18 hours. If smaller size nauplius (instar1) is desired, a harvest time of 18 hours is recommended.

Set up of hatching procedure

Place hatching cone or similarly shaped vessel in well-lit area. Cone should be semi-translucent for ease for ease of harvesting and light transmission. Fill cone with water and adjust salinity to 25 ppt. and the optimum temperature is 28^o C. add the cysts at the rate of 1 gram per liter. Provide adequate aeration to keep cysts in suspension. Depending upon water temperature, cysts should hatch in approximately 18-36 hours. After hatching Brine shrimp, turn off or remove aeration and wait several minutes for the shells and the baby brine shrimp to separate. Newly hatched nauplii will settle to the bottom of the cone or move towards a light source; the shells will float to the surface. Once separated, the nauplii can be siphoned from the bottom with a length of air tubing or gently drained through the bottom of the cone through a valve. The warm incubation temperatures and metabolites from the hatching medium create ideal conditions for a bacteria bloom. Rinsing of the nauplii in a fine mesh net or sieve using clean fresh or salt water is important before feeding them to your fish. Tanks and brine

shrimp hatching equipment should be cleaned and disinfected routinely.

Culture of Moina

The fresh water cladoceran moina is frequently found in ponds. It is readily eaten by bigger fish fry. Moina reproduced by parthenogenesis under favorable condition and forms resting eggs through sexual reproduction under unfavorable conditions. The embryos develop inside the dorsal brood pouch and the young ones hatch out fully formed. It is a filter feeder living on a variety of unicellular fresh water algae. Chlorella species are appears to be the best feed.



Figure 6 Moina

Stock culture

Moina can be collected from ponds and a stock build up starting from a single parthanogenetic female. From a single female kept in a 2 liter beaker containing Chlorella water, it has been possible to obtain about 5000-10000 Moina within 10-15 days. Stock culture maintained in 1-2 liter flask. Good tap water or well water can be used for growing Moina. Salinities above 5ppt are not tolerated by them.

Mass culture

Mass culture done in 100 liter to 1 tone tanks in outdoor. The tanks are cleaned and fill it with fresh water then sterilized with hypochlorite solution. After sterilization inoculated with culture chlorella, fertilized with urea (8g/l), Zinc Superphosphate (4g/l) and Ammonium sulphate (4g/l) give good aeration should be provided. On the second day after the water becomes slightly greenish, inoculate the pure culture of Moina 20-50 per ml were added. The Moina multiplies rapidly and attains a concentration of 10 to 20 thousand individuals per liter in 6-7 days. At this stage 1/3-1/2 the volume can be harvested and replaced by freshwater along with the proportional amount of the above fertilizers or by chlorella water cultured in a separate tank using the same fertilizers. If the latter method is followed 1/3 of the culture volume can be harvested every day.

Appearance of males in the culture is increased in number this will leads to the decline of the population by formation of resting eggs. When this happens it is better to remove all the water leaving only the sediments at the bottom and filling up the tanks with chlorella water again. Moina culture revives in a few days.

Harvesting is done with plankton net in the exponential growth phase when the females are reproducing actively by parthenogenesis. The parthanogenitic females containing 8-12 embryos in the brood pouch are rich I organic matter and are evidently more nutritive than females with resting eggs or the males. Harvested Moina are washed in water and give it as a feed for fish larvae.

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