ROTIFER CULTURE TECHNIQUES FOR MARINE FINFISH LARVAL REARING

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Rotifers are considered as valuable live food for fish and crustacean larvae because of the small size of the rotifer. Several important characteristics of rotifers have contributed to their usefulness as good prey for active larvae of marine fish,

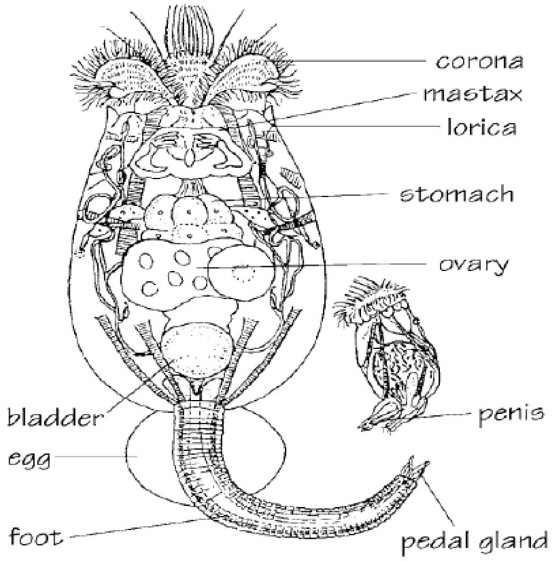
- Nutritional quality: Rotifers have an excellent nutritional profile, if they are fed with right quality feeds
- Small size: They are easily consumed by almost all marine fish larvae
- Relatively slow motility – It helps for easy prey capture by fish larvae
- Good tolerance to marine environment
- Easy to culture in large scale rapidly and inexpensively
- Ability to stay suspended in the water column.

Marine rotifers *Brachionus* spp. are most commonly used for intensive culture of marine finfish larvae in many hatcheries throughout the world. The most common *Brachionus* species used are *Brachionus plicatilis* (L-strain) with a size range of 130 to 340 µm (Average - 240 µm) and *B. rotundiformis* (S-strain) with a size range of 100 to 210 µm (average size-160 µm). There are differences in weight, shape of occipital spines and optimal growth in different temperatures (L-type rotifers have a wider temperature range while S-type rotifers have a higher temperature resistance). S-type rotifers are suitable as first food for fish larvae with a mouth opening smaller than 200 µm at first feeding, such as gilthead seabream, groupers, and rabbitfish.

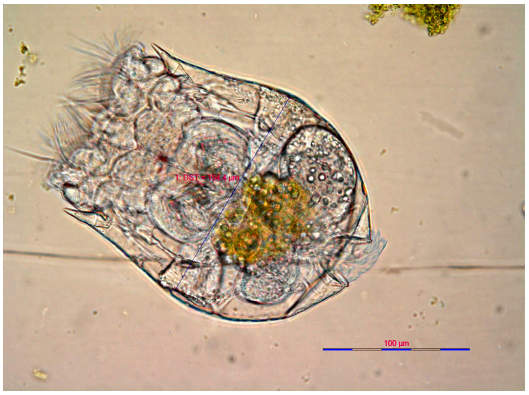
These rotifers are commonly offered to larvae during the first 3–30 days of exogenous feeding. *Rotifers are* supplied at the required concentrations for meeting larval metabolic demands and yielding high survival rates during the larval rearing. Larvae are first fed on a small strain of rotifers, and as larvae increase in size, a larger strain of rotifers is introduced. Rotifers are regarded as living food capsules for transferring nutrients to fish larvae. These nutrients include highly unsaturated fatty acids essential for survival of marine fish larvae. In addition, rotifers treated with antibiotics may promote higher survival rates.

Morphology

Rotifer's body is divided into three different parts namely head, trunk and foot. The corona is found in the head. The corona has an annular ciliation and is retractable, which allows them to move and makes easier the intake of small food particles through a whirling water movement. The digestive tract, the excretory system and the genital organs are in the trunk. The foot is a ring-type retractable structure without segmentation and ends in one or four toes. The body parts of a female and male *Brachionus plicatilis* is depicted in fig 1.



Male and female of *Brachionus plicatilis* strain (Source FAO)



View of rotifer *Brachionus plicatilis* (10 X)

Life history

The life span of rotifers depends on their reproduction cycle. Reproduction frequency of rotifer varies according to the temperature of the culture environment; for example at 25 ºC it is of about 4-5 days and at 20-22 ºC the average life span is 10.5 days. Generally, the larvae become adult after 0.5 to 1.5 days at 25ºC and then the females start to lay eggs approximately every four hours. It is believed that females can produce ten generations of offspring and then die.

The *Brachionus* spp. can reproduce in two different mode of reproduction i.e., both sexually (mictic) and asexually (amictic), depending on the conditions, environmental and also on the rotifer density of the population. During female parthenogenesis, the amictic females produce amictic (diploid, 2n chromosomes) eggs which develop and hatch into amictic females. Under specific environmental conditions the females switch to a more complicated sexual reproduction resulting in mictic and amictic females. The mictic females produce haploid (n- chromosomes) eggs. Larvae hatching out of these unfertilized mictic eggs develop into haploid males. These males are about one quarter of the size of the female; they have no digestive tract and no bladder but have single testis filled with sperm. During the mictic mode, resting eggs are produced that will only develop and hatch into amictic females, after they are exposed to specific conditions. This is probably a mechanism to preserve the survival of the population even under unfavourable conditions

Rotifer culture

The process of rotifer culture can be divided in to 4 different phases.

i. Maintenance of stock cultures

ii. Inoculation phase: This phase is the start of new cultures based on inoculums from stock cultures, or more commonly from production cultures.

iii. Early growth phase: It is the critical phase when food rations and rotifer density are increased gradually.

iv. Late growth or production phase: It is the final phase, before harvest.

Maintenance of stock culture

Maintenance of stock culture is very important in rotifer culture, and it should be kept physically isolated from the production facility of microalgae, rotifers and other zooplanktons culture in order to avoid contamination and transfer of diseases. Algal cultures used to feed the stock cultures of rotifers must also be free from harmful contaminants. Contaminated algal cultures are most easily purified by plating techniques using solid agar. Stock cultures of rotifers can be maintained in small units (0.1-1 l) and the water used must be sterilised. A stock culture is prepared by transferring 5-10 ml of mature stock culture to a beaker 0.1 - 0.5 l of sterilised water. The cultures can be maintained at room temperature, but the feeding and renewal frequencies are lower if the rotifers are kept in the light at low temperature (7-10 ºC). The stock cultures have to be renewed approximately once every month, or even less frequently at low temperatures. If all the stock cultures become contaminated by other zooplankton, single rotifers should be selected carefully under the microscope, and repeatedly washed in sterilised water, and then transferred to small units containing sterilised water and microalgae.

Rotifer culture methods

There are two general methods followed for culturing rotifers:

i. Batch culture: In this method, the given volume of water is added or exchanged each day and the culture is restarted at regular intervals.

Batch culture system normally follows a 4-5 day culture period. In batch culture method, a tank is inoculated with rotifers on day 1. The rotifers are then fed each day; accordingly the volume of the culture is also increased to keep up with rotifer growth. The maximum rotifer density reached in this method normally goes up to 500 rotifers/ml. At the end of the cycle, most of the rotifers are harvested and fed to fish larvae. However, some of the rotifers are kept aside from the harvest for the next tank inoculation. The duration of the cycle can be extended slightly by performing regular water exchanges once a high terminal density is reached. When culture duration is increased, removing 10% to 30% of the water volume on a daily basis can help to keep water quality within desired parameters, however, the culture will eventually need to be restarted due to the accumulation of uneaten feed.

ii. Continuous culture: Recirculation-based technology is employed in this method to increase the density of rotifers cultured while minimizing the need to restart cultures.

In continuous culture method, a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out or harvested. This method permits the maintenance of cultures very close to the maximum growth rate using water recirculation. A typical rotifer recirculation system has units like culture tank, bio filter unit, protein skimmer, algal storage tank and pumps. This system employs a standpipe with 55 mm mesh screen located within the culture tank. The standpipe allows uneaten feed and ciliated protozoans to pass out of the culture tank when the rotifers are cultured in the tank. The waste coming out of the stand pipe travels through a biological filter and foam fractionators (Protein skimmer) before returning back into the culture tank. Flow rates in this type of system are typically two to five tank turnovers per day through the recirculation system. Daily maintenance for this type of system involves cleaning the screened standpipe and floc traps and removal of settled materials from the bottom of the tank. In general, a daily water exchange of 20-30 % of the culture volume should be incorporated for long-term maintenance. This culture method gives production maximum of up to 1,000 rotifers/ml.

Feeding of rotifers

Rotifers feed by grazing actively in the water column, feed on particles of approximately 1 to 10 µm in size. Many species of microalgae such as *Nannochloropsis sp.*, *Pavlova sp.* and *Isochrysis sp.* etc, are good food for rotifers. In addition, there is a number of artificial feed like yeast; algae based rations and other organic feeds are suitable for rotifer culture. Activated baker's yeast has been used successfully as an inexpensive grow-out diet when fed at approximately 0.5g/ million rotifers. However, use of yeast along with microalgae gives better growth. It is important to note that the diet that is devoid of beneficial amino acid, fatty acids and lack certain vitamins is need to be supplemented in order to achieve maximal culture performance.

Water quality parameters in rotifer culture

The culture environment in the tank is one of the factors, which plays a major role in maintaining proper multiplication of rotifers. The water quality parameters such as salinity, temperature, dissolved oxygen, pH, ammonia need to be maintained and the optimum range of these parameters are follows.

Water quality parameters	Optimal Ranges
Salinity	10-35 ppt
Temperature	22-30°C
Dissolved oxygen	>4 ppm
pH	7-8.5
Total ammonia nitrogen	d”5 ppm

Rotifer enrichment

Rotifer enrichment is an important aspect in marine finfish hatchery, because rotifer as such acts as carrier, and nutritional quality of the rotifer depends on the nutritional content of the feed supplied. Rotifers can be fed on a variety of small micron feeds and the resulting rotifers will have the nutritional profile of those feeds. There are several diets available for growing rotifers, but the best diets are marine microalgae. Marine microalgae contain the full spectrum of important nutritional components that is needed for larval development, including fatty acids (especially ARA, EPA, or DHA), sterols, carbohydrates, proteins, and vitamins.

The most commonly used algae for growing rotifers are *Chlorella* and *Nannochloropsis*, which provide high growth rates and healthy rotifers. However, *Chlorella* provides essentially no ARA, EPA and DHA. *Nannochloropsis* contains a high content of EPA, which can be converted to DHA by some fishes. Apart from this, *Isochrysis* is an important feed for rotifers, contains DHA which could be directly used by the fish larvae. However, many fishes require a good amount of DHA concentration in their feed, and to provide sufficient concentration, the rotifers must be enriched with other artificial feed/products that containing exceptionally high amount of DHA. There are different products available in the market for the purpose of enrichment; therefore, suitable products could be selected and used for enrichment. Some of the commonly used products

are products under AlgaMag (Bio-Marine) brand (AlgaMag Protein Plus, AlgaMag Red and AlgaMag- 3050), S. presso (INVE), Red Pepper (Bern Aqua), etc.

The last 12-24 hours of feeding are the most important for determining the nutritional value of the rotifer. This provides substantial enrichment by gut loading of the rotifer with the desired feed. Optimum time need to be identified for proper incorporation of enrichment media into the rotifer tissues and 8 hours of enrichment has been found to be optimum in different studies. Enrichment for more than 24 hours needs to be avoided because; this may lead to wastage of nutrients. Rotifer enrichment can be done in two methods i) The enrichment media can be directly added to the rotifer tank, that is planned for harvest in the next day or eight hours later. ii) The rotifers are harvested first and then the enrichment media added to the concentrated rotifers. The dose of enrichment media used depends on the manufacturer's recommendations.

Feed calculation/determination for rotifer

Generally, feed given to the rotifers depends on the number of rotifers present in the culture tank and number of rotifers needed to be harvested for feeding the fish larvae. Rotifer egg production is high for only the first 3-5 days of their 7-15 days (depending on temperature) of lifespan, so for the best production it is important to harvest at least 25% of the culture each day to keep the population young and reproducing vigorously. In a healthy culture, the number of rotifers produced each day directly corresponds to the amount of feed provided. If more rotifers are needed, the same harvest rate should be maintained and more feed should be added; if less number of rotifers required, less feed should be added to the tank. If feeding rate is changed, then rotifers will take 1-3 days (depending on the magnitude of the change in feed rate) for the culture to reach a new equilibrium and stabilize production.

Rotifer counts

For counting the rotifers, 20-25 ml of sample is collected from middle of the culture tank/near by the aeration where there is good circulation of the water and rotifers in the tank. Add few drops of formalin/vinegar to immobilize the rotifers in a relaxed state, mix and pipette 1 ml from sample onto a Sedgewick-Rafter counting slide, which is etched with a grid of 10 x 10/ 50x20. Count the rotifers in

the grid under microscope. Count the rotifers in the entire grid or middle of two grids and then multiply to get the rotifer in all the grids. The count obtained from Sedgewick-Rafter counting slide is for 1 ml, which is then multiplied accordingly to get the total count in the culture tank. Counting rotifers daily will give the exact stock of the rotifers in the culture tanks.

Important points to be considered in rotifer culture

- When starting a new culture, initial stocking densities should be e"200 rotifers/ ml of culture water. Lower stocking densities will result in delayed start-up time and may help promote the growth of unwanted contaminants.
- Rotifers need a consistent supply of free algae in the water at all times, allowing them to graze continuously. Therefore, frequent feeding need to be given in less concentration, so that feeds could be efficiently utilised and wastage also avoided.
- Batch culture method may be extended up to 6-7 days and during this period bottom should be siphoned at least once to avoid the building up of ammonia in the culture environment.
- Rotifers harvested between 4-5th day after inoculation gives maximum numbers in batch culture method.

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Online sites:

· http://www.angelfire.com/wa/AquariaWeb/_Artemia_.html

· http://www.aquatechgroup.com

· http://reedmariculture.com/support_rotifers.php Reed maricultures site on rotifers

· http://216.25.82.220/Rotifer.PDF, Online scientific article on mass culture of rotifers.