ISSN 0429-9345

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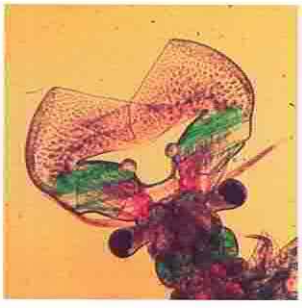

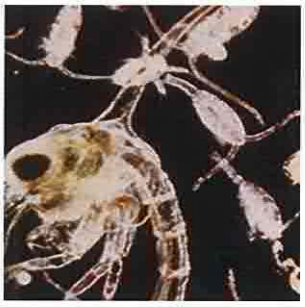

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Rome, 1996

Moreover, if rinsing is performed under water the rotifers will not clog and losses will be limited to less than 1%.

The concentrated rotifers are then distributed in several 15 l bottles filled with 2 l water at a density of 50 individuals.ml^{-1} and a mild tube aeration provided. In order to avoid contamination with ciliates the air should be filtered by a cartridge or activated carbon filters. Fresh algae (*Chlorella* 1.6 x 10^6 cells.ml^{-1}) are supplied daily. Every other day the cultures are cleaned (double-screen filtration) and restocked at densities of 200 rotifers.ml^{-1}. 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.

3.5.3.3. Mass production on algae

Undoubtedly, marine microalgae are the best diet for rotifers and very high yields can be obtained if sufficient algae are available and an appropriate management is followed. Unfortunately in most places it is not possible to cope with the fast filtration capacity of the rotifers which require continuous algal blooms. If the infrastructure and labor is not limiting, a procedure of continuous (daily) harvest and transfer to algal tanks can be considered. In most places, however, pure algae are only given for starting up rotifer cultures or to enrich rotifers (see 3.5.3.1. and 3.6.1.1.).

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 the maintenance of a constant rotifer density by increasing the culture volume (see 3.5.3.4.). 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^{-1}.

3.5.3.4. 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. Since algae have a high nutritional value, an excellent buoyancy and do not pollute the water, they are used as much as possible, not only as a rotifer food, but also as water conditioners and bacteriostatic agents.

In contrast to most European rearing systems, Japanese developed large culture systems of 10 to 200 metric tons. The initial stocking density is relatively high (80-200 rotifers.ml^{-1}) and large amounts of rotifers (2-6 x 10^9) are produced daily with algae (4-40 m^3) supplemented with yeast (1-6 kg).

The mass production on algae and yeast is performed in a batch or semi-continuous culture system. Several alterations to both systems have been developed, and as an example the rearing models used at The Oceanic Institute in Hawaii are described here:

- Batch culture system

  The tanks (1 200 l capacity) are half filled with algae at a density of 13-14 x 10^6 cells.ml^{-1} and inoculated with rotifers at a density of 100 individuals.ml^{-1}. 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

  In this culture technique 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 harvested and the procedure started all over again (i.e. five-day semi-continuous culture system).

The nutritional composition of algae-fed rotifers does not automatically meet the requirements of many predator fish and sometimes implies an extra enrichment step to boost the rotifers with additional nutritional components such as fatty acids, vitamins or proteins (see 3.6.). Also, the addition of vitamins, and in particular vitamin B_{12}, has been reported as being essential for the culture of rotifers (Yu *et al*., 1989).

3.5.3.5. 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*. 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 (see 3.6.1.3.). Better success was obtained with so called ω-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.

3.5.3.6. Mass culture on formulated diets

The most frequently used formulated diet in rotifer culture in Europe is Culture Selco® (CS) available under a 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. However, the diet needs to be suspended in water prior to feeding, which facilitates on one hand the possibilities for automatic feeding but on the other hand requires the use of aeration and cold storage. 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 (Fig. 3.6a.), or in an air-water-lift trap filled with sponges (Fig. 3.6b.).

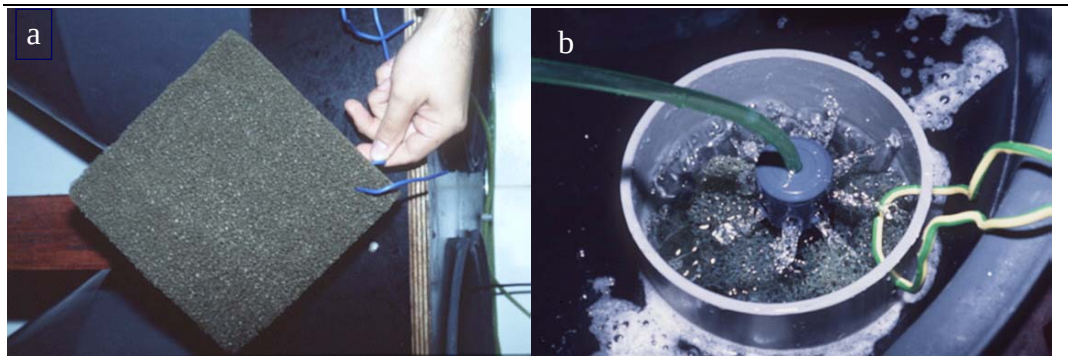

Figure 3.6. Piece of cloth (a) and air-water-lift filled with sponges (b) to trap the floccules in the rotifer tank.

Table 3.2. Feeding regime for optimal rotifer culture in function of the rotifer density using the formulated diet Culture Selco®.

Rotifer density.ml^{-1} (L-strain)	Culture Selco® per 10^6 rotifers.day^{-1} (in g)	Culture Selco® per m^3.day^{-1} (in g)
100 - 150	0.53	53 - 80
150 - 200	0.47	70 - 93
200 - 250	0.40	80 - 100
250 - 300	0.37	92 - 110
300 - 350	0.33	100 - 117
350 - 400	0.30	105 - 120

Table 3.2. (contd.) Feeding regime for optimal rotifer culture in function of the rotifer density using the formulated diet Culture Selco®.

400 - 450	0.27	107 - 120
450 - 500	0.23	105 - 117
> 500	0.25	125
> 1200	0.20	240

Furthermore, all efforts are made to maintain a good water quality with minimal accumulations of wasted food by assuring short retention times of the food particles. This 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 (Fig. 3.7.). Applying this feeding strategy, an optimized feeding regime is developed in function of the rotifer density and the culture performance (Table 3.2.). It should be indicated that this protocol is developed for the L-rotifer strain and should be slightly adapted (less feed) when a S-rotifer strain is used.

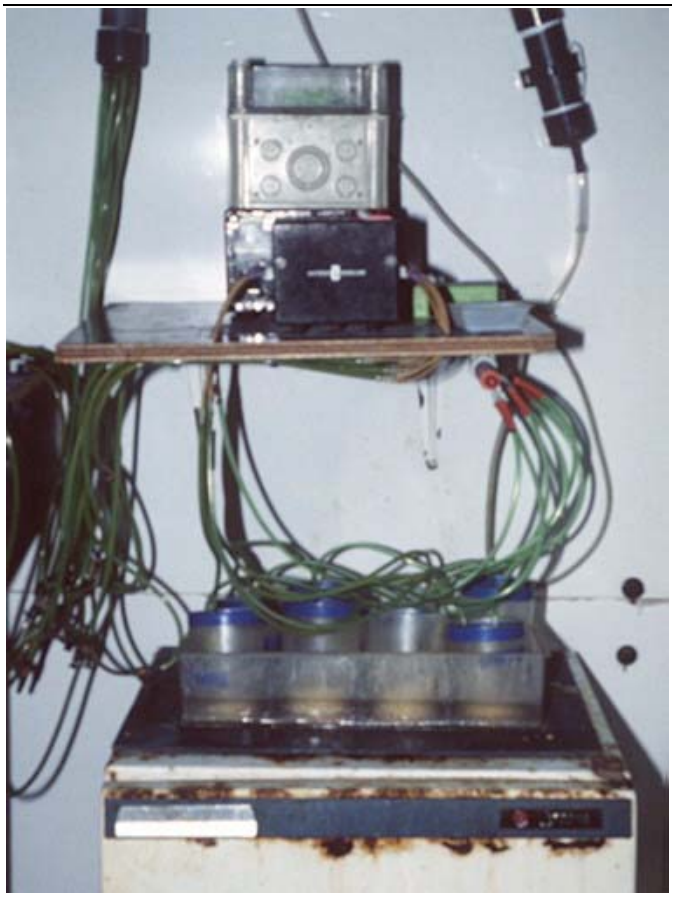

Figure 3.7. Refrigerated feed suspension distributed to the individual rotifer tanks by means of a peristaltic pump.

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 only (Table 3.3.), which is better than for the traditional technique using live algae (and baker's yeast). There is no high variation in production characteristics among the various culture tests and crashes are rarely observed, which most probably is due to the non-introduction of microbial contaminants and the overall good water quality over the culture period. In this respect, it should be emphasized that hygienic precautions should be taken to avoid contacts among different rearing units. All material used during the production (i.e. glass ware) can be disinfected in water baths with NaOCl, HCl or other disinfectants. After each production cycle (4 days) the tanks, airstones and tubing need to be disinfected thoroughly. In order to avoid crashes it is recommended that after approximately one month of culture that the complete system be disinfected and the cultures started again using rotifers from starter cultures.

In commercial hatcheries, peristaltic pumps are not always available. In this case the artificial diet can be fed on a daily basis at a concentration of 400-600 mg/10^{-6} rotifers, and administered in 4 to 6 rations with a minimum quantity of 50 - 100 mg.l^{-1} culture medium. Analogous production outputs are achieved under upscaling conditions in commercial hatcheries (Table 3.3.).

Table 3.3. Growth and reproduction characteristics of rotifers reared on CS under experimental and upscaled conditions.

Experimental	Batch 1	Batch 2	Batch 3
Age of the population		Number of rotifers per ml	
Day 1	200	200	200
Day 2	261 ± 13	327 ± 17	280 ± 12
Day 3	444 ± 65	473 ± 42	497 ± 25
Day 4	581 ± 59	687 ± 44	681 ± 37
Growth rate.day^{-1}	0.267	0.308	0.306
Doubling time	2.60	2.25	2.27

Commercial	Batch 1
Age of the population	Number of rotifers per ml
Day 1	200
Day 2	285
Day 3	505
Day 4	571
Day 5	620

In order to avoid several manual feedings per day, a simple drip-feeding technique can be used as illustrated in Fig. 3.8. A concentrated food suspension is placed in the tank and water is dripped in the food suspension that is gradually diluted and allowed to over-flow into the rotifer tank. Since the overhead tank only contains water the flow rate can be adjusted without danger of clogging. The dimensions of the tank should be made as such that the complete content of the food tank is diluted in 24 h.

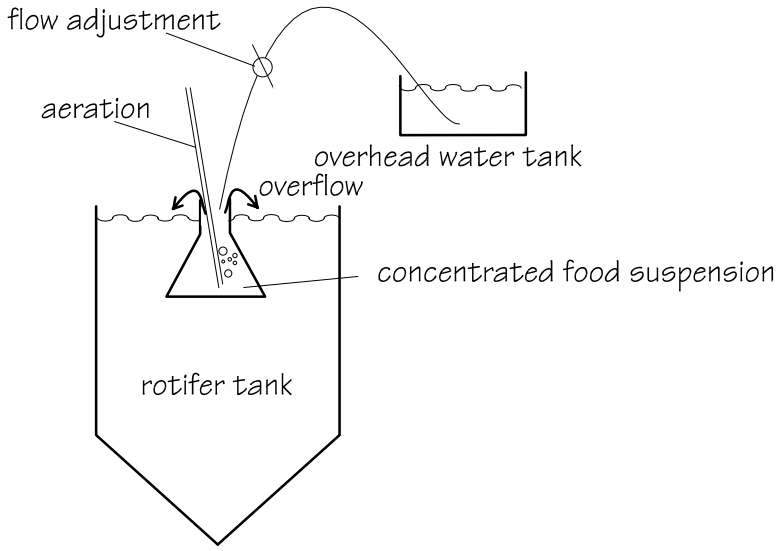

Figure 3.8. Illustration of the drip-feeding technique which can be applied when no sophisticated pumping devices are available.

3.5.3.7. 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^{-1} and to 0.2 g for densities above 1500 rotifers.ml^{-1}. Rearing rotifers at high stocking densities has a direct repercussion on the egg ratio (Fig. 3.9.). This latter is dropping from an average of 30 % at a density of 150 rotifers.ml^{-1} to 10 % at a density of 2000 rotifers.ml^{-1} and less than 5% at densities of 5000 rotifers.ml^{-1}. Maintaining cultures with this low egg ratio is more risky and thus the system should only be used under well controlled conditions.

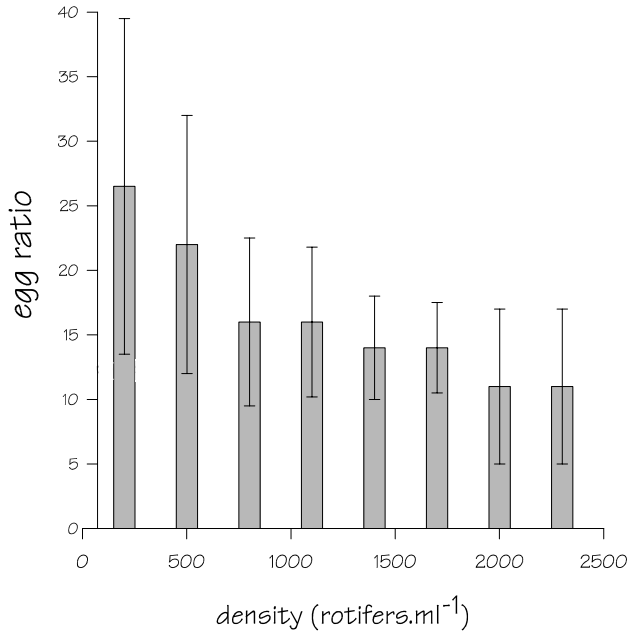

Figure 3.9. Effect of high density rotifer culture on the egg ratio.

High density cultivation of *Brachionus* is also being performed in Japan. In this technique *Nannochloropsis* is being supplemented with concentrated fresh water *Chlorella*, baker's yeast and yeast containing fish oil. Freshwater *Chlorella* is being used for vitamin B_{12} supplementation (± 12 mg.l^{-1} at a cell concentration of 1.5.10^{10} cells.ml^{-1}). In continuous cultures the rotifer population doubles every day. Half the culture is removed daily and replaced by new water. Using this system average densities of 1000 rotifers.ml^{-1} are achieved with peaks of more than 3000 animals.ml^{-1}.

3.5.4. 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 (Fig. 3.10.). 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, specially after enrichment (see Fig. 3.6.4.).

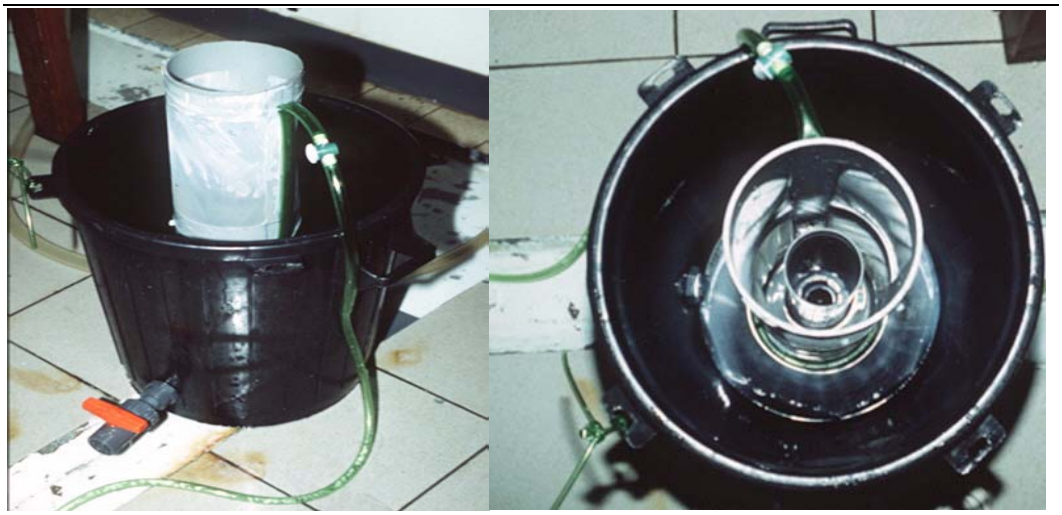

Figure 3.10. Side and upper view of a concentrator rinser containing a filter with a mesh size of 50 µm and equipped with an aeration collar at the bottom.

3.6. Nutritional value of cultured rotifers

3.6.1. Techniques for (n-3) HUFA enrichment

3.6.1.1. 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 (approximately 5.10^6 algae.ml^{-1}) 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* (Fig. 3.11.). However, the culture of microalgae as a sole diet for rotifer feeding is costly due to the labour intensive character of microalgae production. Most of the time the rotifers are boosted in oil emulsions (see 3.6.1.3.) and fed to the predators which are kept in “green water”. This “green water”, consisting of ± 0.2 10^6 algal cells.ml^{-1} (*Tetra-selmis, Nannochloropsis,* or *Isochrysis*) is applied to maintain an appropriate HUFA (but also other components) content in the live prey before they are eventually ingested by the predator (see also 2.5.3.).