CMFRI

Course Manual

Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

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CULTURE OF ROTIFER AND THEIR NUTRITIONAL ENRICHMENT

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Introduction

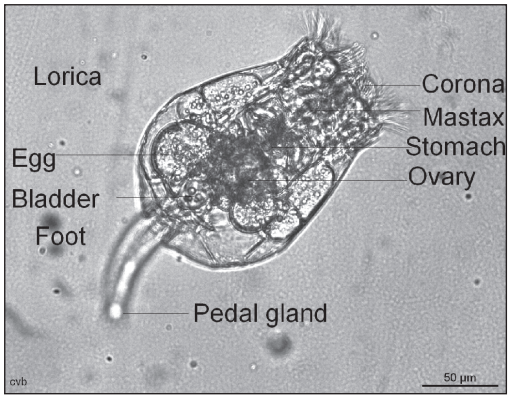
Rotatoria (=Rotifera) belonging to the smallest metazoa consist of 1000 species of which 90% inhabit in freshwater habitats. In the fifties and sixties though *Brachionus plicatilis* was first identified as a pest in the pond culture of eels but soon the Japanese researchers realized that this rotifer could be used as a suitable live food organism for the early larval stages of marine fish. Later in China also *B. plicatilis* is used as food in local shrimp and crab hatcheries. The rotifers are considered as an important live feed in hatchery operation due to their planktonic nature, tolerance to a wide range of environmental conditions, high reproduction rate (0.7-1.4 offspring/female/day), small size and slow swimming nature. The animals reproduce rapidly and can thus contribute to the build up of large quantities of live food in a very short period of time. The possibility of rearing these animals at very high densities (2000 animals/ml) have been reported by (Hirata, 1979) and the growth is assured by plasma increase and not by cell division. More over the filter-feeding nature of the rotifers facilitates the inclusion of specific nutrients essential for the larval predators through bioencapsulation into their body tissues. As a result it became a suitable prey for fish larvae that have just resorbed their yolk sac. The availability of large quantities of this live food source has contributed to the successful hatchery production of more than 60 marine finfish species and 18 species of crustaceans worldwide.

Systematic position:

Phylum	:	Rotifera
Class	:	Monogononta
Order	:	Ploima
Family	:	Brachionidae
Genus	:	*Brachionus*

Morphological characters

The body of rotifer is differentiated in to three distinct parts : head, trunk and foot and head carries the rotatory organ or corona which is easily recognized by its annular ciliation and which is at the origin of the name of the Rotatoria (bearing wheels). The retractable corona assures locomotion and a whirling water movement which facilitates the uptake of small food particles like 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 called the lorica. Males have reduced sizes and are less developed than females; some measuring only 60 mm. The shape of the lorica and the profile of the spines and ornaments allow the determination of the different species and morphotypes.



***Brachionus plicatilis*, female**

Life history

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.

The life cycle of *Brachionus plicatilis* can be closed by two modes of reproduction: parthenogenesis and sexual reproduction. During female parthenogenesis, the amictic females produce amictic eggs (diploid, 2n chromosomes) 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. Although both are not distinguishable morphologically, 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 an over-proportionated single testis which is filled with sperm. Mictic eggs which will hatch into males are significantly smaller in size, while the mictic fertilized eggs are larger and have a thick, faintly granulated outer layer.

The resting eggs will develop and hatch into amictic females after exposure to specific environmental conditions viz. alternations in temperature or salinity or changing food conditions. The density of rotifer population also plays an important role in the determination of the mode of reproduction and it is generally believed that the production of resting eggs is a survival strategy of the population through unfavourable environmental conditions such as drought or cold.

Strain differences

In aquaculture, the most widely used species of rotifer are *B. plicatilis* (L-type), with the lorica ending with 6 occipital spines (Fukusho, 1989) and *B. rotundiformis* or small (S-type), whereas in tropical aquaculture, the SS-type rotifers (Super small-SS Type) 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 µm). Those rotifers, however, are genetically not isolated from S-strains, but are smaller than common S-strains.

Type	Lorica length	Optimum temperature
Large(L type): lorica has obtuse angled spines	Above 200 µm (average 239 µm),	18-25°C.
Small (S type): lorica has pointed spines	100 to 200 µm (average 160 µm).	28-35°C,
Super small (SS type)	60 to 100 µm	24-30°C,

Environmental conditions

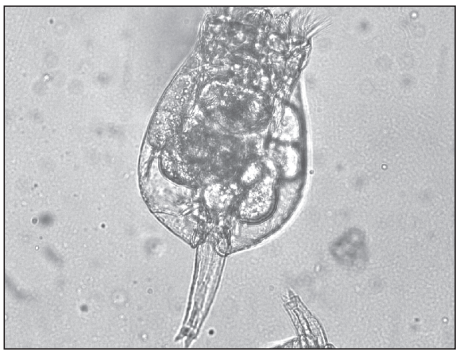
Although *B. plicatilis* can withstand a wide salinity range (1 to 97 ppt), the reproduction can take place at salinities below 35 ppt (Lubzens, 1987). The reproduction activity of *Brachionus* depends on the temperature of the environment and it influences the fecundity of rotifers. Maximum reproduction of *B. plicatilis* occurs at 30° to 34°C. However, the optimal culture temperature for rearing rotifers depends on the rotifer-morphotype. At high temperatures, the cost for food increases and the starving animals consume their lipid and carbohydrate reserves very fast. whereas rearing rotifers below their optimal temperature slows down the population growth considerably. Rotifers can survive in water containing as low as 2 mg/L of dissolved oxygen however the aeration should not be too strong as to avoid physical damage to the population. Rotifers live at pH-levels above 6.6 and best results can be obtained at a pH above 7.5. High levels of un-ionized ammonia NH_3/NH_4^+ ratio are toxic for rotifers but rearing conditions with NH_3-concentrations below 1 mg/L appear to be safe.

Effect of temperature on the reproduction activity of *B. plicatilis*

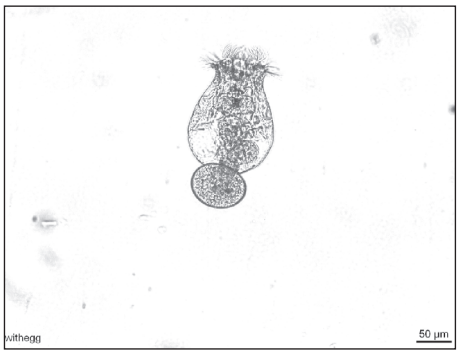
Temperature (°C).	**15°C**	**20°C**	**25°C**
Time for embryonic development (days).	1.3	1.0	0.6
Time for young female to spawn for the first time (days).	3.0	1.9	1.3
Interval between two spawnings (hours).	7.0	5.3	4.0
Length of life (days).	15	10	7
Number of eggs spawned by a female during her life	23	23	20

Rotifer morphotypes, resting eggs and adult female

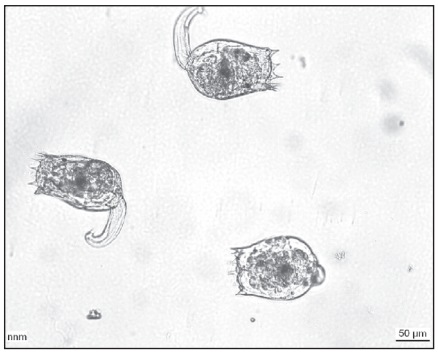
L-TYPE



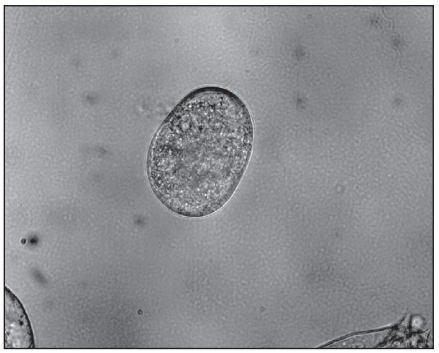
S-Type



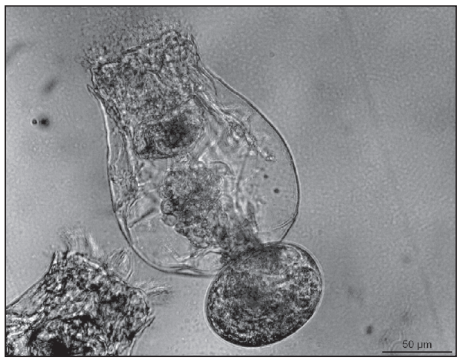
SS Type



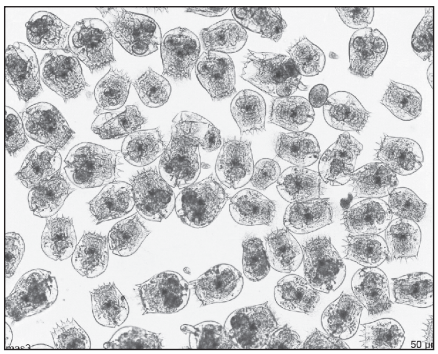
Resting eggs



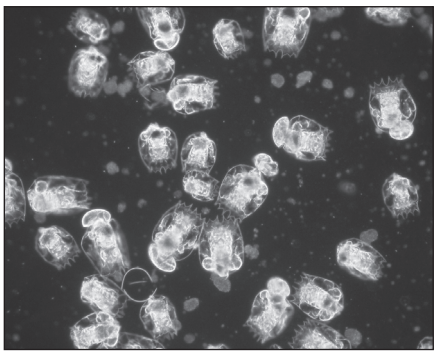
Egg bearing adult female



Non enriched rotifer



Enriched rotifer



Contaminants :Bacteria and Ciliates

Pseudomonas and *Acinetobacter* are common opportunistic bacteria which may be important additional food sources for rotifers. Some *Pseudomonas* species, synthesize vitamin B_{12} which can be a limiting factor under culture conditions (Yu *et al.,* 1988). The dominant bacterial flora associated with the rotifer cultures was V*ibrio* (Verdonck *et al*., 1994) and high numbers of associated bacteria were found after enrichment (Skjermo and Vadstein, 1993) and it can be minimized by appropriate storage (6°C) and adjustment of the rotifer density (Skjermo and Vadstein, 1993). The bacterial counts, *Vibrionaceae* in rotifers can be decrease by feeding the rotifers with *Lactobacillus plantarum* (Gatesoupe, 1991). The supplementation of these probiotic bacteria not only has a regulating effect on the microflora but also increases the production rate of the rotifers.

Invasion of Halotricha and Hypotricha ciliates, such as *Uronema* sp. and *Euplotes* sp., are not desired as they compete for feed with the rotifers under culture conditions. Ciliates produce metabolic wastes which increase the NO_2^-N level in the water and cause a decrease in pH. However, they have a positive effect in clearing the culture tank from bacteria and detritus. The addition of a low formalin concentration of 20 $mg.l^{-1}$ to the algal culture tank, 24 h before rotifer inoculation can significantly reduce protozoan contamination. Screening and cleaning of the rotifers through the use of phytoplankton filters (< 50 µm) so as to reduce the number of ciliates or other small contaminants is an easy precaution which can be taken when setting up starter cultures.

Culture procedures

Intensive production of rotifers is usually performed in batch culture within indoor and extensive outdoor culture . Basically, the production strategy is the same for indoor or outdoor facilities, but higher starting and harvesting densities enable the use of smaller production tanks (generally 1 to 2 m^3) within intensive indoor facilities. In some cases, the algal food can be completely substituted by formulated diets.

Isolation of rotifer for stock culture

Requires strains of rotifer can be collected from the stagnant water bodies, brackishwater ponds or mangrove creeks by sieving water using 50 to 100µm bolting silk cloth. Live samples collected from the field are to be examined under a stereozoom microscope and using a fine dropper, the desire Brachionus species is to be picked up and introduced to glass cavity block containing filtered sterilized seawater having same salinity and pH of the field sample and Chlorella can be used as feed. To avoid contaminants, the inoculum can be disinfected with antibiotics (*e.g.* erythromycin 10 mg/L, chloramphenicol 10 mg/L, sodium oxolinate 10 mg/L, penicillin 100 mg/L, streptomycin 20 mg/L) to kill the free-swimming rotifers and the eggs are then separated with a 50 µm sieve and incubated for hatching and the offspring can be used for starting the stock cultures. The stock can also be disinfected at sublethal doses with 7.5 mg/L furazolidone, 10 mg/L oxytetracycline, 30 mg/L sarafloxacin, or 30 mg/L linco-spectin and the water of the rotifers is completely renewed and the treatment is repeated after 24 h in order to make sure that any pathogens which might have survived the passage of the intestinal tract of the rotifers are killed as well.

Maintenance of Stock culture

The culture water (seawater diluted with tap water to a salinity of 25 ppt) is aerated, pre-filtrated over a 1 µm filter bag and disinfected overnight with 5 mg/L NaOCl. The next day the excess of NaOCl is neutralized with $Na_2S_2O_3$ for neutralization and the water is filtered over a 0.45 µm filter. Stock culture maintained in small vials can be inoculated with initial density of 2 rotifers/ml/L and can be fed marine *Chlorella* every day. After one week the rotifer density should have increased from 2 to 200 individuals/ml. The rotifers are rinsed and a small part of this is used for subsequent 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 500 ml erlenmeyers flask or conical flask and placed 2 cm from fluorescent light tubes (5000 lux). The temperature in the erlenmeyers should not be more than 30°C. The rotifers are

stocked at a density of 50 individuals/ml and fed 400 ml freshly-harvested algae (*Chlorella* 1.6x10^6 cells/ml); approximately 50 ml of algae being added every day to supply enough food. Within 3 days the rotifer concentration can increase to 200 -300 rotifers/ ml. During this short rearing period no aeration is applied.

Once the rotifers have reached a density of 200-300 individuals/ml they are rinsed on a submerged filter consisting of 2 filter screens. The upper mesh size (200 µm) retains large waste particles, while the lower sieve (50 µm) collects the rotifers. The concentrated rotifers are then distributed in several 15 L bottles or columns filled with 2 L water at a density of 50 individuals/m and a mild aeration is provided and algae (*Chlorella* 1.6 × 10^6 cells/ml) are supplied daily. Every other day the cultures are cleaned (double-screen filtration) and restocked at densities of 200 rotifers/ml. After adding algae for approximately one week, the 15 L bottles are completely full and the cultures can be used for inoculation of mass cultures.

Mass production of rotifer on algae

Marine microalgae are the best diet for rotifers and very high yields can be obtained if sufficient algae are available. In most places pure algae are given for starting up rotifer cultures or to enrich rotifers.

Batch cultivation 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 semicontinous culture system in which the maintenance of a constant rotifer density by increasing the culture volume. Extensive culture techniques (using large tanks of more than 50 m^3) as well as intensive methods (using tanks with a volume of 200-2000 L) are applied. In both cases large amounts of cultured microalgae, usually the marine alga *Nannochloropsis*, are usually inoculated in the tanks together with a starter population containing 50 to 150 rotifers/ml.

To start out door culture of rotifer, required tanks 500 l or 100 or 5000 l tanks are cleaned and filled with filtered sea water and the salinity is adjusted to 25 to 28ppt. The sea water is then chlorinated with 5.25% commercial bleaching powder solution @3000ml/100 L and vigorous aeration is provided for 24hrs and subsequently dechlorinated with 150 ppm of sodium thiosulphate solution and keep it for 24 hrs under darkness with vigorous aeration. To this add nutrient mixture of Ammonium sulphate,Super phosphate and urea in the ratio of 100: 10:10g per tonne of seawater. Add inoculum of micro algae *Nannocloropsis* sp. and *Chlorella* sp. (3-5x10^6 cells/ml) at the rate 10% of the total volume of water and leave with aeration for one week. The culture is then inoculated with rotifer 10-15nos/ml or 30-40 nos/ml when the algae reach suitable density. The rotifer grow very rapidly and can be harvested with 7-8 days when the density reach 200 to 300 nos/ml onwards. The rotifer can be harvested with 50µ filter bags by scooping or by draining through 50µ filter sieve fixed on PVC pipe keeping the down side of it immersed in a tub containing water to avoid probable damage while harvesting. The harvested rotifer can be cleaned and washed thoroughly and used for feeding or can be enriched before being fed to the larvae.

In the rotifer culture techniques, thinning method can also be followed rather than subculture method. For this the density is estimated by counting rotifer in 5ml sample fixed in formalin. After thinning the stock, an equal volume of *Chlorella* sp. or *Nannochloropis* sp. can be added to the rotifer tank for further propagation and the process is repeated. After final harvest, the tanks are drained and cleaned and a new culture is started. Normally the culture are run for about 30 days.

Calculation of Chlorella requirement

It is reported that a rotifers consumes 1,50000 Chlorella cells /day and it is considered as the standard rate of feeding. The quantity of algal water required to feed rotifer can be calculated using the following formula.

$$\text{Vol. of Chlorella water} = \frac{\text{No. of rotifer in millions} \times \text{Standard rate of feeding} \times \text{Density of Chlorella in rotifer tank}}{\text{Density of Chlorella of stock tank}} \times 100$$

After adding the Chlorella water in the rotifer rearing tanks, the density of microalgae was determined by using the equation:

$$\text{Density of Chlorella} = \frac{\text{No of cell density already present} \times \text{Quantity of Chlorella water added} \times \text{Density of stock Chlorella}}{\text{Total volume of water}}$$

Mass production on algae and yeast

Depending on the strategy and the quality of the algal blooms, baker's yeast may be supplemented. The amount of yeast fed on a daily basis is about 1 g.million^{-1} of rotifers, although this figure varies depending on the rotifer type (S,L) and culture conditions.

Batch culture system

The tanks (1200 l capacity) are half filled with algae at a density of 13-14 × 10^6 cells/ml and inoculated with rotifers at a density of 100 individuals/ml. The salinity of the water is 23 ppt and the temperature maintained at 30°C. 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).

Semi-continuous culture

The rotifers are kept in the same tank for five days. During the first two days the culture volume is doubled each day to dilute the rotifer density in half. During the next following days, half the tank volume is harvested and refilled again to decrease the density by half. On the fifth day the tank is completely harvested and the procedure is repeated again for mass culture (i.e. five-day semi-continuous culture system).

Mass culture on yeast

Baker's yeast has a small particle size (5-7 µm) and a high protein content and is an acceptable diet for *Brachionus*. Rotifers fed on baker's yeast are usually larger than those fed on live algae. The first trials to replace the complete natural rotifer diet by baker's yeast were characterized by varying success and the occurrence of sudden collapses of the cultures (Hirayama, 1987). Most probably the reason for these crashes was explained by the poor digestibility of the yeast, which requires the presence of bacteria for digestion. Moreover, the yeast usually needs to be supplemented with essential fatty acids and vitamins to suit the larval requirements of the predator organisms. Commercial boosters, but also home-made emulsions (fish oils emulgated with commercial emulgators or with egg-yolk lecithin), may be added to the yeast or administered directly to the rotifer tank. Better success was obtained with so called w-yeast-fed rotifers (rotifers fed on a yeast preparation produced by adding cuttlefish liver oil at a 15% level to the culture medium of baker's yeast) which ensured a high level of (n-3) essential fatty acids in the rotifers (Watanabe *et al*., 1983). The necessity of adding the component in the food of the rotifer or to the rotifers' culture medium was later confirmed by using microparticulate and emulsified formulations (Watanabe *et al*., 1983; Léger *et al.*, 1989). Apart from fresh baker's yeast, instant baker's yeast, marine yeast (*Candida*) or caked yeast (*Rhodotorula*) may also be used.

Mass culture on formulated diets

The most frequently used formulated diet in rotifer culture is Culture Selco® (CS®) available in dry form. It has been formulated as a complete substitute for live microalgae and at the same time guarantees the incorporation of high levels of EFA and vitamins in the rotifers. The biochemical composition of the artificial diet Culture Selco® consists of 45% proteins, 30% carbohydrates, 15% lipids (33% of which are (n-3) HUFA), and 7% ash. Its physical characteristics are optimal for uptake by rotifers: the particle, having a 7 µm particle size, remaining in suspension in the water column with a relatively strong aeration, and not leaching. The following standard culture procedure has been developed and tested on several rotifer strains in 100 l tanks.

Cylindro-conical tanks of 100 l with dark smooth walls (polyethylene) are set up in shaded conditions. The culture medium consists of diluted seawater of 25 ppt kept at 25°C. No water renewal takes place during the 4-day culture period. Air stones are installed a few cm above the cone bottom of the tank to allow sedimentation and possible flushing of waste particles. Food flocculates are trapped in pieces of cloth which are suspended in the water column or in an air-water-lift trap filled with sponges.To maintain a good water quality with minimal accumulations of wasted food by assuring short retention times of the food particles is achieved by using high starting densities of 200 rotifer/ml^{-1} and the distribution of small amounts of feed at hourly intervals; the latter can easily be automated by pumping the feed suspension from a gently aerated stock kept in a refrigerator at 4°C for up to 30 h. Applying this feeding strategy, an optimized feeding regime is developed in function of the rotifer density and the culture performance. Applying this standard culture strategy a doubling of the population is achieved every two days, reaching a harvest density of 600 rotifers.ml^{-1} after four days, which is better than for the traditional technique using live algae (and baker's yeast).

High density rearing

Although high density rearing of rotifers increases the risk for more stressful rearing conditions, and an increased risk of reduced growth rates due to the start of sexual reproduction, promising results have been obtained in controlled cultures. The technique is the same as the one used for the mass culture on Culture Selco® but after each cycle of 4 days the rotifer density is not readjusted. The feeding scheme is adjusted to 0.25-0.3 g/10^{-6} of rotifers for densities between 500 and 1500 rotifers/ml and to 0.2 g for densities above 1500 rotifers/ml. Rearing rotifers at high stocking densities has a direct repercussion on the egg ratio. Maintaining cultures with this low egg ratio is more risky and thus the system should only be used under well controlled conditions.

Nutritional Enrichment of cultured rotifers

Techniques for (n-3) HUFA enrichment

Algae

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 submerged in these algae are incorporating the essential fatty acids in a few hours time and come to an equilibrium with a DHA/EPA level above 2 for rotifers submerged in *Isochrysis* and below 0.5 for *Tetraselmis*.

Formulated feeds

Rotifers grown on the Culture Selco (CS®) have an excellent HUFA composition: 5.4, 4.4 and 15.6 mg.g^{-1} dry matter of EPA, DHA and (n-3) HUFA respectively which is significantly higher than for cultures grown on algae/baker's yeast but comparable in case the latter cultures are subjected to an additional enrichment treatment (Léger *et al*., 1989). The level of total lipids is approximately 18%. Since the use of CS® allows direct enrichment of the rotifers without the need of a cumbersome bioencapsulation treatment, complementary diets such as Protein Selco® (PS) and DHA Culture Selco® (DHA-CS) are in use in order to incorporate higher levels of protein and DHA. The advantage of direct (or long term) enrichment are multiple; in that. the fatty acid profile obtained is stable and reproducible, the lipid content is comparable to that obtained in wild zooplankton, rotifer losses are lower.

Characteristics of some diets and emulsions containing high DHA levels (in mg.g^{-1} DW).

Diets	EPA	DHA	DHA/EPA	S(n-3)HUFA > 20:3n-3
CS	18.9	15.3	0.8	36.4
DHA-CS	16.9	26.7	1.6	45.4

Oil emulsions

One of the cheapest ways to enrich rotifers is by using oil emulsions. Although home-made emulsions can be prepared with egg lecithin and fish oils (Watanabe *et al.,* 1982). Commercial emulsions are generally more stable and have a selected HUFA composition.

Home-made emulsions

The first emulsions were made from (n-3) HUFA rich fish oils (i.e. cuttlefish oil, pollack liver oil, cod liver oil, menhaden oil, etc.) and emulsified with egg yolk and seawater (Watanabe *et al.,* 1982, 1983). Recently, more purified oils containing specifically high levels of the essential fatty acids 20:5n-3 and 22:6n-3 have been used. Since the stability and storage possibility of these products is relatively low they are usually made on the spot and used immediately.

Commercial emulsions

Several emulsified diets are commercially available and based on well-defined formulations. Very popular are the self-emulsifying concentrates (Selco®, Inve Aquaculture NV, Belgium) which can boost the HUFA content of the rotifers in a few hours. In this technique a rotifer suspension containing 200-300 individuals.ml^{-1} is immersed in a diluted oil-emulsion for 6 h, harvested, rinsed and concentrated before being fed to the predators.

In view of the importance of DHA in marine larviculture, considerable efforts have recently been made to incorporate high levels of DHA and/or high ratios of DHA/EPA in rotifers. To date the best results have been obtained using the self-emulsifying product DHA-Super Selco®. Compared to the results obtained with Super Selco®, the boosting of CS-rotifers with this product under standard enrichment practices results in a threefold increase of DHA and total (n-3) HUFA. Furthermore, the evolution of the concentrations of EFA within enriched rotifers after being administered to the predator tanks revealed that EFA levels remain rather constant for at least 7 h under clear water culture conditions at 20°C; with only a 30% drop in DHA being noted after 12 h.

Techniques for vitamin C enrichment

The vitamin C content of rotifers reflects the dietary ascorbic acid (AA) levels both after culture and enrichment. Rotifers cultured on instant baker's yeast contain 150 mg vitamin C/g^{-1} DW, while for *Chlorella*-fed rotifers contain 2300 mg vitamin C/g^{-1} DW.. Problems of operculum deformities can occur due to reduced vitamin C levels. Enrichment of rotifers with AA is carried out using ascorbyl palmitate (AP) as a source of vitamin C to supplement the boosters. AP is converted by the rotifers into active AA up to 1700 mg.g^{-1} DW after 24 h enrichment using a 5% AP (w/w) emulsion.

Techniques for protein enrichment

Protein Selco® is the only enrichment diet especially designed for protein enrichment in rotifers. The high levels of proteins allow the cultures to continue to grow and to develop during the enrichment period. Normally it is used in the same way an oil emulsion and distributed in the tank at a concentration of 125 mg/l seawater at two time intervals of 3 to 4 h.

Harvesting/concentration and cold storage of rotifers

The harvesting and concentrating of non-enriched rotifers should be performed in submerged filters. Harvesting of enriched rotifers should be carried out with extreme care in order to prevent them sticking together in clumps. Instead of pouring enriched rotifers in a bucket it is therefore recommended to siphon them so as to avoid the interference of the air bubbles. Rotifers that can not be fed immediately need to be stored at a cold temperature (4°C) in order to prevent the reduction of their nutritional quality.

Harvesting/concentration of rotifers

Small-scale harvesting of rotifers is usually performed by siphoning the content of the culture tank into filter bags with a mesh size of 50-70 µm. If this is not performed in submerged filters the rotifers may be damaged and result in

mortality. It is therefore recommended to harvest the rotifers under water; concentrator rinsers are very convenient for this purpose Aeration during the concentration of rotifers will not harm the animals, but should not be too strong so as to avoid clogging of the rotifers., this can be very critical.

Production and use of resting eggs

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. Mass production 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 by limiting the food supply or changing the temperature and/or salinity. Resting eggs will sink and need to be harvested from the bottom. In case a lot of waste is trapped at the bottom it is advised to replace the water by brine so that resting eggs 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 rotifers undergo asexual reproduction. The use of cysts is also highly recommended to prevent contamination. Cysts can easily be treated before hatching in order to ensure start cultures free from bacteria and ciliates. The resting eggs could be disinfected with heavy doses of antibiotics, so that the emerging rotifers are essentially bacteria free. The resting eggs can also resist short exposure to disinfectants such as NaOCl or glutaraldehyde.

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