

Rotifers as live feed

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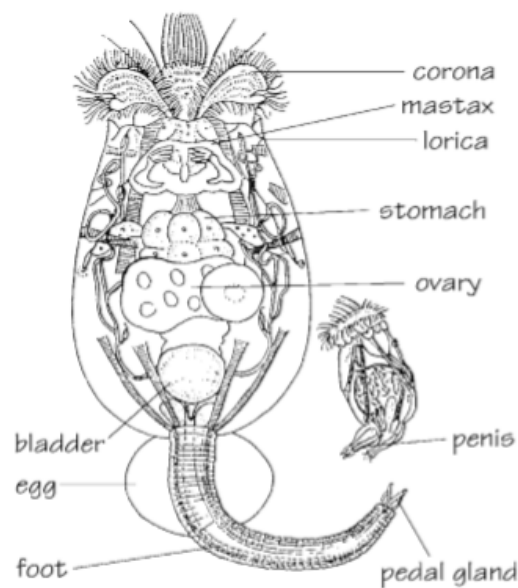
Introduction

The candidate species identified for mariculture includes Cobia, *Rachycentroncanadum*, Asian sea bass, *Latescalcarifer* Silver/ Snubnose pompano, *Trachinotusblochii*, Indian pompano, *T.mookalee*, Orange spotted grouper, *Epinepheluscoioides*, Sea bream, *Lethrinuslentjan*, etc. The larvae of all these species is carnivorous and feeds on zooplankton such as rotifers or copepods. Hence their seed production technique invariably requires the production of the zooplankton such as rotifers, copepods and artemia among which rotifers are the most widely cultured live feed for the seed production of marine fin fishes. The phylum Rotifera (previously known as the Rotatoria) consists of a relatively small group of minute, unsegmented, pseudocoelomate, aquatic invertebrates with bilateral symmetry. Most rotifers are free-crawling or swimming, but sedentary and colonial forms are also known. More than 1000 species have been described, 90 % of which inhabit freshwater habitats. They seldom reach 2 mm in body length. Males have reduced sizes and are less developed than females; some measuring only 60 μm . The body of all species consists of a constant number of cells, the different *Brachionus* species containing approximately 1000 cells which should not be considered as single identities but as a plasma area. The growth of the animal is assured by plasma increase and not by cell division.

Morphology & Anatomy

The rotifer's body is differentiated into three distinct parts, viz; the head, trunk and foot. The head carries the rotatory organ or corona which is easily recognized by its annular ciliation. The retractable corona assures locomotion and a whirling water movement which facilitates the uptake of small food particles (mainly algae and detritus). The trunk contains the digestive tract, the excretory system and the genital organs. A characteristic organ for the rotifers is the mastax (i.e. a calcified apparatus in the mouth region), that is very effective in grinding ingested particles. The foot is a ring-type retractable structure without segmentation ending in one or four toes. The epidermis contains a densely packed layer of keratin-like proteins and is called the

lorica. The shape of the lorica and the profile of the spines and ornaments allow the determination of the different species and morphotypes. Rotifers possess an internal fluid-filled space known as a pseudocoelom that is bound externally by the integument and internally by the epithelial cells of the various organs (digestive, protonephridial and reproductive). There are no respiratory or circulatory systems in rotifers, and the pseudocoelom internal fluid, bathing the internal organs, is equivalent to the circulatory system. Its composition is regulated by the protonephridia and it is replenished by the digestive tract. Rotifers exchange gases and dispose of nitrogenous wastes by diffusion through their body surface. Rotifers are also characterised by the syncytial structure of their body parts. Cell membranes in tissues disappear after embryonic development, forming multinucleated or syncytial tissues. All individuals of a species have a consistent number of nuclei in each organ. This situation, known as eutely, is also found in nematodes. The total number of nuclei, ranging from 900 to 1000, is fixed for life during embryonic development, indicating a limited capacity for repairing damage.



Brachionusplicatilis – Morphology & anatomy (FAO,1996)

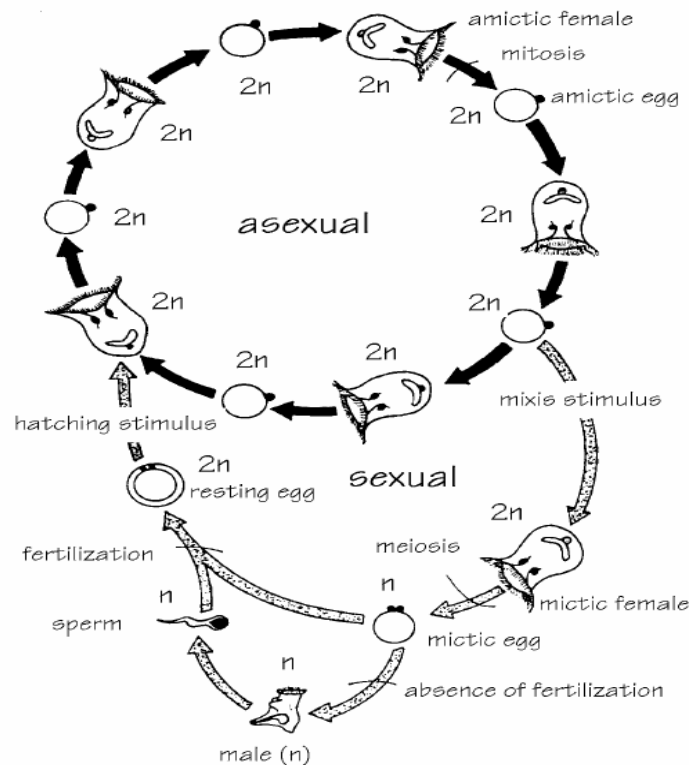
Food & feeding habits

Rotifers were described as mechanical grazers as they were found to graze non-selectively when offered two algal species with different cell size. The most common method of feeding in planktonic brachionid rotifers is by filter feeding. This type of feeding, also described as 'microphagus feeding', is found in rotifers having a developed ciliary corona and a crushing type of mastax. The type of food consumed, particularly its size, is directly dependent on the size and form of the ciliary apparatus and of the mastax. The rotational movement of the cilia directs a water current containing food particles towards the mouth and those that are suitable are swallowed, indicating a sensory mechanism regulating food selection. Food is captured by the corona, enters the mouth opening and passes through the buccal tube to the pharynx. Several studies indicate that food selectivity by suspension feeders such as rotifers is mainly based on prey size. The prey size spectrum for *B. plicatilis* ranges from approximately 1.4 to 4838 μm^3 , or 1.4 to 21 μm^3 of equivalent spherical diameter, with changes in efficiency with different particle size. The filtration or clearance rate is high at low food particle concentrations and declines in a curvilinear manner with the increase in food concentration.

Reproduction

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. Nearly all rotifers seen in nature are females. Males occur only for short periods and in many species have never been observed. Males are known from a limited number of monogonont species, but it is generally assumed that all members of this group are capable of producing males, including the brachionid species, *B. plicatilis* and *B. rotundiformis*. Males are much smaller than females and typically very fast moving. They have a rudimentary digestive gut and a sac-like testis containing free-swimming spermatozoa. A vas deferens leads from the testis to a penis and one or two prostate glands discharge into it. Males attempt copulation with amictic or mictic females and mating occurs at the region of the corona or cloaca. Successful fertilisation occurs in newly emerged females for a very limited period. The female reproductive organs is called Monogononts, as the name implies, composed of a single gonad. The gonad consists of the syncytial ovary that contains the ovocytes, the yolk-producing syncytial vitellarium and the follicular layer that surrounds the ovary and vitellarium and forms

an oviduct leading to the cloaca. The total number of ovocytes is present at birth. Rotifers are generally oviparous, with embryos developing outside the maternal body.



Types of reproduction in *Brachionus plicatilis* (FAO, 1996).

During favourable conditions, the population increases through diploid parthenogenesis (Asexual reproduction), whereby diploid females produce diploid eggs known as amictic eggs. Females are always diploid and males, when they appear, are haploid and very much reduced in size compared with females. Diploid females can either be amictic or mictic and morphologically they are indistinguishable. Amictic females produce parthenogenetically diploid eggs that develop mitotically into females, while mictic females produce, parthenogenetically, haploid eggs via meiosis. Thus, it is easy to obtain genetic clones from cultures originating from one amictic female. If a mictic female does not mate and is not fertilised, the haploid eggs form into males, but a mated mictic female that is fertilised (Sexual reproduction) will form diploid resting eggs (subitaneous eggs or cysts). Parthenogenetically formed eggs (diploid or haploid eggs) will develop immediately into embryos and hatch. Resting eggs will hatch under appropriate conditions into amictic females, after a dormant period. Thus, amictic females produce

parthenogenetically diploid amictic eggs and mictic females produce parthenogenetically haploid male eggs or sexually diploid resting eggs that hatch into diploid amictic females.

In the Brachionidae, the eggs are attached to the body of the female by a thin thread, and the embryos of amictic eggs hatch and are released from the maternal body leaving the egg shells still attached to their mother. Resting eggs at the initial stages of formation cannot be distinguished from those of amictic eggs. In *B. plicatilis*, where the resting eggs are formed outside the female's body, they are also attached by a thin thread to their mother until the end of their formation and later released and sink to the bottom of the culture vessel, pond or lake sediment. However, the resting eggs of *B. rotundiformis* (SM type rotifer strains) that develop within the maternal organism are not released from it. They will sink to the bottom of the culture vessel, pond or lake sediment with the death of their mother and are finally released only after the decomposition of her body. Resting eggs survive for long periods and have been hatched from sediment samples more than 60 years after their formation.

The fecundity of rotifers depends on whether asexual or sexual reproduction takes place. *B. plicatilis* amictic females produced 17–24 eggs during their lifetime, compared with 1–5 resting eggs produced by fertilised mictic females. In addition, the production of amictic eggs is, on average, 10 times faster (about 5 eggs/day) than that of the resting eggs during a similar oviposition period (approximately 108 h). The unfertilised mictic females produced 9–19 eggs, but these did not contribute to the increase in population as they form males. The number of eggs produced by a female is also dependent on the food algal species. The optimal temperature for culturing rotifers depends strongly on the species, as the optimal temperatures for *B. plicatilis* (10–30°C) are lower than those for *B. rotundiformis* (24–35°C). Within each species, differences have been found in the reproductive rates under the same culture conditions, indicating intraspecific variability.

Isolation of rotifer for stock culture

1. Rotifer samples can be collected from a marine or brackish water body with small plankton collecting net (150-300 µm mesh size).
2. The whole sample is then divided into sub samples, observed under microscope, from which healthy rotifer with eggs are collected.
3. The rotifers before being used in the production cycle it should first be disinfected.

4. The disinfection consists of killing the free-swimming rotifers but not the eggs by using a cocktail of antibiotics (e.g. Erythromycin- 10 mg/l, Chloramphenicol -10 mg/l, Sodium oxolate- 10 mg/l, Penicillin- 100 mg/l, Streptomycin- 20 mg/l) or a disinfectant.
5. The eggs are then separated from the dead rotifers on a 50 μm sieve and incubated for hatching and the offsprings can be used for starting the stock cultures.
6. If the rotifers do not contain many eggs (as can be the case) the risk of losing the complete initial stock is high and under such instances, the rotifer should be disinfected at sublethal doses.
7. The rearing water of the rotifers to be completely renewed and the rotifers to be treated with either antibiotics or disinfectants.
8. The treatment is repeated after 24 h in order to be sure that any pathogens which might have survived the passage of the intestinal tract of the rotifers are killed as well.
9. The concentration of the disinfection products differs according to their toxicity and the initial condition of the rotifers.
10. Orientating concentrations for this type of disinfection are furazolidone, 10mg/l, oxytetracycline, 30 mg/l, sarafloxacin, or linco-spectin-30 mg/l.

Maintenance of Stock culture

Small stock cultures are generally kept in closed vials in an isolated room to prevent contamination with bacteria and/or ciliates and further upscaling is essential for the regular mass production of rotifer.

1. The rotifers for stock cultures can be obtained from the wild, or from research institutes or commercial hatcheries.
2. Disinfection of the stock obtained has to be carried out as mentioned in the isolation procedure.
3. The culture water (seawater diluted with tap water to a salinity of 25 ppt) is aerated, pre-filtered over a 1 μm filter bag and disinfected overnight with 5 mg/l NaOCl.
4. The next day the excess of NaOCl is neutralized with $\text{Na}_2\text{S}_2\text{O}_3$ and the water is filtered over a 0.45 μm filter.
5. The vials (50 ml conical centrifuge tubes) which are previously autoclaved and filled with 20 ml of culture water are inoculated with an initial density of 2 rotifers/ml.

6. The vials are exposed to the light of two fluorescent light tubes at a distance of 20 cm (light intensity of 3000 lux on the tubes).
7. The food consists of marine *Chlorella* or *Nannochloropsis* or *Isochrysis*
8. Good cultures of 4 ml ($2-4 \times 10^6$ cells/ml) fresh algae are to be added daily.
9. Shaking is needed mixed with enclosed air to provide enough oxygen for the rotifers.
10. The stock cultures for rotifers are to be kept in a temperature controlled room ($28^\circ\text{C} \pm 1^\circ\text{C}$).
11. After one week the rotifer density would increase from 2 to 200 individuals/ml.
12. The rotifers are rinsed, a small part is used for maintenance of the stock, and the remaining rotifers can be used for upscaling.
13. Furthermore, after some months of regular culture the stock cultures have to be disinfected as described earlier in order to keep healthy and clean stock material.

Mass production of rotifers

Growth phases

Rotifer growth under mass rearing conditions follow different phases, mimicking those of microalgae as described below:

- The lag-phase, when, just after the inoculum, rotifers begin to consume the phytoplankton of their culture medium and the number of both egg-bearing individuals as well as the quantity of amictic eggs increases.
- The log-phase (or exponential phase), where rotifers reproduce very fast and population growth is exponential.
- The transitional phase (or declining growth), where growth rate slows down and egg-bearing rotifers become rarer.
- The decline phase, where almost only old rotifers without eggs are found and their number decreases rapidly as death rate exceeds growth rate.

The quality of the rotifer population to start new cultures is even more important than in the case of microalgae. To be used as inoculum, the rotifer population must still be in the middle of

its log-phase with at least 20% fertility rate (measured as percentage of eggs over total rotifers, populations in their last declining phase, characterised by limited motility, scarce repletion and absence of egg-bearing animals, should always be discarded. With a proper inoculum and under optimal rearing conditions, a rotifer population should reach its harvesting density within 4 to 5 days. Under hatchery conditions, rotifer populations can reach the following densities:

in flasks after 5 to 7 days:

- S-type rotifers, 500 to 700 ind/ml
- L-type rotifers, 150 to 250 ind/ml

in tanks, after 4 to 6 days:

- S-type rotifers, 1000 and more ind/ml
- L-type rotifers, 400 ind/ml

Rotifers and eggs are counted at least in duplicate, alive or fixed in 4% formalin, in screened plastic petridishes (5 cm diam.).

As this microscopic animal is a filter feeder, its nutritional value strictly depends on its food. In hatcheries, the species is first cultured on microalgae, following the same scale-up protocol described for microalgae, then its final mass production is achieved in large tanks where artificial diets are fed to rapidly increasing numbers, improving at the same time their nutritional value. The rotifer *B.plicatilis* is a rather sturdy species able to tolerate a wide range of salinity, temperature and ammonia levels. It can also use several food sources, provided that particle size remains within a 2-20 μm range. Obviously, the highest growth rate is achieved under more restricted environmental parameters, and is closely related to the selected rotifer strain and feeding provided. In particular, good yields are obtained with high dissolved oxygen levels, temperature at 25°C, pH at 7.5-8.5, salinity in a 20-30 ppt range, less than 1 mg/l free ammonia (NH₃), and moderate turbulence. Light is required only when rotifers are fed microalgae.

Preparation of the culture medium

The same procedures and precautions described in the algal production section apply to rotifer culture. The only enrichment added to the rotifer culture medium, be it either a log-phase algal culture or treated seawater plus artificial diet, is represented by the addition of vitamin B₁₂ (cyanocobalamin) as a fertility booster for the rotifers. Its dosage is usually 100 ml of B₁₂ stock

solution per m³ of rotifer culture in tanks, whereas small vessels and bags are fertilized at the rate of 1 ml/litre. In both cases the vitamin is added together with the inoculum.

Mass culture

Rotifer mass culture is carried out in large tanks (1-2t). Because of the very high density achieved (up to 1000 individuals per ml or more), rearing procedures and protocols to maintain strict hygienic conditions have to be applied. Such routine procedures are described below.

There are basically two main mass rearing methods for rotifers: (i) Using algae and baker's yeast as food for the rotifers, and (ii) using an artificial diet, the Culture Selco® produced by INVE SA of Belgium (or similar).

Standard Operating Protocols

Tank preparation

Before starting a new production cycle, normally after the harvest of the previous rotifer culture, prepare the tank as follows:

- Rinse with tap water to eliminate the bulk of organic debris.
- Wash it thoroughly with brush and detergent and rinse it again.
- Wash or spray the tank walls with 500 ppm active chlorine solution.
- After a couple of hours, drain the tank and rinse it well until the chlorine smell is gone.
- Let the tank dry and fill it with sterilized heated water only when needed.
- As an alternative possibility, fill the tank with seawater and sterilize with hypochlorite, then neutralize the residual chlorine with sodium thiosulphate.

Repeat the procedure for the equipment to be used in the tanks: aeration tubing, drain valves and suspended traps. A practical procedure is to assemble all small equipment in the new tank, fill with sea water and sterilize with hypochlorite: the equipment will be disinfected as a consequence.

Inoculation

To be used as inoculum, the rotifer population must still be in the middle of its log-phase with at least 20% fertility rate (measured as percentage of eggs over total rotifers). Never use rotifers which have already reached the last phase characterised by limited motility, scarce repletion and no egg presence. In algae/yeast fed tanks, the initial density of inoculum, one of the most important factors in rotifer culture, should be kept at least at 100 animals/ml, with an optimal density of 150-200 animals/ml. High density cultures with artificial feeding need up to 500 rotifers per ml. With an initial density of 200 animals/ml, rotifer density should reach its peak within four to six days at 25°C.

Feeding

As previously indicated two feeding methods are most widely adopted: 1) a combination of algae and baker's yeast and 2) a totally artificial diet.

Mass culture with algae/yeast as food

The initial method of mass culturing rotifers in hatcheries, makes use of a common and easily available food staple, the bakers' yeast *Saccaromyces cerevisiae*. It is a labour and cost sparing food, which has no nutritional value for rotifers that feed on bacteria associated with the yeast.

Compared to the artificial diets, this method has a lower yield and requires more time, typically one extra day. Density at harvest rarely exceeds 450 rotifers/ml with an average daily increase ranging from 19 to 33%. In addition, rotifers should be enriched with high levels of (n-3) HUFA and vitamins. A major constraint of this method is the absolute necessity to improve the otherwise very poor nutritional quality of yeast-fed rotifers before their distribution to fish larvae, by enriching them.

Procedure

- Fill the tank with sterilized sea water diluted with tap water to obtain a 20 ppt salinity; check the chlorine content of tap water and, if present, neutralize it with an excess of sodium. Take care to leave enough space for the algal cultures to be supplied as food (about 30% of the tank volume).
- Place the air diffusers and switch on the aeration.
- Place the traps for ciliates and impurities.

- Inoculate the tank to achieve an initial density of 150-200 rotifers/ml. This is considered as day 0.
- Add algal culture as 20% of tank volume to provide rotifers with their initial food (as usual, the algae should be in their log-phase and from non contaminated cultures, even if of different species).
- The next day (day 1) fill the remaining 10% volume with algal culture.
- On the tank file, record all information on the culture growth, food distributed and environmental parameters monitored.
- From day 1 on, feed with bakers' yeast according to the recorded rotifer density.

Mass culture with Culture Selco® as food

A different technique based on a compound feed has been developed by the Belgian Company INVE SA. The product, named Culture Selco® (CS) is a dry and complete rotifer diet that does not require algae and is also effective as enrichment medium. Particle size (5 to 7 μm) and physical characteristics ensure an optimal uptake by rotifers. The feed composition includes proteins (>35%), lipids (>15%, of which 23% are PUFA), carbohydrates (30%), carotenoids and other micronutrients as minerals and vitamins A, D3, F and C.

To prepare CS suspend the amount required for a single meal in tap water, up to 50 g CS/l, and mix vigorously for 3 minutes (use a kitchen or better an industrial blender). Mixing or shaking by hand or using a magnetic stirrer is not sufficient to separate the CS cells. Remember that cell agglomerates left in the feed suspension cannot be ingested by rotifers because of their large size. Take care not to overfeed as uneaten feed can also quickly spoil water quality. Feed the daily amount in four to six meals evenly distributed over the 24-h period. In case it would be necessary, the feed suspension can be stored at temperatures below 8°C, and the amount needed for the whole day can be prepared at one time. The feeding ration can thus be distributed from the stored suspension at each meal.

Harvest

At harvest, rotifers are filtered and rinsed before being fed to fish or utilized as inoculum for new tanks. For this purpose, a double submerged filter is used. The inner filter has a mesh size of 300μ to retain larger particles, flocks of agglomerated food particles and ciliates which would rapidly clog the finer filter. The outer filter has a 50 mm filter mesh. Its capacity should be large enough to keep safely the whole rotifer population for the time needed to complete harvest and rinsing. Both filters are placed inside a large wheeled container full of water to avoid pressure build-up from the outgoing water that would smear rotifers against the net. A gentle air bubbling along the inner side of the filter helps to keep the filter free from clogging.

Harvesting and rinsing protocol for round-conical tanks with central drain:

- Prepare the harvesting device, always clean and disinfected with hypochlorite.
- Inject pure oxygen into the tank to be harvested for 10 to 15 minutes to have a supersaturated medium (at 10 ppm DO) in which rotifers could safely stand filtering operation.
- Fix a flex hose to the bottom drainage valve and place the other end into the harvesting Device.
- Open the valve and start filtering.
- Regulate the water flow so that the filter does not clog and the culture water does not overflow; do not exceed 100 l/minute.
- Stop water flow and clean the clogged pre-filter whenever necessary to avoid overflow of rotifer concentrated water.
- While harvesting, check for possible loss of rotifers through the net by sampling some filtered water with a beaker or a Petri dish.
- At the end of filtration, close the valve and rinse for 10 to 15 minutes the rotifers with filtered sterilized seawater at the same temperature as the tank of origin.

Harvesting of rotifers can also be done by the following method:

1. Switch off the aeration for some time (5-10 minutes)
2. Put a light source on the water surface, which will attract the rotifer on the water surface
3. Slowly collect the aggregated rotifers at the water surface along with the water using a bowl
4. Transfer the collected rotifers to a bucket which is prefilled with microalgae upto about 20% of its volume.
5. This can be fed to the larval rearing tanks after ascertaining the density.

Nutritional value and enrichment methods

Rotifers have a limited nutritional value for marine finfish larvae. Their nutritional value can be upgraded by an enriching process before their harvest through feeding them with microalgae rich in PUFA and vitamins such as *Chlorella*, *Nannochloropsis* and *Isochrysis*. It can also be done enriching with specially formulated artificial diets like the above mentioned Selco products. This oil emulsion gives excellent results in terms of high levels of EPA, DHA and vitamin C, which was not possible with the only use of algae. Moreover, labour, time, investment and running costs are spared. Rotifers can be enriched either in their mass culture tanks or after harvesting by placing them in dedicated enrichment tanks. The first method produces an enrichment of the tissues, as it is continuous along the entire culture period. The acquired fatty acids reserves are more stable and are less exposed to a rapid decrease in nutritional value during starvation. This method also saves time and reduces handling losses. The second system is a short term enrichment or, rather, a gut enrichment. It implies the harvesting and rinsing of the rotifers and the preparation of a separate enrichment tank.

Enrichment with algae:

- Use selected algae as specified above; enrich for about 4-6 hrs.
- Maximum rotifer density: 500/ml.
- Microalgae density: *Isochrysis* 5 million cells/ml; *Chlorella* sp. or *Nannochloropsis* sp. 12 million cells/ml.
- Resulting average total PUFA content of enriched rotifers: ± 7 mg/g dry weight.

Enrichment with Selco® products or other similar products:

- Follow the instructions provided by the manufacturer.
- Enrichment should take between 6 to 8 hours.
- Maintain the oxygen level at or above 4 ppm throughout the entire procedure.
- Dry products can be used directly in the production tank, whereas oily products are only fed in enrichment tanks.
- Use an antifoam product during enrichment to prevent rotifers losses by foam aggregation.

- During the enrichment process check frequently rotifer mortality and dissolved oxygen content; the latter should be kept above 80% saturation, with addition of pure oxygen if necessary.

The content of nutrients decreases rapidly in rotifers that are not immediately consumed by fish larvae. In starving rotifers the total PUFA loss reaches 60% after 6h at 18°C. Even in green water, i.e. with microalgae, this loss remains important (about 40% after 6 h). To prevent this degradation in nutritional quality, enriched rotifers not immediately fed to fish should be stored in containers at low temperature as follows:

- Storage time should not exceed 14 hours.
- Temperature should be kept between 5 and 10°C by means of insulated tanks and blue ice or ice bags.
- Rotifer density should not exceed 2500 to 3000 ind/ml.
- Oxygen level should be kept at or above 4 ppm.

Monitoring rotifer populations and health

Check all rotifer cultures daily for both quantitative and qualitative evaluations. From each vessel, flask and tank, take a 1 ml sample and observe under the stereo microscope.

Measure the following parameters:

The physiological and state of health of rotifer cultures can be assessed as follows.

1. Egg ratio
2. Swimming velocity
3. Ingestion rate
4. Viscosity
5. Enzyme activity
6. Diseases

Quantitative parameters:

- Total number of rotifers per ml
- Total number of eggs per ml
- Fertility as percent of total eggs over the total rotifers

Qualitative parameters:

- Average number of eggs per individual (estimate)
- Repletion (presence of food in the stomach, note 0 for empty, + for medium full, ++ when full)
- Motility (++ active, + slow, 0 absent)
- Filtration (activity of the ciliated corona)

In addition the following qualitative parameters of the culture should be checked:

- Presence of foam at the culture surface or sediment on the wall and bottom of the container.
- Presence of other micro-organisms, such as protozoa, fungi, bacterial flocks, etc. (Identification and frequency, note 0 when clean, + for medium contamination, ++ large contamination).

Production and use of resting eggs

Various methods of storing rotifers have been studied. Frozen rotifers are not usually adequate as feed because of leaching of nutrients. Amictic eggs of rotifers can be preserved by cryopreservation in liquid nitrogen after they have been impregnated with cryoprotective agents like dimethyl sulfoxide (DMSO). This method ensures full preservation of genetic traits of importance to aquaculture. Cryopreservation is not a suitable method for preservation of large numbers of rotifers for direct use as feed.

Artificially produced rotifer eggs have been tried as an alternative to daily production of rotifers. The production of these eggs can be manipulated by environmental factors, such as salinity, food quality and quantity, rotifer culture density, exchange of culture media and temperature and varies between *B. plicatilis* and *B. rotundiformis*. The cost of producing resting eggs is very high and therefore not yet been extensively adapted in hatcheries. Using preserved rotifers may eliminate the dependence on daily production of rotifers. Cheaper methods of resting egg production are another field which requires research attention in future.

For the mass rearing of rotifers as larval food the amictic way of reproduction is favoured. The resting eggs/ cysts are relatively large and are ideal for storage and transport and

can be used as inocula for mass cultures. Massproduction of rotifers for cyst production is performed in batch cultures in concrete tanks (Hagiwara *et al.*, 1995; Dhert *et al.*, 1995) or resting eggs are collected from sediments in earthen ponds. Resting egg production can be induced bylimiting the food supply or changing the temperature and/or salinity. Resting eggs will sink and need to be harvestedfrom the bottom. In case a lot of waste is trapped at the bottom it is advised to replace the water by brine so that restingeggs will float and can be collected from the water surface. Dry resting eggs can be stored for more than one year. When placed in seawater, rotifer cysts hatch in about 24 hours at 25°C under light conditions. Newly-hatched rotifersundergo asexual reproduction. The use of cysts is also highly recommended to prevent contamination. Cysts caneasily be treated before hatching in order to ensure that starter cultures are free from bacteria and ciliates. The resting eggscould be disinfected with heavy doses of antibiotics, so that the emerging rotifers are essentially bacteria free. Theresting eggs can also resist short exposure to disinfectants such as NaOCl or glutaraldehyde.

CONCLUSION

The significance of rotifer as alive feed for the larval rearing of various fish species with mariculture potential is widely understood. The basic criteria for selecting a live feed includes qualities such as nutritional value, size, ease of culture etc. Almost all these criteria suits well for rotifer to be qualified as a live feed. A new super small species of rotifer ,*Colurellaadriaticais* also now available for use in marine fish hatcheries (Madhu et al.2016) The size of this rotifer is much smaller than *B. rotundiformis*. Hence the mass culture of this species would be highly beneficial for the hatchery seed production of fishes with very small larval mouth size, such as groupers, damsel fishes, breams etc which are presently being fed on copepods during their early larval stages.

Suggestedreading

Madhu et al (2016). Isolation, Identification and culture of the marine rotifer, *Colurellaadriatica* Ehrenberg,1831(Family: Lepadellidae) from Andaman & Nicobar Islands: A promising live feed for larval rearing of high value shell fishes and fin fishes.JMBAI. 58 (1), pp 5-12

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Artemia – the global live feed for aquaculture

Anikuttan.K.K, G.Tamilmani, M.Sakthivel, P.Rameshkumar, M.Sankar, R.Jayakumar, A.K.Abdul Nazar, Tinto Thomas, G.H.Rao, N.Krishnaveni, Munirasu, Suresh and Ganesan.

Introduction

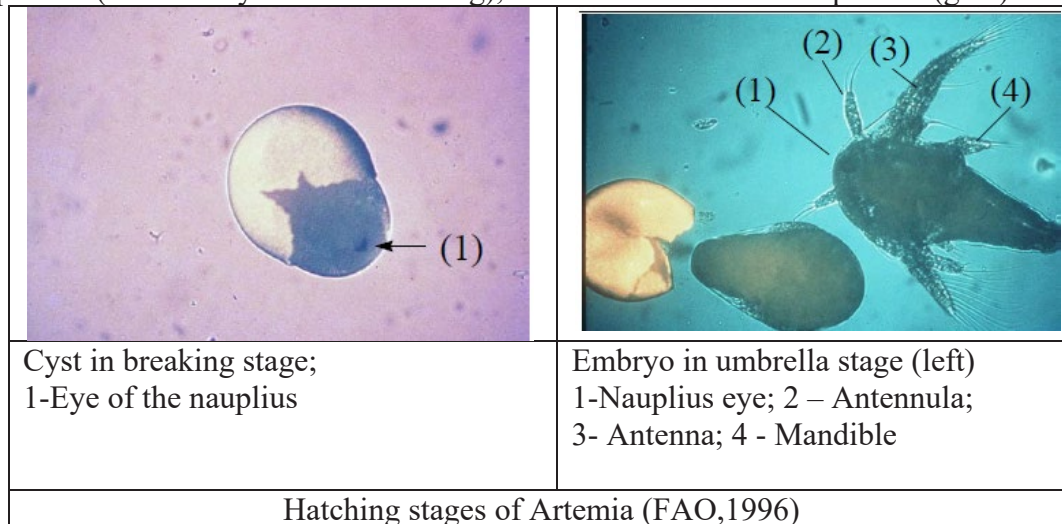
The most popular and globally accepted live feed and life line of aquaculture, *Artemia* is an inevitable component in the hatchery production of most of the species with mariculture potential. The advanced larval stages of these fishes/shell fishes are fed on artemia nauplii. *Artemia salina*, popularly known as brineshrimp are found in salt pans, lakes or evaporation ponds at salinities from 100 ppt onwards. Different geographical strains are available. Over 300 natural biotopes, spread over the 5 continents have been identified. It is characterized by an elongated body with 2 stalked complex eyes in the head region, 11 pairs of thoracic appendages and an abdomen that ends in a furca covered with spines. The unique property of *Artemia* to form dormant embryos, called 'cysts', is the reason for making it the popular live feed. The cysts are available year round in large quantities along the shorelines of hypersaline lakes, coastal lagoons and solar salt works scattered over the five continents. After harvesting and processing, cysts are made available in cans as storable 'on demand' live feed. Upon some 24-h incubation in seawater, these cysts release free-swimming nauplii that can directly be fed as a nutritious live feed to the larvae of a variety of marine as well as freshwater organisms, which make them the most convenient, least labour-intensive live food available for aquaculture. Further, the adults of artemia can be frozen and stored which also can be fed to still bigger juveniles of fishes. So the nauplii as well as adults of artemia can be used to feed the larvae/juveniles of many fishes.

Average proximate composition of <i>Artemia</i> (Leger <i>et.al</i> ; 1987)		
Components	% composition	
	Nauplii	Adults
Protein	52.2 ± 8.8	56.4±5.6
Lipid	18.9±4.5	11.8±5.0
Carbohydrate	14.8±4.8	12.1±4.4
Ash	9.7±4.6	17.4±6.3

Biology

In the natural environment, *Artemia* produces cysts during certain season of the year, that float at the water surface and that are thrown ashore by wind and waves. These cysts are metabolically inactive and do not further develop as long as they are kept dry. Upon immersion in seawater, the biconcave-shaped cysts hydrate, become spherical, and within the shell the embryo resumes its interrupted metabolism. After about 20 hrs the outer membrane of the cyst bursts and the embryo appears, surrounded by the hatching membrane. The development of the nauplius is completed and within a short period of time the hatching membrane is ruptured and the free-swimming nauplius is born. The first larval stage (instar I; 400 to 500 µm in length) has a brownish-orange colour, a red nauplius eye in the head region and three pairs of appendages: *i.e.* the first antennae (sensorial

function), the second antennae (locomotory + filter-feeding function) and the mandibles (food uptake function). The larva grows and differentiates through about 15 molts. From the 10th instar stage on, important morphological as well as functional changes are taking place: *i.e.* the antennae have lost their locomotory function and undergo sexual differentiation. In males they develop into hooked graspers, while the female antennae degenerate into sensorial appendages. The thoracopods are now differentiated into three functional parts namely the telopodites and endopodites (locomotory and filter-feeding), and the membranous exopodites (gills).



Hatching of cysts:

Artemia nauplii is a widely accepted live feed for the advanced stages of fish and crustacean larvae and it can be made available by hatching of their cysts. *Artemia* cysts are commercially available in dried form and it can be stored in anaerobic condition for more than a year. When the cysts are transferred to filtered seawater under illumination and vigorous aeration, the biconcave cysts become spherical, after about 24 hours the cyst shell bursts and within a short period of time the free swimming nauplius will be developed.

When incubated in seawater the biconcave cyst swells up and becomes spherical within 1 to 2 hrs. After 12 to 20 hrs of hydration, the cyst shell (including the outer cuticular membrane) bursts, which is called the breaking stage and the embryo surrounded by the hatching membrane becomes visible. The embryo then leaves the shell completely and hangs underneath the empty shell (the hatching membrane may still be attached to the shell). Shortly thereafter the hatching membrane breaks open and the free-swimming larva (head first) is born. Dry cysts are very hygroscopic and take up water at a fast rate (*i.e.* within the first hours the volume of the hydrated embryo increases to a maximum of 40% water content; However, the active metabolism starts from a 60% water content onwards, provided environmental conditions are favourable.

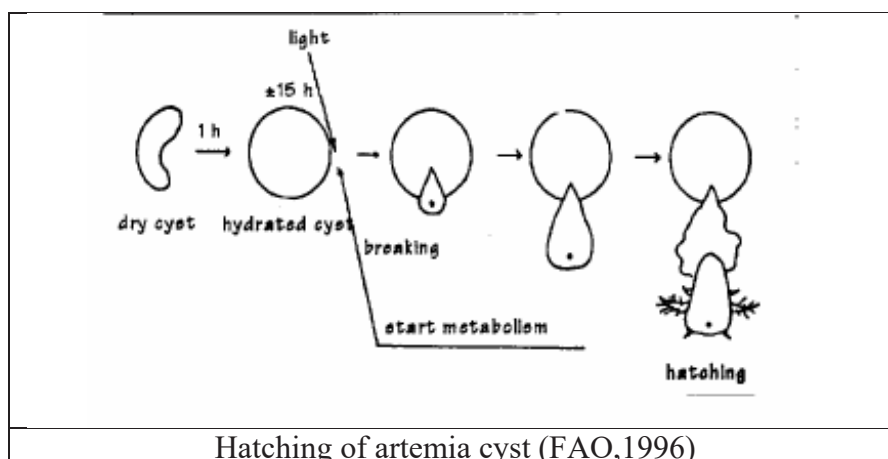
When hatching large quantities of cysts, an impressive bacterial load rapidly develops. Reducing bacterial development during hatching will improve the hygienic status of nauplii and may result in better hatching yields. It can be achieved through simple disinfection of the cysts using liquid bleach solution or through decapsulation.

Disinfection of *Artemia* cysts with liquid bleach

- Prepare 200 ppm hypochlorite solution
- Soak cysts for 30 min. at a density of ± 50 g cysts.l⁻¹;
- Wash cysts thoroughly with tapwater on a 125 μ m screen;
- Cysts are ready for hatching incubation.

Artemia hatching

- Use a transparent or translucent cylindroconical tank
- supply air through open aeration line down to the tip of the conical part of the tank; oxygen level should be maintained above 2 g.l⁻¹, apply strong aeration
- use filtered natural seawater
- maintain the optimum hatching
- hydrate cysts prior to hatching incubation in tap water for 1-2 hours
- incubate cysts at density of 2 g.l⁻¹; for smaller volumes (<20l) a maximal cyst density of 5 g.l⁻¹ can be applied. · Incubate for fixed time period (e.g. 20 hr).
- Remove the aeration prior to harvesting
- Wait to separate between nauplii and unhatched cysts. Nauplii will go down to bottom and unhatched cysts will float.
- concentrate nauplii using a light source
- Sieve nauplii, rinse well with tap water.





A simple hatching arrangement for Artemia cyst

Decapsulation of the cysts:

A complete separation of *Artemia* nauplii from their empty cyst shells is a difficult task and always it may not be successful also. The presence of empty shells is harmful for the larvae too. This can be overcome by a method called Decapsulation of the cyst. The hard shell or chorion of cysts will be removed without affecting the viability of the embryos by short exposure of the hydrated cysts to a hypochlorite solution. This process is called cyst decapsulation. When the cysts are exposed to hypochlorite solution, the hard shell of the cyst dissolves, and a gradual colour change from dark brown to grey and then to orange can be observed. Decapsulated cysts offer a number of advantages compared to the non-decapsulated ones which are listed below:

- Cyst shells are not introduced into the culture tanks. When hatching normal cysts, the complete separation of *Artemia* nauplii from their shells is not always possible.
- Unhatched cysts and empty shells can cause deleterious effects in the larval tanks when they are ingested by the predator: they can not be digested and may obstruct the gut.
- Nauplii that are hatched out of decapsulated cysts have a higher energy content and individual weight (30-55 % depending on strain) than regular instar I nauplii, because they do not spend energy necessary to break out of the shell.
- In some cases where cysts have a relatively low energy content, the hatchability might be improved by decapsulation, because of the lower energy requirement to break out of a decapsulated cyst
- Decapsulation results in a disinfection of the cyst material
- Decapsulated cysts can be used as a direct energy-rich food source for fish and shrimp
- For decapsulated cysts, illumination requirements for hatching would be lower

References

- FAO (1996). FAO Fisheries Technical Paper no.361. Manual on the production and use of live food for aquaculture.
- P.Leger, D.A. Bengston, P.Sorgeloos,K.L.Simpson and A.D.Beck (1987) PThe nutritional value of Artemia: a review. Artemia Research and its Application. 1987, Vol 3. Ecology, culturing, use in aquaculture P.Sorgeloos, D.A, Bengston, W. Decler and E.Jaspers (Eds), Univera Press, Wetteren, Belgium.556p