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Dr. SALIM ALI ROAD  
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LIVE FOOD ORGANISMS - ROTIFER BRACHIONUS PLICATILIS  
AND CLADOCERAN MOINA Sp.

S.K. PANDIAN

Central Marine Fisheries Research Institute,  
Marine Prawn Culture Laboratory,  
Narakkal-682 505.

Fish fry and crustacean larvae are apparently specific to type and size of the feed for their survival and growth. At present successful rearing of fish and crustacean larvae depends upon the availability of suitable live feed organisms. Existing literature reveals that only very few species have been used as live feed organisms of which rotifers and cladocerans are of prime importance in aquaculture industry.

ROTIFER BRACHIONUS PLICATILIS

For early larval stages, rotifers are found to be suitable, initial live-feed which are intensely utilized in hatcheries throughout the world. In Japan, rotifers are given as initial diet for freshly hatched fish fry (of above 2-3 mm in body length) and feeding with rotifer is continued for about 30 days after hatching. For the culture of fish larvae, which are vulnerable to starvation, rotifers are used as initial food in United States, Russia and United Kingdom. The rotifer Brachionus plicatilis is found to be an ideal food for larvae of the blue crab, Callinectes sapidus. At present, without the mass culture of rotifers, larval rearing of marine fishes is virtually impossible in Japan although

artificial microdiets are being gradually developed to replace live-feed. The species Brachionus plicatilis is one of the most commonly used rotifer in aquaculture, serving as an important food source for predatory larvae.

Biology of rotifer Brachionus plicatilis:

B. plicatilis shows important features of a live-food organism such as size suitability, high rate of multiplication within a short period of time, utilization of minute phyto (primary) food as feed and the ability to withstand the adverse condition by producing cyst.

Size of B. plicatilis suits with the requirement of early predatory larvae and it varies with reference to the sex of the species. The length of the (lorica of) female is 165-192 microns while the width is 118-140 microns and the size of the female is bigger when compared to the male which is about 150 microns in length and 55 microns in width. In addition to the suitability of the size, B. plicatilis is having a very high multiplication rate. Under favourable conditions, the above-mentioned rotifers reproduce by parthenogenesis. The average life span of B. plicatilis is observed to be 5.25 days at 28°-30.5°C during which period a parthenogenetic female produces 19 eggs. The doubling time of the population is found to be 0.45 days and theoretically it is calculated that totally 3079 rotifers can be obtained from a single parthenogenetic female during its short life span (Muthu, 1983 a). Rotifer B. plicatilis goes for sexual reproduction during unfavourable conditions. It produces males and subsequently resting eggs to assure the survival of the species during adverse situations. The males, which are diminutive, do not resemble the females and they are without alimentary canal as their purpose of creation appears to be

only for reproduction.

In the parthenogenetic mode of reproduction diploid amictic females produce diploid eggs by mitosis. These eggs normally develop into amictic parthenogenetic females. However, these diploid eggs, under unfavourable conditions, develop into mictic (sexual) females. These mictic females meiotically produce haploid eggs which if unfertilized develop into haploid males and if fertilized develop into thick-shelled diploid resting eggs. These resting eggs hatch into a new generation of amictic females (Fig. 1) (King and Snell, 1977).

Brachionus sp. are filter feeders which consume feed-particles of less than 5 microns in size. They have been cultured by different authors by utilizing a variety of diets such as live Dunaliella, live Chlorella, live Scenedesmus costato-granulatus, live Tetraselmis suecica, live Tetraselmis and powdered Spirulina, yeast, "Torulose" yeast, dry Chlorella powder, powdered commercial Spirulina, Chlorella and methanol-grown yeast and mahua oil cake (Muthu, 1983 a). The algal-bacterial-biomass, developed in ponds which are employed for treatment of piggery waste, has also been used for growing Brachionus rubens. Rotifers are also grown on the algal-bacterial-biomass, produced by fertilizing the water with a combination of fertilizers such as groundnut oil cake, cowdung, urea and superphosphate. When Chlorella or other nanoplankters, which are less than 5 microns in size, are offered as feed, B. plicatilis multiply very fast. The growth is observed to retard when diatoms or green algae which are more than 10 microns in size, are given as feed. Thus size is appeared to be the prime determining factor for the selection of the particular phytoplankton as feed for rotifer. Further fertility is considerably high with algal diet while it is

very low when groundnut oil cake is provided as a direct-feed.

Mass-culture of rotifer B. plicatilis

B. plicatilis is mass-cultured in two ways. In the first method, the feed for rotifer is added to the rotifer tank whenever it is required. In other words, feed preparation and rotifer rearing are separate aspects of the culture programme. Trotta (1980) and SEAFDEC follow this method. In the second method, both rotifer and its feed are cultured together in one and the same container and this method is followed at Narakkal Prawn Culture Laboratory.

Trotta (1980) has made use of a continuous system, in which Chlorella is grown with sterile culture medium in one large bag. This Chlorella-bag has separate inter-connections to a reservoir, containing nutritive medium (for phyto-growth) and a rotifer-bag, which is twice the size of the Chlorella-bag. As soon as Chlorella has attained optimum concentration, it is allowed to flow from the Chlorella-bag into the bottom of the rotifer-bag, which expels a portion of rotifer medium through the outlet at the top of the rotifer-bag. Rotifer will be harvested from the medium, thus expelled. Subsequently, the Chlorella-bag will be refilled with fresh enriched medium, obtained from the reservoir, thereby making the above culture process as a continuous one. In SEAFDEC, 350 ton capacity concrete tanks are separately used for rotifer and Chlorella culture. Chlorella is cultured by fertilizing filtered seawater with inorganic fertilizers such as urea, superphosphate and ammonia. This Chlorella water is siphoned into the adjacent rotifer tank which is already inoculated with a stock culture of B. plicatilis. When the rotifer population attains a density of 200-300 Nos/ml, part of the rotifer medium is

siphoned off through a fine-meshed phytoplankton net to harvest the rotifer. Water level in rotifer tank is restored by replenishing with Chlorella water from the Chlorella tank. After a few days, rotifer will again attain the harvestable density and once again the same process has to be repeated. Thus the whole process goes on continuously until the culture becomes contaminated by unwanted organisms either in the Chlorella tank or in the rotifer tank.

At Narakkal Prawn Culture Laboratory, outdoor containers of 2 ton, 10 ton and 40 ton capacity are used for the mass-culture of B. plicatilis. Initially, feed for rotifer is developed prior to rotifer inoculation in these tanks by fertilizing the filtered (through 50 micron net) seawater with groundnut oil cake (juice), urea and super-phosphate at the rate of 200 gm, 2 gm and 2 gm respectively per ton of water. Profound aeration is necessary and a starter culture of Chlorella is inoculated. After taking these measures to develop the rotifer feed, a starter culture of B. plicatilis is added to the same container when pH of the medium increases above 7. The rotifer starter should be obtained from a healthy population and it is preferable to be with egg-bearing parthenogenetic females. It must be devoid of either males or cyst-bearing females. Inoculation is preferable to be at the rate of females. Inoculation is preferable to be at the rate of 5-10 rotifers per ml of the medium. A Chlorella bloom develops within 2-3 days and the rotifers multiply rapidly, attaining a population density of about 250 Nos/ml within 4-6 days after which harvesting is done everyday in the morning when rotifers swam at the surface. pH and oxygen content of the medium play an important role in rotifer multiplication. Schluter (1980) has observed that at pH above 9.5 and below

4.5 no rotifer is survived and high rotifer densities are associated with a pH value of 6-8. If dissolved oxygen content is above 1.15 mg O<sub>2</sub>/l, the reproduction rate is not inhibited while rotifers ceased to reproduce and die within a few days at 0.72 mg O<sub>2</sub>/l. The presence of a male or a cyst bearing female indicates the existence of adverse conditions in the culture tank. Hence routine observation is essential in order to understand the biological and physical conditions prevailing in the culture tank and the growth pattern of the population.

When Chlorella bloom declines, half the volume of water is replaced with fresh seawater and refertilized with the abovementioned fertilizers at half of the initial dose. The culture process is repeated until the culture gets contaminated with filamentous blue-green algae or ciliates. If contamination occurs, the present culture operation has to be closed and the whole process has to be restarted from the beginning. When this culture method is followed, population may reach upto a high density of 560 rotifers per ml.

#### Utilization of harvested rotifers

The harvested rotifers, have to be washed with clean seawater before giving them as live-feed. However, they can be stored by freezing them into frozen blocks in a deep freezer using 10% glycerin as cryoprotectant.

Nutritional quality of the rotifer, as a live-feed, depends on the feed, used for rotifer culture. The rotifers, cultured with yeast, are quite low in W<sup>3</sup> highly unsaturated fattyacids such as 20:5w<sup>3</sup> and high in monoenoic fatty acids such as 16:1 and 18:1. Those cultured with marine Chlorella contain high amount 20:5w<sup>3</sup> which is one of the required essential fatty acids for marine fish. Hence rotifers

cultured with yeast are always inferior to those cultured with marine Chlorella in their nutritional quality as a live-food. The reason for this difference in nutritional quality is that the baker's yeast, used for mass culture of rotifer, contains no  $w^3$  highly unsaturated fatty acids while the rotifer-feed, Chlorella on the other hand, contains a high level of  $20:5w^3$ . Nutritionally poor rotifer, obtained as a result of feeding with substances such as yeast, can be enriched and made as better ones at the time of offering them as feed to predator. For this purpose, the rotifers which are cultured with nutritionally poor diet, have to be fed with enriched diet such as marine Chlorella, microencapsulated diets, w-yeast and emulsified lipids rich in  $w^3$  highly unsaturated fatty acids, for a period of 3-6 hours (Watanabe et al., 1978).

#### Rotifer resting eggs

One of the possible ways of ensuring good supply of rotifers will be building up of reserve stocks and making use of them when the demand rises. Lubzens et al. (1980) are of the view that this goal can possibly be achieved by finding methods for inducing rotifers to produce resting eggs and finding ways for preserving and hatching resting eggs. It has been reported that changes in environmental conditions such as increase in crowding, cold shock, decrease in food quantity and changes in photoperiod may induce production of males and subsequent formation of resting eggs in rotifers (Gilbert, 1974). Ito (1960) reported that B. plicatilis can be induced to produce resting eggs when transferred from 18‰ chlorinity culture media to lower chlorinity media. Lubzens et al. (1980) have produced males and resting eggs in B. plicatilis by transferring them from 100% seawater (38 ppt) to 25% seawater.



Resting eggs may be preserved for at least 12 weeks by freezing at  $-14^{\circ}\text{C}$  without significant loss of viability. Eggs, dried and kept desiccated at room temperature, retained their viability for upto 3 weeks (Lubzens et al., 1980). Little is known about the stimulus which initiates development of rotifer resting eggs. Pourriot et al. (1980) have observed no or very little hatching of the resting eggs in B. rubens, during the first month after laying, at whatever light and temperature conditions and they are of the opinion that most of the resting eggs have to undergo an obligatory dormancy. Further they observed that hatchability in resting eggs of B. rubens is increased by a dormant period in darkness at low temperature (similar to conditions in winter) followed by illumination and an increase in temperature (similar to spring and summer conditions). When the conditions are favourable, resting eggs hatch out into parthenogenetic females and hatching is achieved by keeping them in well-aerated fresh seawater for 24-48 hours at  $29-30^{\circ}\text{C}$ .

#### CLADOCERAN MOINA Sp.

Cladoceran Moina Sp. are characteristic inhabitants of temporary freshwater pools. Although cladocerans of the genera Daphnia and Moina are freshwater organisms, they have been successfully used in the frozen condition to feed marine animals also. They are utilized as suitable feed for the culture of fish fry and postlarval crustaceans.

#### Biology

Cladoceran Moina is bigger in size when compared to rotifer Brachionus plicatilis. The size of Moina fulfils the feed-size requirement of slightly grown-up fish fry and

postlarval crustaceans while the small sized rotifers form an ideal sized feed to early fish fry and crustacean larval stages. The female Moina with embryos, measures 0.78-1.02 mm in length and 0.43-0.74 mm in width (Muthu, 1983 b). Like rotifer B. plicatilis, cladoceran Moina also possesses desirable features like high reproduction capacity, filter feeding habit, ability to produce dormant cysts during adverse conditions and ready acceptability. Moina also multiplies parthenogenetically when the conditions are favourable and changes over to sexual reproduction at the onset of adverse conditions. The males which appear when conditions become unfavourable, are smaller in size and are 0.54-0.72 mm in length and 0.23-0.37 mm in width. These males mate with the females to result in the formation of dormant cysts and cyst production ensures future survival of the species. The average life-span of Moina is found to be 11 days within which one parthenogenetic female produces about 85 eggs. Forty eight hours after birth, the female releases the first batch of 8-12 youngones, which become as parthenogenetic females. For the rest of its life, the female releases similar batch of youngones at every 24 hours and a total of 42765 animals have been resulted from a single parthenogenetic female within its life period of 11 days. The population doubling time is found to be 0.32 day (Muthu, 1983 b).

### Culture

The culture technique for cladoceran is basically similar to that of rotifer. Cladocerans have been reared in small scale under sterile conditions as monoxenic cultures using Chlamydomonas reinhardtii (Murphy, 1970) and as dixenic cultures with C. reinhardtii and Scenedesmus obliquans (D'Agostino and Provasoli, 1970). However, only few generations of cladoceran can be achieved when algae, grown under

sterile conditions in inorganic media, are used as feed. To overcome this problem, nutritional quality of the algae has to be raised by adding vitamin mixture and liver extract to the algal culture medium. A synthetic biphasic medium, consisting of a liquid phase to supply micronutrients and a particulate phase to provide macronutrients, has been used to rear Moina macrocopa by Conklin and Provasoli (1977). For large scale cladoceran production, culturing in sterile media or biphasic media, is not practicable because of high cost of maintaining such cultures. So simplified and economically viable technology to mass-culture cladoceran gains importance.

By making use of ordinary water, many scientists have mass-cultured cladocerans either by feeding with particular diets or by developing the feed in the medium by enriching with certain fertilizers. Micronized ricebran of less than 60 microns in size and 1% brewer's yeast solution are utilized as prepared feed while fertilization is carried out with swine manure or chicken manure or groundnut oil cake or a combination of groundnut oil cake, urea and superphosphate as done at Narakkal Prawn Culture Laboratory (Muthu, 1983 b). In SEAFDEC, cowdung is used as a manure while in Jepara, a mixture of coconut oil cake and chicken dung is made use of. When the medium is fertilized, algal-bacterial-biomass develops forming as food to the cladoceran. This fertilization-method is basically same for culturing any filter-feeding live feed organism and it is normally carried out in outdoor containers. Any freshwater source, which is free from pollution can be made use of for Moina culture.

At Narakkal Prawn Culture Laboratory, tap water, which is aerated for 2 days to get rid off chlorine, is

initially fertilized with groundnut oil cake, urea and superphosphate at the rate of 250 gm, 2 gm and 2gm respectively per ton of water. Subsequently it is inoculated with a starter Chlorella culture. When the medium gets slightly greenish in colour due to the growth of Chlorella, Moina is to be stocked at the rate of 1 animal per litre of the medium. The groundnut oil cake first stimulates bacterial growth and then induces Chlorella bloom. The cladocerans seem to feed on both the bacteria and the Chlorella and also on the finely divided groundnut oil cake particles, suspended in the water. The cladoceran multiplies very fast and when it reaches a density of 30000-40000 animals per litre in 7-9 days at 29-30°C, it is harvested. In both rotifer and Moina cultures, same procedures are followed for harvesting and utilizing the harvested product. As a result of explosion of Moina population, Chlorella concentration declines and to maintain the optimum Chlorella concentration, partial water change and refertilization with 100 gm groundnut oil cake per ton of water are to be done at interval of 4-5 days. Frequent harvesting and partial change of water increase the yield and prolong the life of the culture.

If the culture-container is not covered with a net, chances are there for the development of mosquito larvae in the culture medium. Sometimes the culture gets contaminated with ciliates or undesirable star-like Pediastrum and filamentous blue green algae, which are unsuitable as feed to cladoceran. Such contaminated cultures are better to be discarded. Fresh cultures can be initiated from the stock of dormant cysts. The viability of these cysts, if kept in dry test tube with cotton plug, is about 2 months and these dormant cysts hatch out into parthenogenetic females in 24-28 hours at 28-30°C when kept for hatching in well aerated freshwater.

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