Live food production in Japan: recent progress and future aspects

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Abstract

Techniques to culture rotifers at high-density (2000 to 20,000 ind./ml) have been developed by utilizing condensed phytoplankton products. Many public hatcheries in Japan have introduced automated systems for culturing and harvesting rotifers. Research has been conducted on the diagnosis of rotifer culture status and chemical treatment for reducing stress on rotifers. Preservation of the resting eggs of rotifers for an extended period or at low temperature (4–8°C) for a limited time has become possible. Thus, appropriately sized rotifers can be provided to fish larvae, according to fish species and growth stage. Techniques to identify the genetic status of rotifer strains have yet to be developed. Practices for culturing copepods in Japan have also been summarized. © 2001 Published by Elsevier Science B.V.

Keywords: Live food; Rotifer; Copepod; Culture

1. Introduction

The rotifer Brachionus has been used worldwide as a live food for the initial stage of larval rearing of marine fishes. These Brachionus species include Brachionus plicatilis O.F. Müller (130–340 μm in lorica length) and B. rotundiformis Tschugunoff (100–210 μm) (Fig. 1). The former species inhabits colder waters (18–25°C), and the latter in...
waters of moderate temperatures (28–35 °C). The ultra-minute *Brachionus* strains (90–150 μm) are common in subtropical and tropical waters. These rotifers are probably included in *B. rotundiformis*; based on assays of male mate recognition (Hagiwara et al., 1995c), mate recognition pheromone (MRP) binding and anti-MRP binding (Kotani et al., 1997). Mate recognition pheromone is localized on the female surface corona and cloaca, and is a 29-kDa glycoprotein (Snell and Hawkinson, 1983; Snell et al., 1995).

Both species are common in brackish waters, but show strong tolerance to high salinity. In Japan, these rotifers were previously known as harmful organisms, which caused “Mizukawari” damage (rapid change of water color) in eel culture ponds. Ito (1960) examined the salinity tolerance of rotifers, and found that these species are very resistant to salinity changes. In other research, Ito (1963) successfully reared larvae of ayu, *Plecoglossus altivelis* Temminck et Schlegel, by feeding freshwater rotifers *B. calyciflorus* Pallas. This encouraged aquaculturists to acclimate *B. plicatilis* and *B. rotundiformis* from brackish water to seawater, and feed them to marine fish larvae. In 1964 and 1965, 50,000 tiger puffer, *Takifugu rubripes* (Temminck et Schlegel), larvae were produced by several public hatcheries in Japan (Japan Sea Farming Association, Yamaguchi Prefectural Fisheries Institute). This was followed by the success, in 1966, of raising more than 10,000 red seabream *Pagrus major* (Temminck et Schlegel), larvae at various Japanese public hatcheries.

Thereafter, the major developments and improvements of rotifer mass culture practices include: (1) introduction of *Nannochloropsis oculata* Droop and baker’s yeast as rotifer diets (Hirata and Mori, 1967); (2) use of agricultural grade fertilizer for the *N. oculata* medium; and (3) establishment of an enrichment culture before feeding fish larvae (Watanabe et al., 1978). For a detailed historical review, see Hirata (1980). Other recent improvements have been the development of condensed phytoplankton products,
such as freshwater *Chlorella*, for rotifer food (Maruyama and Hirayama, 1993), and the development of high density rotifer mass culture technology using condensed phytoplankton products (Yoshimura et al., 1996, 1997). Despite these advances, occasional instability or collapse of rotifer cultures still remains problematic.

In order to provide cultured rotifers as cost-effective feed for fish larvae, it is important to produce sufficient biomass of rotifers, reduce the cost for rotifer production, enhance the stability of rotifer cultures, and provide appropriate size of rotifers to the larvae.

In this paper, we first give a description of *Chlorella*-based rotifer diets. Next, we review the progress in high-density rotifer cultures. Third, we summarize our recent works on diagnosis and regulation of rotifer cultures, as well as on mass preservation of rotifers. Fourth, we describe the significance of the use of appropriately sized rotifers for target fish species. Fifth, we give several examples of the use of copepods as live food in Japan. In conclusion, we indicate several other topics with less research progress. These include elimination of contaminating micro-organisms and establishment of an identification method for rotifer strains.

2. Chlorella-based rotifer diet

The phytoplankton industry developed a condensed phytoplankton product for feeding mass-cultured rotifers, which eliminated the need for space in the hatchery for growing microalgae. Among those products, condensed freshwater *Chlorella* was the most popular in Japan. It is available in an 18-l volume with the density of 20 billion cells/ml at a cost of 15 000 Japanese yen (US$140–150). During the manufacturing process, *Chlorella* cells receive enrichment with vitamin B₁₂, which is essential for rotifers (Scott, 1981; Hirayama and Funamoto, 1983).

A characteristic of *Chlorella* is that it can be grown with an organic carbon source, such as glucose or acetic acid, which significantly reduces the costs for lighting (see Table 1 of Hirayama et al., 1989 for an example of a *Chlorella* culture medium). Another feature of *Chlorella* is its short doubling time, as compared with *N. oculata*. The doubling time of *N. oculata* is 16 to 20 h and it needs light for growth, but the doubling time for *Chlorella* can be shortened to 3 h by selecting a suitable *Chlorella* strain. The doubling time does not change even when the *Chlorella* are cultured with an organic carbon source without light. Recently, the Chlorella Industry Company of Japan succeeded in incorporating *n* − 3 HUFA in *Chlorella* cells (Hayashi et al., in press). The product can be used for mass culture of rotifers, and the harvested rotifers can be fed directly to fish larvae, which eliminates the labor for enrichment culture. In addition, fortified DHA and vitamin C (added as an antioxidant) serves to enhance the rotifer population growth (Satuito and Hirayama, 1991; Hayashi et al., in press).

By using a microalgae product such as this, rotifer culture technique has also been improved. For example, high-density mass-culture systems using the batch culture method or continuous culture method have been developed. The product is also useful for mass production of rotifer resting eggs.
3. High-density culture of rotifers

The development of a high-density rotifer culture system resulted in a decrease in the space needed at the hatchery facility for live food production. By conventional methods, a 55-m³ mass-culture tank was needed to produce 10 billion *B. rotundiformis*, a common level of rotifer production in Japanese public hatcheries. To feed these rotifers, *N. oculata* was cultured in a 200-m³ tank. By feeding the condensed *Chlorella* product, the density of *B. rotundiformis* can be increased to between 5000 and 8000 individuals/ml, using only one 4-m³ tank to produce 10 billion rotifers. If equipment to supply oxygen and control the pH is available, it is possible to raise the rotifer density up to 20 000 ind./ml, and to decrease the culture volume to 1 m³ (Yoshimura et al., 1996). At this density, removal of foam and solid substances is also important (Yoshimura et al., 1997). The Fukuoka Mariculture operates two 1-m³ rotifer cultures with a 2-day batch culture, producing about $1 \times 10^{10}$ rotifers daily. Yoshimura’s group also reported a simple method for rotifer counting: sample rotifers from the mass culture tank, centrifuge them at 5000 rpm, measure the volume, and estimate the number of rotifers (Yoshimura et al., 1997).

Rotifers can also be produced at high densities in a continuous culture system (Fu et al., 1997), a system that has been introduced in several public hatcheries in Japan. With this system, Fu et al. (1997) maintained *B. rotundiformis* densities ranging from 3000 to 6000 ind./ml, and *B. plicatilis* densities from 1100 to 2200 ind./ml. The continuous culture system is more advantageous for maintaining the environment of rotifer culture water (James and Razeq, 1986, 1989; Walz, 1983), and it seems to have great potential to spread as a future style of rotifer mass-culture practice. Detailed comparisons in culture stability, labor input, cost of the culture unit and its maintenance, and rotifer productivity among different styles of rotifer culture methods should be conducted to provide aquaculturists with data that will be useful to develop the most desirable culture method for each hatchery.

4. Diagnosis of rotifer cultures

After the work of Ito (1960), studies were conducted to obtain suitable temperatures (Hirayama and Kusano, 1972), diet (Chotiyaputta and Hirayama, 1978) and salinity (Hagiwara et al., 1988) for culturing rotifers. Even under optimal levels of temperature, salinity and diet, however, culture collapse can occur due to the increase of unionized ammonia (Yu and Hirayama, 1986), and the presence of certain bacteria (Yu et al., 1990) and protozoans (Maeda and Hino, 1991; Hagiwara et al., 1995b; Jung et al., 1997; Cheng et al., 1997). The viscosity of rotifer culture water increases with the accumulation of dissolved organic substances, and the higher viscosity causes a decline in the rotifer population (Hagiwara et al., 1998). Hence, it is important to assess the daily status of rotifer mass cultures, which contain many environmental stressors.

Rotifer egg ratio, ingestion rate and swimming speed are good indices of rotifer condition (Snell et al., 1987; Juchelka and Snell, 1994). As with more highly evolved animals, the impairment of physiological functions in rotifers may be one of the first
effects of environmental stress. This impairment could result in changes in feeding and swimming behavior, and eventually can lead to reduced survival and reproduction (Juchelka and Snell, 1994). Thus, the measurements of feeding and swimming behavior could enable aquaculturists to conduct more rapid diagnosis of rotifer cultures. In toxicity testing, Snell and Janssen (1998) indicated that the measurement of rotifer ingestion rate is more appropriate for estimating the population growth potential than swimming speed. Recent studies have been conducted for establishing a technique to measure the physiological status of rotifers. An example is estimating the tolerance to environmental stressors. Koiso and Hino (1999) reported that the percent swimming rotifers among egg-bearing amictic females after 3-h exposure to 70 ppt seawater correlated with the daily population growth rate. For the direct measurement of physiological functions of zooplanktons, in vivo enzyme analysis in freshwater cladocerans and rotifers has been developed for a chronic toxicity test in ecotoxicology (reviewed by Snell and Janssen, 1995). We apply this technique for evaluating the culture status of marine rotifers.

Fig. 2 shows *B. plicatilis*, viewed under 100× magnification through a compound microscope (Fig. 2A), and through a fluorescent microscope (Fig. 2B,C). Rotifers were exposed to a substrate of enzymes, such as esterase and glucosidase. These substrates are non-fluorescent, but are cleaved by endogenous enzymes to yield fluorescent products. Since the enzyme activity of the rotifer changes with its growth, measurement of enzyme activity of mass-cultured rotifers would yield large variations in the estimated values. Instead, in our technique, culture water is obtained by sieving rotifers with a 45-μm mesh, and newly hatched rotifers from resting eggs are inoculated into the test water. By using neonates from rotifer resting eggs, we can provide physiologically identical animals to test the stress of experimental water. de Araujo et al. (2000) described in detail the procedure for enzyme activity measurement. In order to obtain the basic information about this method, we first measured the enzyme activity change of rotifers under stressed conditions. The effects of environmental stressors on rotifer
reproduction and enzyme activity are shown in de Araujo et al. (2000). In this study, *B. plicatilis* were exposed for 2 h to a series of stressors: free ammonia concentration, seawater viscosity and different levels of *Euplotes* contamination. Rotifer glucosidase and esterase activities decreased with increasing free ammonia and viscosity. Glucosidase and phospholipase activity decreased with heavier contamination of *Euplotes*. Enzyme activities highly reflect the physiological changes in rotifers, and it is thus possible to assess the physiological condition of rotifers rapidly by measuring enzyme activity.

5. Stabilization of rotifer cultures

While monitoring the status of rotifer cultures, it is important to develop techniques to stabilize the cultures. Previous reports clarified optimal external conditions of rotifer population growth. Placing a filter in the culture tank is useful for reducing the solid

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Population growth</th>
<th>Mictic reproduction</th>
<th>Body size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH (porcine)</td>
<td>+ +</td>
<td>+ +</td>
<td>n.e.</td>
</tr>
<tr>
<td>GABA</td>
<td>+ +</td>
<td>+ +</td>
<td>+</td>
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<tr>
<td>5-HT</td>
<td>+</td>
<td>+ +</td>
<td>n.e.</td>
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<tr>
<td>JH</td>
<td>n.e.</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>HCG</td>
<td>+</td>
<td>n.e</td>
<td>±</td>
</tr>
<tr>
<td>E$_2$</td>
<td>n.e.</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>20-HE</td>
<td>n.e.</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>T$_3$</td>
<td>n.e.</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GH (human)</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>FSH</td>
<td>n.e.</td>
<td>–</td>
<td>n.e.</td>
</tr>
<tr>
<td>LH</td>
<td>–</td>
<td>–</td>
<td>n.e.</td>
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<tr>
<td>LH-RH</td>
<td>–</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>Carp pituitary</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>cAMP</td>
<td>–</td>
<td>–</td>
<td>n.e.</td>
</tr>
<tr>
<td>PGE$_1$</td>
<td>n.e.</td>
<td>n.e.</td>
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</tr>
<tr>
<td>PGE$_2$</td>
<td>n.e.</td>
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<tr>
<td>PGF$_{1a}$</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>PGF$_{2a}$</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>PGA$_1$</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>PGI$_1$</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
</tbody>
</table>

GH (porcine), porcine growth hormone; GABA, gamma aminobutyric acid; 5-HT, serotonin; JH, juvenile hormone; HCG, human chorionic gonadotropin; E$_2$, estradiol-17β; 20-HE, 20-hydroxycyedysone; T$_3$, tri-iodothyronine; GH (human), human growth hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; LH-RH, luteinizing hormone-releasing hormone; Carp pituitary, carp pituitary extract; cAMP, cyclic AMP; PG, prostaglandins.

+ +, positive effect ($P < 0.01$); +, positive effect ($P < 0.05$); –, negative effect ($P < 0.05$); n.e., no effect ($P > 0.05$).
substance, bacterial biomass, and protozoan contamination in rotifer culture water (Yoshimura et al., 1997; Balompapueng et al., 1997b), but rotifer culture collapses still occur.

We have tested the effect of the addition of hormones and neuro-transmitters to rotifer cultures. We started by screening 20 species of vertebrate and invertebrate hormones, including two neurotransmitters. Among the tested chemicals, we found a positive effect on rotifer population growth with the addition of porcine growth hormone (GH), human chorionic gonadotropin (HCG), and gamma aminobutyric acid (GABA) (Gallardo et al., 1997). Table 1 summarizes the results of hormone screening studies in our laboratory. As an example, Fig. 3 shows that the addition of GH at 0.0025 and 0.025 IU/ml significantly enhances the rotifer population growth (data based on Gallardo et al., 1997). GH is effective on rotifer population growth when environmental stressors are low, such as under optimal food conditions with a low concentration of free ammonia (Gallardo et al., 1999). GABA is effective when rotifers are exposed to a high concentration of free ammonia (3.1 µg/ml) or low food density (7 x 10^3 N. oculata cells/ml). The effects of GH and GABA were also produced in the F1 and F2 offspring, which were not directly treated with hormones (Gallardo et al., 1999).

Rotifer sexual reproduction is also influenced by the addition of hormones. Addition of juvenile hormone (JH) to rotifer culture water causes mixis induction under optimal and sub-optimal food conditions, while serotonin (5-HT) increases mixis induction at low food conditions and increases population growth at high levels of free ammonia (Gallardo et al., 2000a). By HPLC analysis, we recently found that GABA and 5-HT are present in rotifers (Gallardo et al., in press(b)).

We have applied the GABA results to rotifer culture practice; namely, in mass cultures and enrichment cultures in hatcheries. In mass cultures, GABA is most effective when added to rotifer cultures at the lag phase of population growth. To save in the cost

![Fig. 3. Population growth of B. plicatilis treated with porcine growth hormone. Level of significant difference with control: * P < 0.05, ** P < 0.01. (data based on Gallardo et al., 1997).](image)
of using GABA, rotifers may be concentrated at high densities and then treated with GABA for 24–48 h (Gallardo et al., in press(c)). In enrichment cultures, the addition of GABA to rotifers was effective at 24 h before enrichment. It was not effective when GABA was added together with enrichment chemicals (Gallardo, 1999). It remains unknown what effect GABA, GH, JH or 5-HT treated rotifers would have on fish larvae, and further experiments are needed. Furthermore, effluents containing these hormones need to be carefully monitored for effects on indigenous species in receiving waters.

6. Use of appropriate rotifer strains for fish larvae

It is generally accepted that the suitable size of prey for fish larvae varies according to the larval mouth size (Shirota, 1970), and fish larvae select larger prey with their growth (Ivlev, 1961). Although many researchers have reported larval rearing trials of marine fishes, only a few studies have been conducted to compare the appropriate rotifer size among fish species and among different growth stages (Oozeki et al., 1992). As described in the Introduction, the size of rotifers varies among species and strains. Euryhaline brachionid rotifers range in size between 90 and 340 μm. When rotifers of different sizes are fed to fish larvae, larvae show different growth responses. In recent research, we reared threeline grunt, Parapristipoma trilineatum (Thunberg), larvae for 15 days from posthatch (Ueda et al., 1998). Larvae fed B. rotundiformis (lorica length, 90–210 μm in range) grew better during the first 7 days, while the larvae fed B. plicatilis (160–320 μm in range) reached the same size on day 7 and grew better thereafter. It is important to accumulate information in this aspect using different fish species.

In order to promote larval growth rate, and to conduct efficient larval rearing, it would be useful to select rotifer strains of a suitable size, according to the larval species and the larval developmental stage. For this purpose, it would be important to preserve various rotifer strains of different sizes and provide them when necessary.

7. Preservation of rotifers

For a short-term storage, rotifers can be preserved for periods varying from a few days to 3 or 4 weeks at temperatures ranging between −2 and +8 °C, while resting eggs can be used for long-term preservation (Berghahn et al., 1990; Lubzens et al., 1990). For preserving B. plicatilis and B. rotundiformis at 4 °C, the selection of rotifer strains, the culture medium exchange, and temperature acclimation at 15 °C before transfer to 4 °C are important for both of these species (Lubzens et al., 1990; Assavaaree et al., in press). For B. plicatilis, the percent survival after 30 days of preservation at 4 °C was up to 60%, when the appropriate strain was selected (Figs. 4 and 5; data based on Assavaaree et al., in press). The S- and SS-type B. rotundiformis are weak under 4 °C, and we have not observed survival for more than 15 days. When we compared rotifer survival on day 5 at 4 °C, some strains showed 75% survival, but for tropical SS type rotifers, survival generally remained at a very low level. We are continuing studies.
Fig. 4. Percent survival of six *B. plicatilis* strains on day 30 after preservation at 4 °C. Columns and vertical bars indicate mean rotifer survival of three replicates and standard deviation, respectively. Means with a common letter are not significantly different at 95% probability Tukey test (data based on Assavaaree et al., in press).

To increase survival of SS type rotifers by increasing the temperature, trials to mass-preserve rotifers in a hatchery (Goto Branch, Japan Sea Farming Association) were conducted using 30-l containers. *B. plicatilis* could be preserved for 15 days in 30-l containers.

Fig. 5. Percent survival of eight *B. rotundiformis* strains on day 5 after preservation at 4 °C. Columns and vertical bars indicate mean rotifer survival of three replicates and standard deviation, respectively. Means with a common letter are not significantly different at 95% probability Tukey test (data based on Assavaaree et al., in press).
Fig. 6. A typical resting egg formation pattern in the rotifer *Brachionus* population, relative to the three types of females, males and resting eggs.
tanks by changing the initial rotifer density from 2000 to 20 000 ind./ml. Although the percent survival was lowest when rotifers were stocked at 20 000 ind./ml, the actual number of rotifers that survived was highest (300 000 rotifers).

For long-term storage of rotifers, we have conducted mass production trials of rotifer resting eggs. The significant factors for mass production of resting eggs include selection of strain and regulation of environment (Lubzens, 1987; Hagiwara and Hirayama, 1993; Hagiwara, 1994; Hagiwara et al., 1997). We produced 10 billion resting eggs in a 10-day culture trial using 50-m³ tanks (Hagiwara et al., 1993), and preserved the resting eggs by canning (Balompapueng et al., 1997a). For this product, the resting eggs were canned with optimal water content and air pressure for storage.

Resting eggs are the product of rotifer sexual reproduction, which is initiated by the appearance of mictic females (mixis). The mixis is induced by, thus far, unspecified accumulated substances in the rotifer culture water (Hino and Hirano, 1976). By conducting axenic rotifer cultures, Hagiwara et al. (1994) showed that mixis is induced in the presence of certain bacteria and with the addition of a water-soluble extract of rotifers. It appears that an excess amount of rotifer extract inhibits mixis induction. Based on this knowledge, we introduced semi-continuous cultures to maintain the concentration of mixis-inducing substance, resulting in efficient resting egg production (Balompapueng et al., 1997b).

Fig. 6 illustrates a typical example of population growth stages resulting in resting egg formation by fertilized mictic females. With the increase of rotifer density, the mixis rate or sexual reproduction rate also increase. After abundant males appear, fertilization proceeds, and resting eggs are produced as the final product of sexual reproduction. Several problems are involved in this process. For B. plicatilis, optimal temperature for sexual reproduction induction is low (10–15 °C), and under low temperature, a longer time is necessary for rotifer population growth and resting egg production. For B. rotundiformis, the optimal temperature of sexual reproduction is much higher. At higher temperatures, water quality tends to decline, and cultures may collapse before we observe resting egg formation. Very recent progress in this area is the further improvement of the efficiency of resting egg formation by temperature manipulation. For B. plicatilis, we first adjusted the temperature to a low level to enhance sexual reproduction in the rotifer population, then increased the temperature to promote fertilized rotifers to produce resting eggs (Kogane et al., 1997). For B. rotundiformis, the temperature was first set at a high level, but decreased thereafter to avoid the decline in water quality (Assavaaree et al., unpublished). For B. plicatilis, resting egg formation was slow at 15 °C, and very few resting eggs were produced after 7 days. By increasing the temperature to 25 °C on day 5, we harvested 15 to 20 million resting eggs on day 7. For B. rotundiformis, the number of resting eggs produced was also improved by decreasing the temperature as the culture progressed.

8. Use of copepods as live food

Several reviews are available about the use of copepods in European hatcheries (Støttrup and Norsker, 1997; Støttrup, 2000). Here, we summarize the use of copepods in Japanese hatcheries.
In 1970s and 1980s, the harpacticoid copepod, *Tigriopus japonicus* Mori, was commonly mass-cultured in Japan (Fukusho, 1980). Without conducting enrichment culture, the nutritional value of copepods is excellent, being rich in \( n-3 \) HUFA, with an especially high DHA content (Watanabe et al., 1978).

Besides harpacticoid copepods, *Eurytemora pacifica* Sato was used at Aomori Prefectural Fisheries Station for feeding Pacific cod (*Gadus macrocephalus* Tilesius) larvae in the late 1980s (Anonymous, 1988). *E. pacifica* was collected by pumping up coastal seawater at night under the light, and used as inoculants for 8-m\(^3\) copepod cultures. Prior to the culture inoculation, 1 kg of fresh baker’s yeast was added to seawater and kept aerated for a month. Conditioned-sea water prepared in this way contains various species of phytoplankton, protozoans and bacteria. In most cases, copepod culture started with 300 ind./l and reached 3000 ind./l after 7 days at about 20 °C. Nauplii stages dominate in the culture, which offers 100–400 \( \mu \)m range of live food for cod larvae. Table 2 shows the results of the larval rearing trials. The cod larvae-fed copepods showed much higher survival rates.

The use of copepods has markedly diminished in Japan since the technological development and improvement of microcapsule diets or products for nutritional enrichment of rotifers and *Artemia*. Nevertheless, harpacticoid copepods readily contaminate rotifer mass cultures. Their feces stay long as solid substance in rotifer cultures and disturb the harvest of rotifers. Hence, in most hatcheries in Japan, contaminating copepods are regarded as noxious animals rather than as a food source for fish larvae (Hagiwara et al., 1995a). Studies on interspecific relations between rotifers and *T. japonicus* indicated that *T. japonicus* often reproduce best in rotifer cultures, probably utilizing rotifer feces or associated bacteria flora as food sources (Fukusho, 1980; Hagiwara et al., 1995b). Jung et al. (1997) reported that contaminating *T. japonicus* suppressed the population growth of rotifers, depending on the composition of bacteria flora.

For some fish species with smaller mouth sizes, young nauplii of copepods can be provided as useful food source. Research conducted at the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC) in the Philippines showed that nauplii of copepods are effective for feeding larvae of the red-spotted grouper, *Ephinephelus coioides* (Hamilton), (Doi et al., 1997a,b; Toledo et al., 1997).

### Table 2
Survival of pacific cod larvae by feeding rotifers, brine shrimp and nauplii of *E. pacifica*. Three thousand newly hatched larvae were stocked and reared for 40 days in a 500-l tank (data from Anonymous, 1988)

<table>
<thead>
<tr>
<th>Feeding regime</th>
<th>Trial</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotifer → Artemia</td>
<td>I</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>25</td>
</tr>
<tr>
<td>Rotifer → Copepoda</td>
<td>I</td>
<td>68</td>
</tr>
<tr>
<td>→ Artemia</td>
<td>II</td>
<td>49</td>
</tr>
<tr>
<td>Copepoda → Artemia</td>
<td>I</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>67</td>
</tr>
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</table>
The copepods used in these studies included *Acartia tsuensis* Ito, *A. sinjiensis* Mori, and *Pseudodiaptomus annandalei* Sewell. Ohno and Okamura (1988) developed a mass culture method for *A. tsuensis* in 24-m³ outdoor tanks.

Resting eggs of copepods can be preserved at 4 °C for a long period (Marcus and Boero, 1998). Yamada et al. (unpublished data) obtained *A. steueri* Smirnov and *A. omorii* Bradford from coastal seawater of Nagasaki and transferred them to 30-m³ tanks which contained *Chaetoceros gracilis* Schwä. After a week, bottom sediments were siphoned out and resting eggs were separated. Resting eggs were viable after storage at 4 °C, and hatchability was kept at more than 90% for more than a year. Studies to establish the feeding scheme utilizing copepod nauplii are currently in progress.

9. Conclusion

As described in Section 4 of this paper, changes in bacteria flora or the contamination of protozoans, such as *Euplotes* and *Uronema* are the major cause of rotifer culture collapse. But available techniques to exterminate the contamination of harmful bacteria and protozoans from rotifer cultures are limited. The use of continuous culture systems will provide less risk against the sudden change of bacterial flora in rotifer cultures. Maeda and Hino (1991) could exterminate 80% of *Euplotes* contamination by employing a circulating system for 10-l rotifer cultures, equipped with a coarse filter material with pore size about 5 mm. Rotifer population growth was not improved, however. The mechanism of harmful effects of protozoans, such as *Euplotes* or *Uronema* on rotifer population growth has not been clarified, but it may be related with the function of coexisting bacteria (Maeda and Hino, 1991). Another method Maeda and Hino (1991) developed was to inoculate *Artemia* into rotifer cultures. By inoculating *Artemia* (1.2–7.0 mm in body length) at 1 ind./ml, *Artemia* effectively ingested protozoans, and rotifer population growth was recovered. But several researchers (Hagiwara et al., 1995a,b,c; Jung et al., 1997) reported that *Artemia* outcompete rotifers when they are mix-cultured under food limitation conditions. Developing effective chemical treatments to eliminate harmful protozoans will be a desirable solution.

The selection of rotifer strains is important for rotifer mass culture practice, since rotifers have strain specific features in size (Fu et al., 1991) and population growth potential (Rumengan and Hirayama, 1990). It is probable that the resistance to environmental stress during mass culture or during preservation process varies among rotifer strains. Little work has been conducted, however, in order to characterize such biological functions of each rotifer strain. Based on this information, it will be useful to develop desirable rotifer strains for larviculture, through inbreeding or appropriate biotechnological methods using strains with useful characteristics.

As the specific characteristics of different rotifer strains show further importance in larviculture, techniques to identify *B. plicatilis* and *B. rotundiformis* strains should be developed. For this purpose, currently available methods are allozyme analysis (Fu et al., 1991) and analysis of satellite DNA sequence (Boehm et al., 2000). Development of more simple techniques, such as the use of DNA probes that hybridize with the strain specific sequence of bases, will be desirable for larviculture practices.
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References


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