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Raising rotifers for use in aquaculture

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Abstract

Of the three Brachionus species used in aquaculture, *Brachionus rubens, B. calyciflorus* and *B. plicatilis*, the latter is most widely used in raising marine fish and shrimp larvae due to its tolerance to the marine environment. In freshwater aquaculture the use of *B. rubens* and *B. calyciflorus* is limited, probably because inert food products are readily available as feed for freshwater larvae.

The rotifer *Brachionus plicatilis* is used in large numbers as the first food organism in intensive cultures of marine fish and shrimp larvae. An adequate supply of these rotifers relies on mass cultures. The reproductive rate of rotifers in these cultures depends on food quality and quantity, salinity, temperature and pH of the medium. Removal of waste products from culture tanks leads to higher and more efficient production of rotifers over extended periods of time. Rotifers have to be enriched with polyunsaturated fatty acids, which are essential for proper development and survival of marine fish and shrimp larvae.

The future use of preserved rotifers and their resting eggs may help to overcome unforeseen failures of live cultures and may lead to more efficient use of these organisms in raising freshwater and marine fish and shrimp larvae.

Introduction

Rotifers are considered to be an excellent food source for newly hatched fish larvae [Howell, 1973], even better than copepods [Theilacker & Kimball, 1984]. In freshwater aquaculture, *Brachionus calyciflorus* and *Brachionus rubens* have been proposed as food for fish larvae [Galkovskaya, 1963; Groeneweg & Schluter, 1981; Schluter & Groeneweg, 1981], and their limited use is probably due to convenient inert food being available to feed freshwater fish larvae.

In the marine environment inert food is unavailable and the euryhaline rotifer, *Brachionus plicatilis*, is extensively used as a food organism for a large variety of finfish and crustacean larvae [for references see Hirata, 1980; Lubzens, 1981; James *et al.*, 1983; Kafuku & Ikenoue, 1983]. This rotifer, once considered a pest in Japanese eel ponds [Hirata, 1980], is now indispensible in raising marine fish larvae because of its size (130–320 μm), relatively slow motility, caloric value, and the possibility of artificially manipulating its nutritional qualities [Watanabe *et al.*, 1983; Theilacker & Kimball, 1984].

Rotifers must be cultured at relatively high densities to provide an adequate supply of food for mariculture hatcheries producing vast numbers of fingerlings [Kafuku & Ikenoue, 1983]. Fish larvae production in Japan is probably limited by the number of rotifers raised in these hatcheries [Hirata, 1980]. It has been estimated that 40000 to 100000 rotifers are required to feed one fish larva from the day it hatches until it can use another source of food [Okauchi *et al.*, 1980; Kafuku & Ikenoue, 1983]. Clearly, the production of millions of fish larvae, which takes place in many countries today, [Gordin, 1980; Hirata, 1980; James *et al.*,

1983; Kafuku & Ikenoue, 1983] involves the production of vast numbers of rotifers. The rotifer is looked upon as a living food capsule which transmits adequate supplies of macro- and micronutrients, vitamins, or even antibiotics to the fish larvae [Gatesoupe, 1982]. Providing rotifers in adequate numbers during crucial periods is the main problem of most marine hatcheries.

Many hatcheries practice mass cultivation of rotifers (Table 1). In initiating mass cultivation of rotifers, the following problems must be considered:

1. A rotifer strain must be selected which will be an appropriate size for the larvae and suitable to the culture conditions.
2. The food quality and quantity must be maintained at an adequate level.
3. Water quality parameters in culture tanks (i.e. salinity, temperature, pH) must be controlled and waste products must be removed.

The main aim is to obtain large numbers of rotifers at predictable qualities and production rates, although the economic cost of achieving these aims must be taken into consideration.

Rotifer culture

1. Rotifer strain

In choosing the rotifer strain, selection of body size is very important. Prey selection by fish larvae is size dependent, and preferred size increases as the larvae grow [Hunter, 1980]. For example, newly hatched larvae of *Sparus aurata* preferred small rotifers (191 μm × 138 μm), while larvae more than 6 days old selected larger (258 μm × 171 μm) rotifers [Helps, 1982]. Small rotifers must also be supplied in greater numbers than larger ones, to meet the metabolic demands of the developing larvae [Hunter, 1980]. In Japan, cyclic changes in rotifer size in outdoor tanks were reported by Fukusho & Iwamoto [1980]. During July, when temperatures were high, S-type rotifers (lorica length 205 – 304 μm) prevailed, while during February, when low temperatures were recorded, L-type rotifers (lorica length 306 – 347 μm) were most common. Fukusho & Iwamoto [1980] attributed these differences in rotifer size to cyclomorphosis, while Snell & Carrillo [1984] suggested that they may have been due to clonal replacement. Data presented recently by Okauchi & Fukusho [1985] support the assumption that L and S type rotifers are genetically different.

Cyclomorphosis was also disclosed in a study made by Serra & Miracle [1983] on *Brachionus plicatilis* collected from Spanish lagoons. Using allometric coefficients, a wider dispersion in size was revealed in field collected rotifers when compared to laboratory cultured ones. The analysis of results suggested that allometric coefficients were influenced by environmental factors, mainly temperature and salinity. As in Japan, the largest rotifer forms appeared in winter and the smallest in summer, with intermediate sizes in autumn and spring. Also in this study, it was not known whether the diversity in size was associated with phenotypic variation of parthenogenic lineages or with clonal displacement.

Alloenzyme variation in six gene systems confirmed that clones showing variation in size were genetically distinguishable [Serra & Miracle, 1985]. Genetic variation was also resolved among 17 strains of *Brachionus plicatilis* originating from different geographical locations [Snell & Winkler, 1984], and cultured under identical laboratory conditions. Isozyme analysis of five rotifer enzymes showed that geographic proximity of strains was not always reflected in electrophoretic patterns. Among five strains collected in Tampa Bay, Florida, U.S.A., three were 93% similar to strains collected in the Soviet Union and Austria. One strain (WP) appeared genetically distinct from all the other tested strains.

Following the work of Yufera [1982] and Snell & Carrillo [1984], which showed clearly that lorica length in *B. plicatilis* is genetically determined, it is now possible to culture a large variety of size specific strains of *B. plicatilis* to suit the various demands of fish larvae.

Only small modifications in size (up to 15%) were possible by changing diet or salinity, but it was possible to encourage growth of large or small rotifers, which differed in their specific growth rate, by manipulation of food concentration [Endo & Mochizuki, 1979]. Also, the effect of temperature

on rotifer size was found to be strain dependent [Snell & Carrillo, 1984].

In choosing the appropriate rotifer strain for mass culture, its reproductive rate at the available environmental conditions should be carefully considered. Rotifer strains used for the above-mentioned genetic studies vary considerably in their reproductive potentials, with r values from 0.55 to 1.15 [Snell & Carrillo, 1984]. Larger stock cultures must be kept of rotifers with low reproductive rates than of those with fast reproduction rates. It is therefore essential that a rotifer strain is chosen which will reproduce rapidly under the available environmental conditions.

2. *Food quality and quantity*

Brachionus plicatilis is a filter feeder and may be fed on a variety of food types, including algae, yeast, bacteria or inert food such as microcapsules. In filter feeding rotifers, the size of ingested particles is thought to be closely related to that of the mouth opening [Pourriot, 1977], and a positive correlation between body size and the maximum size of particles ingested has been shown [Hino & Hirano, 1980]. Similarly, a low correlation between body size and the minimum size of these ingested particles has also been reported [Hino & Hirano, 1984]. These results agree with those obtained on several other zooplankton species [Bogdan & Gilbert, 1984].

a. Algae. Chotiyaputta & Hirayama [1978] showed that, of two algal species, *B. plicatilis* preferred *Chlamydomonas* sp. over *Olisthodiscus* sp., and algae in their exponential phase of growth over senescent ones. While some studies have suggested that algal diet has little effect on reproductive rates [Ito, 1960; Theilacker & McMaster, 1971; Scott & Baynes, 1978], others [Hirayama *et al.*, 1979; Okauchi & Fukusho, 1984] reported that different species of algae resulted in substantially different reproductive rates. Of the eight species examined by Hirayama *et al.* (1979), two were evaluated as excellent food (*Synechococcus* sp. and *Chlorella* sp.), four were average (*Chlamydomonas* sp., *Monochrysis lutheri, Dunaliella tertiolecta* and *Cyclotella cryptica*), and two were found inadequate (*Eutreptiella* sp. and *Nitzschia closterium*). *Tetraselmis tetrathele* was recently reported to yield higher reproductive rates than *Chlorella* sp. [Okauchi & Fukusho, 1984]. These authors also showed a delay in reproduction following a transfer of rotifers fed on *Tetraselmis tetrathele* to *Chlorella*. This delay was not observed if rotifers were first fed on *Chlorella* and later on *T. tetrathele*.

Mixed diets containing two different algae (*Chlorella* and the blue-green *Schizothrix*) were found to increase the reproductive rate of *B. plicatilis* substantially (by an average of 2.7 times) compared to those obtained by feeding rotifers on either alga alone [Snell *et al.*, 1983a]. The blue-green alga was found to contain a heat labile substance whose effect was mediated through the medium; ingestion was not required.

The reproductive rate and survival of *B. plicatilis* depends on the concentration of food in the culture medium [Hirayama *et al.*, 1973, 1979; Endo & Mochizuki, 1979; Lubzens, 1981; Snell *et al.*, 1983a; Yamasaki *et al.*, 1984]. Although maximal conversion rates in rotifers fed on *Chlorella saccharophila* var. *saccharophila* were observed in cultures containing 10^6 cells ml^{-1}, maximal population growth was obtained in cultures containing at least 3×10^6 cells ml^{-1} [Yamasaki *et al.*, 1984]. Rotifers cultured on *Chlorella stigmatophora* exhibited maximal reproductive rates in algal concentrations from 2 to 8×10^6 cells ml^{-1}, depending on the salinity of the culture [Lubzens, 1981].

The amount of food that should be provided to rotifer cultures depends on rotifer density, salinity and algal species. Hirayama & Ogawa [1972] showed that filtration rates of *B. plicatilis* change with temperature, chlorinity and food concentration. Satiated rotifers maintained under optimal conditions (algal food concentrations 2.13×10^6 cells ml^{-1}, temperature 22 °C, chlorinity 7.8‰, pH 8.0) ingested 200 cells min^{-1} $individual^{-1}$. Although rotifer density was found to have little effect on ingestion rate at this food concentration, almost all the algae are consumed in less than one hour in cultures maintained at a density of 200 rotifers ml^{-1}. Food must either be supplied initial-

ly at much higher concentrations or several times a day, preferably by continuous feeding, in order to support dense rotifer populations.

In evaluating the conditions prevailing in rotifer mass cultures, Yufera & Pascual [1985] showed that filtration and ingestion rates were dependent on algal concentration and varied among four algal species. With *Nannochloris oculata, Nannochloris maculata* and *Nannochloropsis oculata*, filtration rates decreased with increasing cell concentrations, reaching a constant level at concentrations exceeding 60–80 $\mu g\ ml^{-1}$. Bimodal ingestion rates were observed by increasing food concentrations. These rates increased with food density, reaching a plateau at 15–30 $\mu g\ ml^{-1}$ and increased again after the food concentration exceeded 50–100 $\mu g\ ml^{-1}$. Rotifers fed on *Nannochloropsis gaditana* showed a decrease in filtration and ingestion rates at food concentrations above 40 $\mu g\ ml^{-1}$. Food consumption and utilization were also found to depend on the rotifer strain. At moderate algal concentrations, the small rotifer strain (Bs) was found to consume about 0.16 ng $ind^{-1}\ min^{-1}$, while the larger size strain (S-1) consumed 0.30 ng $ind^{-1}\ min^{-1}$. Bs strain rotifers ingested each day about three times their own dry weight, while S-1 rotifers consumed only one and a half times their own dry weight and yielded more biomass for the same food quantities [Yufera *et al.*, 1983].

To meet food requirements of rotifers, the volumes of algal cultures required for feeding the rotifers are 5–10 times greater than the volumes of rotifer cultures, depending on the algal concentrations obtained [Hirata, 1980]. Using algae alone as food for rotifers would have made mass production of rotifers prohibitively expensive. Furthermore, rotifer cultures would have to depend on another live organism with all its associated culture problems.

b. Yeast. A major breakthrough in rotifer culture was made by Hirata & Mori [1967], who found that rotifers could be raised on baker's yeast (*Saccharomyces cerevisiae*) which is inexpensive and commercially available in most countries.

Rotifers cultured exclusively on baker's yeast were found to have lower reproductive rates than those cultured on algae and could not be maintained for long periods [Hirata, 1979, 1980; Yufera & Pascual, 1980]. The yeast used by Japanese workers lacked vitamin B12 and cystine which are essential for rotifer growth [Scott, 1981; Hirayama & Funamoto, 1983]. Providing yeast and *Chlorella*, in a ratio of 1:1, resulted in rotifer reproductive rates similar to those obtained by feeding *Chlorella* alone. This showed that yeast could partially replace the more expensive *Chlorella* in rotifer cultures. Following this work, several species of marine yeast were isolated and found to be an adequate food for rotifers [Furukawa & Hidaka, 1973; Kawano *et al.*, 1976]. Matsuda *et al.*, [1980] isolated a marine yeast which, when fed to rotifers, resulted in population growth rates two to three times higher than those achieved with *Chlorella*. However, the use of these marine yeasts is rather limited since they require special culture techniques, making them relatively expensive.

Using baker's yeast as food for rotifers, which are later fed to marine larvae, requires special attention. Rotifers fed exclusively on baker's yeast have insufficient amounts of highly unsaturated fatty acids, mainly eicosapentaenoic acid (20:5 ω3) and docosahexaenoic acid (22:6 ω3), which are essential for survival and development of marine larvae [(see review by Watanabe *et al.*, 1983]. Although these acids can be synthesized in yeast fed rotifers either *de novo* or through elongation and desaturation of precursor molecules, this process is rather slow and rotifers do not accumulate sufficient amounts [Lubzens *et al.*, 1984a, 1985a]. Enrichment of rotifers with these essential acids can take place by feeding them for 8–24 hours on algae, ω-yeast or a suspension of egg yolk and fish oil containing these acids [Watanabe *et al.*, 1983].

c. Bacteria. The bacteria present in the culture tanks of *Chlorella* fed rotifers were also consumed by the animals and this improved their reproduction [Ushiro *et al.*, 1980; Yamasaki & Hirata, 1984]. Yasuda & Taga [1980] found that at least two strains of marine bacteria (*Pseudomonas* P-1 and P-7) supported rotifer growth. Recently, the possible use of inexpensive supplies of bacteria produced in wastewater treatment plants or from alcohol fer-

mentation slops has been examined with promising results [Hino *et al.*, 1981; Fukuhara *et al.*, 1982]. The nutritional quality of rotifers raised on these bacteria awaits analysis.

d. Inert food. The search for inexpensive convenient and reliable food sources for rotifer cultures led several workers to examine powdered algae or synthetic food sources. Spray dried *Chlorella* fed to rotifers was less effective than live algae, but better than caked yeast [Hirayama & Nakamura, 1976]. Similarly, the use of freeze dried *Spirulina* or *Platymonas suecica* resulted in low density rotifer cultures [Person-le Ruyet, 1975]. Gatesoupe & Robin [1981] showed that successful use of commercially available *Spirulina, Chlorella* and methanol grown yeast depended on the salinity of the rotifer culture and the amount of food provided. Low salinity (18‰), and adjustment of food level to rate of increase in rotifer number, resulted in a daily production of 35–55 rotifers ml^{-1} day^{-1}, with poor food conversion ratios. Higher food conversion ratios were achieved in formulated diets containing vitamins and other additives in addition to yeast and algae.

Although microencapsulated diets did not support rotifer cultures over extended periods of time [Teshima *et al.*, 1981], this feeding method may permit the enrichment of rotifers with micronutrients required by fish larvae.

3. Salinity

Rotifer reproductive rates are strongly influenced by the salinity of the medium [Ito, 1960; Ruttner-Kolisko, 1972; Pascual & Yufera, 1983; Lubzens *et al.*, 1985b]. *Brachionus plicatilis* tolerates a wide range of salinities from 1–97 g l^{-1} [Walker, 1981]; its reproductive rate is most optimal at 4–35‰, depending on strain and culture conditions. Filtration rates are reduced at high salinity [Hirayama & Ogawa, 1972], and this may account, at least in part, for the lower reproductive rates. Thus, the amount of food provided to rotifers has to be adjusted according to the salinity in which they are cultured.

In choosing the salinity for culturing rotifers, the salinity of the predator must be taken into account. Transferring rotifers from low to high salinity or vice versa may result in death of rotifers. Transfer should be gradual to allow rotifers to adapt to new salinity [Gatesoupe & Luquet, 1981; Pascual & Yufera, 1983]. By culturing rotifers at a salinity of 20‰, relatively high reproductive rates are obtained. Rotifers from this culture may then be transferred to fish larvae maintained either in fresh water [Lubzens *et al.*, 1984b] or in 40‰ salinity sea water. In the first case rotifers have to be transferred for about one day to 10‰ salinity sea water, while in the second case they must first be transferred to 30‰ salinity sea water.

Raising rotifers at salinities lower than 35‰ results in mixis in certain strains [Lubzens *et al.*, 1985b]. This results in lower yields and the possible collapse of the culture. However, this can be avoided by the isolation of clones which do not respond to salinity changes in this way [Lubzens *et al.*, 1984a].

4. Temperature

Optimal culture temperature for rotifers is dependent on the strain being cultured [Snell & Carrillo, 1984]. Hirayama & Kusano [1972] recommended culturing rotifers at the optimum temperature for reproduction, 25 °C. Using a thermophilic small-sized rotifer strain, Pascual & Yufera [1983] observed maximal densities and greatest yield in rotifers raised at 30 to 35 °C.

Temperature also affects the biochemical composition of rotifers [Scott & Baynes, 1978]. At 18 °C, rotifers consumed their food slowly and maintained relatively high levels of lipids and carbohydrates for a long period. However, at 28 °C, food was consumed rapidly, and the biochemical composition of the rotifers changed quickly when the food was gone.

5. Water quality

Accumulation of excretory products produced by

rotifers (feces, urea, ammonia and phosphates) and excess uneaten food contribute to the deterioration in water quality in tanks of rotifers cultured for extended periods.

Hirata & Nagata [1982] showed that *B. plicatilis* fed on *Chlorella* excretes ammonia, urea and phosphates ($1.41 \pm 0.87 \times 10^{-4}$ µg NH_4-N, 1.17 ± 1.31 µg urea−N and $0.27 \pm 0.29 \times 10^{-4}$ µg PO_4-P, respectively, hour^{-1} individual^{-1}). While these excretory rates increased with the amount of food available and the number of eggs produced, they decreased rapidly in rotifer cultures containing more than 100 individuals ml^{-1}. These results were obtained in 1 litre beakers where temperature (20 ± 0.2 °C), salinity (20‰) and illumination (16:8 photoperiod) were kept constant.

Little information is available on the toxicity of the nitrogeneous compounds (i.e. ammonia, urea, nitrite and nitrate) that may accumulate in culture tanks. Lincoln *et al.*, [1983] reported that free ammonia levels of 17 mg l^{-1} were found to be toxic to rotifers. Furukawa & Hidaka [1973] observed relatively high levels of ammonia (40−50 mg l^{-1}) in their culture tanks. Slow accumulation of NO_3-N, NO_2-N and NH_4-N levels in rotifer tanks was observed in cultures maintained for 70 days, reaching levels of 170, 716 and 2310 µg l^{-1}, respectively [Hirata *et al.*, 1983]. Preliminary observations have shown that while ammonia levels of 9−12 mg l^{-1} NH_4-N (at pH 7.6−7.8) did not affect rotifer reproduction, nitrite (NO_2-N) levels of 90−140 µg l^{-1} resulted in collapse of some cultures [Lubzens, Holtkamp & Gordin, unpublished]. Groeneweg & Schluter [1981] showed that growth of *B. rubens* was not affected by high levels of nitrites (10−20 mg l^{-1}), probably as a result of adaptation. The interrelationship among the nitrogeneous excretory products in culture tanks requires further detailed attention.

6. *Rotifer culture methods*

A large array of culture methods, some adapted to specific local requirements, is found in the literature (Table 1). These can be sorted into three basic methods: (1) batch cultures, (2) semicontinuous cultures, and (3) 'feedback' culture systems.

a. Batch cultures. In using this method, rotifers are introduced at low density into dense algal cultures ('green water') and harvested once all the algae are consumed. The duration of these cultures and the rotifer densities obtained depends on the initial concentration of algae. Using this method, rotifers are either cultured in large outdoor tanks where volumes may be 10000 litres and rotifer densities are rather low [Kawano *et al.*, 1978; Rothbard, 1979; Watanabe, 1982; James *et al.*, 1983], or in indoor plastic bags (50 litres) where rotifer densities are much higher [Trotta, 1980, 1981]. An early version of this method called for daily harvest of 1.5 ton tanks, where 10−30% of the rotifers were removed to be used as food, and the remaining rotifers served to inoculate new algal culture tanks [Hirata, 1979, 1980].

b. Semicontinuous cultures. This method is an extension of the previous one. Rotifers are inoculated at low density into dense algal cultures, but once algae are consumed, rotifers are either fed on yeast or on an external supply of algae until densities exceed 100 rotifers ml^{-1}. Every 1−3 days, a certain volume of the culture is removed and replaced by sea water containing algae or pure sea water. In the latter case, rotifers are fed on yeast. About 10−30% of the water is removed from the culture tanks, depending on the rotifer reproductive rate. Using this method, rotifers are maintained at relatively high densities. After several harvests, rotifer reproductive rates decrease, probably as a result of accumulated excretory products and uneaten food. At that time, all the rotifers are harvested and used as food or to inoculate new cultures. Attaching a biological filter to these culture tanks extends the harvest period for 2−3 months [Fujita, 1979; Witt *et al.*, 1981; Watanabe, 1982; James *et al.*, 1983]. The culture volumes used in this method vary greatly from a few hundred litres to 200000 litre cultures [Fujita, 1979; Hirata, 1980; Watanabe, 1982]. Relatively higher rotifer densities are obtained in smaller volume cultures.

Table 1. Daily production of *Brachionus plicatilis* in mass culture systems at various volumes and salinities.

Reference	Production rotifers l^{-1} day^{-1}	Maximum rotifer density ml^{-1}	Salinity ‰	Volume litre	Food organism
Theilacker & McMaster 1971	5387	N.S.*	33	464	*Dunaliella* sp.
Furukawa & Hidaka 1973	16300	1000	N.S.	1000	Marine yeast
Cruz & Millares 1974	24719	360	diluted sea water	14	*Nannochloris* sp.
Devauchelle & Girin 1974	24250	150	N.S.	250	*Tetraselmis* sp.
Alessio 1974	40000	55	N.S.	600	A mix of: *Dunalliella salina* *Platymonas suecica* *Chroomonas fragarioides* *Phaeodactylum tricornutum* *Chlorella ovalis* *Glenodinium* sp.
Amat 1975	42571	300	33	60	*Tetraselmis* sp.
Millares & Gonzalez 1975	31000	420	35	20 – 100	*Platymonas* sp.
Person-le Ruyet 1975	55000	340	N.S.	100	*Tetraselmis suecica*
Hirayama & Nakamura 1976	28500	434	22	12	*Chlorella* sp.
Kawano *et al.* 1978	59633	1100	N.S.	1090	*Chlorella* dried bread yeast
	7143	90	N.S.	10000 or 20000	*Chlorella* marine yeast
Hirata 1979	57000	530	N.S.	550	*Chlorella* sp.
Fontaine & Revera 1980	34000	583	N.S.	500	Turulose yeast
Hirata 1980	80000	514	N.S.	500	*Chlorella*, yeast
Trotta 1980	18860 – 34780	± 300	N.S.	30	*Chlorella* sp.
Yufera & Pascual 1980	63733	540	36	70	*Nannochloris* sp. *Tetraselmis suecica*
Gatesoupe & Luquet 1981	122000	790	33	60	*Tetraselmis suecica*
Witt *et al.* 1981	47143	219	15	1400	*Nannochloris*
	22955	100		2200	*Nannochloris*
Reguera *et al.* 1982	51750	786	36	750	Bread yeast
Ortega *et al.* 1983	77000	230	N.S.	6000	*Tetraselmis* sp., yeast
Trotta 1983	57000	428	36	50	*Tetraselmis suecica*
Meragelman, Lubzens & Minkoff (unpublished)	70000	500	40	200	Baker's yeast

* N.S. – Not Specified.

c. 'Feedback' culture systems. Culturing rotifers at high densities results in the accumulation of high levels of particulate matter and excretory products. Hirata [1974] described a simple method for removal of particulate matter, by passing the rotifer culture through a gravel bed. A more efficient method was achieved by using a stream unit with partitions through which the culture was circulated 20 times each day [Hirata, 1979]. Feces and accumulated particulate matter were removed every few days from the stream unit into a decomposer tank, where bacterial decomposition took place. The decomposed matter was used as fertilizer for algal cultures, which were then used to feed the rotifer cultures.

Rotifers in this system are cultured in 500 litre tanks attached to a 150 litre stream unit. Rotifers are inoculated at low density, and harvesting begins

once rotifer density has reached 100–150 rotifers ml^{-1} and may continue to increase to 500 ml^{-1}. At this density a daily harvest of 80.5×10^6 rotifers was recorded for at least 20 days [Hirata, 1979]. By recirculation of waste products accumulated in rotifer tanks, the apparent conversion rate, calculated as the amount of food supplied to the weight of harvested rotifers, was 89.2% in wet weight and 29.0% in caloric value.

By using these culture methods, production rates ranging from 5387 to 122800 rotifers l^{-1} day^{-1} were recorded from various locations (Table 1). In general, higher rotifer densities in cultures lead to higher yields per volume. Thus, relatively low yields were obtained in large outdoor tanks (10–20 tons), where rotifer concentration was relatively low [Kawano *et al.*, 1978].

7. Use of preserved rotifers

Unpredictable events occasionally result in failure of rotifer cultures, leaving the fish culturist without an available food source. In extreme cases this may lead to a loss of one year's production. The use of preserved rotifers may help to overcome the consequences of such disastrous events. Four methods for preserving rotifers can be considered: (1) freezing, (2) cryopreservation, (3) refrigeration, and (4) production of resting eggs.

Frozen rotifers have been successfully used as food for shrimp larvae [Yamasaki & Hirata, 1982], although their nutritional quality probably deteriorates upon thawing as a result of leaching [Grabner *et al.*, 1981]. No record is available on the successful use of frozen rotifers as food for marine fish larvae, although this method is routinely used in the fish hatchery at the Kagoshima Mariculture Center, Tarumizu City, Japan (Fujita, personal communication).

Attempts to preserve rotifer using cryopreservation have met with limited success [King *et al.*, 1983; Euteneuer *et al.*, 1984]. This method, which is relatively expensive, may prove valuable in the preservation of important isolated clones over extended periods of time.

Live rotifers were found to survive refrigeration (4 °C) for at least 22 days, at densities of 2000 individuals ml^{-1}. During this period rotifers were fed *Isochrysis* sp. and the water was changed every four days [Lubzens, Kolodny & Perry, unpubl. obs.]. This method would permit short term storage of surplus rotifers or the advance preparation of rotifers in anticipation of high demands in the hatchery.

Long-term preservation of live rotifers can be achieved by using their resting eggs, which are available from natural deposits [Snell *et al.*, 1983b] or from laboratory cultures [Lubzens, 1981]; they may be hatched under controlled conditions [Blanchot & Pourriot, 1982; Minkoff *et al.*, 1983]. Today the production efficiency of these eggs is rather low [Lubzens, 1981], making them unattractive for commercial production and widespread use. Research into factors controlling resting egg production will permit their use, if not directly as food for larvae, at least as a reserve stock for initiating large scale rotifer cultures.

Conclusions

The extensive use of *Brachionus plicatilis* as food for marine fish and shrimp larvae led to the development of several methods for its mass cultivation, the most efficient and reliable one being the 'feedback' system of Hirata [1979]. In the search for conditions leading to high production of rotifers, the effects of environmental factors such as food quality and quantity, temperature and salinity on the rotifer's physiology have been studied extensively. In order to overcome unforeseen failures of live cultures, the use of cryopreservation, refrigeration and resting egg production has been examined. Economic considerations will dictate the future use of rotifers as food for marine larvae or their replacement by inert particulate foods, as used today in freshwater fish cultures.

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