

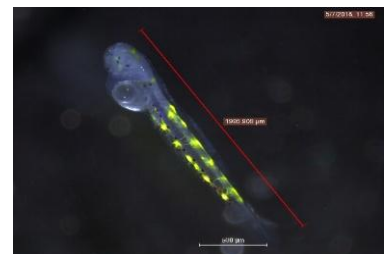
Culture Techniques of Rotifer and Artemia

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Live feed refers to small, microscopic living organisms which are used to feed fish larvae especially during the early larval stages. Live food organisms include all plants (phytoplankton) and animal (zooplankton) lives grazed upon by economically important fishes. Phytoplankton are generally eaten by zooplankton and forms the basis of the food chain. Live foods can swim in water column and are constantly available to fish and shellfish larvae and are likely to stimulate larval feeding response. Most of the fish and shellfish larvae in nature feed on small phytoplanktonic and zooplanktonic organisms. The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food for feeding fish larvae, fry and fingerlings. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids and hence are commonly known as “living capsules of nutrition”.



Upon hatching fish eggs, larvae obtain their nutrition from yolk sac for the first few days. Generally, there are two types of fish larvae: precocial and altricial. Precocial larvae are those that when yolk sac is exhausted, appear as mini adult exhibiting fully developed fins and mature digestive system. Such fish can ingest and digest formulated diet as first food. But altricial larvae (most of marine fish



larvae) having less yolk - reserves remain in a relatively undeveloped state with small mouth gape when yolk sac is exhausted. They also possess an undeveloped digestive system lacking a stomach. These types of larvae cannot digest formulated diet and has to be provided with live feeds appropriate to their mouth gape. Live feeds are able to swim in water column and thus constantly available to the larvae. In addition the movement of live feed in the water is likely to stimulate larval feeding responses since evolutionary history has probably adapted them to attack moving prey in nature.

SELECTION OF LIVE FEED

When selecting food to be fed to the larvae, the following points should be considered:

1. The food must be perceived by the larvae.
2. The size of food must be such that it can be accommodated by the mouth of the larvae.
3. The feed should have high nutritional quality especially Highly Unsaturated Fatty Acids (HUFA) essential to the growth and survival of the larvae.
4. The feed can be digested by the larvae.
5. Food organisms must be hardy.
6. They must be able to reproduce rapidly and can mass produced under controlled condition

ROTIFERS

Rotifers are very small organisms mostly ranging from 0.1 to 0.5 mm belongs to the Phylum Rotifera. Rotifers were found to be suitable as first feed for marine fish larvae in Japan in the late sixties and early seventies. Rotifers became ubiquitous in all mass rearing trials after their successful use in the mass rearing of the red seabream (*Pagrus major*) in Japan. The introduction of rotifers marked the first regular successes in the mass larval rearing of several marine species of economic value such as grey mullet (*Mugil cephalus*), sole (*Solea solea*), gilthead seabream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*) and flounder (*Paralichthys olivaceus*), milkfish (*Chanos chanos*) etc.. during seventies and eighties. They are now the first food of the initial larval stage of many fish species grown in commercial marine hatcheries.

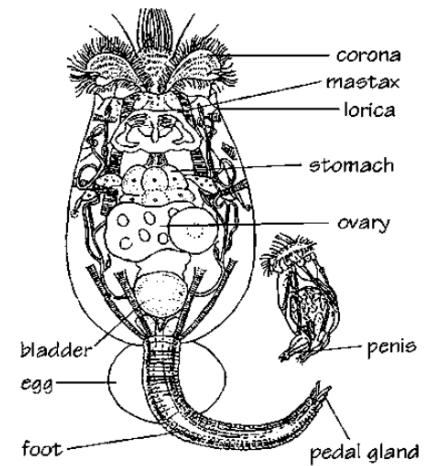
Morphology of rotifer

The success of rotifers as a culture organism are manifold, including their planktonic nature, tolerance to a wide range of environmental conditions, high reproduction rate. Moreover, their small size and slow swimming velocity make them a suitable prey for fish larvae that have just resorbed their yolk sac but cannot yet ingest the larger *Artemia* nauplii. However, the greatest potential for rotifer culture resides, however, is

the possibility of rearing these animals at very high densities. Even at high densities, the animals reproduce rapidly and can thus contribute to the buildup of large quantities of live food in a very short period of time. Last, but not least, the filter-feeding nature of the rotifers facilitates the inclusion into their body tissues of specific nutrients essential for the larval predators.

Rotifers are capable of reproduction through both sexual and asexual methods. The life span of rotifers has been estimated to be between 3.4 to 4.4 days at 25°C. Generally, the larvae become adult after 0.5 to 1.5 days and females thereafter start to lay eggs approximately every four hours. It is believed that females can produce ten generations of offspring before they eventually die.

Euryhaline rotifer species, *Brachionus* are used extensively used for marine fish larval rearing in many hatcheries throughout the world. *B. plicatilis* (L type) and *B. rotundiformis* (S-type) are the two common species used for larviculture of marine fishes. The differences among the two types can be clearly distinguished by their morphological characteristics: the lorica length of the L-type ranging from 130 to 340 μ m (av. 239 μ m), and of the S-



B. rotundiformis



B. plicatilis

type ranging from 100 to 210 mm (av. 160 mm). Moreover, the lorica of the S-type shows pointed spines, while of the L-type has obtuse angled spines. Another strain. SS-type rotifers (Super small rotifers) are preferred for the first feeding of fish larvae with small mouth openings (rabbitfish, groupers, and other fish with mouth openings at start feeding of less than 100 mm). Those rotifers, however, are genetically not isolated from S-strains, but are smaller than common S-strains.

Stock culture

Relying only on mass cultures of rotifers for reinoculation of new tanks is too risky an approach because the threat of mass mortality. 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 rotifers as fast as possible are generally maintained on algae.

- Sterilized seawater of 25 ppt salinity is used
- Inoculation of the tubes (50 ml) is carried out with an initial density of 2 rotifers.ml⁻¹.
- The tubes are placed at a distance of 20 cm from fluorescent tubes (light intensity of 3000 lux)
- 4 ml of microalgae is added daily
- After one week the rotifer density should have increased to 200 individuals/ml. The rotifers are rinsed, a small part is used for maintenance of the stock, and the remaining rotifers can be used for upscaling.

Upscaling of stock cultures to starter cultures

The upscaling of rotifers is carried out in static systems consisting of conical flask of 500 ml placed 2 cm from fluorescent light tubes (5000 lux). The rotifers are stocked at a density of 50 individuals/ml



and fed 400 ml freshly-harvested algae (10^6 cells.ml⁻¹). Next two days rotifers are fed approximately 50 ml of algae. Within 3 days the rotifer concentration can increase to 200 rotifers/ml.

Mass culture

Batch culture system is probably the most common method of rotifer production in marine fish hatcheries. The culture strategy consists of either the maintenance of a constant culture volume with an increasing rotifer density or the maintenance of a constant rotifer density by increasing the culture volume. Batch culture system normally follows a 4–5-day culture period. Initially a tank (0.5-1 ton) is inoculated with rotifers (≥ 200 nos./ml). The rotifers are then fed each day with microalgae (10^6 cells/ml) and the volume of the culture is also increased to keep up with rotifer growth. Many species of microalgae such as *Nannochloropsis*, *Chlorella*, *Pavlova*, *Isochrysis* etc, are used for feeding rotifers. Rotifer densities reaches up to 500 nos. /ml after 4-5 days. Rotifers are harvested after the culture period using 300 μ and 50 μ mesh sieves, so that dust free rotifer will be collected on the 50 μ mesh sieves. Both filters are placed inside a container full of water to avoid damage to the rotifers due to pressure of outgoing water. A gentle air bubbling along the inner side of the filter helps to keep the filter free from clogging.

Ideal water quality parameters	
Salinity	10 – 35 ppt
Temperature	20- 28°C
Dissolved Oxygen	>3 ppm
pH	7.5-8.5
Ammonia	<0.5 ppm

A part of the harvested rotifers are used as the inoculum for the next culture.

Rotifers are also cultured using a combination of microalgae and baker's yeast and promotes better growth of rotifer cultures. The tanks are half filled with algae (10^6 cells/ml) and inoculated with rotifers at a density of 100 nos./ml. The first day active baker's yeast is administered two times a day at a quantity of 0.25 g/ 10^6 rotifers. The next day the tanks are completely filled with algae at the same algal density and 0.375 g baker's yeast per million rotifers is added twice a day. The next day the rotifers are harvested, and

new tanks are inoculated (i.e. two-day batch culture system). These harvested rotifers need to be enriched with microalgae or enrichment diets. Rotifers are often cultured at temperatures and salinities different from the larval rearing tanks. So they must be acclimated for at least 6 hours to larval rearing conditions before introducing into larval rearing tanks.

Enrichment

Rotifers have a limited nutritional value for fulfilling the nutritional requirement of marine finfish larvae. The high content of the essential fatty acid eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3) in some microalgae (e.g. 20:5n-3 in *Nannochloropsis occulata* and 22:6n-3 in *Isochrysis galbana*) have made them excellent live food diets for boosting the fatty acid content of the rotifers. Rotifers are enriched by stocking the harvested rotifers @ 500 nos./ml in microalgae cultures (10^6 cells/ml) for 4-6 hrs before feeding to larvae. It can also be enriched with specially formulated artificial diets like Selco products/Algamac/commercial oil emulsions as per manufactures or using homemade emulsions.

Enrichment includes:

- Short-term enrichment
- Long-term enrichment

Enrichment food:

- Microalgae
- Oil emulsions
- Formulated diets

Commercial enrichment products



Fresh Chlorella



Instant microalgae



AlgaMac



Selco® S.parkle

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ARTEMIA

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp *Artemia* constitute the most widely used food item. It is also called as brine shrimp or sea monkey. The total length is about 8-10mm for adult males and 10-12mm for females, the width including legs is about 4mm for both sexes. The widely used species of *Artemia* is *A. salina*. The females can produce eggs either as a result of mating or via parthenogenesis. The thin shelled eggs hatch immediately, and thick shelled eggs can remain in dormant state and forms cysts. These cysts are metabolically inactive, do not further develop as long as they are kept dry and can be stored up to 2 years. Upon immersion in seawater, the biconcave-shaped cysts hydrate, become spherical, and within the shell the embryo resumes its interrupted metabolism. In less than 24 hrs, the outer membrane of the cyst bursts and the embryo appears surrounded by the hatching membrane. While the embryo hangs underneath the empty shell, the development of the nauplii is completed and within a short period of time the hatching membrane is ruptured and the free-swimming nauplii comes out. This freshly hatched nauplius I stage (Instar I) (length of 400-500 μ) is popularly used for feeding the larvae.



Each gram of *Artemia* cyst contains 200000 to 300000 eggs and atleast 50% will hatch within 20-24hrs on proper hydration. To estimate the amount of *Artemia* required one must consider both the volume of the tank and the expected number of *Artemia* the larvae will consume. Based on the stage or the age of the larvae, estimate a daily *Artemia* requirement per ml. *Artemia* nauplii are usually maintained in the larval culture tank at densities of 0.5 to 2 per ml for most species of finfishes. The requirement of *Artemia* nauplii should be calculated based on the volume of larval rearing tank and the species under culture. Also, *Artemia* nauplii left over in the tank should be determined daily by taking water samples from the larval rearing tank to back calculate the requirement of nauplii/l and requirement of cysts in g for producing the required amount of nauplii.

Weight of *Artemia* cyst required =

Total volume of all rearing tanks (in ml) X No. of *Artemia* required per ml

Percentage hatch rate X No. of cysts per gram

Artemia nauplii when 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.

Procedure for hatching *Artemia* cysts in hatchery

Artemia cysts are hatched into nauplii following the standard procedure involving the following steps

1. Hydration of cyst

Artemia cysts (<100 g/l) are put into container containing low salinity water or freshwater at 25°C for 1 hour with aeration. After an hour, hydrated cysts are filtered through 100- 125 µm sieve. Hydration steps ensures the spherical shape of the cyst, which allows for easy removal of cysts.



2. Decapsulation

Decapsulation procedure ensures disinfection of cysts, complete removal of shell from the hydrated cysts, minimize illumination requirements for hatching, reduces hatching time and increases nutritional value of nauplii.

a. Using Sodium hypochlorite: Hydrated cysts are kept in a beaker filled with 5% sodium hypochlorite solution @ 15 ml for every 1 g cyst. In order to prevent the temperature rising above 300 °C, the beaker containing the hydrated cyst and sodium hypochlorite are kept in a trough containing cool water or ice. In about 10 minutes, chorion of hydrated cysts gets dissolved and is then filtered through a 100 µm sieve.

b. Using Calcium hypochlorite: The scoop net containing hydrated cysts are kept in a bucket filled with water containing calcium hypochlorite having 200-250 ppm of chlorine. In about 10 minutes, chorion of hydrated cysts gets dissolved and is then filtered through a 100 µm sieve.

Note: The decapsulated cysts should be thoroughly washed in tap water or seawater for about 10 minutes until no chlorine smell can be detected. To ensure complete removal of chlorine, the cysts are dipped in 0.1% sodium thiosulphate solution or 0.1N hydrochloric acid.

3. Hatching/ Incubation of decapsulated cyst

Decapsulated *Artemia* cysts are hatched in a cylindroconical FRP tanks/jars having transparent bottom with continuously aerated from the bottom with air-lines. Decapsulated cysts are stocked @ 5 g/l of seawater in smaller volume tanks (< 20 L) to 2 g/l of seawater in tanks with large volumes. The optimum water quality parameters required for hatching of cysts is 27-30°C temperature, 8-8.5 pH, 25-35 ppt salinity, 1000-2000 lux light and saturated dissolved oxygen concentration

4. Harvesting of nauplii.

The decapsulated cysts hatch out after a period of 16-24 hrs. Harvesting is done by taking advantage of phototactic movement of *Artemia* nauplii. The top of the cylinder is closed with a lid and illumination is provided at the bottom transparent part. After 5-10 minutes, the nauplii gets accumulated at the bottom. The outlet of the tank at or near bottom is opened and water is sieved through 100-125 µm net to harvest the hatched out nauplii. Then harvesting net should be submerged in water all the time so as to prevent physical damage of the nauplii. They are then rinsed thoroughly with water to remove possible contaminants and hatching metabolites.

***Artemia* enrichment**

Since *Artemia* have poor content of essential fatty acids, eicosapentaenoic acid (EPA: 20:5n-3) and even more importantly docosahexaenoic acid (DHA: 22:6n-3), modification of

the nutritional value of *Artemia* for aquaculture purposes has been done for years. Taking advantage of the primitive feeding characteristics of *Artemia* nauplii, it is possible to manipulate the nutritional value of HUFA-deficient *Artemia*. Since second instar stage (about 8 h following hatching) are non-selective particle feeders, simple methods have been developed to incorporate different kinds of products into the *Artemia* prior to feeding to predator larvae. This method of bioencapsulation, also called *Artemia* enrichment or boosting, is widely applied in marine fish and crustacean hatcheries for enhancing the nutritional value of *Artemia* with essential fatty acids.

Artemia are non-selective filter feeders and therefore will ingest a wide range of foods. The main criteria for food selection are particle size, digestibility, and nutrient levels. The best feeds for *Artemia* are live microalgae and the combinations of live phytoplanktons fed to *Artemia* cultures have demonstrated superior enrichment characteristics (i.e., increased HUFAs). In addition to live algae, *Artemia* cultures can be enriched by feeding a wide variety of processed foods, including yeasts, fish meal, soybean powder, egg yolk, and micronized rice bran. *Artemia* are enriched with commercially available enrichment medium (DC DHA Selco, algamag, ORI-N3 Skretting). The enriched, *Artemia* are rinsed, concentrated, counted, and then fed to larvae.

Counting rotifer and *Artemia*

Collect a representative sample of the rotifer or brine shrimp stock using a small beaker or glass container.

Mix the sample thoroughly using a pipette.

Transfer 1 mL of the mixed sample into a 10 mL graduated cylinder and add 9 mL of seawater (SW) to prepare a 10-fold dilution.

Mix the diluted sample thoroughly and transfer it to a small beaker. Withdraw five 1 mL aliquots and place each into a counting chamber. Add a few drops of Lugol's solution to stain and immobilize the rotifers or brine shrimp.

Using a stereomicroscope, count the number of rotifers or brine shrimp in each 1 mL aliquot and calculate the average density (individuals/mL) of the diluted sample.

Multiply the average density by 10 to account for the 10-fold dilution and determine the actual density (individuals/mL) of the original stock culture.

Volume of rotifer/*Artemia* to be transferred for feeding larvae in tanks

$$V = \frac{\text{Desired feeding rate (ind/ml) x vol of rearing tank (ml)}}{\text{Density of harvested rotifer/*Artemia* (ind/ml)}}$$