Part II
Review of the Research Status of Zooplankton Production In Japan

Kunihiko Fukusho
National Research Institute of Aquaculture
Nansei, Mie, 516-01
JAPAN

ABSTRACT

A short history of marine zooplankton culture research and the status of their mass culture technology are introduced in the present paper. Copepods and cladocerans are excellent feed for larvae and juveniles of marine finfish and various studies have been conducted to establish the technology for their mass culture. However, only the copepod, Tigropus japonicus can be successfully mass cultured. This has been accomplished by combining its culture with that of Brachionus plicatilis and using baker’s yeast or yeast as feed. Two to three kg (wet weight) of T. japonicus can be harvested daily and the maximum density attained is 10,100 - 22,048 ind./liter in outdoor 200-m³ tanks. However, the production cost is fairly high and microparticulated diets of high quality which approximate the size of copepods (1 mm) have been developed. Therefore, no facility produces copepods intensively.

The rotifer, B. plicatilis is the most important zooplankton for the mass production of marine finfish and prawns. At present, it is possible to produce 1.2 trillion rotifers (nearly 2.5 tons wet weight) in one season at a fish farming station (April to June). The developmental phases of rotifer culture technology might be categorized as follows: 1) introduction as a feed organism, 2) development of mass culture technology, 3) evaluation and improvement of nutritional value, 4) accumulation of biological and genetic information, 5) investigation into the nutritional requirements of rotifers, 6) environmental control of rotifer culture tanks and 7) automation and mechanization of mass culture.

COPEPODS AND CLADOCERANS

Copepods and cladocerans are excellent feed for both cultured and wild organisms (Hirano and Oshima 1963, Shirotta 1975), and various kinds of studies have been carried out on these zooplankton. Most of the investigations were related to ecology, physiology, and life history until 1960-1965 in Japan, although a few studies on their culture were attempted (Matsudaira 1957). Studies on the mass culture of copepods and cladocerans were begun around 1965 to satisfy the requirement for live feeds for the early stages of marine finfish larviculture (Hirano 1966, 1984; Iwaski and Kamiya 1977).

The Fisheries Agency, the Ministry of Agriculture, Forestry, and Fisheries (MAFF),

*Present address: Nikko Laboratory, National Research Institute of Aquaculture, Chugushi, Nikko, Tochigi, 321-16, JAPAN
designed and organized a research project on the mass culture of copepods and cladocerans. Five prefectural institutes of fisheries and four universities joined the project which began in 1972 and terminated in 1978. The results of the project's research were compiled into a book (Anraku 1979) including a bibliography of 175 papers, which detailed basic research on the mass culture of copepods and cladocerans. The project's goal was to mass culture each species at a density of 5,000 ind./liter, 50 times their density in nature. The promising species for mass culture are listed in Table 1. Omori (1973) also listed the names of copepods found to be promising for mass culture. The most successful species during the course of the project was a copepod, *Tigriopus japonicus*. The maximum density attained was 3,000 ind./liter in combination culture with the rotifer, *Brachionus plicatilis*. The copepods and rotifers were cultured in 200-m³ outdoor tanks and baker's yeast was the feed.

The Plankton Society of Japan and the Oceanography Society of Japan held a symposium, "Plankton Symposium in Cultivation and Mass Culture of Zooplankton" in 1973. Twelve papers were presented and the proceedings was published as the Bull. Plankton Soc. Japan, Vol. 20, No. 1 in 1973. In that volume, Fujita (1973) and Kitajima (1973) emphasized the importance of mass culture and reviewed the technology for mass zooplankton culture.


Of all the species of copepods investigated, only *T. japonicus* can be cultured in large quantities (Figs. 1 and 2). For *T. japonicus*, combined culture with rotifers is an effective technique. Outdoor large tanks (200 m³) are employed with a special yeast (omega yeast) as feed. Two to three kg (wet weight) are harvested daily and the maximum density attained is 10,100 - 22,048 ind./liter (Fukusho 1980). However, the amount of yeast used to produce 1 kg of *T. japonicus* is 5.1 - 7.5 kg, and the production cost is fairly high. Therefore, no facility produces the copepod intensively, although they attempt a thinning harvest of the *T. japonicus* which multiply in rotifer tanks and feed them to larvae and juveniles of marine finfish.

Cladocerans, like rotifers, reproduce by parthenogenesis only when the environment is favorable, and multiply in a short period of time. High hopes are thus placed on the invention of mass culture techniques for marine cladocerans and information on their biology has been accumulated (Iwasaki et al. 1977, Onbe 1974, Takami and Iwasaki 1978). How-

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However, no technique for their mass culture has been found yet. This is in contrast to *Moina macrocorpa*, whose production is well established. This species is fed to marine finfish larvae and juveniles (Suizu 1987).

Technology for the mass production of rotifers is almost established, and microparticulate diets have been developed as a substitute for 1-mm sized live food. Therefore, the role of copepods and cladocerans is actually becoming less important for the mass production of marine finfish.

**TINTINNID CILIATES**

Tintinnid ciliates, which are consumed by larval fish in the wild, are candidates for mass production, and biological information has been accumulated (Taniuchi 1978). To date, tintinnids are not, however, used intensively at mass production facilities in Japan.

**ROTIFERS, BRACHIONUS P LICATILIS**

Great progress in production technology for marine finfish fry has been based upon the successful introduction of the rotifer, *Brachionus plicatilis* O.F. Müller, as a food organism. It was in 1965 that the rotifer was first utilized to feed red sea bream, *Pagrus major*, larvae and its high food value was confirmed. At present, it is possible to produce 1.2 trillion rotifers (nearly 2.5 tons in wet weight) for the
production of red sea bream fry and other marine finfish at a fish farming center during a mass production season (April to June).

The history of the development of mass production technology may be found in previous papers (Fujita 1983, Fukusho 1983, 1989a, 1989b, Hirata 1979). The seven developmental phases might be categorized as follows.

**Introduction as a Feed Organism**

The rotifer was originally recognized as a noxious zooplankton which often reached high densities in eel culture ponds and caused the sudden death of eels due to rapid consumption of dissolved oxygen (Ito 1960). However, the rotifer was later cultured and utilized to feed larvae of Ayu, *Plecoglossus altivelis*, and red sea bream (Hirata 1965).

**Development of Mass Culture Technology**

The rotifer was initially cultured in small indoor tanks (smaller than 1 m$^3$) at high densities. Larger tanks (nearly 100 m$^3$), with various kinds of feeds, are presently used. The following feeds or combinations of feeds are commonly used at fish farming centers: 1) *Nannochloropsis oculata*, which used to be called “*Chlorella sp.*,” 2) baker’s yeast, 3) ω yeast, 4) *Tetraselmis tetrahele*, 5) a combination of 1 and 2, and 6) a combination of 1 and 3. The developmental history and a classification of the culture methods adopted in Japan are precisely explained in earlier papers (Fukusho 1989a, 1989b).

**Evaluation and Improvement of Nutritional Value**

Baker’s yeast was introduced as feed for cultured rotifers in 1967 (Hirata and Mori 1967). The introduction allowed the mass production of rotifers in larger tanks. However, the nutritional value of the rotifers fed baker’s yeast was low, therefore we began to analyze rotifers raised on various feeds. Highly unsaturated fatty acids (ω3 HUFAs), especially 20:5ω3, was shown to be essential for survival and growth of marine finfish larvae (Watanabe et al. 1983). Methods to improve the nutritional value of rotifers were also established (Watanabe et al. 1983). A special yeast, “ω yeast,” which contains sufficient levels of ω3 HUFAs, was developed and has been intensively used at mass production facilities (Imada et al. 1979).

**Accumulation of Biological and Genetic Information**

Information on the life history, physiology, mode of reproduction, ecology, taxonomy and genetics of *B. plicatilis* has been accumulated. A symposium, “The Rotifer *Brachionus plicatilis* — Biology and Mass Culture” was held in Fukuyama, Hiroshima. The proceedings was compiled in 1983 (Japan Sci. Soc. Fish. ed., Koseisha-Koseikaku, Tokyo, 161 pp.).

The Fisheries Agency, MAFF, organized a research project on the biology and mass production of rotifers. Research was conducted from 1980 through 1986. The seven prefectural institutes of fisheries (Aomori, Kanagawa, Ishikawa, Hiroshima, Nagasaki, Kumamoto, and Okinawa) joined the project. As a first step, a bibliography containing 393 papers was published. The
results of the project were finally compiled in 1989 as a book: "A Live Feed — the Rotifer, Brachionus plicatilis" (Fukusho and Hira- yama eds., Koseisha-Koseikaku, Tokyo, 240 pp.) with five chapters and references to 439 papers.

Several monographs on rotifer biology were also published (Hirano 1987, Nagata 1985).

Nutritional Requirements of Rotifers

Precise and up-to-date information is presented by Professor Hirayama in this volume (Hirayama and Satuito, this volume).

Environmental Control in Rotifer Culture Tanks

Dr. Maeda presents a paper on micro-fauna in rotifer tanks and discusses biological control of the environmental conditions (Maeda and Hino, this volume).

Automation and Mechanization for Mass Culture

Mr. Morizane presents a paper on mechanization and automation to save labor in rotifer production (Morizane, this volume). The Ehime Prefectural Fish Farming Center is used as an example.

REFERENCES


Improving the Design of Mass Culture Systems for the Rotifer, *Brachionus plicatilis*

Terry W. Snell
Department of Biology
University of Tampa
Tampa, FL 33606
U.S.A.

**ABSTRACT**

Larviculture of many marine finfish is dependent upon rotifers as a larval feed. To be useful in aquaculture, sufficient quantities of high quality rotifer biomass must be produced cost-effectively and reliably. Several aspects of mass culture design provide opportunities for improving rotifer production. Comparison of batch and continuous cultures suggests that chemostats offer the best chance for reliable production of highly nutritious rotifer biomass. Improvements in three areas of rotifer culture could yield substantial progress in biomass production. The causes of mass culture instability and sudden crashes need to be identified. Nitrogen waste excretion and un-ionized ammonia toxicity probably play an important role. Early detection of stress in mass cultures by monitoring reproductive traits, swimming activity, and enzyme inhibition could be useful in reducing the probability of a crash and lessening its impact. Better rotifer feeds are becoming available in the form of high quality dried algae and improved digestibility yeast. Finally, techniques for improving culture management including faster upscaling, optimizing harvest rates, identifying better strains, and manipulating the bacterial composition of mass cultures are discussed.

**INTRODUCTION**

Rotifer culture has become a critical element in the larval rearing of many marine finfish (Hirata 1979, Kafuku and Ikenoue 1983, Lubzens 1987, Lubzens et al. 1989, Fukusho 1989a, b). As a result, a great deal of effort has been directed toward defining requirements for rotifer mass production. For rotifer biomass to be useful in aquaculture, sufficient quantities must be produced cost-effectively, the biomass must be of high nutritional quality, and its production must be reliable. Unpredictable availability of rotifer biomass is currently one of the major factors limiting fry production (Hirayama 1987, Fukusho 1989a). Several aspects of mass culture design provide opportunities for improving the quantity, quality and reliability of rotifer biomass production for fish larviculture.

There are a number of considerations in optimizing rotifer culture system design. These include culture procedures like methods

*Present address: School of Biology, Georgia Institute of Technology, Atlanta, GA 30332-0230
for maintaining stock cultures of algae and rotifers, procedures for upscaling, culture medium, tank size, tank shape, aeration level, inoculation density, food type, feeding schedule, harvest method, harvest schedule, and post-harvest enrichment.

Design optimization attempts to minimize rotifer transfers, labor input, production time, and costs. At the same time, biomass production, nutritional quality, and production reliability are maximized. Selection of an optimal design for rotifer culture can simplify biomass production and reduce technician errors, whereas suboptimal design can increase costs, decrease reliability and render an aquaculture venture economically infeasible. My primary objective in this paper is to examine several aspects of rotifer mass culture and identify how they could be incorporated in improving production system design.

A wide variety of culture systems have been employed for rotifer culture including batch, semi-continuous and continuous cultures (Lubzens 1987). Nearly every aquaculture facility has its own variation on one of these themes which is tailored to site-specific materials and conditions. While this ad hoc approach has produced great variety, it has not resulted in an optimal design based on well characterized principles of rotifer culture. After 25 years of practical experience with commercial rotifer culture, it is time to systematically identify critical elements of rotifer biology and how they can be exploited in an optimally designed culture system.

Optimal rotifer culture design is based upon a thorough understanding of the interaction of construction materials and their configuration with biological factors. The best materials are identified by their durability, effectiveness and low costs. The critical biological factors are identified by a thorough understanding of rotifer response to mass culture environments. This requires that the physiological responses of cultured animals be well characterized. The size, population growth rate, tolerance of crowding, excretion products and rates, and response to low food levels of a cultured strain will influence system design. These characteristics will also indicate which water quality parameters need to be monitored and to what level of precision.

**TYPE OF CULTURE SYSTEM**

**Batch vs. Continuous**

Batch culture is an extensive method in which a culture is inoculated and allowed a growth period before the entire volume or a portion thereof is harvested. This approach has been the most reliable because of its technical simplicity and built-in redundancy, but it is the least efficient. Continuous mass cultures are smaller than batch cultures and more intensively managed. The most advanced design of continuous culture is the chemostat, which has been applied to aquaculture by James and his colleagues (James et al. 1988, James and Abu-Rezeq 1989a, b). In this approach, algae and yeast are supplied continuously at a predetermined rate. The culture is diluted by a certain volume each day and this volume is harvested to obtain rotifer biomass. Chemostat mass cultures have yielded the greatest rotifer biomass production per unit of effort thus far recorded in aquaculture.

**Batch Culture Example**

An example of the current methodology for large scale batch culture as practiced in Japan is provided by Fukusho (1989a). He
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describes a typical Japanese hatchery using 100-m³ tanks for rotifer culture and a diet of microalgae, usually *Nannochloropsis*, and baker’s yeast. After a growth period of a few weeks, rotifer densities of about 100/ml of the L-type *B. plicatilis* are reached and the mass culture is then harvested for several days. Six 100-m³ tanks hold a standing crop of rotifers from which 1 - 2 billion can be harvested daily. Using this system, 600 m³ of water must be managed to produce about one billion rotifers per day.

Chemostat Culture Example

An example of the chemostat continuous culture methodology is provided by James and Abu-Rezeq (1989a) (Fig. 1). The standard culture volume in their system is 1 m³. A diet of *Nannochloropsis* MFD-2 strain is provided at 20 million cells/ml and baker’s yeast at 0.3 - 0.4 g/million rotifers/day. The algae are cultured in a separate chemostat at about 50 million cells/ml (James et al. 1988) and diluted to the appropriate density in a mixing reactor before introduction into the rotifer tank. A dilution rate of 0.5/day is used for the L-type strain which allows 500 liters to be harvested from a 1-m³ chemostat each day. The average production from this system is 187 million rotifers/day. The system has been run for several months sustaining these yields without major technical difficulty (James, pers. comm. 1990).

Production from 1-m³ chemostats is currently sufficient to meet the rotifer needs of most small- to medium-sized hatcheries. For example, producing one billion rotifers per day requires six to seven 1-m³ chemostats with the yields cited above. This means that 6 - 7 m³ of water can be managed to produce one billion rotifers per day, which is about 100-fold less than the batch culture method (Fukusho 1989a). Intensively managing much smaller volumes of water results in substantial labor and cost savings for the chemostat method. In addition, the nutritional quality of the rotifiers can be more tightly controlled. James and Abu-Rezeq (1989a) reported that

![Figure 1. System design for the mass culture of rotifers in chemostats (from James and Abu-Rezeq 1988b).
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the ω3 fatty acid composition of chemostat raised rotifers provided adequate quantities of essential fatty acids for marine fish larvae without further enrichment.

Very large marine fish hatcheries require the production of about 20 billion rotifers/day (Fushimi 1989). The chemostat method could supply this biomass of rotifers, but it would take 100 - 140 1-m³ tanks. To meet the rotifer requirements of very large hatcheries, 10-m³ chemostats would be preferable so that 12 - 14 could yield 20 billion rotifers/day. Ten-m³ chemostats represent a 10-fold upscaling of existing systems. However, the basic principles of chemostats are well developed, so a modest amount of research should provide the information necessary to accomplish this task. James and Abu-Rezeq (1989b) reported no differences in production when upscaling 100-liter chemostats to 1 m³. In my view, the application of knowledge of rotifer physiology to chemostat culture can save hatchery space, time, materials, costs, and improve consistency. This approach, therefore, offers the best hope for reliably supplying adequate quantities of high quality rotifer biomass for larviculture.

**BIOLOGICAL FACTORS INFLUENCING ROTIFER CULTURE DESIGN**

**Introduction**

Mass culture techniques for rotifers have improved substantially over the past 25 years (Lubzens 1987, 1989), yet rotifer biomass production remains a primary limiting factor in seedling production of many marine finfish (Hirata et al. 1983). There are three areas of research which could yield considerable improvements. The first is identification of the reasons for rotifer mass culture instability and sudden crashes. The second is development of improved, cost-effective feeds which could replace live algae. The third is development of more effective culture management procedures to maximize rotifer production.

**Mass Culture Instability**

Unexpected population crashes occur occasionally in rotifer mass cultures with catastrophic consequences for hatcheries. After a crash, the amount of rotifer biomass is greatly diminished and it is of such poor quality that it is unlikely to sustain fish larvae. Reduced growth and high larval mortality result which can cause serious economic losses for hatcheries. Several attempts have been made to identify the causes for rotifer culture crashes and water quality degradation has become a primary focus of concern (Hirata et al. 1983). There are two major contributing factors to water quality degradation in rotifer cultures: bacterial decomposition of uneaten food (Hirayama 1987) and metabolic wastes excreted by the rotifers (Hirata and Nagata 1982, Hirayama 1990). Both problems are exaggerated in high density cultures by the use of inert feeds like yeasts and the packing of large numbers of animals into relatively small volumes of water.

Nitrogen wastes are among the most important in suppressing rotifer production, especially un-ionized ammonia (NH₃) (Yu and Hirayama 1986). Un-ionized ammonia is toxic to most aquatic animals in the low parts per million range (Russo 1985). It is produced as a by-product of bacterial decomposition of organic material. In addition, rotifers excrete half of their dissolved nitrogen wastes as ammonium (NH₄⁺) (Hirata and Nagata 1982). Depending on temperature, salinity, and pH,
a proportion of this ammonium is converted to toxic un-ionized ammonia.

The significance of un-ionized ammonia as a limiting factor in rotifer cultures is suggested by the fact that rotifers excrete the other half of their nitrogenous wastes as urea (Hirata and Nagata 1982). This is very unusual for an aquatic animal, but would be predicted for populations adapted for growth at high population densities. Under these conditions, ammonia toxicity could be reduced by excreting a portion of nitrogenous wastes as urea which is much less toxic. Urea excretion could therefore be interpreted as an adaptation for minimizing exposure of rotifer populations to ammonia toxicity. Since nitrogen metabolism is believed to play a central role in rotifer population growth, a complete nitrogen budget for B. plicatilis has been described (Nagata 1989).

Mass culture instability likely results from physiological stress of rotifers, resulting in a rapid decline in reproductive rate (Hirayama 1990). Early detection of stress in mass cultures could be important to aquaculturists so that preventative action can be taken. Several things could be done to minimize the likelihood of a crash and lessen its impact. These include adjusting the feeding rates or changing food type to reduce the amount of organic material available for bacterial degradation, lowering culture pH and temperature shifting the chemical equilibrium of ammonia away from the un-ionized form, making water exchanges to dilute toxic metabolites, and preparing back-up cultures to shorten the time of upscaling should re-starting of the culture be required.

Identification of the physiological responses to stress in rotifer mass cultures has proven useful in their management. The effects of un-ionized ammonia on the reproductive performance of rotifers have been characterized. Yu and Hirayama (1986) found that the effective concentration of un-ionized ammonia (EC50) that reduced population growth rate (r) to 50% of the control was 13.2 mg/liter and 7.8 mg/liter for net reproduction (R0). Un-ionized ammonia significantly depressed r and R0 at concentrations as low as 2.1 mg/liter. These authors also found a correlation between culture density and un-ionized ammonia concentration. Rotifer population densities less than 100/ml were observed only when un-ionized ammonia was less than 0.5 mg/liter. Densities of less than 60/ml were observed when un-ionized ammonia concentrations exceeded 1.4 mg/liter. When un-ionized ammonia concentrations peaked at 3.6 mg/liter, rotifer density was less than 10/ml. Snell et al. (1987) described the effects of un-ionized ammonia on the egg ratio of B. plicatilis, reporting an EC50 of 7.3 mg/liter. These results demonstrate that un-ionized ammonia concentrations in rotifer mass cultures can reach levels that significantly impair production.

Besides these reproductive characteristics, swimming activity has been shown to be a good indicator of un-ionized ammonia toxicity. An EC50 of 2.3 mg/liter was found for un-ionized ammonia and a 17% depression of swimming activity was detectable at concentrations as low as 0.32 mg/liter (Snell et al. 1987). Swimming activity, therefore, is more sensitive to un-ionized ammonia than the reproductive traits thus far examined and swimming activity can be assayed in a few minutes. Rotifer swimming quickly reflects changes in the status of mass cultures and its observation is an effective early indicator of stress. The difficulty of using swimming activity is that it is time consuming to quantify and requires direct observations which are
labor intensive. Other stress indicators more amenable to automation would be useful in managing rotifer mass cultures.

One approach that holds promise as a stress indicator is enzyme inhibition assays. These are general indicators of stress that have been effectively employed in aquatic toxicology. Several toxicants have been shown to inhibit digestive enzymes like lipase, acylase and chymotrypsin in *B. plicatilis* (Snell and Moffat, in preparation). Enzyme activity is detected *in vivo* using fluorescent probes which are quantified with a fluorometer. The protocol is to remove 20 - 40 rotifers from mass culture and expose them to a fluorescent labelled substrate for 5 - 10 minutes. The animals are then washed, placed into a cuvette, and their fluorescence determined. The intensity of fluorescence is directly proportional to the level of enzyme activity in a dose-dependent manner. We believe that enzyme inhibition assays based on fluorescence are promising indicators of stress in rotifer mass cultures and could be useful as culture management tools.

While un-ionized ammonia is probably a major contributor to rotifer mass culture instability, other compounds may also be important. Nitrite (NO₂⁻) is another nitrogen compound commonly toxic to aquatic animals at relatively low concentrations (Russo 1985). Lubzens (1987) suggested that nitrite toxicity for *B. plicatilis* occurs at concentrations of 90 - 140 mg/liter. In contrast, Groeneeweg and Schluter (1981) reported no nitrite toxicity for *B. rubens* at concentrations of 10 - 20 mg/liter.

Further investigation of these and other compounds is warranted to develop tools for monitoring their activity. Identification of chemical compounds limiting mass culture growth will simplify water quality monitoring and make it more effective. The more thorough our understanding of rotifer population growth and its requirements, the better aquaculturists will be at producing high quality feed for their fish larvae.

Cost-Effective Feeds for Rotifer Mass Culture

The most expensive part of rotifer culture is feed. Fushimi (1989) estimated the cost of rotifer biomass production using large-scale batch methods to be US$ 4.50/million rotifers. Of this, 72% was for feed, 50% for live algae and 22% for yeast. In addition to the cost of live algae, there are several other reasons for replacing all or part of the live algae in the rotifer diet. It is sometimes difficult to match algal production with rotifer consumption. Variations in the quality of algal cells is common, leading to changes in rotifer yield. Contamination of algal mass cultures is a persistent problem. Maintenance of productive algal mass cultures is not technically simple and therefore prone to technican error. For these reasons, aquaculturists have examined a wide variety of inert feeds for their ability to support rotifer growth. A second major line of investigation has developed to identify treatments which can improve the digestibility of yeast.

Replacements for live algae

Dried algae

Recent advances in microalgae mass culture and biomass preservation have introduced new options for rotifer culture. Spray drying of algal biomass has proven effective in commercial production. This technique yields a dried product that retains much of the nutritional quality of live cells (Biedenbach et al. 1990, Snell et al. 1990). Although spray dried algae now is commercially available, its cur-
rent cost of about US$ 170/kg may be too expensive for large-scale rotifer culture.

An example of how dried algae can be used for rotifer culture was provided by Snell et al. (1990). The alga, Nannochloropsis salina, was cultured by Earthrise Farms (California, California), the biomass harvested, and preserved by various methods. The effectiveness of this algal product to support rotifer population growth was tested using a standardized population growth bioassay with B. plicatilis. Algal biomass was preserved using three types of freezing, saline, and two types of spray drying. Rotifer population growth rates (r) on the frozen and saline preserved algae were only 35% that of live cells. Spray dried N. salina yielded growth rates which were about 70% those of live cells, despite the fact that the spray drying process was not fully optimized.

Side-by-side comparisons were made between live N. salina cells and spray dried N. salina, dried Tetraselmis suecica (Cell Systems Ltd., Cambridge, England), Microfeast L-10 yeast (Provesta, Bartlesville, Oklahoma), Culture Selco (Artemia Systems, Belgium), and 7B yeast (Fleischmann, New York) (Fig. 2). Live N. salina was provided at 5 × 10^6 cells/ml, dried N. salina and T. suecica at 100 µg/ml, and yeast at 80 µg/ml. Both dried algal products yielded better rotifer growth than the yeasts, but only about 70% as high as live N. salina cells. Therefore, with current production techniques, rotifer diets composed exclusively of dried algae cannot match the biomass yield of live algae diets. Dried algae, however, can replace 80-90% of live cells without productivity loss (Snell et al. 1990). I expect that the quality of dried algae will continue to improve over the next few years and its cost to decline.

Yeast

Yeast as a sole diet for rotifer mass culture has a number of problems. Yeast-fed rotifers are not nutritionally adequate for most marine fish larvae (Watanabe et al. 1983). Furthermore, yeast-fed rotifer cultures are prone to instability and precipitous crashes in population density (Hirayama 1987). While inadequate as a sole diet, several authors have reported that yeast can replace a substantial portion of live algae cells without significantly diminishing rotifer biomass production. An illustration of yeast replacement of live algae can be seen in Figure 3. The yeast used in these experiments was Microfeast L-10, but other types of yeast worked as well. Yeast and algae were provided at 90 µg/ml and a standard population growth bioassay using B. plicatilis was conducted. For Nannochloropsis, 90% of live algae cells could be replaced with yeast without significantly reducing population

![Figure 2. A comparison of rotifer growth on diets of dried algae and yeast. Live NS - live Nannochloropsis salina cells, dried NS - spray dried N. salina cells, dried Tet - dried Tetraselmis suecica cells, MF - Microfeast L-10, AS - Culture Selco, 7B - Fleischmann's yeast. r is population growth rate in offspring/female/day.](image-url)
growth rate. Yeast as a sole diet yielded a growth rate only about 25% that of live cells. For *Isochrysis*, 90% of live algae cells also could be replaced without significantly diminishing growth rate, but this figure was only 80% for *Tetraselmis*.

**Improving yeast diets**

The inadequacy of yeast as a sole diet for *B. plicatilis* could be explained by its poor nutritional quality and its lack of digestibility. Hirayama and Funamoto (1983) found that sterile yeast was unable to support rotifer population growth. The presence of bacteria was required, perhaps to supply needed vitamins or growth factors. An alternative explanation is that bacteria "pre-condition" yeast cells rendering them more susceptible to rotifer digestive enzymes. Perhaps the variability in growth of yeast-fed rotifers is related to the type of bacterial populations that develop in culture tanks. Certain types of bacteria are more likely to facilitate rotifer digestion than others.

Recent work has suggested that untreated baker's yeast is relatively indigestible for *Artemia*, but chemical treatment can improve its digestibility (Coutteau et al. 1990). Treatment with 2% 2-mercaptoethanol cleaves disulfide linkages among proteins in yeast cell walls, making them more permeable and susceptible to cleavage by digestive enzymes. Growth of *Artemia* nauplii on diets of thiol-treated yeast was compared to growth on the green alga *Dunaliella tertiolecta*. Nauplii length after eight days was not significantly different on diets of treated yeast or algae. Survival, however, was 68% and 95% for yeast and algae, respectively. The factors determining digestibility are not well understood, but it is clear that some aquacultural feeds are poorly digested by filter feeding invertebrates. Additional research is needed on cost-effective treatments that can improve yeast digestibility.

![Graph](image)

**Figure 3.** Replacement of live algae by yeast. The X-axis is the percent algae of a 100 μg dry weight/ml diet. The remaining portion of the diet is Microfeast L-10 yeast. r is population growth rate in offspring/female/day. Vertical lines indicate standard error.

**Improving Culture Management**

As new information becomes available, aquaculturists should be prepared to modify their culture procedures to take advantage of improved methods. Several ideas recently have appeared in the literature which could improve rotifer culture management and should be considered for incorporation into standard practices.

**Faster upscaling**

The cost of keeping stock cultures for upscaling to mass cultures is considerable. Stock culture maintenance requires technician time and results in errors which are magnified during upscaling. Upscaling from test tubes to
10,000 liters takes weeks, so matching live feed production with larval requirements is not simple. An alternative to maintaining rotifer stock cultures is to inoculate mass cultures by hatching resting cysts. Animals hatched from cysts are asexual females which begin reproducing with doubling times of 16 - 20 hours at 25°C. Rotifer cyst hatching can be exploited by aquaculturists so that large inocula are available without the need for stock culture maintenance (Snell and Hoff 1988).

**Optimizing harvest rates**

Application of fisheries management principles to harvesting semi-continuous rotifer cultures suggests certain guidelines for producing maximum yield. A computer simulation of rotifer population growth illustrated how different harvest rates affect yield (Snell and Hoff 1989). They showed that over a 30-day culture period, maximum yield is obtained by starting the harvest early and harvesting no more than 30% of the population daily (Fig. 4). Beginning harvests later and harvesting a larger proportion of the culture resulted in lower yields. These guidelines provide estimates of optimal harvest rates and reduce the amount of trial and error required to determine how to best manage rotifer cultures.

**Improving rotifer strains for aquaculture**

Many investigations of phenotypic traits in *B. plicatilis* have revealed abundant genetic variation in natural populations. As of yet, there has been no systematic effort to selectively breed particular characteristics into aquacultured strains. A variety of rotifer strains are utilized at different hatcheries, depending on local conditions. These strains are not readily available from a central source, so they are not widely shared among aquaculturists. Reports of larger and smaller strains of *B. plicatilis* in natural populations have appeared in the literature (e.g. Koste 1980). No effort has been organized, however, to collect these strains and domesticate them. Great potential for exploiting rotifers in aquaculture remains untapped because genetic characteristics have yet to be manipulated. This approach has been extremely successful in other areas of agriculture.

**Manipulating bacterial composition of mass cultures**

Recent work has shown that bacteria added to rotifer cultures are capable of enhancing yields. Yu et al. (1989, 1990) found that B12-producing bacteria introduced into mass cultures could greatly enhance rotifer production. Gatesoupe et al. (1989) described the effect of two food additives containing live lactic bacteria on rotifer production. One additive increased rotifer production 23%. The other additive did not increase production, but improved the dietary value of rotifers for Japanese flounder larvae. Manipulation of bacterial populations in rotifer mass cultures.

![Figure 4. Optimizing harvest rates. Harvest begins on day 0-25 and continues daily until day 30 when the culture is terminated. Percent harvest is the portion of the population removed each day. The total biomass harvested is the sum of the rotifers removed each day times 3 µg/rotifer.](image-url)
has the potential to substantially increase rotifer production, but more research is necessary before this potential can be fully realized.

CONCLUSIONS

- Rotifer production is currently a limiting component of marine fish larviculture.
- Improvements in mass culture design have the potential to substantially increase the quality, quantity and reliability of rotifer biomass production.
- Continuous culture using chemostats offers the most promise for improving rotifer mass culture performance.
- Mass culture instability results from physiological stress in high density mass cultures. Understanding the causes of this stress and developing techniques for monitoring it are important for increasing rotifer production.
- The replacement of a large portion of live algae in rotifer diets with inert feeds is now possible. Advances in the commercial production of dried algae and a reduction in its cost will be of great benefit to aquaculturists raising rotifers. Techniques for improving the digestibility of yeast could make this feed far more effective for rotifer mass culture.
- Methods for improving rotifer culture management like faster upscaling, optimizing harvest rates, selecting genetically superior strains, and manipulating bacterial populations in mass cultures can considerably increase rotifer production.

LITERATURE CITED


Practical Approach to High Density Production of the Rotifer, *Brachionus plicatilis*

M. Refik Orhun, Stephen R. Johnson, Donald B. Kent
Hubbs-Sea World Research Institute
1700 S. Shores Road
San Diego, CA 92109
U.S.A.

and

Richard F. Ford
San Diego State University
Department of Biology
5178 College Avenue
San Diego, CA 92182
U.S.A.

ABSTRACT

A practical approach to the intensive, high density culture of the rotifer *Brachionus plicatilis* using two low-cost 8.5-m$^3$ tanks is presented. Partial automation of harvesting (mean = 4.6%/day) and water exchanges (20% of pool volume/day) as well as waste removal (350 liters every other day) is described. Average rotifer densities were maintained at 540 rotifers/ml over culture periods of 8 - 60 days. A total of $113.3 \times 10^9$ rotifers were produced in 17 separate trials during 288 days of culture, yielding an overall average production rate of 25.7 rotifers/ml/day. Activated baker’s yeast was the primary food source with supplemental additions of microalgae at a rate of 50 liters/m$^3$ of rotifer culture.

INTRODUCTION

Interest in the culture of ornamental and consumable marine fish species for commercial aquaculture, bioassay work and fisheries enhancement has resulted in the need to develop methods for the intensive culture of zooplankton. The culture of the rotifer *Brachionus plicatilis* is a vital part of many experimental and large-scale marine fish hatchery programs. Various larval fish feeding methods have been reviewed by Fukusho (1983) and Lubzens (1987). Although they pointed out that culturing rotifers solely on microalgae would be costly and require extensive algal culture space, James et al. (1983) reported that supplemental additions of microalgae to rotifer cultures enhances production. This enhance-
ment may result from the removal of waste products from the rotifer culture system by the algae with a resulting increase in production rates (Hirata et al. 1983). This paper deals with the practical application of experimental results toward the design, management and operation of a high density rotifer production system.

METHODS

Rotifer production reported here supported the intensive culture of larval white seabass (Atractoscion nobilis) at the Hubbs-Sea World Research Institute/San Diego State University Marine Laboratory in San Diego, California from May through December of 1988. A total of 17 production trials in two pools (8.5 m$^3$ each) were carried out during this time period.

The culture system was located in a 12 x 9-m polyethylene-covered greenhouse where the two 3.65-m diameter aboveground pools (12 x 3-ft. swimming pools) were employed as the rotifer production tanks. These consisted of a steel reinforcing wall and a polyvinyl plastic liner. Two identical pools filled to 3,500 liters were used for microalgae culture and were also located in the same greenhouse.

Seawater from Mission Bay was supplied by a centrifugal pump that pressurized the water through a sand and gravel filter and two serial cotton filter cartridges (5.0- and 1.0-$\mu$m pore size). Water temperatures in the rotifer cultures fluctuated from 23 to 28$^\circ$C, and in the winter months introduction of heated seawater and use of electric immersion heaters were required to maintain this temperature range. Vigorous aeration was supplied to the rotifer pools by a 2-hp blower to maintain the particulate matter in suspension and the dissolved oxygen levels above 4.0 ppm.

A 450-ml sample was obtained in the morning from each culture tank, allowed to settle for 10 minutes, and then decanted. The rotifers were then counted using a Coulter Particle Counter (Model ZM). A 400-$\mu$m aperture was used and the instrument was adjusted to count particles with volumes equivalent to spherical particles with diameters 100 - 200 $\mu$m.

An initial inoculum of Salton Sea strain rotifers was obtained in 1982 from the Southwest Fisheries Center in La Jolla. Relatively small pools, 1.0 m$^3$, were used to provide inocula for the 8.5-m$^3$ production pools. Many production trials were stocked with the entire harvest available from previous trials. Cultures were usually inoculated into the production pools at densities greater than 350 rotifers/ml, with only three of the 17 trials stocked at less than 100 rotifers/ml. These cultures were usually maintained at densities greater than 500 rotifers/ml.

Rotifers were fed baker's yeast at feeding rates decreasing relative to increasing rotifer density (i.e., less than 150 rotifers/ml fed at 1.0 g yeast/10$^6$ rotifers; 150 - 250/ml at 0.7 g/10$^6$; more than 250/ml at 0.4 g/10$^6$). The dry yeast was vigorously mixed with 10 liters of water and introduced to the culture tank in two equal feedings at noon and midnight. The midnight feeding was accomplished using a timer-operated valve that allowed the prepared yeast suspension to overflow into the rotifer culture tanks.

Turbidity was used as an indicator of food conditions in the culture tanks. High turbidity in the morning indicated that the yeast offered at the last feeding was not entirely consumed. Overfeeding with yeast has been observed to result in a marked decline in
population densities of rotifer cultures maintained on yeast (Hirayama 1987). Clear water indicated either underfeeding or the consumption of the yeast by proliferating ciliate populations (various free-swimming and stalked species) coexisting in the rotifer production system.

Menhaden oil (Zapata Haynie Corp., Reedville, Virginia) was emulsified in warm water with either raw egg yolk or soy bean lecithin and then added directly to the culture tanks at rate of 3% of the weight of the yeast, "direct method" (Watanabe et al. 1983). The vitamins E and C were also added to the cultures at 0.06% and 1.0% of the weight of the yeast fed, respectively.

Microalgae, 300 - 400 liters/day of Tetraselmis suecica, Nannochloropsis sp., or Isochrysis galbana (Tahitian strain), were supplied to each production tank (respective average densities were $0.5 \times 10^6$, $10.0 \times 10^6$ and $1.0 \times 10^6$ cells/ml). The transfer from the algae culture tanks into the rotifer tanks was made with a 0.5-hp magnetic drive pump (140 liters/min.).

Approximately 20% (1,700 liters) of the culture water was replaced daily with filtered seawater. For harvesting, the aeration was turned off for one hour to allow debris to settle. A floating, subsurface intake head (see Fig. 1) placed in the tank and attached to a common laundry pump (6-pen impeller) moved rotifers suspended in the water column into a harvester tank at a rate of 80 liters/min.

The harvester tank (see Fig. 1) consists of a rectangular fiberglass tank ($91 \times 61 \times 56$ cm) divided into two compartments (2:1) by a slanted, screened (70-µm mesh) partition. The rotifer slurry is pumped into the larger of the two compartments. The rotifers are concentrated as they are retained in the larger compartment by the divider screen while water flows to waste through a three-piece, standpipe drain (50.8 mm diameter) in the smaller compartment. An aeration collar fabricated from PVC pipe (12.7 mm diameter) prevented the screen from clogging.

After the transfer of rotifers was completed, the standpipe was shortened while the rotifers were rinsed thoroughly with filtered seawater. The harvester was angled with the input side down, so that when the standpipe was removed, several liters of water with concentrated rotifers remained which could then be drained into a 10-liter container. Rotifers were either returned to the production tanks or sampled, counted, and transferred to the larval feeder tanks for further nutritional enhancement.

The rotifer harvest was automated using timers programmed to turn off the aeration and then activate the harvesting pump one hour later. The floating intake was placed in the tank at the end of the previous day. The harvesting pump was automatically turned off after a predetermined period of time and resulting

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Figure 1. Harvesting tank.
volume of water, leaving the rotifers ready for manual rinsing.

Every other day the sides and bottom of each tank were scrubbed and a two-blade propeller attached to a 0.5-hp motor was used to create a vortex. The resulting circular motion of the water consolidated detritus in the center of the pool for efficient removal with a magnetic drive pump attached to a clear PVC intake (see Fig. 1), which allowed observation of the debris. Approximately 350 liters of water with suspended debris was removed with this procedure. The tanks were subsequently refilled with algae and seawater to 8.5-m$^3$ volume.

RESULTS

Table 1 is a summary of the data collected from the 17 separate production trials performed on the two available pools. The average culture duration of a pool was 30.2 days, ranging from 8 - 60 days. The total days of culture for the two pools combined was 513 days. The pools were maintained at average densities of 540 rotifers/ml ranging from 391 - 644 rotifers/ml. The average daily standing crop of rotifers in the pools was 4.47 x 10$^9$ individuals. The average percentage of female rotifers carrying eggs was 32.3% (range 24.9 - 39.6%).

Rotifers were harvested only when there was a need to feed fish larvae, consequently they were not removed every day. The pools were harvested on an average of 20.6 days (total of 350 harvesting days for the two pools combined) or for 68.3% of their culture duration. The calculated average daily harvest per pool was 202 x 10$^6$ rotifers (range 59 - 383 x 10$^6$ rotifers) or 4.6%/day (range 1.5 - 10.2%). Average actual daily harvest was 305 x 10$^6$ rotifers per pool or 6.8%/day ranging from 2.2 - 12.0%. Figure 2 shows the daily standing crop of rotifers and the numbers of rotifers harvested in one representative trial (pool 13) from which rotifers were harvested for more than 80% of the culture duration.

Total rotifer production $P (= N_t - N_0 +$ total harvest; Gatesoupe and Robin 1982, James et al. 1983) averaged 210 x 10$^6$ day/pool. Average production was 25.7 rotifers/ml/day (range: -6.0 - 47.1 rotifers/ml, see Table 1). A total of 113 x 10$^9$ rotifers was produced in the 288 culture days. This represents a harvesting rate of 393 x 10$^6$ rotifers per day for both pools combined (17.0 m$^3$).

During the 288 days, the total dry weight production of rotifers was estimated to be 30.6 kg calculated from an observed average dry weight of 0.27 µg/rotifer (SD = 0.019 µg). Totals of 826 kg of yeast and 163 m$^3$ of algae were fed to the rotifers. Assuming a dry weight algal density to water volume coefficient of between 0.1 and 0.2 g/liter, the approximate percentage of algae in the rotifer diet was 2 to 4%, and the calculated overall gross conver-
### Table 1. Summary of the data from two 8.5 m$^3$ rotifer production pools over 288 days.

<table>
<thead>
<tr>
<th>Pool No.</th>
<th>Mean rotifer density ± SD (#/ml)</th>
<th>Initial rotifer density (#/ml)</th>
<th>Culture duration /No. of harvests (days)</th>
<th>Average percent daily harvest</th>
<th>Average daily harvest (x 10$^6$)</th>
<th>Average rotifer production (#/day/ml)</th>
<th>Percentage of egg carrying females (%)</th>
<th>Average amount of algae fed (liters/day)</th>
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<tr>
<td>1</td>
<td>542 ± 275</td>
<td>39</td>
<td>56/26</td>
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<td>217</td>
<td>29.5</td>
<td>39.6</td>
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<td>29/5</td>
<td>1.5</td>
<td>59</td>
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<td>34.7</td>
<td>321</td>
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<tr>
<td>3</td>
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<td>4</td>
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<td>163,096$^2$</td>
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</table>

$^1$Rotifers were added from harvests of pool 16.
$^2$Total algae fed for all pools during the 288 day culture period.

Expansion efficiency [total dry weight rotifers produced/(total dry weight yeast + total dry weight algae)] was approximately 3.6%.

**DISCUSSION**

As stated, the rotifers were harvested only on demand and therefore potential production from this system can be assumed to be higher than the observed average harvest of 4.6%/day. According to Snell and Hoff (1989), a harvesting rate of rotifer cultures of about 20 - 30% per day should be optimal. As reported by James et al. (1983), supplementing rotifer cultures with 0.05 m$^3$ of algae/m$^3$ culture enhances rotifer production. In addition to enhancing rotifer reproduction, algal supplements may also improve water quality by removing rotifer metabolites. Removal of waste products is mandatory in high density production systems as build up of metabolites
is most likely a major cause of both sudden standing crop decline (culture crashes) and diminished growth rates.

The main advantage of a high density production system is that it allows the culturists to withstand sudden losses or decreases in rotifer production. The primary disadvantage is that most of the food is used for maintenance of the population. As a result, the conversion efficiency based on the amount of rotifers produced is relatively low.

The automation of the harvest and water exchanges is greatly eased by the hardness of the rotifer and its ability to withstand the physical stress of water velocities at pumping rates of 5-8 m³/hour.

Facultative pathogenic bacteria such as Vibrio sp. may reside in high density cultures and special attention must be given to tank hygiene in order to prevent the transfer of disease bacteria via the rotifers to the fish larvae. We observed the transmission of Vibrio sp. via the rotifers to larval fish and experimented with antibiotic treatments as described by Gatesoupe (1982 and 1987) and Tabata (1982 and pers. comm.). But, we found that thorough washing with seawater as described by Foscarini (1988) is the preferred procedure as excessive treatments with antibiotics is costly and may affect larval fish development.

Acknowledgments

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