produced *Monochrysis lutheri* in a connected series of three glass carboys. The first carboy had a 20-liter capacity, the second and third were 10 liters. Total culture volume was 30 liters. The system was nonaxenic, and runs lasted an average of six weeks. Cell density increased with each successive carboy, and production was maximized ($2.1 \times 10^{11}$ cells/day) at a harvest rate of 10 liters/day. Note that daily production from the 30-liter system equalled the harvest of a 1,000-liter batch culture with a cell density of $2 \times 10^8$ cells/liter. An equivalent yield by batch culture would have required ten days of scale-up prior to harvest.

A relatively simple, inexpensive turbidostat system is also described in Sorgeloos et al. (1976) (see Fig. 6). The authors grew a number of species, including *T. suecica* and *Dunaliella viridis*. Little production data is given, however. Finally, Camacho et al. (1990) reported their first results from an outdoor continuous culture of *Tetraselmis* sp. in which the pond acted as a chemostat.

### 3.0 ROTIFER (*Brachionus plicatilis*)
#### PRODUCTION SYSTEMS

### 3.1 Biology and Life History

Rotifers comprise a phylum of microscopic, filter-feeding metazoa (multi-cellular organisms). Composed of approximately 1,000 cells, these animals filter small particles out of the water column by means of a ciliated

![Figure 4. General arrangement of the culture vessel designed by Laing and Jones (1988). Reprinted with permission of Elsevier Science Publishers, B.V.](image)

![Figure 5. Arrangement of the support frame for the vessel in Figure 4 (from Laing and Jones (1988). Reprinted with permission of Elsevier Science Publishers, B.V.](image)
corona located on the anterior portion of the body. The corona may also be used for locomotion; however many species spend the majority of their lives attached to a substrate. *Brachionus plicatilis* is one of the planktonic, or unattached varieties. Geographic strains of adult *B. plicatilis* range in size from approximately 125 to 300 μm in length.

*Brachionus plicatilis* can reproduce either sexually (mictic reproduction) or, as is more common, asexually (amictic reproduction). A female rotifer reproducing asexually simply produces clones, genetically identical copies of herself. A change in the rotifers' environment such as a sudden increase or decrease in salinity or temperature, however, can trigger mixis (sexual reproduction) (also see Snell and Boyer 1988). At this time, males are produced, as are special resting eggs, analogous to *Artemia* cysts. Normally, aquaculturists promote only amictic reproduction because 1) the rate of amictic reproduction is faster than mictic reproduction, 2) males, which are only produced during mixis, are inferior nutritionally due to the lack of a functional digestive system (Meragelman et al. 1985), and 3) the onset of mixis can cause culture collapse (Meragelman et al. 1985).

In some cases, however, a culturist may want to induce mixis to acquire resting eggs. These cysts, which are relatively large (their volume is almost 60% that of a normal adult female rotifer, Pourriot 1990), are ideal for storage and transport. See Snell and Hoff 1988 for a discussion of the use of cysts as inocula for mass cultures. While mixis may

---

*Figure 6. Schematic diagram of the turbidostat from Sorgeloos et al. (1976). (A) algal culture vessel; (B) stock medium; (C) dispensing vessel; (D) electronic control unit. Reprinted with permission of the American Society for Microbiology and the author.*
be brought about by a number of factors, including sudden changes in salinity or exposure to extremely high or low salinities, high population densities, and changes in the type or amount of food (Lubzens et al. 1985, Snell 1986, Snell and Boyer 1988), most experimenters induce mixis by manipulating salinity. For example, Hagiwara and Hino (1989) promoted mixis in a population of *B. plicatilis* by culturing them at a fairly low salinity, 14.5 ppt. Some clones, however, are known to be exclusively amictic (Meragelman et al. 1985, James and Abu Rezeq 1990).

Depending on conditions, an amictic female may produce 20 or more eggs during her seven to 10 day lifetime (Hoff and Snell 1989). She carries all of her eggs attached to the posterior portion of her body until they hatch.

Some life history characteristics of *B. plicatilis* that were fed a variety of algal diets were recently investigated by Korstad et al. (1989). Animals did best (had the highest fecundity) when raised on a pure diet of *Isochrysis galbana* (Tahiti strain), and at 20-22°C yielded the following mean results: 21 offspring/female, a reproductive period lasting 6.7 days, a 10.5 day life-span and a mean adult length of 234 μm.


### 3.2 Rotifers as Feed

*Brachionus plicatilis* culture has become an indispensable aspect of many marine finfish hatcheries. For example, Lubzens (1987) states that “providing rotifers in adequate numbers during crucial periods is the main problem of most marine hatcheries” (p. 246), while Kafuku and Ikenoue (1983) stress that “the results of artificial seed production directly reflect upon the success of rotifer culture” (p. 209). And, although we will be primarily interested in the culture of rotifers for fish, *B. plicatilis* is also widely used as a feed for larval crustaceans — mainly shrimp, prawns and crabs; see, for example, Fontaine and Revera (1980), Lovett and Felder (1988), and Samocha et al. (1989) (Table 1).

The mass production of *B. plicatilis* is the topic of this section. Much of the information to follow has come from research and production facilities in Japan, a country that is prominent in the field of marine finfish culture and rotifer mass production.

*Brachionus plicatilis* is an excellent first feed for larval fish because of its 1) small size, 2) slow swimming speed and habit of staying suspended in the water column, 3) ability to be cultured at high densities (2,000 ind./ml has been reported, Hirata 1979), and 4) high reproductive rate. Furthermore, rotifers can easily be enriched with fatty acids, antiboitics, etc. and used to transfer these substances to larvae (Lubzens et al. 1989) (Table 6). There are other rotifer species that possess some or all of these characteristics, but *B. plicatilis* is used widely in mariculture because it is able to thrive in a wide range of salinities.

Rotifers are in such great demand at finfish hatcheries that producing adequate quantities can be an overwhelming task. Commonly, rotifers are offered to finfish lar-
Table 6. Principal characteristics of *B. plicatilis* that make it an excellent first food for larval fish.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small size</td>
<td></td>
</tr>
<tr>
<td>Slow speed, planktonic nature</td>
<td></td>
</tr>
<tr>
<td>Tolerance of high densities</td>
<td></td>
</tr>
<tr>
<td>High reproduction rate</td>
<td></td>
</tr>
<tr>
<td>Living “biocapsules” for delivering substances to larvae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>123 - 292 μm&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>up to 2,000 ind./ml reported&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>r = 0.7 - 1.4 offspring/female/day&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Snell and Carrillo 1984.  
<sup>2</sup>Reported in Hirata 1979.  
<sup>3</sup>Hoff and Snell 1989.

vae for seven to 30 days after exogenous feeding has begun (Lubzens et al. 1989). Anywhere from 40,000 to 173,000 rotifers are needed to feed one fish larva from hatching until it can utilize another type of food (Okauchi et al. 1980, Kafuku and Ikenoue 1983), although the exact number depends on the species of fish cultured and also on the size of the rotifers.

Not all of the rotifers that must be supplied are consumed, however. It is necessary to supply more rotifers than the fish will eat; just how many more depends on the predatory ability of the larvae being cultured (Fukusho 1989b). This is because the rotifers must be maintained at a density high enough to allow the fish to feed efficiently (see Theilacker and McMaster 1971, Hoff and Snell 1989). Rotifer concentrations should not, however, be too high. This could cause the fish to ingest so much food that they are not able to assimilate it all (Lubzens et al. 1989). Kafuku and Ikenoue (1983) estimate that the number of rotifers given to one red sea bream (*Pagrus major*) larva is almost three times the amount actually eaten. The production of one million red sea bream larvae in Japan requires almost 20 billion rotifers/day, on average (Kafuku and Ikenoue 1983).

3.2.1 Culture history

In Japan, *B. plicatilis* was first investigated because it was a serious hindrance to the culture of Japanese eels (*Anguilla japonica*). Sudden, rapid increases in rotifer populations degraded water quality in the outdoor ponds, killing the eels. In Japan, the phenomenon is known as “mizukawari.” For this reason, rotifers were regarded as major aquacultural pests for more than 100 years (Hirata 1979, Fukusho 1989a).

The first research was done by Takashi Ito (see Ito 1955, 1957a, 1957b, 1960), who discovered that *B. plicatilis* is an excellent food for larvae of the marine fish, ayu (*Plecoglossus altivelis*) (Table 7). Ito’s pioneering work laid a foundation for the culture of economically important fishes in Japan. According to Hirata (1979), mass culture of *B. plicatilis* in Japan began at the Yashima Station of the Seto Inland Sea Farming Fisheries Association (SISFFA) around 1964. Rotifers were fed “marine Chlorella,” recently re-identified as *Nannochloropsis oculata* according to Fukusho (1989a) and Hirayama et al. (1989), and cultured according to the “daily tank-transfer” method (see Section 3.4.1).

It was 1965 when rotifers were first used to feed the commercially important red sea
bream, whereupon their “high nutritional value was confirmed” (Fukusho 1989a p. 69). The next big breakthrough was Hirata and Mori’s discovery that rotifers could be cultured on baker’s yeast (Saccharomyces cerevisiae), a less expensive and more convenient form of food (Hirata and Mori 1967, cited in Hirata 1979). However, the widespread use of yeast to produce rotifers in Japan did not occur until the 1970s (Fukusho 1989a) (Table 7).

In the United States, one of the early papers detailing quantitative studies of the mass culture of B. plicatilis was published by Theilacker and McMaster (1971). They tested growth in response to a number of different algal foods and also determined that B. plicatilis was an excellent food source for larval anchovies (Engraulis mordax).

Today, rotifers are mass produced in hatcheries all over the world. According to Nagata (1989), B. plicatilis is widely used in the larval culture of more than 60 species of marine finfish and 18 crustaceans worldwide. Furthermore, production is reaching new heights in Japan where, for example, in 1982 approximately 2.5 tons of rotifers were grown at just one fish farming center. These rotifers allowed the production of 6.32 million red and black sea bream (12.1 - 16.0 mm in length) and 4.04 million crabs (Portunus trituberculatus) at that location (Fukusho 1989b). Further, in 1987 an “average” Japanese commercial marine hatchery was reported to produce more than one million red sea bream fingerlings/year (Hirayama 1987). Recently, research has focused on the bacterial environment of rotifer cultures (Gatesoupe 1990; Maeda, this volume).

### 3.2.2 Strains cultured

Strain selection is important for a number of reasons. The following can all vary among strains: reproductive rate, size, optimum culture conditions (including temperature and salinity), and frequency of mixis (Lubzens et al. 1989, Lubzens 1987, Fukusho 1989a, Meragelman et al. 1985, James and Abu-Rezeq 1989c, Fushimi 1989). Reproductive rate should, of course, be maximized while frequency of mixis should be minimized. Furthermore, size is an important factor because different target species and developmental

<table>
<thead>
<tr>
<th>Table 7. Milestones in the culture history of B. plicatilis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Milestone</strong></td>
</tr>
<tr>
<td>1. Mizukawari investigated by Takeshi Ito</td>
</tr>
<tr>
<td>2. B. plicatilis' value as a larval fish feed discovered, mass culture techniques explored</td>
</tr>
<tr>
<td>3. Hirata's &quot;daily tank transfer method&quot; used at the Yashima Station of the Seto Inland Sea Farming Association</td>
</tr>
<tr>
<td>4. B. plicatilis discovered to be excellent food for red sea bream (Pagrus major) larvae</td>
</tr>
<tr>
<td>5. Hirata and Mori discover that baker's yeast is a suitable food source</td>
</tr>
<tr>
<td>6. Baker's yeast used extensively in mass culture</td>
</tr>
</tbody>
</table>

See text for references.
stages within species have different optimum food sizes. Fukusho (1989a) cites a need to breed "super large" and "super small" rotifers in order to accommodate the needs of different species and ages of fish larvae. Because different strains may perform better at different temperatures and salinities, local conditions, as well as those required by the target species, should be taken into consideration when selecting a strain to be cultured.

Two of the best known *B. plicatilis* strains or morphotypes are the "large" (L-) and "small" (S-) types recognized in Japan. James and Abu-Rezeq (1989b) determined that the mean dry weights of S-and L-type rotifers are .22 and .33 μg/rotifer, respectively. The ecology and culture characteristics of these types are also well known; they have different temperature tolerances and optima, grow at different rates, etc. (Fukusho 1989a, also see James and Abu-Rezeq 1989b, 1990).

### 3.2.3 General culture conditions

*Brachionus plicatilis* is a relatively hearty species, able to withstand a wide range of salinities, and to some degree, temperatures. Individuals are also fairly tolerant of high levels of ammonia; concentrations may reach 6 - 10 ppm without causing death. Importantly, rotifers can also be cultured on a wide variety of food types, as long as they are composed of particles of the appropriate size, approximately 2 - 20 μm. However, the rotifers' nutritional value is strongly affected by their diet.

To maximize production per unit volume, one must choose a suitable strain and provide conditions under which the reproductive rate is highest. It is difficult to make generalizations, but good yields are often realized at 25°C and at salinities from 10 - 20 ppt (see Table 8). Microalgae is commonly used as feed, often in conjunction with baker's yeast. In Japan, for example, "marine Chlorella" (*Nannochloropsis oculata*) is the most popular feed type. Initial algal densities may reach 10 to 20 million cells/ml. *Tetraselmis tetrathele* is another species gaining popularity in Japan.

*Brachionus plicatilis* populations can become quite dense. 100 - 200 ind./ml is not uncommon, and densities exceeding 1,000 rotifers/ml are achieved under some conditions.

### 3.3 Problems and the Need for Design Studies

Fukusho (1989a) cites "... prevention of a rapid population decrease during rotifer production" (p. 69) as one of the important areas for future research. The rapid population decrease to which he is referring is otherwise known as a "crash." Rotifer culture crashes occur quite often in modern hatcheries (Meragelman et al. 1985, Fushimi 1989), leaving a culturist without food for his/her valuable larvae. Such collapses could conceivably lead to loss of an entire year's production (Lubzens 1987); hence a number of recent papers have dealt with this issue (Yu and Hirayama 1986, Hirayama 1987, Snell and Hoff 1988, Fushimi 1989, Lubzens et al. 1989, Nagata 1989, Yu et al. 1989, Comps et al. 1991). Snell et al. (1987) suggested that culturists monitor the physiological state of their rotifers by measuring swimming activity and/or egg ratios as an early warning of culture collapse. Droop (1975) tried to prevent the occurrence of rotifer crashes by growing axenic populations of *B. plicatilis*. He concluded, however, that his system would probably be impractical in a commercial setting.
Table 8. A generalized set of conditions for culturing *B. plicatilis*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acceptable Range</th>
<th>Optima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>20 - 30(^1)</td>
<td>21 - 25(^2), 35(^3)</td>
</tr>
<tr>
<td>Dissolved oxygen (ppm)</td>
<td>6 - 10(^5)</td>
<td>2 - 7(^4)</td>
</tr>
<tr>
<td>[NH(_3) + NH(_4)] (mg/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>1 - 60(^6)</td>
<td>10 - 20(^6); 16(^5); 20 (S-type), 30 (L-type)(^7)</td>
</tr>
<tr>
<td>Inoculation density (ind./ml)</td>
<td>505, 100 (extensive)(^8)</td>
<td>100 - 1,000 (intensive)(^8)</td>
</tr>
<tr>
<td>Feeding amount (temperature dependent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Nannochloropsis oculata</em> (cells/rotifer/day)</td>
<td></td>
<td>100,000 - 150,000(^9)</td>
</tr>
<tr>
<td><em>Dunaliella</em> (cells/ml)</td>
<td></td>
<td>1,000,000(^10)</td>
</tr>
<tr>
<td><em>Chlorella</em> (cells/ml)</td>
<td></td>
<td>1,500,000(^10)</td>
</tr>
<tr>
<td><em>Terraselmis suecica</em> (cells/ml)</td>
<td>.4 - 1.2(^11),(^9)</td>
<td>500,000(^2)</td>
</tr>
<tr>
<td>Baker’s yeast (mg/rotifer/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light intensity (lux)</td>
<td></td>
<td>2,000</td>
</tr>
<tr>
<td>Photoperiod (light hours:dark)</td>
<td>5 - 10</td>
<td>18.6(^6)</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.5 - 8.5(^6)</td>
</tr>
</tbody>
</table>

\(^1\)Hirayama and Kusano (1972).
\(^2\)Theilacker and McMaster (1971).
\(^3\)Pascual and Yufera (1983).
\(^4\)Fukusho (1989a).
\(^5\)Owens et al. (1990).
\(^6\)Heff and Snell (1989).
\(^7\)James and Abu Rezaq (1990). These are salinities recommended for marine finfish hatcheries. Best growth, however, was achieved at 5 ppt for both strains.
\(^8\)Kusaka and Ikenoue (1983).

The causes of culture failures are not well understood (Table 9) (see Hirayama 1987 for review). Meragelman et al. (1985) cite water quality problems and advocate periodic dilution or water exchange as a means of partially overcoming the problem. They also state that there is a need for continuous feeding apparatuses which are not available in most culture facilities. These, the authors state, could improve water quality and reproduction rate, and lower the incidence of culture crashes. Along the same lines, Hirata (1980, cited by Nagata 1989) and Hirayama (1987) hypothesized that the accumulation of waste products was a major contributor to culture crashes. Lubzens (1987) blames culture crashes on "unpredictable events" and lists several ways to soften their impact by preserving (freezing, etc.) the rotifers.

Yu and Hirayama (1986) pinpoint unionized ammonia as an important factor restricting reproduction of cultured rotifers.
Table 9. Postulated causes of sudden *B. plicatilis* culture crashes.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water quality</td>
<td>Meragelman et al. 1985</td>
</tr>
<tr>
<td>Accumulation of waste products</td>
<td>Hirayama 1987, Hirata 1980</td>
</tr>
<tr>
<td>“Unpredictable events”</td>
<td>Lubzens 1987</td>
</tr>
<tr>
<td>Un-ionized ammonia</td>
<td>Yu and Hirayama 1986</td>
</tr>
<tr>
<td>Declining water temperatures</td>
<td>Kitajima et al. 1981, Kitajima 1983</td>
</tr>
<tr>
<td></td>
<td>(both cited by Yu and Hirata 1986),</td>
</tr>
<tr>
<td></td>
<td>Fushimi 1989</td>
</tr>
<tr>
<td>Mictic reproduction</td>
<td>Meragelman et al. 1985, Snell 1987</td>
</tr>
<tr>
<td>Viral infection</td>
<td>Comps et al. 1991</td>
</tr>
</tbody>
</table>

and as a possible cause of mass culture instability. Other research has been directed toward nutritional deficiencies; a lack of vitamin B12, other vitamins, or free amino acids (Fyhn 1989), and toxins produced by bacteria. Noting that crashes are especially common in yeast-fed cultures, Fushimi (1989) reports on a system in which the frequency of culture collapse was lowered as a result of recycling culture water.

Reguera (1984) reported that ciliate contamination reduces yields of cultured rotifers, and Kitajima et al. (1981), Kitajima (1983, both cited by Yu and Hirayama 1986) and Fushimi (1989) suggested that declining water temperatures may sometimes be responsible for sudden population decreases in rotifers. Finally, Comps et al. (1991) recently detected viral lesions and isolated virions which they named RBV (Rotifer Birna-like Virus) from a culture with declining productivity.

A second area in need of attention is the cost of rotifer mass production. Few studies have been published on this topic in the United States; however, Girin (1979) estimated that the production cost of rotifers was $2/g dry weight. Assuming that one L-type rotifer weighs .33 μg dry weight, this comes out to $2/3,030,000, or $0.66/million rotifers. He noted that the high cost of culturing algae for feed made rotifers even more expensive than *Artemia* cysts. In 1987, Duerr and his colleagues produced $9 \times 10^8$ *Tetraedrimeris*-fed rotifers per month at a cost of $0.65/million rotifers (Duerr, pers. com.).

Fushimi (1989) presented the results of a 1981 Hiroshima Fish Hatchery Center Report that examined the cost of mass producing rotifers at that facility. The study found that 49.6% of the total cost of cultivation was for algae, *Nannochloropsis oculata*, while the remainder could be accounted for as follows: 22.2% for baker’s yeast cultured in a fatty acid emulsion (“ω yeast”), 20.7% for electricity, 6.3% for equipment and supplies related to cleaning, filtration, aeration and other culture maintenance, and 1.2% for miscellaneous expenditures. Rent was not included in the calculations. The cost of production at that facility in 1981 was substantially higher than Girin’s 1979 production estimate: ¥598, approximately US$4.50, per million rotifers.

As with microalgal production systems, it is possible to produce rotifers less expensively and more efficiently by means of design improvements. For instance, continuous cul-
tures are much more efficient than batch and semi-continuous ones, but the latter predominate despite the fact that rotifers have been cultured continuously in chemostats (e.g., Droop 1975, Scott 1980, James and Abu Rezeq 1989a, 1989b, 1990) with promising results. Furthermore, improvements in the types of culture enclosures used have already made growing rotifers less expensive. For example, Trotta (1981, 1983) reduced the cost of culturing both rotifers and algae by growing them vertically in disposable plastic bags. No sterilization was necessary and the bags took up relatively little floor space, see Section 3.6.1. Finally, automation is another means of lowering expenses by reducing the amount of labor needed. Fushimi (1989) reports on several ways in which the culture of rotifers in Japanese hatcheries has been improved through the invention and use of new machines.

3.4 General Principles

3.4.1 Types of culture

As with microalgae, there are many recognized techniques for culturing rotifers. One early culture method was termed the “daily tank-transfer method” by its creator Hachiro Hirata (1979). Rotifers grown in “Chlorella”-rich tanks were continually transferred to fresh tanks of the same size after most of the algae in the original tanks was consumed. Although it was inefficient, this was the sole method of rotifer production before Hirata and Mori introduced yeast as a food for rotifers in 1967 (Hirata 1979).

Fukusho (1989b) discusses rotifer culture techniques commonly employed in Japan. First, production may be extensive, in a large 50 to 150-m³ tank, or intensive, in small, 1 to 2-m³ tanks. As the former method is now more popular, Fukusho lists and describes four “large tank” methods and only one “small tank” method of rotifer culture currently used in Japan. His classification is based on tank size, harvest method (batch or semi-continuous), and feed type (Table 10).

Yoshida (1989) and Fushimi (1989) also discuss some of the varied culture techniques employed in Japan (Table 11). In addition to discussing the use of batch and semi-continuous culture, Yoshida (1989) describes an example of a “combined batch and semi-continuous” technique, while Fushimi (1989) reports on a “method of re-using water” for culturing rotifers. The latter has been used in some batch cultures at the Fukui Prefecture

<table>
<thead>
<tr>
<th>Table 10. Summary of <em>B. plicatilis</em> culture techniques used in Japan (from Fukusho 1989b).</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Semi-continuous culture in large tanks using only <em>N. oculata</em> as feed</td>
</tr>
<tr>
<td>2. Semi-continuous culture using <em>N. oculata</em> in combination with plain yeast or enriched yeast</td>
</tr>
<tr>
<td>3. Batch culture in large tanks using <em>N. oculata</em> in combination with plain yeast or enriched yeast</td>
</tr>
<tr>
<td>4. Batch culture in small tanks using <em>N. oculata</em> in combination with plain yeast or enriched yeast</td>
</tr>
<tr>
<td>5. Feedback method</td>
</tr>
</tbody>
</table>
Table 11. Culture methods and production of rotifers in Japan (from Yoshida 1989).

<table>
<thead>
<tr>
<th>Facility Name</th>
<th>Culture Method and <em>B. plicatilis</em> strain</th>
<th>Culture Tanks</th>
<th>Temp. (°C)</th>
<th><em>B. plicatilis</em> inoculation density (N/ml)</th>
<th>Average Daily Production (10^6)</th>
<th>Amount of &quot;Chlorella&quot; used per 10^8 rotifers (m^3)</th>
<th>Amount of yeast used per 10^8 rotifers (kg)</th>
<th><em>B. plicatilis</em> production per 1 ton of culture water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miyazaki Prefecture Marine Testing Site</td>
<td>Batch S-type</td>
<td>4 tanks 0.5 t panlite</td>
<td>27-32 (heated)</td>
<td>100-200</td>
<td>1.5</td>
<td>0.27</td>
<td>0.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Hiroshima City Marine Promotion Association</td>
<td>Batch L- and S-type</td>
<td>7-8 tanks 26 t concrete</td>
<td>6-29 (unheated)</td>
<td>80-150</td>
<td>62.6</td>
<td>0.009, concentrated Chlorella (0.28 m^3)</td>
<td>omega yeast (0.225)</td>
<td>2.4</td>
</tr>
<tr>
<td>Nagasaki Prefecture Fisheries Public Corporation</td>
<td>Batch S-type</td>
<td>4 tanks 80 t concrete</td>
<td>23-24 (heated)</td>
<td>80</td>
<td>25.7</td>
<td>0.69</td>
<td>0.64</td>
<td>0.6</td>
</tr>
<tr>
<td>Yamaguchi Prefecture Foreign Fish Hatchery Center</td>
<td>Semi-continuous L- and S-type</td>
<td>4 tanks 23 t and 21 t concrete</td>
<td>25-28 (heated)</td>
<td>100-130</td>
<td>61 (32)</td>
<td>0.75</td>
<td>0.28</td>
<td>1.7</td>
</tr>
<tr>
<td>Nagasaki Prefecture Marine Experiments Site of Culture Research Center</td>
<td>Semi-continuous L- and S-type</td>
<td>4 tanks 10 t concrete</td>
<td>27-32 (heated)</td>
<td>300</td>
<td>15-20</td>
<td>0.25</td>
<td>0.27</td>
<td>3.7</td>
</tr>
<tr>
<td>Nagasaki City Aquatic Center</td>
<td>Semi-continuous L-type</td>
<td>2 tanks 200 t concrete</td>
<td>18-25</td>
<td>31</td>
<td>17.4</td>
<td>1.26</td>
<td>0.65</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Fish Farming Center to prevent culture crashes. Note that in Japan they often refer to semi-continuous culture as “the thinning method” and to batch culture as either the “repeated stocking method” or the “total harvesting method.”

Like Fukusho, Kafuku and Ikenoue (1983) also specify intensive and extensive as the two main culture types in Japan. They state that the former employs .5 to 1-m^3 vessels stocked at 500 to 1,000 rotifers/ml. Extensive culture, by contrast, uses 5 to 20-m^3 tanks stocked with approximately 100 rotifers /ml. Semi-continuous culture in a “canvas cage” that is suspended in a larger tank from planks is yet another type of culture sometimes used in Japan (Fukusho et al. 1976, Kuronuma and Fukusho 1984).

In general, though, most culture methods are simply classified as either batch, semi-continuous, or continuous (for definitions of these culture types see previous section on the types of microalgal culture). Lubzens (1987) describes two examples of batch culture. She states that large vessels, 10,000-liter outdoor tanks, may be used to grow rotifers at low densities, or small vessels, e.g. 50-liter indoor plastic bags, may be used to culture them at high densities (also see Trotta 1980, 1981, 1983). As was the case with microalgal culture, batch culture of rotifers is the most reliable method but also the least efficient in
terms of the labor, time and facilities needed to culture a given number of rotifers (Trotta 1980, Fushimi 1989).

More rotifers can be produced from the same volume of culture water with semi-continuous and continuous systems. According to Lubzens (1987), semi-continuous rotifer culture employs vessels ranging from a few hundred liters to 200,000 liters. Relatively high densities can be obtained in the smaller volume cultures. However, as was the case for microalgal cultures, build up of waste products, and in this case, uneaten food, and contamination are problems in semi-continuous systems. This fact tends to make them less reliable than batch cultures. Note, however, that in Japan they have devised several means of filtering rotifer culture water, see Section 3.5.10. Meragelman et al. (1985) describe a semi-continuous system developed in Israel. Batch-type upsampling in small vessels is used to produce the inocula for 200-liter tanks. Rotifers are fed mainly baker’s yeast, and can be harvested repeatedly for 14 - 22 days.

As discussed in the previous section on microalgal culture, continuous cultures are the most efficient way to produce a consistent supply of high-quality algae and rotifers. Since continuous culture apparatuses must be maintained under strictly defined conditions, however, they are almost always “closed” and indoors. This limits their size somewhat, and may add to the cost of operations.

Droop (1975) described a continuous chemostat culture of *B. plicatilis* but expressed doubt about its commercial value. By contrast, James and Abu-Rezeq (1989a, 1989b, 1990) apparently achieved a high-degree of success with indoor, vertical, 1 and 100-liter continuous chemostat cultures of *B. plicatilis*. L- and S-type strains were fed Nannochlorop-sis and baker’s yeast. The S-type rotifers had the highest yield and the best food conversion ratio. The authors stated that the average yields obtained, 308 and 186 million/m²/day for S- and L-type rotifers, respectively, were “considerably higher than in any conventional rotifer production systems reported to date for aquacultural purposes” (James and Abu-Rezeq 1989b p. 297) and proposed that their system be adapted for large-scale rotifer production. No information about the long-term operation of the system was provided, however, in either paper.

Other culturing techniques worthy of mention include the Galveston method and feedback culture. The Galveston method is a technique whereby rotifers are grown in fiberglass tanks (1.5 x 1.6 x 6 m) in an open shed. Unfiltered seawater (5 m deep) is used, and torulose yeast is the feed type. Rotifers are harvested from the top of the water column by means of a skimmer. At this facility, rotifers were grown mainly for shrimp larvae which will eat frozen rotifers. Thus, a portion of the harvest was often frozen for later use (Fontaine and Revera 1980).

Feedback culture was pioneered by Hachiro Hirata and his colleagues in Japan (Hirata 1974, Hirata et al. 1979, 1983). Rotifer wastes are treated by bacteria and the liberated nutrients are used to fertilize microalgae which is cultured in a separate tank. The algae, in turn, are fed to the rotifers. According to Hirata et al. (1979), rotifer densities of 500/ml can be reached, and the system can produce 80.5 million rotifers/day for at least 20 days. Yields with this method ranged from 5,387 - 122,800 rotifers/liter/day. Hirata considers this the “most efficient and reliable” of the culture methods (Hirata 1979 p. 252).
3.5 General Requirements and Considerations

3.5.1 Nutritional quality and feeds

3.5.1.1 Choosing an appropriate feed

The type of feed used for culturing rotifers can have a profound effect on the cost of operations, the amount of labor and time required to culture a given amount of rotifers, and their nutritional value. When choosing a feed, one must consider both the requirements of the rotifers as well as the needs of the target species. It is fair to say that there are two standards by which to judge rotifer feed quality: one can measure how well the rotifers survive and propagate, or one can look at the survival and growth rate of the target species raised on the rotifers. Generally speaking, *B. plicatilis* have broad nutritional requirements. These animals ingest many types of feed, including bacteria, so long as it is of the appropriate particle size. Rotifers do, however, require vitamin B12 (Yu et al. 1989) and may also require vitamin A (Fukushima 1989a).

By contrast, the nutritional requirements of fish larvae are relatively specific. This, in addition to the problems previously discussed, such as needing a feed type that is 1) small enough to pass through their tiny mouths, 2) suspended in the water column, and 3) easily captured complicates the task of culturing larval marine finfish. It is convenient then, that rotifers, because of their non-selective feeding habits, can be packed with selected nutrients and even antibiotics and used to “deliver” them to the larvae.

“Essential” nutrients are those which an organism cannot manufacture itself. For example, carnivorous marine fishes have a notoriously high requirement for long-chained “highly unsaturated fatty acids” or “HUFAs” (Watanabe et al. 1983). While some species can synthesize long-chained fatty acids from short-chained fatty acids, many marine fishes cannot. Certain feeds contain high levels of HUFAs making them particularly valuable as rotifer feed.

Other factors to consider when selecting a feed type include the stability of culture required (for example, yeast-fed rotifer cultures seem to be especially prone to crashes) as well as the availability and cost (including labor, space, energy, etc.) of purchasing or producing the feed. Depending on the geographic and economic setting, certain feeds will be more expensive than others.

3.5.1.2 Feed types

*Microalgae*: Phytoplankton, the first feed used in rotifer cultures, is probably still the principal component of most cultured rotifer diets. I. Hirata (1989) notes that there are several benefits derived from using algal feeds, specifically *Nannochloropsis oculata*. He cites the algae’s “ability to clean the culture water” as a major reason for using *N. oculata* as rotifer feed.

Many species of algae may be used, the choice being largely dependant on what is available, what the culturist has worked with before, ease of culture under local conditions, and the exact nutritional requirements of the rotifers and the target species. Species high in ω3 HUFAs such as *Nannochloropsis* spp. are regarded as very good feeds.

The most commonly used species are *Nannochloropsis oculata*, sometimes referred to as “marine Chlorella,” and *Tetraselmis tetrahele* in Japan, and *T. suecica* and *Isochrysis galbana* elsewhere. Additionally, highly concentrated *Chlorella vulgaris*, sometimes referred to as “freshwater Chlorella,” (Hirayama et al. 1989) and freeze-dried algae
have been used successfully to feed rotifers in Japan.

The primary drawback to using phytoplankton is the huge amount of labor, time, and facilities that must be devoted to producing the large quantities needed to feed rotifers. Additionally, some algae-fed rotifers may require HUFA supplementation (Foscarini 1988). For example, to grow a 500-liter culture of *B. plicatilis*, one needs approximately 5-10 times that volume, or 2,500 - 5,000 liters of algae (Hirata 1980 cited in Lubzens 1987). When culturing *N. oculata* for rotifers, the Finfish Program at The Oceanic Institute (OI) grows algae in successively larger containers, beginning with test tubes and ending with 25,000-liter tanks (Fig. 7); 25,000 liters feeds the OI rotifers for three days (batch culture). The steps are essentially the same when growing *Tetraselmis tetrathele*, except the outdoor tanks are inoculated with 160-liter cylinder cultures. OI may use 5,000 - 8,000 liters of algae (density: 10 - 20 million cells/ml *N. oculata*, 300,000 - 500,000 cells/ml *T. tetrathele*) to feed 1,000 liters of rotifers. Finally, six 1,000-liter tanks, harvested daily, may feed 250,000 fish fry. The loss of energy with each step up the trophic pyramid is quite evident.

Recent research has focused on trying to shorten the [algae → rotifer → fish] food chain. Ideally, the rotifers themselves would be replaced by manufactured feeds, but the highly specialized diet of marine larvae has slowed progress in this area. The next best option is to find something easier and less expensive than algae to feed the rotifers. That’s where yeast has substantially simplified rotifer culture.

**Yeast.** Marine yeast (*Candida* sp.), as well as baker’s yeast (*Saccharomyces cerevisiae*) and “caked yeast” (*Rhodotorula* sp.) have all been successfully used for rearing rotifers. Baker’s yeast was the first to be tried and has been used with the greatest degree of success. It is now a very common feed type for rotifers, replacing algae altogether in some hatcheries. Often yeast is used as a backup when algae cultures are poor. According to Fukusho (1989b), the advantages of baker’s yeast over *N. oculata* include the following:

"1) a stable supply of *Chlorella* is difficult due to the heavy dependence of production yield on weather, 2) baker’s yeast is much more labour-, time- and cost-saving compared with *Chlorella*, 3) the yeast is easy to feed, and 4) easy to store" (p. 293).

Interestingly, it is now widely believed that yeast contain no nutritional value for rotifers. Rather, it is most likely that the bacteria associated with the yeast, as well as any contaminating phytoplankton that may be present, are nourishing the “yeast-fed” rotifers (Fukusho 1989a, Hirayama and Funamoto 1983).

Problems encountered with the use of yeast, especially baker’s yeast, include more frequent rotifer culture crashes and poor survival in target species that have a high HUFA requirement. The latter problem has been
alleviated by several means. In some cases, the rotifers are given a mixed diet of algae and baker’s yeast. Alternatively, the animals may be grown to harvest density on yeast and given a species of algae high in HUFAs for a few hours or days prior to harvest.

So-called “ω-yeast” has also been developed to boost the ω3 HUFA content of rotifers. ω-yeast is simply baker’s yeast that has been cultured with emulsified ω3 HUFAs. In Japan, these are often derived from cheap, readily available squid liver oil. The ω3 HUFAs are eventually transferred to the fish, via the rotifers and the yeast. Finally, the rotifers can also be enriched by adding ω3 HUFA-rich emulsions directly to the rotifers (e.g. Ostrowski and Divakaran 1990). This is usually done after they have been harvested and are in concentrated form. The latter method is the easiest means of enriching rotifers; however it can cause clumping of the rotifers and degradation of the larval rearing tank water quality (Hoff and Snell 1989). A number of commercial post-harvest enrichment solutions are now available in the U.S. (e.g. Selco).

Bacteria. A fairly recent topic for research is the use of bacteria as feed for B. plicatilis. Jian-Ping Yu recently authored several bacteria-related papers that dealt with the positive effects of vitamin B12-producing bacteria (Yu et al. 1989, 1990). According to Yu et al. (1990), addition of vitamin B12-producing bacteria can greatly enhance the growth of cultured B. plicatilis. They calculated that one strain of B. plicatilis (“Thai strain”) had an average lifetime requirement of 1.0 pg vitamin B12/rotifer, while a larger strain (“Nagasaki L strain”) required 1.5 pg/rotifer.

Photosynthetic bacteria, in conjunction with baker’s yeast, have also been used to feed semi-continuously cultured rotifers in 5-m³ indoor tanks in Japan (Fushimi 1989, see also Gatesoupe et al. 1989, and Fukusho 1989a). Finally, a few other feed types are reported to have been used to grow rotifers: alcohol fermentation mother liquor, activated sludge, and microparticulate diets have all been used on a trial basis in Japan (Fukusho 1989b).

See Lubzens (1987) for a discussion of the different types of rotifer feeds, including algae, yeast, bacteria, and inert feeds (spray dried Chlorella, freeze dried Spirulina and Platymonas [= Tetraselmis] suecica, and microencapsulated diets) and Coves et al. (1990).

3.5.1.3 Feed amount and frequency of feeding

Both the amount of food supplied and the frequency with which it is provided can also affect the nutritional quality and growth rate of rotifers. Ingestion rates are known to be correlated with the size of the particles offered, their concentration, the past feeding history of the rotifers and any chemical stimulants that may be released by the food (Fukusho 1989a). Thus, the amount of food given depends on the type of feed.

As is the case when feeding rotifers to fish larvae, microalgae must be provided at a density great enough to allow the rotifers to feed efficiently. In most cases, however, rotifer densities are high enough that the number of cells or g/ml does not limit rotifer growth. Rather, it is the absolute quantity of feed provided/rotifer/day that is the most important parameter. Because microalgal rations are commonly measured in cells/rotifer/day, this value will vary greatly with the cell size of the particular algae being used (Table 8). For example, N. oculata cells are approximately 2 μm in diameter, whereas cells
of *T. tetrahele* may measure as much as 30 μm across.

Because of the high degree of variability in culture techniques, it is difficult to generalize about the amount of food required to feed a given number of rotifers. Methods vary greatly among the different fish farming centers in Japan, however, as many as 20 million or so *N. oculata* cells/ml may be supplied at the start of a culture. Feeding rates are estimated to be 100,000 - 150,000 *N. oculata* cells/rotifer/day (Table 8).

Foscarini (1988) reports that at the Kagoshima and Miyazaki Prefectural Aquaculture Centres, *B. plicatilis* are fed a combination of baker's yeast (0.4 mg/rotifer/day) and *N. oculata* (.5 - 1 million cells/rotifer/day). Alternatively, a fish farming center may rely more heavily on baker's yeast, providing 1 - 1.2 g/million rotifers/day split into two daily feedings (Fushimi 1989). It is important not to overfeed with yeast, especially in semi-continuous cultures, because poor water quality will result. In general, the consumption rate of both algae and yeast should be monitored closely and used to determine the feeding amount (Kafuku and Ikenoue 1983).

The daily ration will also depend on temperature (Nagata 1989) and salinity (Lubzens 1987). The daily number of *Chlorella saccharophila* cells required at, for example, 10°C is about 56% less than that required at 20°C (Nagata 1989).

Finally, feeding frequency is an important factor affecting rotifer quality and growth rate (Hirata 1980 cited in Meragelman et al. 1985, Lubzens 1987, Lubzens et al. 1989). Most of the nutritional value of rotifers comes from their gut contents, partially digested and highly concentrated phytoplankton, yeast, bacteria, etc., not from their own tissues. Hence, if rotifers are deprived of food prior to harvest, their nutritional quality will be poor:

"The rapid loss of organic material from rotifers which are deprived of food is generally perceived as one of the main factors causing poor growth and high mortalities in fish larval cultures" (Lubzens et al. 1989 p. 394).

3.5.2 Dissolved oxygen

The amount of aeration needed to maintain an optimum DO level in rotifer cultures depends on temperature, rotifer density and the feed type. In general, if microalgae are used as the sole feed source, the amount of aeration that must be provided is lower than if yeast are being fed. This is because algae, given sufficient light, produce oxygen, while yeast and associated bacteria consume it.

Fukusho (1989a) reported that at 20°C, both L- and S-type rotifers consume 7.07 x 10^{-5} ml oxygen/day. That rate increases to 10.04 x 10^{-5} ml/day at 25°C, and to 16.48 x 10^{-5} ml/day at 30°C. This is due to the rotifers' elevated metabolic rates at high temperatures. Thus, the potential for DO problems increases quickly as temperatures climb. Hirata and Yamasaki (1987) looked into the relationship between oxygen consumption and "food availability" in *B. plicatilis*. Consumption ranged from 1 - 7 ml /ind./hour (= 2.4 - 16.8 x 10^{-5} ml/rotifer/day), and was found to increase with increased feeding.

In terms of the amount of aeration needed to supply the necessary oxygen, Fushimi (1989) states that 60 - 100 liters of air/min/m³ must be provided to the latter stages of an *ω*-yeast-fed culture of rotifers. Yeast was supplied at the rate of 1.2 g/day/million rotifers; rotifer density was approximately 1,000 ind./ml. Finally, Hoff and Snell (1989) recommend "moderate to low aeration."
In Japan, aeration may be provided by several means, including blowers, air stones, air-lift pumps, and PVC piping into which holes have been drilled. The latter may be 13 mm in diameter with 1 - 2-mm holes spaced 50 cm apart. The size of the holes will determine bubble size that, in turn, influences the efficiency with which oxygen diffuses into the water. The piping is arranged on the tank bottom so as to provide an even supply of air (Yoshida 1989). The amount of air pumped through these pipes must be carefully regulated, however, to prevent the distribution of settled impurities.

Often, a combination of air stones and air-lift pumps or PVC piping is used (Yoshida 1989). In one example, an air-lift pump was used in a .5-m³ tank to provide 15 liters/min. aeration while an attached air stone gave an additional 12 - 13 liters/min. In another example, 9 - 12 spherical air stones, each providing 8 liters air/min., were the sole means of aeration for a 10-m³ rotifer culture tank.

### 3.5.3 Light

Indoors, rotifers are cultured either with constant or part-time illumination; 2,000 lux is within the range of intensities often used (Ito 1960, Hoff and Snell 1989). Hoff and Snell (1989) also recommend a light:dark cycle of 18:6 hours. However, according to Fukusho (1989a), for *B. plicatilis* "the optimum lighting condition has not yet been well defined." He indicates that the beneficial effect of light noted by many researchers may be an indirect one. That is, the light may promote rotifer growth by stimulating growth of photosynthetic bacteria and microalgae in the rearing tanks.

### 3.5.4 pH

*Brachionus plicatilis* can withstand a fairly wide pH range (5 - 10 has been reported), however the "optimum" pH range for culture is reported to be 5 - 9 by Fukusho (1989a), and 7.5 - 8.5 by Hoff and Snell (1989). This "optimum" may vary, however, depending on the type of feed (Furukawa and Hidaka 1973). Yu and Hirayama (1986) postulated that pH indirectly influences rotifer population growth by its effect on the amount of un-ionized ammonia nitrogen in the culture water. In their experiments, higher densities of rotifers were found at the relatively low pH range of 7.3 - 7.8. Finally, Epp and Winston (1978) found no relationship between population growth and pH within the range of 6.5 - 8.5.

Fushimi (1989) reports on an example of rotifer mass culture in which the pH was maintained at 8.0 - 8.2 with hydrochloric acid and sodium hydroxide. Yoshida (1989), by contrast, described a case where the pH could be maintained at 7.0 or higher as a result of aeration and the effects of *N. oculata*.

### 3.5.5 Temperature

The "optimum" temperature – the temperature at which *B. plicatilis* has the highest growth rate – will depend on the strain being cultured (Snell and Carrillo 1984, Fukusho 1989a). Fukusho (1989a) observed that rotifers "appear" in Japanese eel ponds in the spring of the year when water is 17 - 20°C. They propagate rapidly in summer and fall, and disappear in the winter when the water temperature falls to below 10°C, overwintering as resting eggs.

Theilacker and McMaster (1971) state that maximum reproduction occurs between 30° and 34°C. However, this is not necessarily the temperature range recommended for
culture. Because of the temperature requirements of the algae they were using as feed, these authors cultured *B. plicatilis* at 21 - 25°C. In general, the recommended temperature range for culturing rotifers is between 20 and 30°C (Table 12).

Fushimi (1989) describes the heating mechanisms employed at some fish farming centers in Japan. Oil boilers are used for large tanks, whereas electric heaters are sufficient for cultures in small tanks. Heating is only necessary in the fall and winter in temperate areas of Japan such as Kyushu and Shikoku, but heaters must be used year-round in cooler areas like Tohoku and Hokkaido (Fushimi 1989).

3.5.6 Salinity

*Brachionus plicatilis* is known for its ability to tolerate a wide range of salinities. As early as 1957, *B. plicatilis* was categorized by Ito as a brackishwater species, being found in eel ponds having a chlorinity of 0.232 - 12.928 ppt.

According to Hoff and Snell (1989), salinities ranging from 1 - 60 ppt may be tolerated by *B. plicatilis*, but 10 - 20 ppt will give the best growth. Salinity may have a large effect on reproductive rate. In fact, different strains and clones have been found to have different salinity optima (see, for example, Lubzens 1987). However, a culturist must also take into consideration the salinity at which the target species will be grown. For example, rotifers cultured at 20 ppt should be acclimated for a day at 30 ppt before being fed to fish larvae in 40 ppt seawater (Lubzens 1987). Otherwise the rotifers will be stressed and stop swimming (also see Lubzens et al. 1989, Hoff and Snell 1989). James and Abu Rezeq (1990), while discussing the salinity tolerances of S- and L-type rotifers in a chemostat culture system, observed that salinities of 20 and 30 ppt were conducive to S-type and L-type rotifer production, respectively.

Recall also that rotifer filtration rates, an indirect measure of feeding rates, vary with salinity and are reduced at high salinities (Hirayama and Ogawa 1972 cited in Lubzens 1987). Furthermore, James and Abu Rezeq (1990) also found that the ω3 HUFA content in L-type rotifers was highest for those cultured in 30 ppt water while 15 - 20 ppt water was correlated with higher ω3 HUFA content in the S-type rotifers tested. Finally, lorica lengths for both strains were significantly greater at 5 ppt than at 30 ppt (James and Abu Rezeq 1990).

3.5.7 Un-ionized ammonia

The concentration of un-ionized ammonia ([NH₃]) is largely a function of [NH₄], temperature, and pH. The published literature contains relatively few accounts of the effects on un-ionized ammonia on rotifer growth, although Coves et al. (1990) state that “high levels of ammonia ([NH₃] + [NH₄]) are generally found in Brachionus tanks” (p. 233), and that *Brachionus* sp. “appear to be fairly resistant to ammonia” (p. 233). Hira et al. and Nagata (1982, cited in Lubzens 1987) showed that *B. plicatilis* raised on *N. oculata* concret ammonia, urea and phosphates (1.41 ± 0.87 x 10⁻⁴ μg NH₄-N, 1.17 ± 1.31 μg urea-N, and 0.27 ± 0.29 x 10⁻⁸ μg PO₄-P/hour/ind., respectively). Yu and Hira (1986) found a correlation between high levels of un-ionized ammonia ([NH₃]) and low densities of rotifers in mass culture. They further investigated the acute and chronic effects of un-ionized ammonia on rotifers' intrinsic rate of population growth and reproduction rate, and implicated un-ionized ammonia in the “unexpected sudden decrease
or suppressed growth” of cultured rotifers. The authors concluded that un-ionized ammonia levels “can be one of the restrictive factors affecting the increase of the rotifers in mass production.” See Snell et al. 1987 for a discussion of the effects of un-ionized ammonia on *B. plicatilis* swimming activity. Finally, Hoff and Snell (1989) recommend that free ammonia concentrations not exceed 1 mg/liter.

### 3.5.8 Filtration of culture water

Debris that accumulates during the high-density culture of rotifers can be detrimental both to the health of rotifers and to the larvae that feed on the rotifers (Fushimi 1989). Removing this debris can enhance water quality in the rotifer and larval rearing tanks and also reduce clogging of nets during harvesting. Furthermore, the number of pathogenic bacteria introduced into finfish larval rearing tanks can also be reduced by filtration (Fushimi 1989).

Hirata (1979) cited a need to remove “wastes” by filtration when rotifer population densities reached 100 - 500 ind./ml. In 1974, he designed and tested a gravel filter to accomplish this task. Rotifer feces were said to have adhered to the gravel. Furthermore, Mori (1970, cited in Hirata 1979) cultured rotifers with a stone filter system, and Kureha et al. (1977, cited in Hirata 1979) used a plastic rough filter system and found the plastic to be superior to the gravel. Others pass their rotifer culture water through a separate, non-agitated settling basin by means of an air-lift pump. Feces settle on the bottom of the basin and are drained off daily (Yoneta et al. 1973, cited in Hirata 1979).

Fushimi (1989) places a high priority on the development of automatic filtering equipment, mostly to aid in the harvest of the rotifers. He states that it is difficult to remove impurities from the large tanks often used in semi-continuous culturing, but that two general types of filtering equipment are used in high-density batch cultures. One type of filter is inserted directly into the tank. The other consists of a separate filtering tank attached to a main culturing tank. An example of the former is the “saranlock” filtering mat (0.5 - 1-m²) used at the Hiroshima and Nagasaki stations. Mats are placed on the bottom of tanks and washed daily. The filter material in the mat must be removed and washed every one to three days (no further description is given). Use of these mats has been shown to decrease the number of *Vibrio* harvested with the *B. plicatilis* from $10^6$ to $10^4$.

Fushimi (1989) also reports that “kinran,” material that is also used as goldfish egg-laying nests, crushed oyster shells, plastic and vinilock filters (30 cm x 30 cm), can all be used to filter impurities from rotifer culture water. Finally, Yoshida (1989) provides a diagram of an “indirect” filtering device used in .5 m³ rotifer tanks. An air-lift pump delivers water through a 30-liter “polybucket” filter that has been filled with 6 kg of shells.

### 3.5.9 Monitoring

“To control the culture process one must know the condition of the *B. plicatilis* being cultured” (Fushimi 1989).

A time-consuming but important aspect of rotifer culture is the regular assessment of animal health and population density. Of course, other parameters such as pH, DO, temperature, salinity, food density and ammonia concentration should also be monitored and kept within predetermined levels. Conscientious management of culture conditions will help ensure rapid population growth and prevent culture collapse.
Most culturists monitor their rotifers at least once a day. The usual method is to remove a fixed quantity of culture water and observe it under a microscope. The number of rotifers is noted, as is their activity and the presence of any contaminants such as protozoa. It is important to have an accurate count so the amount of feed can be regulated and to determine the timing of harvest. Additionally, the rate of reproduction can be used as an indicator of culture health. Foscarini (1988) reported that rotifer culture densities are checked three to four times a day at fish farming centers in Japan.

Snell et al. (1987) proposed two means by which culturists could accurately assess the health of their rotifer cultures. They devised two techniques for determining whether rotifers were under stress. The first is a test whereby the swimming activity of a single rotifer is observed in a 1-ml chamber. A grid with 1-mm squares is placed under the chamber, and the number of squares entered is recorded for 30 seconds. The second technique simply calls for counting the number of eggs carried by each female. Decreases in swimming activity and egg ratios signalled that the rotifers were being stressed.

3.5.10 Harvesting

Unlike microalgae, rotifers are ordinarily separated from their culture medium and concentrated before being used as feed. This is mainly to reduce the amount of debris and dissolved organic matter, and the number of bacteria and other foreign organisms introduced into the larval rearing tanks. Dissolved organic matter, often found in high concentrations in dense rotifer cultures, is an ideal substrate for bacteria. To further reduce contamination, harvested rotifers are often rinsed thoroughly with clean seawater before they are offered to larvae.

Harvesting is accomplished by passing the culture water through fine nylon or silk netting. In Japan, they use different netting depending on whether they are harvesting L- or S-type rotifers; 80 - 100 μm mesh size is used to harvest L-type rotifers, 50 - 70 μm mesh netting is needed for S-types. They also have two different types of nets: “streamer” or “sleeve” nets and, nets that are suspended from a frame (Fushimi 1989). It's possible to lose a significant portion of rotifer production during harvesting. Not only does a certain percentage pass through the net, but some are also killed in the process.

According to Fushimi (1989), the harvesting process can be very time consuming; hence new, mechanized means of harvesting are being tested in Japan. One machine is simply a large container with netting. A motor moves the netting back and forth, thus reducing the chances of the net becoming blocked. More elaborate devices that rinse the rotifers in addition to separating them from the culture water have also been tested. For example, a revolving drum that has long bristles on its outer circumference has been tried with success. The drum rotates, trapping B. plicatilis in the bristles. After a time, the flow of culture water is stopped and the rotifers are rinsed off the bristles and subsequently concentrated. A culture with a density of 100 B. plicatilis/ml can be concentrated to 12,200/ml with a mortality rate of only 12.4% (Fushimi 1989).

3.5.11 Storage

There are several reasons one might want to store rotifers, including:

- maintenance and/or transport of stock cultures;
short-term preservation of harvested rotifers that are to be used as live feed; and

- longer-term preservation of dead, harvested rotifers that are to be used as feed.

Addressing the first of these purposes, Snell and Hoff (1988) reviewed the benefits of using resting eggs as inocula, thereby eliminating the need for traditional stock cultures. The eggs require no maintenance and can be stored for long periods of time; commercially produced cysts have a shelf life of approximately one year. Their cost in 1988 (from Florida Aqua Farms Inc.) was $6/1,000 cysts or $20/10,000 cysts (Snell and Hoff 1988).

Alternatively, adult rotifers can be maintained in much the same manner as algal stock cultures. The temperature is kept fairly low (18°C in an example given by Coves et al. 1990). This, in conjunction with small rations of feed, discourages rapid population growth. Finally, on a related topic, Dr. Esther Lubzens at the National Institute of Oceanography in Haifa is actively pursuing the possibility of cryopreservation as a means of preserving strains of rotifers, especially those not known to produce resting eggs, for extended periods of time (Lubzens 1987, Lubzens et al. 1989, also see Toledo and Kurokura 1990).

Second, the ability to store live rotifers after harvest would add flexibility to the production process. The negative impact of variations in daily output would be dampened and the chances of larval starvation decreased. However, rotifers are at their best nutritionally just after they have been fed. Since, storing live rotifers would most likely necessitate starving them, little progress has been made toward the short-term preservation of live rotifers. See Berghahn et al. 1989.

Alternatively, rotifers could be killed immediately after feeding by freezing or some other means, stored indefinitely, and fed to larvae in that state. The principal drawbacks to this approach are those that apply to feeding larvae any inert type of feed: 1) the feed will settle rapidly, fouling the water; and 2) many types of finfish larvae either will not eat dead rotifers or will do so at a rate significantly lower than normal. While Fontaine and Revera (1980) reported that all larval and postlarval forms of crustaceans accepted frozen rotifers and appeared to do as well on live rotifers, this was not the case for finfish larvae, which they observed to eat only live rotifers. According to Foscarini (1988), however, red sea bream larvae raised in Japan are fed partially with frozen rotifers 15 - 20 days after hatching.

### 3.6 Design Examples

Some examples of the types of system designs used to grow large quantities of rotifers will be presented. They should give the reader a feel for the different techniques being used around the world to grow *B. plicatilis*. Section 3.6.1 describes semi-continuous and batch culture as it is practiced in Japan and elsewhere, while Section 3.6.2 has some examples of continuous systems.

#### 3.6.1 Semi-continuous and batch systems

Coves et al. (1990) describe the semi-continuous culture of *B. plicatilis* in 0.5 - 2-m³ cylindrical polyester tanks (see Fig. 8) at IFREMER centers in France. Production usually occurs at 25 - 27°C in temperature-controlled rooms. *Platymonas (= Tetraselmis) suecica* is the primary feed for stock cultures, but *Pavlova lutheri* and *Chlorella* sp. may be used in addition to *T. suecica* during full-scale production. The culturists’ reper-
toire also includes baker's yeast, "yeast + fish oil emulsion" (analogous to ω-yeast), and an artificial diet known as "PM1," composed of spray dried Spirulina and Toprina, corn starch, cod liver oil, methionine, D-glucosamine hydrochloride, choline chloride, and a vitamin premix. Production rates and food conversion ratios vary depending on the feed type(s) and amount.

Trotta (1983) designed a batch rotifer culture system that utilized 50-liter, vertical polyethylene bags — this is a modification of the system described in Section 2.5.2. Rotifers were added to dense cultures of T. suecica, grown until most of the food was eaten and then harvested. Densities of 400 ind./ml were achieved with this closed, indoor system. Using inocula grown in continuous systems (described in Trotta 1980), 56 bags produced more than 160 million ind./day.

As was previously stated, it is difficult to make generalizations about the rotifer culture techniques and production system designs used in Japan. It seems as though every fish farming center has a different setup — tanks which are different shapes and sizes, some indoors, some outdoors, different harvesting and feeding regimes, etc. A few specific examples will be given here.

Kafuku and Ikenoue (1983) give an example of a simple rotifer culture system that is equipped with an air-lift pump for aeration and a filtering apparatus. A somewhat more complex system is that described in Kuronuma and Fukusho (1984). This 60-m³ square tank is equipped with a filtering device, air stones, an overflow tube, a feeding tank (for baker's yeast) and a collection tank. The overflow pipe carries rotifers to the harvest tank. Both "Chlorella" (probably Nannochloropsis oculata) and baker's yeast are used as feed.

Figures 9A-C show the Kagoshima Prefecture Fish Farming Center's larval rearing system (Fukusho 1989c, Fushimi 1989). It was designed to be "fully automated and economical." A 1,296-m² room on the second floor houses six square 100-m³ rotifer production tanks (2.5 m deep, each with a 1-m³ filter attachment) and a 50-m³ reserve culture tank. The first floor contains a 10-m³ B. plicatilis collection tank and two 25-m³ tanks that hold concentrated N. oculata. Nannochloropsis oculata production takes place outdoors in ten, 300-m³ tanks (22 m x 14 m x 1 m). Figures 9

![Figure 8. Vessel used for rotifer culture at IFREMER centres in France (from Coves et al. 1990).](image)

Original published by Technique et Documentation. Reprinted with permission from Ellis Horwood, Ltd.
B and C from Fukusho (1989c) illustrate the transfer of *N. oculata* to the rotifer tanks and that of rotifers to the fish larval tanks. Rotifer production is semi-continuous, with a daily harvest rate of approximately 30%. In one case, from the end of April to the beginning of June, a total of 40.15 ± 19.11 billion rotifers were produced. The average density was 94.1 ± 29.3 ind./ml (Fushimi 1989).

Small tanks, however, may also be used. Figure 10 illustrates a highly automated system designed to grow *B. plicatilis* semi-continuously in ~.5-m³ tanks. Photosynthetic bacteria and baker’s yeast are used as feed. In one case, daily production averaged 290 million rotifers, and in 30 days totaled 8.567 billion (Fushimi 1989). Another system employing ~.5-m³ tanks is described in Yoshida (1989) (Fig. 11). Four circular polycarbonate tanks, each in its own water bath are housed in a temperature-controlled room (air and water temperature: 27 - 32°C) at the Miyazaki Prefecture Experimental Aquaculture Center. Note the air-lift pump (40 mm diameter), air stones and filtering bucket. Both *N. oculata* and baker’s yeast were used as feed. In three to four days, the density increased from 100 to 400 S-type rotifers/ml. Each tank was reported to yield 3 x 10⁸ ind./m³, using 0.4

---


A: Reprinted with permission of Koseisha-Koseikaku, Tokyo.

B and C: Reprinted with permission of Fuji Technology, Ltd.
m$^3$ of \textit{N. oculata} and 0.9 kg of baker's yeast in the process.

Finally, an example of large-scale batch culture is described in Fushimi (1989). This system is used at the Hiroshima Prefecture Fish Farming Center. \textit{Brachionus plicatilis} are cultured outdoors in eight, unheated 150-m$^3$ tanks. Each rotifer culture period lasts five days, and S- and L-type rotifers are produced alternately. \textit{Nannochloropsis oculata} and \(\omega\)-yeast were used as feed. Tanks averaged 18.25 billion and 24.1 billion \textit{B. plicatilis}/day for L- and S-type rotifers, respectively.

\subsection{Continuous systems}

The chemostat culture systems described in James and Abu Rezeq (1989a and b) and their yields were discussed in Section 3.4.1. Figure 12 is a schematic diagram of the rotifer chemostat designed by the authors. Algae (\textit{Nannochloropsis} strain MFD-2) was chemostatically cultured in 200-liter capacity translucent vertical tubes (30 cm diameter), while the rotifer culture apparatus consisted of a 1-m$^3$ chemostat unit and a 500-liter mixing reactor from which the algae were metered.
into the rotifer tank. Temperature was 25°C. The results obtained from this small system were promising, and a scaled-up version may prove to be suitable for commercial operations.

**LITERATURE CITED**


Hirata, H. and W. Nagata. 1982. Excretion rates and excreted compounds of the rotifer Brachionus


Ito, T. 1957b. Studies on the "mitukawari" in eel-culture ponds. VI. The relations between the growth of Brachionus plicatilis and the quantity of phytoplankton. Report of Faculty of Fisheries. 2 (3): 502-516.


