TECHNIQUES FOR THE MASS CULTURE OF ROTIFERS AND MOINA

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INTRODUCTION

Successful hatchery production of fish and shellfish seeds for aquaculture depends on the availability of suitable live feed organisms. The rotifer *Brachionus* sp., cladoceran, *Moina* sp., and brine shrimp *Artemia* sp., have high reproductive potential, short generation period, high nutritive value and capacity to live and grow in high density. These are favourable characters for the mass culture of the above organisms under controlled conditions. Among these species *B. plicatilis, M. brachiata* and *A. salina* (heterosex and partheneo genetic strain) have been most successfully utilized in fish and shellfish hatcheries all over the world. This paper deals with the methods for the mass production of rotifers and cladocerans carried out under controlled conditions at the Regional Centre of Central Marine Fisheries Research Institute (CMFRI), Mandapam Camp.

ROTIFER

Rotifers are available in the intertidal regions along the sea coast, brackiswater channels, pools and ponds where they grow naturally. Isolation is done by micropipette method under binocular microscope or through the sub-culture method. Stock culture can be developed within a few days from the isolated culture.

MASS CULTURE

Techniques for the continuous mass culture of euryhaline rotifer *B. plicatilis* have been developed and perfected. High pressure filtered seawater is pumped into a tank and fertilized with ground-nut oilcake, urea and super phosphate at the rate of 250g, 10g and 5g per tonne respectively. This medium is inoculated with *Chlorella* sp. on the same day and the rotifers are introduced 1000-10000 nos./l on the second day; vigorous aeration is given from the start of the culture, since the dense concentration of algae and rotifers requires large quantity of oxygen especially during night time. Chicken drops or pig manure can also be used as fertilizers.

The planktonic, euryhaline and filter feeding rotifer multiplies very fast by parthenogenesis under ideal conditions and attains maximum concentration (4-5 x 105 rotifer.litre) within 5-7 days. It grows very well at salinity levels 20-30 ppt and temperature range between 26 and 34°C. It is better to harvest daily 25 to 50% of the culture with a replacement of harvested volume of fresh algal culture. Handnet of 60 micron meshsize may be used for harvesting in the morning hours when they reach the surface. Harvesting at regular intervals helps the culture for good maintenance

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for a long period and should be carried out during the exponential growing phase with 3 or more eggs attached to the body of the rotifers. The nutritive value of the rotifers will be very high during this stage. The harvested rotifers are washed in clean sea water and may be fed directly as fresh or stored by mixing with 10% glycerin in a deep freezer at 14°C for later use.

Another method involves culturing *Chlorella* sp. in a 50 t cement tank with above rate of fertilization to achieve 20-40 million cells/ml concentration. These micro algae are filtered and passed into 5 t tanks. They are inoculated with stock rotifers.

During unfavourable conditions such as high density of rotifer, low oxygen level and scarcity of food, amictic female produces male. By sexual reproduction, the resting eggs get released and settle at the bottom of the culture tank. These cysts are collected along with sediments, dried and stored in the dessicator at room temperature. They can also be stored in a deep freezer at 14°C for 3-4 months. The cysts can be safety transported. the dried cysts hatch out within 36-48 hours after hydration with sea water. The resting cysts can be induced to hatch out by sudden reduction of the salinity in the culture medium.

High temperature reduces the length, width and DLS (distance between lateral spines) of the rotifer. The distance between the median spines (DMS) changes with salinity. The size of the rotifers is bigger (130-140/u in length) at low temperature (below 20°C) and smaller (100/210u) at higher temperatures. *B. plicatilis* is more tolerant to temperature and salinity fluctuations.

Antibacterial treatment

As the rotifer may harbour bacteria such as *Vibrio, Pseeudomonas*, *Moraxella*, *Cytophaga* and *Flavo* bacterium, antibacterial treatment is required. Sodium nifurstyrenate (NFS-Na) at the rate of 5 microgram/ml with ultra violet radiation is most effective.

Nutritive value

The nutritive value of the rotifers depends on the diet they are given. Microalgal diet such as *Chlorella* sp. *Tetraselmis* sp., *Dunaliella* sp. give better nutritive values. Marine fish and crustacean larvae require poly unsaturated fatty acids (essential fatty acids) (PUFA) for the developmental stages and these can be met through the enriched rotifers. The high initial algal density enhances the fatty acid enrichment in rotifers. The rotifers can also be enriched by using micro encapsulated (3-5 micro size) particles of fish oil (cod liver oil) or any other oil extract from the marine sources.

EAA (Essential aminoacid) such as proline and methionine are rich in rotifers fed with 37.5×10^6 *Chlorella* sp. cell concentration/ml. Basic aminoacids such as histidine and lysine are more in 25×10^6 fed rotifers. The nutritional quality of rotifers could vary with different cell densities of *Chlorella* sp. utilised in the culture system. The yeast (yeast supplemented with fish oil) are fed to increase the nutritional qual-

ity of rotifer. Concentration of protein at the exponential and stationary phase of growth in the rotifer varies from 34 to 52% depending upon feeding treatment. This can meet the protein requirement of fish and crustacean larvae (30-50%)

For commercial enrichment, super selco, dry selco or protein selco may be used for 3-6 hours along with fresh water *Chlorella* sp. or baker's yeast, which contains high quantity of α^3 PUFA. Several enrichment techniques using yeast, micro encaposultated particles or oil based emulsion are available.

MOINA

Moina can be collected from brackishwater channels, pools and freshwater ponds. A stock culture can be built up from a single parthenogenetic female. The adult releases young ones at the rate of 10-12 nos./day and the young ones become adult in 18-24 hours and begin to reproduce. It is possible to obtain 42,000 nos. within 12 days from a single female.

MASS CULTURE

The filtered freshwater is pumped into a tank (1-10 tonne) and fertilized with ground-nut-oil cake (250g), urea (10g) and super phosphate (5g) per tonne of water. Freshwater *Chlorella* sp. is inoculated on the same day with a cell concentration of 30-50 million/ml (5-10 litre/tonne). Vigorous aeration should be provided. On the second day after the water becomes slightly greenish the culture of *Moina* is inoculated from the stock culture with stocking density of 1-5 nos/litre. The plankton multiply rapidly by feeding on the *Chlorella* sp. and finer particles of ground-nut-oil cake and attain the maximum concentration of 25,000 - 30,000 nos./litre within 5 to 7 days. At this stage 30 to 50% of the population can be harvested and replaced by freshwater with proportional amount of the above fertilizer or with *Chlorella* from a separate tank. Harvesting can be done daily morning or evening, when they swarm at the surface by a zooplankton net. *Chlorella* sp. bloom can be also be obtained by fertilizing with chicken drops or pig manure.

During unfavourable conditions such as high population. low oxygen level, scarcity of feed, the parthenogenetic females release the male. After mating, the female produces the dormant cyst in the brood-pouch. The released cysts settle at the bottom of the tank and can be collected along with the sediments, dried in the room temperature and stored in an airtight container for 3 to 4 months. This can be easily transported without risk. Embryonic development starts when the dry cysts are hydrated with freshwater. The hatchlings come out within 24-36 hours.

Harvesting

Harvesting is done by using a zooplankton net in the exponential growing phase when the females start reproducing. Parthenogenetic females containing 8-12 embryos in the broodpouch have rich inorganic matter and more nutritive values than

females with resting eggs or the males. Harvested *Moina* are washed with freshwater and fed to the larvae or are preserved by mixing equal volume of 10% glycerin and frozen ones (small blocks) in a deep freezer for future use.

Nutritive value

Moina fed with fish algae are nutritionally richer in PUFA than the ones grown on yeast or commercial single cell proteins.

The enrichment of harvested *Moina* is essential before feeding the fish and crustacean larvae. Enrichment of PUFA (cod liver oil or squid oil) can be done as mentioned for the rotifers. The harvested *Moina* 200-300 nos./ml. are mixed with enrichment medium along with freshwater *Chlorella* sp. for 3-4 hours.

Conclusion

Since *Moina* are freshwater parthenogenetic animals the $\alpha 3$ PUFA contents are naturally inadequate to feed the fish and crustacean larvae. So, the enrichment is inevitable.