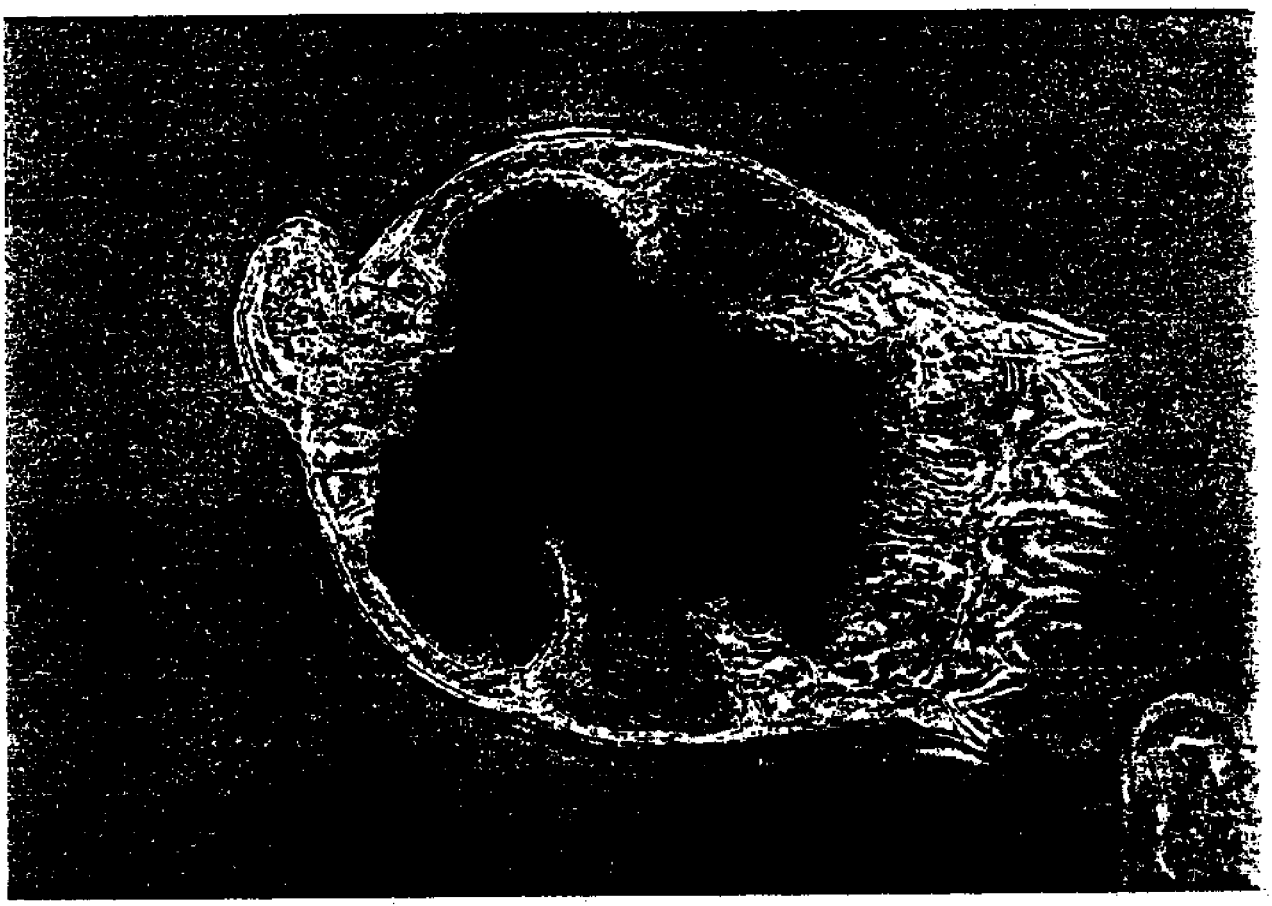
Rotifer and Microalgae Culture Systems

Proceedings of a U.S.–Asia Workshop



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Cover photo of *Brachionus plicatilis* by Vernon Sato.

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PREFACE

The Asian Interchange Program (AIP) was founded at The Oceanic Institute to facilitate the exchange of applied aquacultural information and technology between the United States and Asia. This program is funded by the National Oceanic and Atmospheric Administration through a grant administered by the University of Hawaii Sea Grant College Program (Grant # NA90AA-D-SG483). Each year the AIP staff organizes a workshop, bringing together researchers, commercial producers and extension specialists from a particular field of aquaculture for a direct, reciprocal exchange of information. Many of the Asian participants in this year's workshop are from Japan, the country in which large-scale live feeds production is most advanced. Other participants were selected from South Korea, the People's Republic of China, Thailand, Taiwan, Singapore and the United States.

Microalgae and/or rotifers are indispensable as feed in today's intensive marine finfish, crustacean, and bivalve hatcheries. Although most of the major biological issues related to culturing microalgae and rotifers have been resolved, the task of growing enough feed to supply the needs of a commercial bivalve, shrimp or finfish hatchery can be unreliable and unnecessarily expensive. The workshop focused on the techniques and hardware used to culture rotifers and microalgae in Asia and the United States. This volume summarizes the findings of the workshop and also makes some generalizations about how live feeds production systems are currently designed and how they might be improved to alleviate the problems of unreliability and high cost.

The Workshop

Twenty-one participants attended AIP's second annual workshop at the East-West Center in Honolulu, Hawaii (Fig. 1). Participants met for four days, from January 28 - 31, 1991. Researchers and producers presented papers during the morning sessions; afternoons were spent in smaller discussion groups. In the discussion groups, participants described their culture situations; provided general descriptions of their systems, culturing procedures and culture conditions; talked about the issues of reliability and cost; and estimated their production levels and the resources needed to conduct operations. Japanese, Chinese and Korean interpreters were present to facilitate communications; simultaneous interpretation services were provided during the paper presentations and final discussion group.

Because of the promising results obtained from a rotifer chemostat developed in Kuwait by Charles James and its relevance to the present topic, information about the experimental-scale system appears in the rotifer discussion group summaries. Terry Snell provided details of the chemostat and its operation during the discussion group sessions, and Charles James subsequently reviewed the data sheets and provided supplemental information.

Workshop Participants. *Bottom* (left to right): Geon Gil Pi, Sung Bum Hur, Cheng-Sheng Lee, Ray Gladue, Mi-Seon Park, Huei-Meei Su, Masachika Maeda, Tsuneo Morizane; *Top*: Xing-Qian Chen, Clyde Tamaru, Vernon Sato, Kevan Main, Masaaki Fukuda, Jim Donaldson, Connie Arnold, Don Kent, Kelly Rusch, Wendy Fulks, Adithya Padala, Hassanai Kongkeo, Terry Snell, Jiao-Fen Chen, Kunihiko Fukusho, Masanori Okauchi, Kazutsugu Hirayama and Lian Chuan Lim.

The Proceedings

This volume is divided into three sections: the background review, contributed papers presented at the workshop, and discussion group summaries. In addition there are appendices containing the names and addresses of workshop participants and a workshop agenda.

The background review and discussion group summaries were written by the editors. The background review was distributed to participants prior to the workshop. It provided everyone with a common baseline of information related to the design and operations of live feeds production systems and also served as a starting point for discussions and presentations to be made at the meeting.

Papers appear in the order in which they were presented at the workshop. Kunihiko Fukusho presented "A review of the research status of zooplankton production in Japan." His talk covered the history and culture techniques used to produce copepods, cladocerans, ciliates and rotifers as feed for marine finfish larvae in Japan. His presentation was followed by "Improving the design of mass culture systems for the rotifer *Brachionus plicatilis*." This paper was given by Terry Snell and covered a variety of topics including batch vs. continuous culture, mass culture instability, cost-effective feeds and improving culture management. Don Kent of Hubbs-Sea World Research Institute in San Diego described the production of rotifers for larval white seabass in 8,500-liter pools. Rotifers were cultured at an average density of 540/ml, activated baker's yeast served as the primary feed type and harvesting was partially automated.

Citing culture crashes and high labor requirements as the most important issues related to producing rotifers, Tsuneo Morizane outlined microalgae and rotifer culture procedures used in Japan and also discussed automation and mechanization at existing and planned facilities. A great deal of progress has been made in the area of automatic rotifer harvesting/concentrating equipment. Attention was then switched to Hawaii for Clyde Tamaru's talk that focused on the culture of rotifers for striped mullet at The Oceanic Institute. The authors (Clyde Tamaru, Cheng-Sheng Lee and Harry Ako) explored the nutritional value of rotifers cultured on a variety of feeds and also experimented with different mullet and rotifer densities. Their work has resulted in extremely high survival rates for this economically valuable fish. Jiao-Fen Chen was the next speaker. In her paper titled "Commercial production of microalgae and rotifers in China," she detailed techniques used in China's commercial hatcheries. Sung-Ji Industry is a young Korean company founded to produce marine finfish seed. Mr. Geon Gil Pi represented Sung-Ji at the workshop and gave a paper in which he detailed his company's rotifer culture procedures. One of the largest obstacles the company faces is producing enough *Nannochloropsis* to feed its rotifers. In Texas, rotifers are produced on both commercial and experimental scales to feed red drum larvae. Connie Arnold, in a paper co-authored with Joan Holt, summarized four culture techniques used at the University of Texas Mariculture Program using the following feeds either singly or in combination: baker's yeast, emulsified oil, *Isochrysis galbana*, and *Tetraselmis chuii*.

Recently there has been a trend toward viewing rotifer cultures from an ecosystem perspective; attention is being paid to the lesser known residents of tanks, bacteria and protozoa, in an attempt to understand the mechanisms allowing for coexistence. This may eventually allow

culturists to manipulate the ecosystem to promote rotifer growth. Masachika Maeda and Akinori Hino's contribution to the workshop represents an important step toward understanding the complex rotifer-protozoan-bacterial dynamics of rotifer cultures. I-Chiu Liao, Mao-Sen Su and Huei-Meei Su contributed a detailed overview of rotifer and microalgal production in Taiwan. They have provided production data, culture histories and culture techniques for the production of live feeds in Taiwanese crustacean, mollusc and finfish hatcheries.

Kazutsugu Hirayama is one of the foremost researchers in the field of rotifer nutrition. Along with Cyril Glenn Satuito, he documented experiments in which the nutritional value of baker's yeast was improved with the addition of different vitamin combinations and/or squid liver oil. They also included data on vitamin B_{12}-producing bacteria and the amino acid contents of three varieties of ω3 HUFA-supplemented baker's yeast ("ω-yeast"). In a related paper, Sung Bum Hur presented the results of his research on the optimum species of phytoplankton for rotifer culture in warm and cold seasons in South Korea. He investigated not only the growth dynamics of different microalgal species at different temperatures, but also the nutritional value of the different species for rotifers and the value of the rotifers fed to Japanese flounder larvae.

Many of the hatcheries in Thailand are small "backyard" operations producing *Penaeus monodon* seed. Hassanai Kongkeo addressed the conference on the live feeds culture techniques used in these hatcheries and described how the government assists farmers by providing free *Chaetoceros calcitrans* and *Skeletonema costatum* starter cultures and by simplifying mass culture techniques. The situation in the People's Republic of China is somewhat different. Xing Qian Chen and Li Juan Long summarized the research status of live feeds production in China and also described commercial rotifer and microalgae production techniques. Mollusc and shrimp hatcheries in China usually produce microalgae and rotifers extensively. In many instances, mixed cultures of microalgae are grown by fertilizing filtered seawater. This culture is then harvested to feed larvae, or rotifers may be added directly to the medium at a density of 1 - 5/liter. Lian Chuan Lim addressed the conference next, providing a detailed summary of the production of green mussel eggs and larvae, microalgae and rotifers in Singapore. Green mussel eggs and larvae are needed for first feeding greasy grouper and golden snapper larvae, diatoms are widely cultured for *Penaeus merguiensis* and *P. monodon*, *Tetraselmis tetrathele* and *Nannochloropsis oculata* are produced for rotifers, and the rotifers, in turn, are given to Asian seabass fry.

The focus of the workshop then shifted to expert systems technology and its application to aquaculture. Adithya Padala, in a paper co-authored with Stephen Zilber, began by explaining the expert system concept and presented an example whereby such a system was designed to automatically and intelligently monitor an intensive culture of tilapia. Following this, Jim Donaldson described the "Commercial production of microalgae at Coast Oyster Company," the world's largest oyster company. The Quilcene, Washington hatchery produces *Skeletonema* sp., *Chaetoceros calcitrans* and *Thalassiosira pseudonana*. Cultures are upscaled to 20,000-liter tanks that are inside a greenhouse and provided with special lighting.

In the third paper to explicitly treat automation in the culture of live feeds, Kelly Rusch discussed a bench-scale, micro-computer automated chemostat that produced *Chlorella minutissima* in three 20-gallon growth chambers. Overall performance was good over a five-month

period, and the researchers at Louisiana State University's Civil Engineering Aquatic Systems Laboratory are currently developing a production-scale automated chemostat.

In Japan, microalgae belonging to the genera *Chaetoceros*, *Isochrysis*, *Pavlova*, *Nannochloropsis* and *Tetraselmis*, as well as a variety of benthic diatoms are cultured to feed a variety of molluscs, crustaceans and other invertebrates. In his presentation, Masonori Okauchi detailed the uses of these species and then provided an overview of mass microalgal production techniques used in Japan. In another algae-related paper, Vernon Sato described the phytoplankton production system used at The Oceanic Institute to support finfish larval rearing research. *Nannochloropsis oculata* and *Tetraselmis tetrathele* are batch cultured year-round in outdoor 25,000-liter tanks to feed rotifers.

Ray Gladue's paper represented a novel approach to solving the problem of high algae production costs. He gave an overview of heterotrophic microalgal production techniques and provided some examples in which this technology has been successful in producing large volumes of relatively inexpensive microalgae. The nutritional value of such algae for rotifers and other cultured animals is still being investigated, however. Finally, Mi Seon Park explained the culture techniques of microalgae and rotifers in South Korea and described how they are used to feed bivalves, crustaceans, and a variety of finfish species. South Korea presently has 88 hatcheries; 78 are private and 10 are owned and operated by the government.

Acknowledgements

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We are indebted to all of the participants for providing papers and the information in the discussion group summaries. Furthermore, Kunihiko Fukusho, I-Chiu Liao, Ruiyu Liu, Byung-Ha Park and Cheng-Sheng Lee helped in the identification and selection of participants. Paul Bienfang, Charles Brownell, Eirik Duerr, Charles James, Cheng-Sheng Lee, Miguel Olaizola, Kelly Rusch, Vernon Sato, Terry Snell and Clyde Tamaru reviewed portions of the text. Rick Mulcahy translated the Japanese material cited in the Background Review and Hongja Harrison translated one of the workshop papers.

We would also like to acknowledge Patti Killelea-Almonte, Stephanie Frank, Esma Harper and Pat Pablo for proofreading and editorial assistance and Julie Burkhead and Michelle Lewis-Mumford for administrative support. Finally, we must mention the interpreters, Stella Guillory, Hongja Harrison, Joanne Kija Koo, Lynette Shi, Taeko Wellington and Masako Yamatani, who persistently facilitated communications for the duration of the workshop.

Part I

The Design and Operation of Commercial-Scale Live Feeds Production Systems

1.0 INTRODUCTION

Progress in the commercial culture of many marine animals is currently being hampered by an inconsistent supply of seed. This is due, in part, to the difficulty and expense associated with securing large, predictable quantities of high-quality live feeds, especially microalgae and rotifers (Droop 1975, Horstmann 1985). Live animals and plants are used as feed for many types of commercially important aquatic organisms, and although research continues, inert feeds have not fully replaced live feeds.

In particular, **microalgae** are of great importance to the commercial culture of bivalves (larvae, juveniles and adults), crustaceans (mostly the early larval stages), zooplankton, and to a lesser degree, finfish (larvae and/or adults, see Horstmann 1985, De Pauw and Pruder 1986) (Table 1). Primary producers such as algae form the base of the trophic pyramid, and as such constitute the largest link in the food chain. **Rotifers**, specifically *Brachionus plicatilis* Müller, are indispensable in the intensive culture of marine larval finfish and additionally serve as feed for a number of other taxa. This species' small size, euryhaline nature, rapid reproduction rate, and ability to be grown in dense cultures make it extremely valuable as a first feed for fish.

The large-scale, intensive production of microalgae and rotifers suffers from two major problems: it is expensive and often unreliable. To improve the cost-effectiveness and dependability of live feeds production, The Oceanic Institute's Asian Interchange Program has chosen a broad-based approach. Rather than concentrate solely on small, well-defined problems such as preventing culture contamination or finding more cost-effective nutrient media, we have decided to step back and view the live feeds production system as a whole. In this manner we hope to 1) discern ways in which systems can be improved through better design, and 2) encourage researchers and producers to focus on design as a means of

Table 1. The use of microalgae and rotifers to feed commercially important species.

	Bivalves			Crustaceans			Marine Finfish		
	Larvae	Juveniles	Adults	Larvae	Juveniles	Adults	Larvae	Juveniles	Adults
Microalgae	X	X	X	X			+	+	+
Rotifers				+			X		

X = an important food source

\+ = used for the culture of some species

solving problems. **Design optimization**, then is our approach to tackling the problems of cost-effectiveness and reliability in live feeds production.

This paper will:

- identify the major issues surrounding the intensive, large-scale culture of microalgae and rotifers;
- present examples of different system designs that have been used to produce live feeds; and
- identify important knowledge gaps in live feeds system design research.

This is accomplished through a compilation and evaluation of information from the published literature, including some translated from Japanese.

There are two major sections, one addressing the culture of microalgae, the other rotifers. Each section discusses the uses of microalgae or rotifers and their problems, principles (including upscaling, population dynamics, culture types, and species/strains cultured) and general requirements (equipment and supplies, culture medium/nutrients, etc.). Examples of some system designs conclude Sections 2.0 and 3.0, respectively.

1.1 Definitions

If this paper is to serve as a common baseline, it is important to begin by clearly explaining the terms to be used. The concept of **design** includes the material, shape, size and arrangement of essential system components (see Kinne 1977, p. 580). The "essential system components" we will be primarily concerned with are 1) culture enclosures and 2) devices such as harvesters which have been used to automate live feeds production. The type of culture practiced (e.g., batch, semi-continuous, continuous) also features prominently in our concept of "design." As we shall see, decisions about design cannot be made without consideration of a number of other factors, including site selection, species selection (both **target species** and **feeds species**), production goals, and economics.

Inasmuch as **operations** are inseparable from design considerations, and because there is a need for more detailed descriptions of operating procedures for live feeds production facilities (e.g., Fox 1983), they will be considered also. The term **commercial-scale** will be used interchangeably with **large-scale** to describe a range of aquacultural systems large enough to be economically feasible (see Table 7 in Huguenin and Colt 1986). The size of such an operation can vary immensely depending upon the species and life stage cultured. For example, a system set up to supply phytoplankton to a bivalve nursery would need to be much larger than one constructed for a bivalve or shrimp hatchery. The efficiency of a particular operation will also affect its size. Finally, a system large enough to be economically feasible in one country or economic setting may be much too small to be operated profitably in another. Hence, "commercial-scale" is a somewhat nebulous term, used to denote a system that is larger than experimental- and pilot-scale (for a given target species and life stage).

To define our topic further, the live feeds culture we are talking about is **intensive** in the sense of De Pauw et al. (1984). That is, we have tried to limit discussion to those systems in which 1) aquaculturists exercise a relatively great degree of control over the species cultured, and 2) the feeds species are cultured separately from the target species.

As we have stated, this paper considers two categories of live feeds: microalgae and

rotifers. **Microalgae** (used here interchangeably with the terms **phytoplankton** and **algae**), in the strict sense, are unicellular eukaryotic algae. The term may also be stretched to include some of the cyanobacteria such as *Spirulina*. Finally, the most commonly cultured species of **rotifer** for feed is ***Brachionus plicatilis***, and unless otherwise noted, discussion will be limited to this species.

1.2 Designing Live Feeds Production Systems

Several authors have recognized the need for more design-related studies. For example, Persoone and Claus (1980) noted that

> "the bioengineering of the mass culturing of marine algae . . . is still in its infancy. Comparative research is needed between different technologies to determine their respective productions and their respective costs" (p. 282, also see Terry and Raymond 1985).

Much the same could be said for large-scale rotifer production systems. In short, design optimization studies of commercial-scale live feeds systems are rare.

Contributing to the problem is the fact that designs used for experimental- and pilot-scale units are usually inappropriate for larger systems because of

- logistical problems:

> "The approaches and techniques that are practical on a small experimental basis may not be rational or even reasonable at much larger scales and the converse is also true. These scale-up problems can arise in bulk handling of materials such as animals, water and feeds, which in laboratory situations are easily transported and held in small containers. Performing necessary life support functions can also become complicated, since individually monitoring the distribution of water, providing food and checking general health for large numbers of animals each day quickly becomes prohibitive. Even the routine maintenance and cleaning of culture units, while trivial in the laboratory, becomes a major problem with increased scale" (Huguenin and Colt 1986 p. 510),

- the prohibitive cost of materials (Ukeles 1977, Persoone and Claus 1980); and/or
- the relationship of surface area to volume (i.e., a vessel of a given shape and size cannot simply be scaled up because the ratio of surface area to volume will decrease; this is especially important for algal cultures that need to be illuminated).

This problem of scale has been noted by a number of authors (e.g., Persoone et al. 1980, De Pauw and Pruder 1986, De Pauw and Persoone 1988, Hoff and Snell 1989).

The task of designing a large-scale live feeds production system is a complex one (Goldman 1979b, Terry and Raymond 1985). Size, number, shape and arrangement of culture enclosures will depend on the type of culture to be practiced (e.g., batch or semi-continuous), site characteristics (such as illumination, water quality and temperature), the target species cultured (volume of feed needed, specific nutritional requirements, etc.), the feed species to be produced and production goals. These considerations, in turn, are interdependent (see Fig. 1). Finally, economic factors are usually an overriding concern. Hence, the sections to follow on microalgae and rotifer culture system design consider the general principles and requirements for producing these organisms prior to any detailed discussion of design.

2.0 MICROALGAL PRODUCTION SYSTEMS

Since the 1940s there has been interest in the mass production of microalgae. Microal-

gae have been cultured as a source of oils, polysaccharides, fine chemicals and oxygen. They have been exploited for soil conditioning, eutrophication control, waste-water treatment, and consumption by humans, livestock and aquatic organisms (see Goldman 1979a for an excellent discussion of the history and applications of microalgal culture; also see Kinne 1977). At one time, mass culture of algae was seen as a solution to the world protein shortage and numerous other global problems (Burlew 1953). In most cases, however, large-scale microalgal production has been found to be economically infeasible due, in large part, to the expense and difficulties associated with separation of the cells from the culture medium and processing (drying, freezing, etc.; Droop 1975, Becker and Venkataraman 1980, Venkataraman et el. 1980).

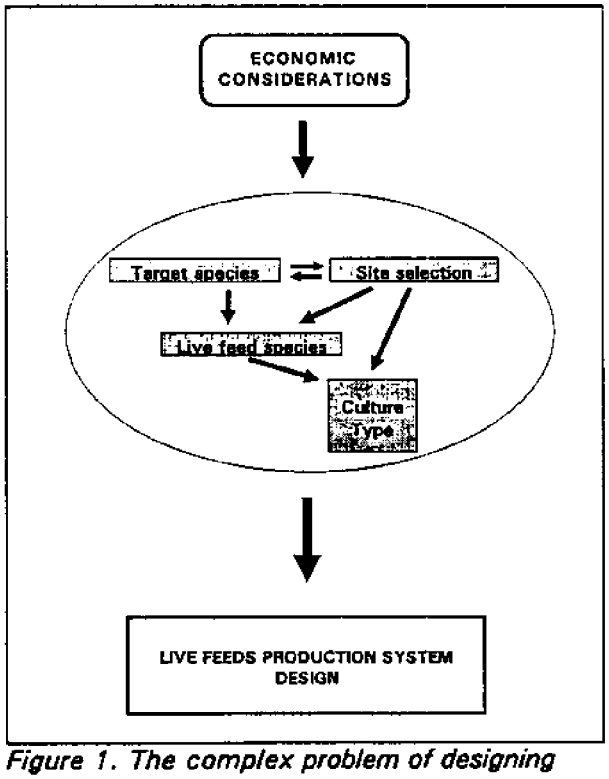

Figure 1. The complex problem of designing live feeds production systems.

An exception is the cultivation of microalgae to feed aquatic organisms, i.e., its use as **feed** as opposed to **food**. In most cases, harvesting and processing is not necessary (Droop 1975, Taub 1975). Microalgae, still suspended in their culture medium, can be fed directly to the primary consumer although circumstances may make some type of processing advantageous (see Fox 1983, Sommer et al. 1990). Hence, if the economic value of the "target species" is great enough, large-scale culture of microalgae can be economically feasible. **Unless otherwise noted, "microalgal culture" will be used in this paper to refer to the large-scale cultivation of algae to feed aquatic animals.**

2.1 Microalgae as Feed

Although much effort has been expended trying to find substitutes, microalgae remain prominent in the culture of many aquatic animals, especially marine species (Horstmann 1985) (Table 1). For example, some species of fish consume algae as adults and/or benefit indirectly from the presence of algae in their tanks (Jones 1970 cited in James et al. 1988; also see Stanley and Jones 1976, Buri 1978). Additionally, the intensive larviculture of many species of marine fish depends on a large supply of rotifers, which are usually raised on microalgae. Finally, tremendously large volumes of algae are required for the nursery culture of bivalve molluscs, while smaller amounts are used in the larviculture of commercially valuable molluscs and crustaceans. This paper will focus on the culture of phytoplankton for bivalve and shrimp hatcheries and in connection with the production of rotifers.

2.2 Problems and the Need for Design Studies

Of the work that has been done in the area of design optimization, most has resulted in small, laboratory-scale units that can not be scaled up to accommodate the needs of a commercial operation (but see Canzonier and Brunetti 1975). In fact, even **descriptions** of the design and operations of large-scale microalgal production units are relatively scarce. As a first step toward designing better production facilities, aquacultural biologists, engineers and economists need to take a critical look at existing systems, their **yields, operating costs, reliability**, etc. and consider ways of improving performance through better design. Only in this manner can we prevent repeating the mistakes of the past.

Even the production of a relatively small amount of algae can demand substantial resources. For instance, Taub (1975) estimated that even though millions of larval bivalves can be reared on a few grams of algae, "as much as 20 - 40% of the shellfish hatchery may be devoted to algal culture" (p. 1). Estimates like these inspired Ukeles (1980) to describe large-scale production of microalgal biomass as a "serious impediment to the future development of shellfish aquaculture" (p. 288).

To be more specific, the most important problems encountered in the large-scale production of microalgae as feed for bivalves, crustaceans and rotifers may be classified as either 1) economic in nature, or 2) related to the dependable output and consistent quality of large volumes of algae (Ukeles 1980). With regard to the first, De Pauw et al. (1984) estimated that monospecific algal cultures produced indoors or in a greenhouse range in cost from US$120 - 200/kg dry weight. While costs may be lower for some operations, there is no question that commercial production costs for phytoplankton are high (Taub 1975; Ukeles 1976, 1980; Laing and Utting 1980; Persoone and Claus 1980; Horstmann 1985, De Pauw and Persoone 1988; James et al. 1988).

The economic woes of cultivators stem from the fact that most microalgal culture today is labor intensive and requires a great deal of space (inside and/or outside). Additionally, the cost of energy (for lighting, pumping, aeration/mixing and heating/cooling) and nutrients is high. De Pauw and Persoone (1988, also see Helm et al. 1979) reported the following cost breakdowns for culturing algae by the bloom induction technique: labor (50 - 85%), pumping (4 - 24%), nutrients (4 - 20%) and mixing (5 - 8%) of the total production costs. Better system designs could help reduce expenditures. For example, money could be saved through design features that reduce labor costs by improving the efficiency of algal transfer during scale-up and harvesting (feeding the primary consumers). Automation is another means of reducing labor requirements. For example, computer technology has recently been used to facilitate automation in microalgal cultures (Hill et al. 1985, Rusch 1989, Wangersky et al. 1989). Finally, systems should be designed to promote maximal growth with minimal expenditures for energy, nutrients, labor, materials and space (Table 2).

The problem of **reliability** has two components: maintaining a **regular supply**, and producing algae of consistently high **quality**. All live cultures are subject to occasional failure ("crashes"). We can consider two types of culture failure: one in which the organisms do not multiply as predicted, and another in which the entire culture simply dies.

Table 2. Some of the problems associated with microalgal culture and examples of potential design-related solutions.

Problems	Potential Design-related Solutions
COST	
Labor intensive	■ Improvements in the efficiency of transfer/harvesting
High energy costs	■ Designing more cost-effective culture enclosures and techniques; e.g., optimizing culture depth, flow rate/residence time, mixing regime, etc.
High cost of nutrients /culture media	■ Automation
RELIABILITY	
Regularity of supply	■ Designing systems over which culturists have the highest degree of control while balancing costs
Consistency of quality	■ Consider designing and implementing larger, more cost-effective continuous culture devices

Either would result in starvation of the target species for some period of time. Depending on the particulars of the operation, the consequences could be minor, or they could be devastating. Many facilities often keep **back-up cultures**, an expensive, but prudent precaution. The frequency of culture collapse may even prohibit the large-scale production of certain species of algae altogether: "a major handicap in the cultivation of algae is our inability to grow selected species with known food value in substantial volumes (hundreds of m^3)" (De Pauw et al. 1984 p. 126). Similarly, De Pauw and Pruder (1986) state that "the consistent production of large quantities of desirable algae remains an elusive goal" (p. 95). Note that these authors previously defined a "modest" algae requirement as 5×10^{12} cells/day.

As one might imagine, the likelihood of crashes increases as the amount of control a culturist has over factors such as illumination, temperature, nutrient quantity, etc. decreases. Hence, crashes are particularly problematic in outdoor cultures, where one usually has little control over these important variables:

> "Upscaling the cultures . . . to larger volumes (mostly in outdoor enclosures) and thus leaving the artificially protected environment of (semi) sterility, rapidly leads to collapse of the culture or take-over by other species better adapted to the prevailing outdoor conditions" (De Pauw et al. 1984 p. 126).

With regard to culture **quality**, the nutritional value of algae (when grown in non-continuous systems) changes with the state of the culture. Culture age/growth phase, light intensity, temperature, nutrient limitation and source, and cell density can all affect the chemical composition of algae (De Pauw and Persoone 1988, also see Volkman et al. 1989). The problem of consistent nutritional quality is also especially pronounced in outdoor cultures. So, we must design systems that, in addition to producing the required volume of feed in an economical fashion, can turn out a high quality product with a high degree of reliability (Table 2).

2.3 General Principles

2.3.1 Upscaling

Most commercial-scale production today is probably unialgal semi-continuous or batch culture. Algae from stock cultures are "grown up" in successively larger enclosures until harvest. The following description of The Oceanic Institute's (OI) shrimp hatchery algae upscaling protocol demonstrates some important principles (also see Ukeles 1971 pp. 55-59, Guillard 1975 pp. 120-122, Fox 1983 pp. 22-37, Treece and Yates 1988 pp. 37-42 and Hoff and Snell 1989 pp. 28-33).

Stock cultures of the diatom *Chaetoceros gracilis* are maintained in screw-top test tubes in a 22°C room. Test tubes containing 10 ml of a mixture of nutrient medium and filtered seawater are inoculated with one drop of the stock culture and allowed to grow for three days; illumination is provided with fluorescent tubes at all stages. In this time, the cells will have multiplied, reaching a density of approximately two million cells/ml. The contents of one test tube are then used to inoculate a sterile 500-ml flask to which enriched seawater has been added. After two days, there may be as many as 3 - 5 million cells/ml in the flasks. One flask is enough to inoculate a 20-liter carboy. Carboy cultures are also grown for two days, to approximately two million cells/ml. Finally, 15 - 20 liters of a carboy culture are added to one 150-liter cylinder. Two more days are needed to bring the density back up to two million cells/ml (for a total of nine days from stock to final culture), at which time the cylinder can be harvested to feed *Penaeus vannamei* larvae.

While upscaling, it is crucial to inoculate a new container with a sufficiently large amount of algae. De Pauw and Persoone (1988) recommended starting with 5 - 10% of the total volume, or an initial concentration of 10^5 to 10^6 cells/ml. There are two reasons for this: to ensure rapid population growth; and for open cultures, to prevent unwanted species of algae, zooplankton, protozoa and/or bacteria from outcompeting, grazing, or otherwise harming the desired species of algae. Moreover, phytoplankton should be transferred while in the log phase of population growth to ensure rapid multiplication (see discussion below).

Another vital aspect of upscaling is the preparation of culture vessels. Although time consuming, this step is necessary to help prevent contamination in closed systems and to forestall serious contamination troubles in open systems. Hoff and Snell (1989) for example, recommend that small containers be 1) washed in detergent, 2) rinsed in hot water, 3) acid cleaned with 30% muriatic acid, 4) rinsed again in hot water, and 5) dried before use. Other culturists sterilize their test tubes, flasks, and even carboys in autoclaves or by other means. Because vessel preparation is so time consuming, disposable culture vessels such as sturdy polyethylene bags are gaining popularity (e.g., Baynes et al. 1979, Trotta 1981).

2.3.2 Population dynamics

Depending upon whom you ask, there are three to five generally recognized "phases" of population growth. For microalgae, these are thought to correspond to the nutritional state of the cells (Droop 1975). First is the **lag phase** in which cells in culture have just begun to absorb the nutrients present in the medium. Reproduction is slow, as is net population growth. Upon absorption of nutrients, the population enters the **log phase** (or **exponential phase**) in which reproduction is extremely fast (population growth is exponential). The

transitional phase or **phase of declining growth** comes next. Net population growth proceeds, but at a slower pace (some authors may refer to this as the late log phase). Finally, the **stationary phase**, in which there is no net population growth, follows the growth phases. If the culture is allowed to continue, cell death will follow (some authors consider this to be a separate phase).

Generally, it is best to harvest cells during the log phase, and to use these cells as inocula for other cultures. Log phase inocula will divide more rapidly than cells taken from other phases, thus they yield cultures that are, in general, more viable. Also, the biochemical composition, and hence the nutritional quality of algae varies with the stage of population growth. For example, Flaak and Epifanio (1978, cited in De Pauw et al. 1984) reported that log phase cells contain a relatively greater proportion of protein than cells in other growth stages, and that stationary phase cells have a higher proportion of carbohydrates.

2.3.3 Types of culture

Microalgal cultures may be coarsely divided into **indoor** and **outdoor systems**. Indoor cultures typically produce small volumes of algae under controlled conditions. There is some overlap. For example, the early stages of large, outdoor unialgal cultures are almost always grown indoors where it is relatively easy to prevent takeover by predators, competitors and disease. Furthermore, illumination, temperature, and nutrient levels can all be controlled within strict levels, allowing for very predictable growth. In general, then, the specifics of culturing microalgae indoors have been worked out. This is in sharp contrast to outdoor mass culturing:

> "One of the major disappointments of algal mass culturing has been an inability to control algal speciation in outdoor cultures, except in unique chemical environments . . . the large size and openness of outdoor algal systems makes it virtually impossible to inoculate with and maintain a desired species in culture for extended periods" (Goldman 1979a p. 14);

and,

> ". . . virtually all attempts to grow specific algal species outdoors for sustained periods have failed . . . due to the rapid generation periods of algae, certain species tend to dominate through natural selection regardless of which alga is used as an inoculum. Invariably, the weed species such as *Chlorella, Scenedesmus* and *Micractinium* in freshwater culture and *Phaeodactylum* and *Skeletonema* in marine systems tend to become dominant over time" (Goldman 1979b p. 134, also see Goldman and Mann 1980).

One can also distinguish between **open** and **closed** cultures (see Ukeles 1980). Closed cultures are maintained in tubes, flasks, carboys, bags, etc. This is in contrast to phytoplankton cultured in uncovered pools or ponds (indoors or outdoors). Open cultures are more readily contaminated:

> "The possibility of unsuitable algae, predator populations, and the growth of disease-producing bacteria appearing in the algae is an ever-present danger with the consequence of mortality in the grazing population and/or loss of the food supply" (Ukeles 1980, p. 296).

However, for producing large quantities of algae, open cultures are the only practical systems at this time.

Some indoor, closed cultures are **axenic** (also referred to as "sterile"): that is, they are free of foreign organisms such as bacteria. Axenic cultures require that glassware, tubing, water, pipettes, nutrient media, etc. all be scrupulously sterilized — care is taken at every step to avoid contamination (see Ukeles 1980 for a detailed description of the maintenance of axenic algal cultures). There is, of

Table 3. The primary types of microalgal culture.

Culture type	Advantages	Disadvantages
Indoors	A high degree of control (predictable)	-Expensive
Outdoors	Cheaper	-Little control (less predictable)
Closed	Contamination less likely	-Expensive
Open	Cheaper	-Contamination more likely
Axenic	Predictable, less prone to crashes	-Expensive, difficult
Nonaxenic	Cheaper, less difficult	-More prone to crashes
Continuous	Efficient, provides a consistent supply of high-quality cells, automation, highest rate of production over extended periods	-Difficult, usually only possible to culture small quantities, complex, equipment expenses may be high
Semi-continuous	Easier, somewhat efficient	-Sporadic quality, less reliable
Batch	Easiest, most reliable	-Least efficient, quality may be inconsistent

course, an increased expense associated with sterilization; however, axenic cultures may be less prone to failure and are essential for many types of algal research. Furthermore, some primary consumers may be successfully cultured only with axenic microalgae. However, it is impractical for commercial operations to produce large volumes of microalgae axenically.

Axenic and nonaxenic cultures notwithstanding, we shall consider the following three basic types of phytoplankton culture: continuous, semi-continuous and batch. Droop (1975) defines the two most common types of **continuous** culture (chemostats and turbidostats) as "steady-state continuous flow cultures in which the rate of growth is governed by the rate of supply of the limiting nutrient" (p. 71). These are delicately balanced systems, often axenic, in which the culture organisms are harvested continually and receive constant nutrient replenishment. It is very important to adjust the rate of "wash out" in continuous systems so that the rate of harvest is a bit slower than the maximum specific growth rate (Taub 1970, Gold 1973).

Turbidostats simultaneously harvest algae and add fresh medium when cell counts in the culture vessel exceed a certain level. Hence, the culturist sets the cell density at a certain value, and the wash out rate is continually adjusted automatically to keep the density from changing (see Sorgeloos et al. 1976, Laing and Jones 1983).

Chemostats, by contrast, act on the principle of limiting a vital nutrient, such as nitrate. When the concentration of that nutrient drops below a certain level, a fixed quantity of medium containing algae is removed, and a fixed quantity of nutrient is added. Hence, it is the growth rate (regulated by the supply of a limiting nutrient), not the cell density, which remains constant in chemostats (see Droop 1975, James et al. 1988).

There are many advantages to continuous algal cultures, including a steady supply of high-quality, log-phase cells; a greater rate of production; and automation (Table 3) (also see Taub 1970, 1975, 1980; Droop 1975). Furthermore, continuous cultures, like axenic cultures, may be preferred for research purposes. From a commercial production standpoint,

however, illumination and temperature must be maintained within tightly defined limits, hence these systems are almost always housed indoors (but see Camacho et al. 1990). This is one reason continuous cultures are, for the most part, only feasible for the production of relatively small amounts of microalgae. Advances are being made, however, in the adaptation of continuous culture technology to the large-scale production of microalgae. James et al. (1988), for example, report on an indoor, vertical chemostat system, five 200-liter "translucent tubes", with which they achieved extremely high yields. It is unclear, however, how long the authors were able to maintain those yields.

If a great deal of algal biomass is required, semi-continuous or batch culture is usually employed. In **semi-continuous** cultures, a given population is allowed to grow until it reaches a desired cell density. Then it is partially harvested, and fresh medium is added. The culture is grown up again, partially harvested, etc. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable, especially outdoors. Competitors, predators and/or other contaminants and metabolites eventually build up, rendering the culture inviable. A further drawback is the variability in the nutritional quality of the cells produced.

The alternative is **batch** culture, complete harvest when the population reaches its maximum or near-maximum density. The entire volume may not be needed at once, so it may take several days to harvest a tank. Used both indoors and outdoors, this technique is considered by many to be the most reliable method of algal production. Once a working protocol has been established at a site, there is little uncertainty about how long a tank will last (even outdoors), and contamination is less troublesome than it is in the older stages of semi-continuous cultures. However, because a given tank is harvested completely, it will yield less algae than will a tank of the same size run semi-continuously. This is one reason batch culture requires more tanks than semi-continuous culture. Also, the quality of cells produced in batch cultures is not as predictable as those produced in continuous cultures (Taub 1970, 1980). One important variable may be the timing of harvest. For example, Liao et al. (1983) reported that *Skeletonema costatum* batch cultures harvested in the morning were better feed for shrimp larvae than cells that were harvested in the afternoon of the same day.

The decision to use semi-continuous or batch culture may depend on many factors, among them: experience, level of quality and consistency needed, available space and facilities, the target species, the algal species and site characteristics. Some environments are more stable than others with regard to temperature, illumination, water quality and other variables, hence they may be more suited to semi-continuous culture than less stable sites. The trade-off seems to be one of reliability. Semi-continuous systems can realize savings over batch systems in that fewer tanks and labor are needed to produce the same volume of algae. Finally, Taub (1970) contends that batch cultures are less efficient than continuous cultures because a given population must be maintained long after the maximum specific growth rate has been attained (and growth rate is declining). See Table 3 for a list of advantages and disadvantages of the major types of algal culture.

Less common culture types include single-species or multi-species systems (e.g. Hirata 1974, 1979; Pruder 1975; Pruder and Bolton 1978; Hirata et al. 1983) that employ

feedback culture. The waste products of primary and/or secondary consumers provide nutrients for the algae. These may or may not be closed recirculating systems that have the added benefit of conserving water (Epifanio et al. 1975 cited in Ukeles 1980). Finally, bloom induction may also be considered as a different culture type (see discussion below).

2.3.4 Species

Chlorella, *Chaetoceros*, *Isochrysis*, *Nannochloropsis*, *Dunaliella*, and *Tetraselmis* are the genera of algae most commonly cultured for aquacultural purposes (also see Persoone and Claus 1980, Ukeles 1980, Laing and Millican 1986, and Loosanoff and Davis 1963 for lists of species commonly used to feed bivalve larvae; see Liao et al. 1983 for a discussion of species choice for penaeids; and see Guillard 1975 and De Pauw and Persoone 1988 and Gladue, this volume for a listing of some commonly cultured species and the classes to which they belong). Species are usually chosen on the basis of size, nutritional value and ease of culture. A species must also be non-toxic. Naked flagellates like *Isochrysis galbana* seem to be particularly good feed for bivalves (Ukeles 1980), whereas taxa with thick cell walls (like *Chlorella*) apparently cannot be digested by bivalves. Moreover, mixed diets containing several species of microalgae have been reported to give better results for some organisms (Davis and Guillard 1958 cited in Brown et al. 1989, Hu 1990; but see Laing and Millican 1986).

Not all species lend themselves to large-scale culture, hence those that are both nutritious and relatively simple to culture tend to be used most frequently (see Witt et al. 1981). Also, some species or geographic strains within species are better suited to certain geographic regions. For example, for the nursery culture of bivalves, Christine Claus (1981) lists *Skeletonema costatum*, *Phaeodactylum tricornutum*, *Tetraselmis suecica* and *Pavlova lutheri* as species that have been successfully mass-cultured in temperate climates. *Bellerochia spinifera*, *Chaetoceros simplex*, *Thalassiosira pseudonana* and *Isochrysis galbana* (Tahiti strain), on the other hand, have been grown in subtropical conditions.

Not only must a culturist decide between species of algae, there can also be significant differences between strains (genetically distinct groups within species), or even clones (genetically identical descendants of one asexually reproducing organism). For example, some algae have been selected to grow in the absence of so-called "essential" vitamins (Ukeles 1976) or under some other stress. Other strains have simply been found to exhibit good or poor growth under certain conditions. That intraspecific variation can be great is attested to by the fact that researchers often report the strain and/or clonal type of the species studied, along with the source of the stock culture.

2.3.5 A history of the culture of phytoplankton as an aquacultural feed

In 1910 Allen and Nelson cultured diatoms monospecifically to feed a variety of invertebrates (Ryther and Goldman 1975) whereas Bruce et al. (1939, cited by Ukeles 1980) are reported to have been the first to isolate and maintain unialgal cultures (in this case *Isochrysis galbana* and *Pyramimonas grossii*) to feed oyster larvae.

The first successful attempts at culturing microalgae for shrimp were carried out to feed *Penaeus japonicus* larvae. Dr. Fujinaga (also known as "Dr. Hudinaga") of Japan pioneered two very different techniques (detailed in De Pauw and Pruder 1986). With the first method,

a desirable species of algae was isolated from natural seawater and then, with the addition of nutrients, light and air, allowed to multiply. For shrimp, Fujinaga grew mainly the diatom *Skeletonema costatum*. This is probably how most microalgae are grown in aquaculture today. Under the correct conditions, this method can produce large, fairly dense, unialgal cultures.

The second technique has been termed **bloom induction**. Dr. Fujinaga adopted this method in 1946. Addition of inorganic fertilizer to coarsely filtered, illuminated, natural seawater was found to result in multispecific algal blooms. Similar techniques were also used by Loosanoff (1951) and Loosanoff and Davis (1963) for feeding bivalves. Originally, fertilizer was added directly to the shrimp rearing tanks. Not only did algae grow, but with time, populations of zooplankton such as rotifers and copepods would often become established. These organisms were ideal feed for the later omnivorous shrimp stages, the late zoeae and myses (also see Yang 1975).

One variation on this theme is the "Wells-Glancy method" used early in the history of bivalve larviculture (Glancy 1965, cited in Ukeles 1980). Natural seawater was coarsely filtered either by centrifugation or by passing it through fine mesh cotton bags. It was then placed in a greenhouse-type enclosure, whereupon blooms of plankton eventually resulted (fertilizer was not added, see Figs. 2 and 3 in Ukeles 1980).

As one might imagine, the bloom induction and Wells-Glancy techniques are much easier and cheaper than monospecific culture. In general, though, the lack of control over the species composition of induced blooms makes this technique less reliable, hence the predominance of unialgal culture. Some research in Belgium, however, has produced promising results for the bloom induction technique. Growing feed for juvenile bivalves, researchers were able to control the dominant phytoplankton species by manipulation of culture conditions, especially nutrients, while at the same time avoiding serious infestation by zooplankton (De Pauw et al. 1983, De Pauw and de Leenheer 1985; cf. Dunstan and Tenore 1974, Srna 1976, Goldman and Mann 1980, Riva and Lelong 1981).

2.4 General Requirements and Considerations

The most important parameters regulating algal growth are nutrients, light, pH, and turbulence (Persoone and Claus 1980). In this section, we will discuss all of these factors, in addition to temperature, salinity, monitoring, equipment and supplies. In most cases, the "most optimal" parameters as well as the tolerated ranges for these variables are species specific, hence only broad generalizations can be made here. It should also be noted that the effects of factors such as nutrient quantity and quality, pH, salinity, temperature, light and turbulence are often interdependent. For example, a parameter that is "optimal" for one set of conditions is not necessarily optimal for another.

2.4.1 Culture medium/nutrients

Most of the microalgae grown as feed for commercially important aquatic animals are marine or brackishwater species. Hence, they require a culture medium with a chemical composition similar to that of seawater. Water containing toxins such as oils, pesticides, organics, or unchelated heavy metals should be avoided. For most outdoor mass cultures, natural seawater is the only economically feasible culture medium. Seawater used in

both indoor and outdoor cultures is usually filtered to rid it of algae, zooplankton, and protozoa. The degree of filtration may be site-specific (Fox 1983), however, water for outdoor cultures is usually not filtered as finely as that destined for indoor use. The former is commonly passed through a filter ranging in size from 2.0-0.4 μm, while the latter may be filtered down to 0.2 μm or less. Additionally, water for indoor cultures may also be sterilized via an autoclave, UV radiation, or some other means. This reduces the possibility of culture collapse due to bacterial contamination. For small operations, it may be preferable to use artificial seawater (see Ukeles 1976, 1980; Kaplan et al. 1986; Boussiba et al. 1988), depending on the need for uniformity, the quality of the natural seawater available and the amount of money available.

Besides carbon, the principle nutrients phytoplankton require are nitrogen and phosphorus, in an approximate ratio of 6:1 by weight, respectively (e.g. Valero et al. 1981). Additionally, diatoms require silicate. Trace minerals (iron, copper, zinc, cobalt, manganese, and molybdenum) and vitamins (especially B_{12} and thiamine, and sometimes biotin) can also be added, and are necessary in most axenic cultures. Other additives like chelating agents may also be used (see Ukeles 1976, Fabregas et al. 1987), but they are usually expensive. Whether or not these are used will likely depend upon the species of algae to be grown, the cost-effectiveness of using the additive, and the philosophy of the culturist. It should also be noted that there are now commercially available nutrient solutions that can reduce preparation labor.

Numerous published descriptions of algal nutrient media exist; however, one of the most recent and comprehensive is that compiled by Borowitzka (1988). Additionally, Guillard (1975) provides detailed instructions on making stock solutions for the major elements, trace elements, vitamins, etc. and for making soil extract (see also Ukeles 1976). Finally, see Fox (1983) for a detailed discussion of enrichment media used in shrimp hatcheries.

The OI shrimp hatchery uses F/2 Guillard's medium (Guillard 1975) containing $NaNO_3$, NaH_2PO_4 x H_2O, and Na_2SiO_3 x H_2O as the source of major nutrients, along with a commercial vitamin mix, EDTA, and sodium metasilicate. However, for economic reasons, the complexity of the culture medium is usually inversely proportional to the size of the culture volume. Thus, large, outdoor cultures are usually supplied with only the barest essentials, and agricultural-grade rather than laboratory-grade fertilizer is often used (see Gonzalez-Rodriguez and Maestrini 1984).

2.4.2 Light

Light is the source of energy which drives photosynthesis. In order to maximize yield, one must maximize the efficiency with which available light is converted into algal biomass. Maximum culture depth and cell density are the key variables regulating light utilization efficiency (Roels et al. 1977, Goldman 1979b, Richmond et al. 1980).

With respect to the illumination regime to be used, intensity, spectral quality, and photoperiod are all important considerations. Indoor microalgal facilities usually have fluorescent tubes (80 watts is common) arranged to provide maximal illumination of the culture vessels (although the heat produced by the lights must also be taken into consideration). Hoff and Snell (1989) state that an intensity in the range of 2500 - 5000 lux is optimal, and Guillard (1975) recommends 3500 and 4500 lux for stock cultures of *Thalas-*

siosira pseudonana under continuous and 14 hours/day illumination, respectively (see Table 4). Plants are known to utilize only a portion of the spectrum of visible light (PAR). "Cool White" fluorescent bulbs are commonly used.

Cultures may be kept in continuous light, or timers may be used to maintain a fixed schedule of light and dark hours (photoperiod). The amount of incident light a culture needs depends on its cell density and increases as populations increase (Guillard 1975). Of course, outdoors the natural sunlight and photoperiod is used. However, direct sun may not be tolerated (see Pruder and Bolton 1978), depending on the intensity of the sun (which, in turn, varies with season and weather conditions), the species or strain being cultured and the density of the culture (Guillard 1975).

When, during upscaling, an algal culture is transferred outdoors, the cells often suffer from photic shock. This is because they are not adapted to light of such a high intensity, and they require a period of time to adapt. On the other hand, in greenhouses, supplemental light may be needed (e.g. Loosanoff 1951, Loosanoff and Davis 1963). Also see Lorenzen (1980).

2.4.3 pH

A hydrogen ion concentration (pH level) that is too high or too low will slow algal growth by disrupting cellular processes. The optimum pH range for most of the species cultured falls between 7 and 9. The "most optimum" range, furthermore, is reported to be 8.2 - 8.7 (Ukeles 1971) (Table 4). However, Kaplan et al. (1986) found that as long as the concentration of Fe^{3+} was high enough, *Isochrysis galbana* grew equally well within the range of 5.0 - 9.0 pH.

Complete culture collapse can result from a failure to properly monitor and maintain an acceptable pH. Fortunately, this is easily accomplished in moderately dense cultures through aeration, a process which serves other purposes as well (see below). The addition of carbon dioxide, naturally present in air, serves to increase the buffering capacity of the culture medium and prevent the pH from getting too high (becoming too alkaline, see below).

Table 4. A generalized set of conditions for culturing microalgae.

Parameters	Range	Optima
Temperature (°C)	16 - 27[1]	20 - 24, 18 - 22[2]
Salinity (ppt)	12 - 40 (for neritic flagellates)[3]	20 - 24[3]
Light intensity (lux)	1,000 - 10,000 (depends on vol., density)[2]	2,500 - 5,000
Photoperiod (light hours:dark)		16:8 (minimum)[1] continuous (maximum)[1]
pH	7 - 9	8.2 - 8.7[4]

[1]Hoff and Snell (1989).
[2]Le Borgne (1990).
[3]Ukeles (1976).
[4]Ukeles (1971).

2.4.4 Aeration/mixing (turbulence)

Aeration is beneficial for three reasons. First, air is a source of carbon in the form of CO_2, which is fixed during photosynthesis. Second, adding CO_2 provides essential pH stabilization. CO_2 addition must keep pace with assimilation, or else the pH of the medium will rise. This is because the balance maintained between bicarbonate, carbon dioxide, and hydroxide ions [$HCO_3^- \leftrightarrow CO_2 + OH^-$] serves to buffer the water against pH changes. Air, which is approximately 0.03% CO_2 by volume, may or may not be enriched with additional CO_2 before it is added to a culture. The need for extra CO_2 will depend on the density of the culture, pH, light intensity and growth rate (Ukeles 1971), but because of the expense, CO_2 is usually supplemented only in extremely dense cultures.

The third benefit derives from the fact that for many cultures, aeration is the sole means of mixing. Agitation is essential for a host of reasons (see Goldman and Ryther 1977, Goldman 1979b, Persoone et al. 1980, Richmond et al. 1980, Terry and Raymond 1985, Oswald 1988). It keeps both nutrients and cells evenly distributed, promoting uniform nutrient and light absorption. The supply of light is often a limiting factor in dense cultures, and mixing helps decrease the loss of production due to self-shading and/or photo-inhibition (a decrease in photosynthesis due to an excess of light). For outdoor cultures, an adequate level of mixing can also prevent thermal stratification, and the resulting precipitation of heavy metals, as well as the settling and subsequent decay of organic matter. See Persoone et al. 1980 for descriptions and results of experiments with continuously mixed, discontinuously mixed, and non-agitated *Chlorella saccharophila* cultures. Mixed cultures yielded approximately 30% more algae than non-mixed cultures. Also see Molina et al. 1990.

Not all species can tolerate vigorous mixing, however. The intensity of agitation should be adequate but not so intense as to slow or prevent growth. Guillard (1975) recommends gentle aeration for a day or two after inoculation, followed by increasing amounts of mixing as the culture grows.

Bubbling air as a means of mixing may be more appropriate for small-scale cultures than for larger ones (Persoone et al. 1980). Note, however, that there can be a trade-off between the efficiency of mixing and the efficiency of aeration. Large bubbles achieve the highest degree of mixing, whereas small bubbles are best for diffusing gases to a liquid medium.

Common alternatives for large vessels include jet pumps, paddle wheels, continual circulation of the water mass and air-lift pumps. Vessels that are shaped differently will likely have different optimal means of ensuring adequate aeration and mixing:

> " . . . for each type of culturing unit the most economic aeration regime for which the algal output is maximal, should be determined" (Persoone et al. 1980 p. 520).

2.4.5 Temperature

When nutrients are present in excess, temperature and illumination are the sole limiting factors in algal cultures (Goldman 1979b). However, Goldman (1979b) indicated that temperature is not as important as sunlight in controlling productivity. Furthermore, Payer et al. (1980) investigated the direct and indirect effects of temperature on a number of species and strains of algae in order to select those that would be suitable for production in Thailand. In addition to finding strain-specific results, they concluded that

> "a control or at least an observation of temperatures of outdoor mass culture seems to be necessary in a few specific cases only" (p. 399).

Temperature, however, can be important in determining which species will predominate in open, outdoor cultures (Goldman and Ryther 1977, Goldman 1979a, Goldman and Mann 1980, De Pauw et al. 1980, and Witt et al. 1981).

With regard to the effect of temperature on growth, temperature tolerance may vary with the nutritional composition of the medium, the species and the strain cultured (Table 5) (Ukeles 1976). Each species is regarded as having minimum, maximum, and optimum temperature ranges for growth. In general, though, the most commonly cultured species of microalgae tolerate temperatures between 16 and 27°C. Twenty to 24°C may be considered "optimum" (Guillard 1975, Hoff and Snell 1989) (Table 4). Temperatures lower than 16°C will slow growth, but those higher than 35°C are lethal for a number of species (Hoff and Snell 1989). For outdoor cultures, it is very important to choose a species of algae that will tolerate the range of temperatures likely to prevail at the culture site. For nonaxenic, indoor systems like that at the OI shrimp hatchery, temperatures may be kept somewhat below the optimum level for algal growth in order to discourage the growth of bacteria. This may be accomplished by several means, for example, air conditioning the culture room and/or using water baths.

According to Ukeles (1971):

> "The optimum temperature for growth will vary with species and to some extent is a complex factor that depends on other environmental conditions. Cultures should be maintained at the lowest temperature that is consistent with good yield to avoid encouraging bacterial growth" (p. 58).

Finally, Kaplan et al. (1986) determined that the optimal temperature for culturing a strain of *Isochrysis galbana* — that with which they achieved the highest yield — was 27°C, whereas temperatures above 32°C and below 19°C caused a marked reduction in yield.

2.4.6 Salinity

The tolerance of marine phytoplankton to changes in salinity is considered to be extremely broad. Most species grow best at a salinity that is a bit lower than that of their native habitat. Tolerated and optimum salinities have been investigated by a number of authors for

Table 5. Growth response of different microalgal species at various temperatures (°C) (from Ukeles 1961).

Species	No Growth	Growth less than control	Growth equal to control at 20.5°C	Growth less than control	No growth
Monochrysis lutheri	8 - 9	12	14 - 25	27	29 - 35
Isochrysis galbana	8 - 9	12	14 - 22	24 - 25	27 - 35
Phaeodactylum tricornutum	—	—	8 - 24	27	29 - 35
Dunaliella euchlora	8 - 9	—	12 - 35	—	39
Platymonas (= *Tetraselmis* sp.)	—	8 - 9	12 - 32	—	35
Chlorella sp. (isolate #580)	8 - 9	12	14 - 35	—	—
Chlorella sp. (UHMC isolate)	8 - 9	12	14 - 29	—	32 - 35

a variety of commercially important species (also see Table 4). For example:

Most neritic flagellates, (those inhabiting the relatively shallow waters over the continental shelf), are reported to grow in salinities ranging from 12 - 40 ppt., but 20 - 24 ppt. is optimal (Ukeles 1976; also see Duerr and Mitsui 1982). *Isochrysis galbana* grew well within the range of 5 - 60 g/liter NaCl (15% the salinity of seawater to twice the salinity of seawater; Kaplan et al. 1986). Additionally, Fabregas et al. (1984) concluded that good *Tetraselmis suecica* growth occurred between 25 and 35 ppt. (coupled with concentrations of 2 - 8 m*M* $NaNO_3$). Finally, Laing and Utting (1980) found 15 - 25 ppt. and 25 - 30 ppt. to be optimal salinities for *I. galbana* and *T. suecica*, respectively.

2.4.7 Monitoring

> "Culture crashes can happen overnight, so it is important to closely monitor cultures for early warnings of a crash like declining cell densities or changes in culture color. Algal cells from declining populations also have little nutritional value for zooplankton" (Hoff and Snell 1989 p. 23).
>
> "High quality control in food cultures may be maintained by frequent observations of cultures, both macroscopically and microscopically, as well as density and pH measurements" (Ukeles 1971 p. 59).
>
> "Color and pH are often guidelines to the condition of the algal culture. If the color appears a somewhat opaque gray and the pH is lower than about 7.5, it is likely that the bacterial population is too high to attempt a rescue of the algal culture" (Ukeles 1980 p. 296).

The aforementioned attest to the fact that monitoring (especially pH, nutrient levels, contaminant levels and density) is a vital component of any algal production system. The primary motivation, of course, is the prevention of culture collapse and the maintenance of high quality live feeds. Cell densities can be monitored in a variety of ways, but Secchi disks and hemacytometers are probably the most commonly used devices. Secchi disks are quick and easy to use, but hemocytometers provide more accurate measurements (also see Valero et al. 1981).

2.4.8 Equipment and supplies

The equipment needed to culture microalgae is highly dependent on the type and scale of culture. For details on the materials needed to grow axenic cultures see Stein (1973) and Guillard (1975). Guillard provides descriptions and evaluations of a variety of common culture vessels used for growing small, indoor cultures of algae as well as guidelines for the selection and arrangement of heating and cooling units. A list of "various materials," pasteur pipettes, cotton stoppers, etc., required by an indoor algal culture facility is also included.

Justice et al. (1972) tested the effects of different materials (e.g. pyrex vs. kimax glassware, surgical Tygon tubing vs. regular Tygon tubing, etc.) on algal growth. Additionally, the equipment and supplies needed for a small-scale, simple algal culture system are outlined in Hoff and Snell (1989), while Davis (1971) gives some guidelines for the selection of piping, pumps, and heat exchangers.

Regarding the types of large culture containers to be used, tanks, ponds and even commercial swimming pools have been tried successfully:

> "Pools and ponds may be natural or man made; the bottom may be natural or lined with cement, asphalt or synthetic materials such as polyethylene or polyvinyl-chloride sheets. Tanks are usually rectangular, square or circular, and built on a solid base, either at ground level or excavated; the commonly used materials are reinforced concrete, fibre glass, plywood, bricks coated with cement,

resins and plastic sheets" (De Pauw and Persoone 1988 p. 206).

Culture depths in such large-scale systems are typically .25 - 1 m but may be greater than 1.5 m. Finally, De Pauw and Persoone recommend that algal culture systems have draining devices and be easy to clean.

2.5 Design Examples

Some examples of algae production system designs will be presented here. The list is not comprehensive, rather it is meant to introduce the reader to some of the many designs which have been used, either experimentally or on a larger scale. Section 2.5.1 describes relatively simple systems, modifications of which have been used since the early days of controlled microalgal culture. The examples in Sections 2.5.2 - 2.5.4, by contrast, have been proposed as improvements to the traditional techniques. In most cases, they were developed to increase production efficiency and/or decrease cost while maintaining reliability and high yields.

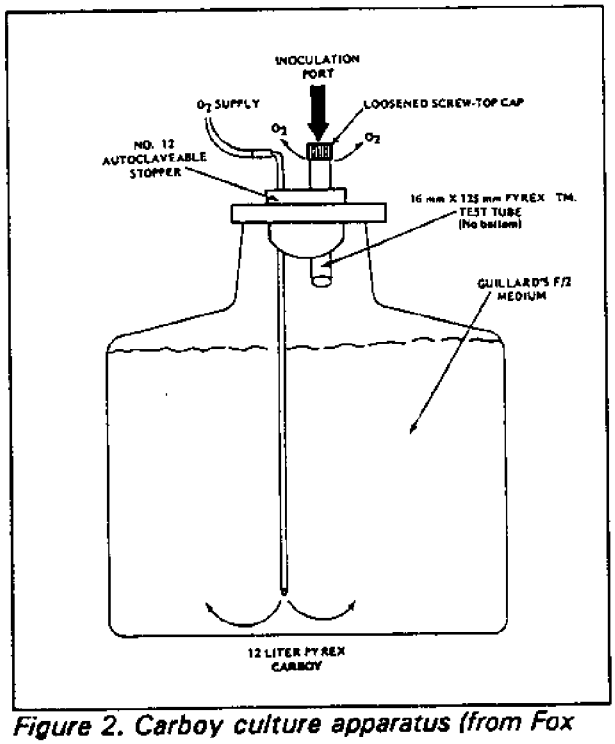

Figure 2. Carboy culture apparatus (from Fox 1983).
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2.5.1 Commonly used systems

Indoor, closed algal culture systems are often used to produce relatively small amounts of monospecific microalgae. Depending on the particular system, cultures may be axenic or non-axenic, and they may be run as batch, semi-continuous or even continuous systems. One of the simplest types of indoor culture employs 10 to 20-liter glass or plastic carboys as primary culturing vessels (see Fig. 2). The resulting algae can be used as feed or as inocula for larger cultures. Lighting is provided by fluorescent tubes; the temperature is kept fairly constant; aeration is provided; and a high-grade nutrient medium is used. Several carboys may be kept on shelves, backlit with fluorescent bulbs (Fig. 3).

When more algae is needed, open, semi-continuous or batch cultures are the simplest and most common type of system employed. Tanks ranging in size from .5 m^3 to 200 m^3 or more can be kept in the open air or in covered sheds or greenhouses. They are inoculated with indoor cultures and provided with agricultural-grade nutrients, natural illumination and aeration. Contamination and fluctuations in sunlight and temperature are potential problems that necessitate keeping backup cultures. To facilitate transfer and harvest, the tanks may be connected to each other and/or to the target species' culture vessels by piping.

2.5.2 Semi-continuous and continuous culture in polyethylene bags

Examples of this type of system are described in Baynes et al. (1979) and Trotta (1981). Baynes et al. (1979) detail the design and operation of a system that employs vertical, 480-liter capacity plastic bags to grow *Pavlova lutheri*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, and *Dunaliella tertiolecta* to feed rotifers. Their culture vessel design is based on that used by Seasalter Shellfish Co. Ltd. (depicted in Farrar 1975). Bags were created from tubing that was heat-sealed on both ends and suspended from a frame. Care is taken to avoid contamination at all steps; sterile medium and air + CO_2 are used.

Good results were obtained with both indoor and outdoor units. The outdoor cultures could be operated semi-continuously for six to eight weeks before contaminants reduced algal growth rates. Improvements over "traditional" culture methods included the fact that the closed cultures were easy to manage and the cost of materials, bags and frames, was low. Additionally, the vessels could be used indoors or outdoors and took up little horizontal space. One drawback was that the relatively large diameter of the bag, 600 mm, prevented the growth of dense cultures because self-shading occurred. Whereas densities of 16,000 *Pavlova lutheri* cells/μl could be cultured indoors in 20-liter (300-mm diameter) flasks, densities of 8,000 and 6,000 cells/μl were the maximum obtained from outdoor and indoor polyethylene bags, respectively.

A similar system was designed by Trotta (1981). This time, however, the bags were

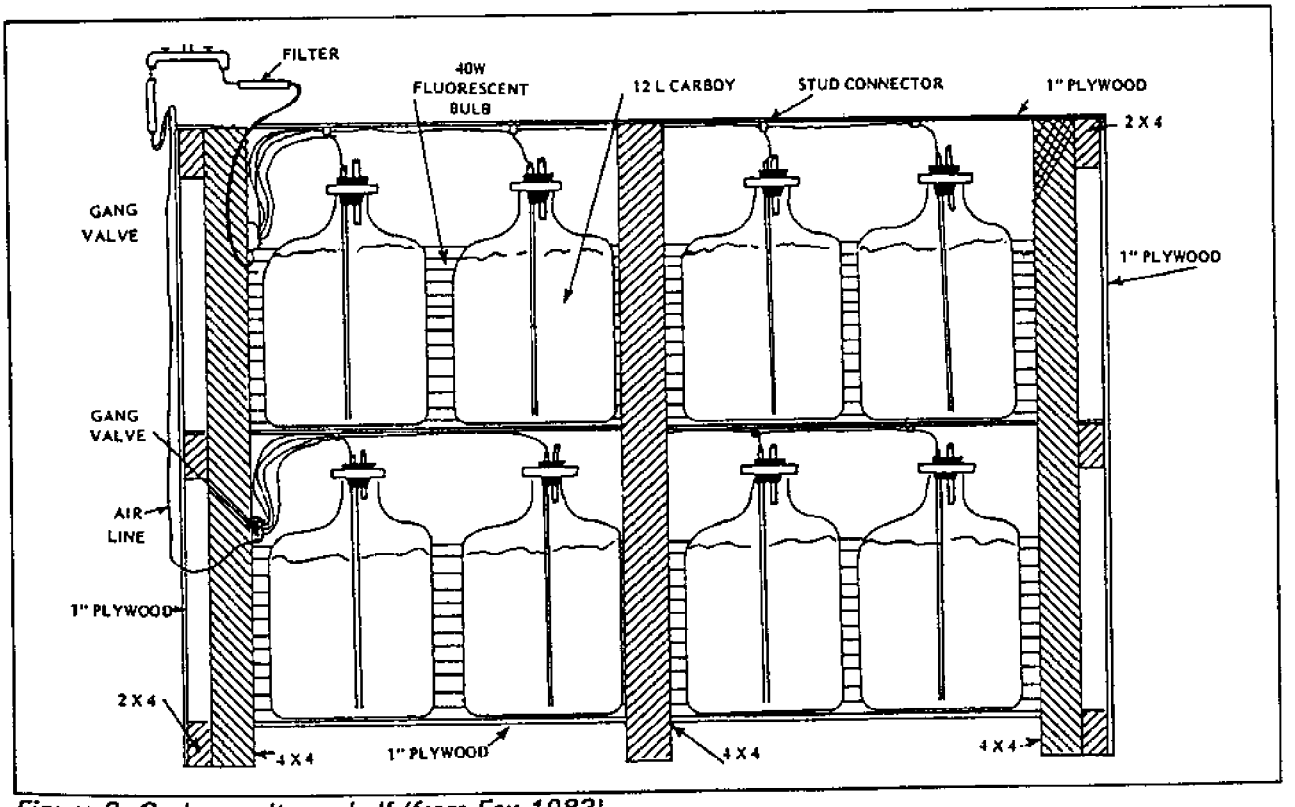

Figure 3. Carboy culture shelf (from Fox 1983).
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only 50 liters in volume and the algal cultures were run continuously (indoors only) with an automatic supply of culture medium and air. These vessels were small enough to be suspended from hangers; no frame was needed. Benefits were the same as those mentioned above — cheap materials that needed no sterilization and took up little floor space, with the added bonus of automation.

2.5.3 Internally illuminated vessels

These containers were designed to provide maximal light exposure to closed, cylindrical cultures. For example, Helm et al. (1979) designed and tested a 200-liter vessel in which they grew *Tetraselmis suecica* semi-continuously. The primary aim was to reduce the labor input per unit volume of algae produced while maintaining reliability. Their container was constructed largely of "white pigmented glass-fibre." The white surface reflected light that emanated from an inner, transparent acrylic tube. The latter held four 80-watt, 150-cm long, "daylight" fluorescent lamps. After some experimentation, the cultures could be run semi-continuously for more than 70 days, and the amount of labor needed was estimated to be only 3 hours per day for a ten-vessel system. The total cost of production was also estimated for a system having ten vessels, each of which produced 52 liters of *T. suecica*/day (cell density: 1,000 cells/μl). This system would be large enough to supply the majority of feed to an oyster hatchery producing one million 5-mm juveniles per month. The cost per 520 liters was £15.64, or by today's exchange rate, US$8.30.

A modification of this system is that described by Laing and Jones (1988) for the production of *T. suecica* and *Isochrysis* aff. *galbana* (also see Laing and Jones 1983). This time a 40-liter, internally illuminated, turbidostat vessel was devised in which the algae were contained in "polyethylene tubing supported around a core of six 80 W fluorescent lamps" (see Figs. 4 and 5). *Tetraselmis suecica* cultures could be run continuously for 24 - 69 days, whereas cultures of *I. galbana* lasted only 18 - 24 days. Average yields were .56 x 10^{11} cells/day for 33 days and 5.21 x 10^{11} cells/day for 21 days for *T. suecica* and *I. galbana*, respectively.

Finally, Knowles and Edwards (1971) described a 330-liter "water-jacketed vat" system in which they cultured a variety of algal species for bivalves. They could be operated either as batch cultures or semi-continuously (the latter was found to be more economical). The three internal lights (each 250 watts, 21.1 cm long and 8.9 cm in diameter) were enclosed in glass pockets attached to the lid of the vat. One container provided 780 liters of *T. suecica* (mean cell density: 800,000 cells/ml) over a two-week period. Prolonging the life of these semi-continuous cultures resulted in algae that was unsatisfactory in quality.

2.5.4 Other continuous systems

Canzonier and Brunetti (1975) reported on an unusual system in which they produced *Phaeodactylum* and *Chlorella* continuously for more than eight months. They utilized 35 1.5-m long sections of thin-walled, soft-glass tubing, 1-cm internal diameter, connected with Tygon plastic tubing. Illumination was supplied by a bank of fluorescent tubes. Best yields were obtained at a harvest rate of 3 - 4 liters/day; cell density averaged one million cells/ml. Advantages of this system included the high degree of automation and the ease with which it could be scaled up and adapted to fit a variety of spatial configurations.

Palmer et al. (1975) described a continuous culture apparatus in which they

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 (2.1×10^{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 2×10^{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.

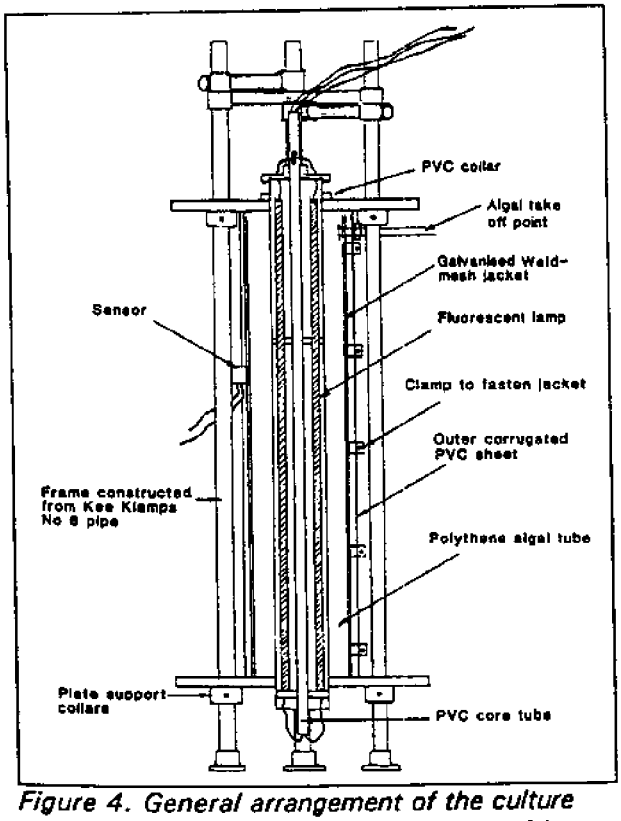

Figure 4. General arrangement of the culture vessel designed by Laing and Jones (1988). Reprinted with permission of Elsevier Science Publishers, B.V.

3.0 ROTIFER (*Brachionus plicatilis*) PRODUCTION SYSTEMS

3.1 Biology and Life History

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

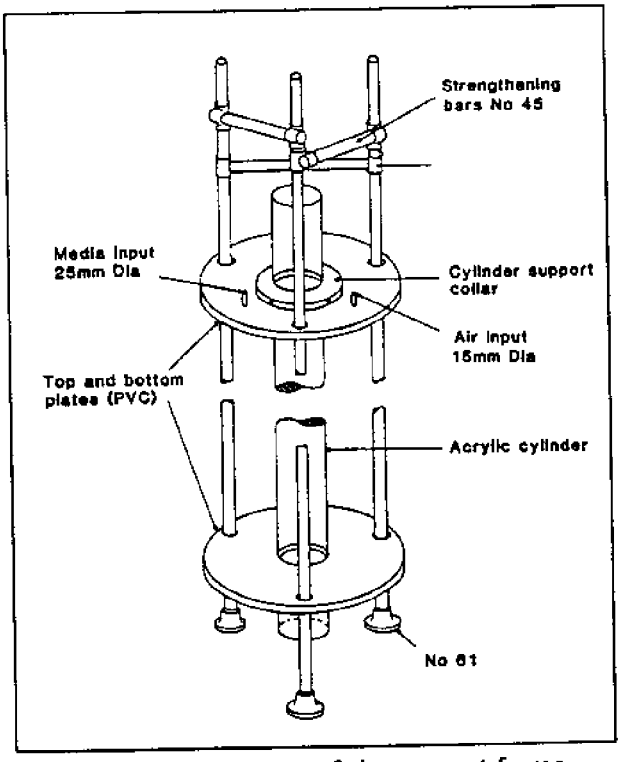

Figure 5. Arrangement of the support frame for the vessel in Figure 4 (from Laing and Jones (1988). Reprinted with permission of Elsevier Science Publishers, B.V.

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

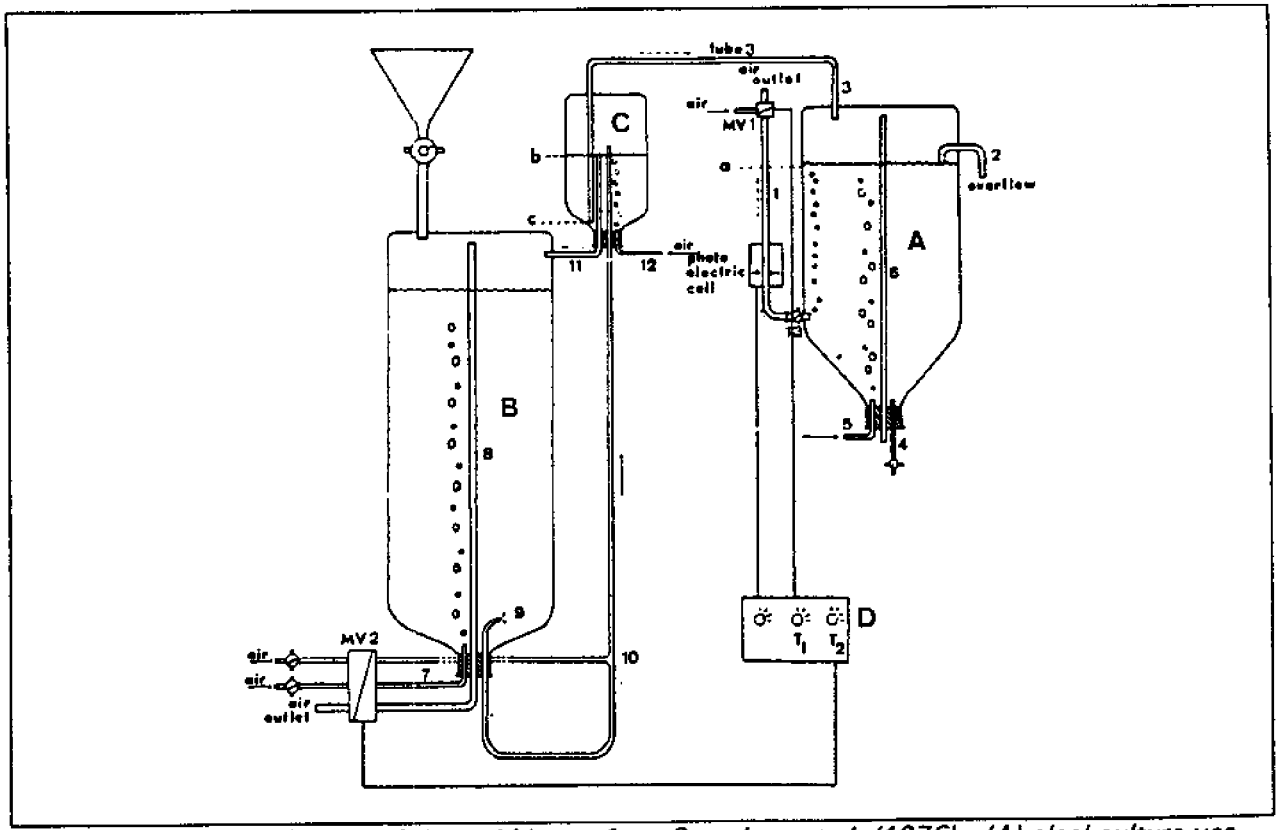

Figure 6. Schematic diagram of the turbidostat from Sorgeloos et al. (1976). (A) algal culture vessel; (B) stock medium; (C) dispensing vessel; (D) electronic control unit.
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be brought about by a number of factors, including sudden changes in salinity 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.

See Salt (1987) and Korstad et al. (1989) for additional information about rotifer feeding behavior. For papers regarding various aspects of reproduction and the production and hatching of resting eggs, the reader is directed to Pourriot and Snell (1983), Snell and Childress (1987), Snell and Boyer (1988), Ben-Amotz and Fishler (1982), Hino and Hirano (1977), Minkoff et al. (1983), Hagiwara and Hino (1989), Hagiwara et al. (1989), Serrano et al. (1989) and Snell (1986, 1987).

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, antibiotics, 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.

Small size	123 - 292 μm[1]
Slow speed, planktonic nature	
Tolerance of high densities	up to 2,000 ind./ml reported[2]
High reproduction rate	r = 0.7 - 1.4 offspring/female/day[3]
Living "biocapsules" for delivering substances to larvae	

[1]Snell and Carrillo 1984.
[2]Reported in Hirata 1979.
[3]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 altivelia*) (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 7. Milestones in the culture history of *B. plicatilis*.[1]

	Milestone	Approximate Date
1.	*Mizukawari* investigated by Takeshi Ito	Early 1950s
2.	*B. plicatilis*' value as a larval fish feed discovered, mass culture techniques explored	1960
3.	Hirata's "daily tank transfer method" used at the Yashima Station of the Seto Inland Sea Farming Association	1964
4.	*B. plicatilis* discovered to be excellent food for red sea bream (*Pagrus major*) larvae	1965
5.	Hirata and Mori discover that baker's yeast is a suitable food source	1967
6.	Baker's yeast used extensively in mass culture	1970s

[1]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*.

Parameters	Acceptable Range	Optima
Temperature (°C)	20 - 30[1]	21 - 25[2], 35[3]
Dissolved oxygen (ppm)		2 - 7[4]
[NH_3 + NH_4] (mg/liter)	6 - 10[5]	
[NH_3] (mg/liter)		< 1[6]
Salinity (ppt)	1 - 60[6]	10 - 20[6]; 18[5]; 20 (S-type), 30 (L-type)[7]
Inoculation density (ind./ml)	505, 100 (extensive)[8]	100 - 1,000 (intensive)[8]
Feeding amount (temperature dependent)		
Nannochloropsis oculata (cells/rotifer/day)		100,000 - 150,000[9]
Dunaliella (cells/ml)		1,000,000[10]
Chlorella (cells/ml)		1,500,000[10]
Tetraselmis suecica (cells/ml)		500,000[5]
Baker's yeast (mg/rotifer/day)	.4 - 1.2[11, 9]	
Light intensity (lux)		2,000
Photoperiod (light hours:dark)		18:6[6]
pH	5 - 10	7.5 - 8.5[6]

[1]Hirayama and Kusano (1972).
[2]Theilacker and McMaster (1971).
[3]Pascual and Yufera (1983).
[4]Fukusho (1989a).
[5]Coves et al. (1990).
[6]Hoff and Snell (1989).
[7]James and Abu Rezeq (1990). These are salinities recommended for marine finfish hatcheries. Best growth, however, was achieved at 5 ppt for both strains.
[8]Kafuku and Ikenoue (1983).
[9]Fushimi (1989).
[10]Pourriot (1990).
[11]Foscarini (1988).

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.

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

and as a possible cause of mass culture instability. Other research has been directed toward nutritional deficiencies; a lack of vitamin B_{12}, 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 x 10^8 *Tetraselmis*-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-m^3 tank, or **intensive**, in small, 1 to 2-m^3 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 10. Summary of *B. plicatilis* culture techniques used in Japan (from Fukusho 1989b).

1.	Semi-continuous culture in large tanks using only *N. oculata* as feed
2.	Semi-continuous culture using *N. oculata* in combination with plain yeast or enriched yeast
3.	Batch culture in large tanks using *N. oculata* in combination with plain yeast or enriched yeast
4.	Batch culture in small tanks using *N. oculata* in combination with plain yeast or enriched yeast
5.	Feedback method

Table 11. Culture methods and production of rotifers in Japan (from Yoshida 1989).

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

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 upscaling 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 *Nannochloropsis* 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/m^3/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 .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 B_{12} (Yu et al. 1989) and may also require vitamin A (Fukusho 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 ω 3 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 tetrathele* 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

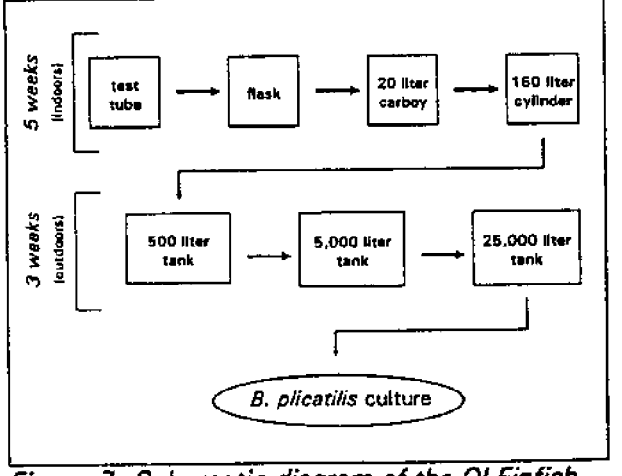

Figure 7. Schematic diagram of the OI Finfish Program's Tetraselmis tetrathele *upscaling protocol.*

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 B_{12}-producing bacteria (Yu et al. 1989, 1990). According to Yu et al. (1990), addition of vitamin B_{12}-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 B_{12}/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-m^3 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. tetrathele* 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 nl /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/m^3 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-m^3 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-m^3 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 Tohuku 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 ([NH_3]) is largely a function of [NH_4], 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 (NH_3+ + NH_4) are generally found in *Brachionus* tanks" (p. 233), and that *Brachionus* sp. "appear to be fairly resistant to ammonia" (p. 233).

Hirata and Nagata (1982, cited in Lubzens 1987) showed that *B. plicatilis* raised on *N. oculata* excrete ammonia, urea and phosphates ($1.41 \pm 0.87 \times 10^{-4}$ μg NH_4-N, 1.17 ± 1.31 μg urea-N, and $0.27 \pm 0.29 \times 10^{-4}$ μg PO_4-P/hour/ind., respectively). Yu and Hirayama (1986) found a correlation between high levels of un-ionized ammonia (NH_3) 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-m^2) 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 "*kin-ran*," 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-m^3 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 as 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-m^3 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-m^3 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-m^2 room on the second floor houses six square 100-m^3 rotifer production tanks (2.5 m deep, each with a 1-m^3 filter attachment) and a 50-m^3 reserve culture tank. The first floor contains a 10-m^3 *B. plicatilis* collection tank and two 25-m^3 tanks that hold concentrated *N. oculata*. *Nannochloropsis oculata* production takes place outdoors in ten, 300-m^3 tanks (22 m x 14 m x 1 m). Figures 9

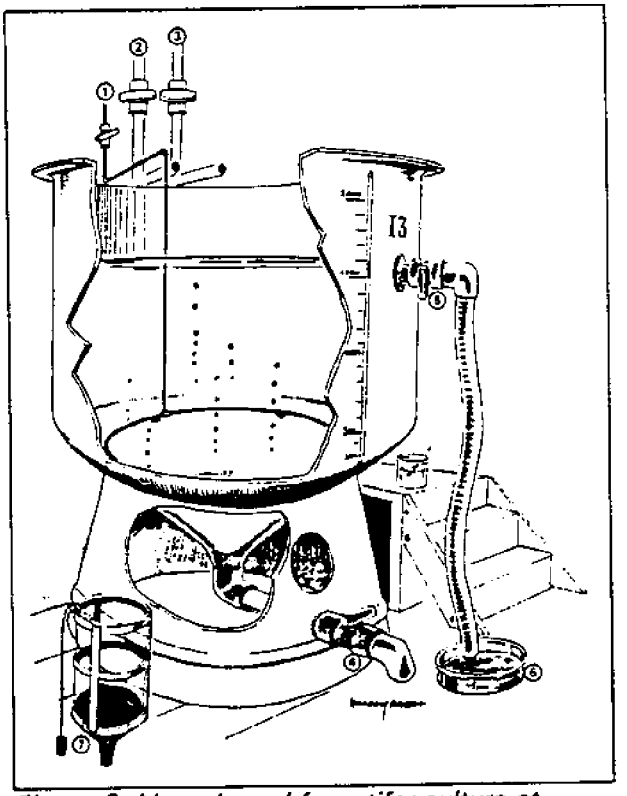

Figure 8. Vessel used for rotifer culture at IFREMER centres in France (from Coves et al. 1990). (1) compressed air; (2) seawater [salt reduced]; (3) live algae; (4) draining trap; (5) harvesting tap and hose; (6) round stainless filter; (7) cylindro-conical filter.
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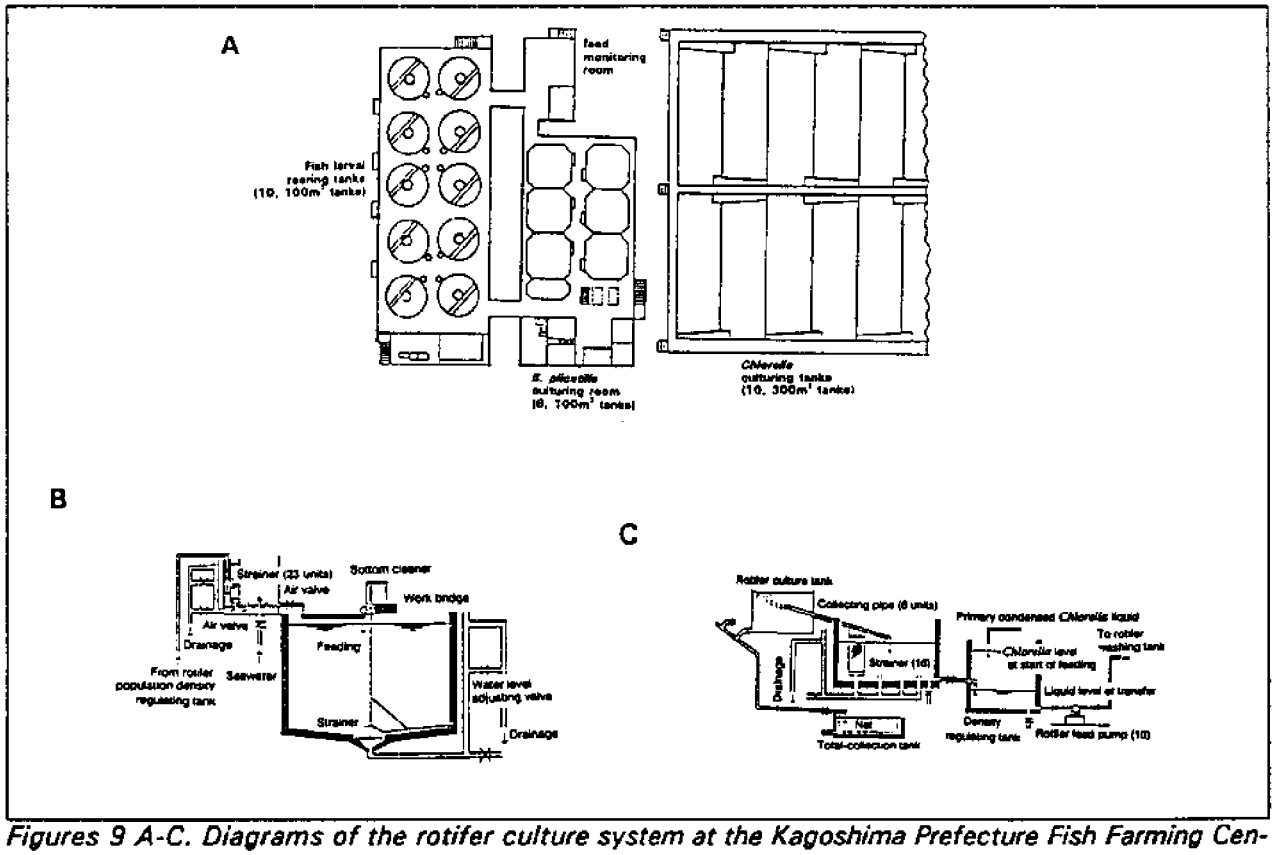
B and C from Fukusho (1989c) illustrate the transfer of *N. oculata* to the rotifer tanks and that of rotifers to the fish larval tanks. Rotifer production is semi-continuous, with a daily harvest rate of approximately 30%. In one case, from the end of April to the beginning of June, a total of 40.15 ± 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-m^3 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-m^3 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 10^8 ind./m^3, using 0.4



Figures 9 A-C. Diagrams of the rotifer culture system at the Kagoshima Prefecture Fish Farming Center (A is from Fushimi 1989, B and C are taken from Fukusho 1989c). A: An overview of the larval rearing section. B: Larval rearing tank and rotifer washing apparatus. C: Rotfier collection/feeding system.

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B and C: Reprinted with permission of Fuji Technology, Ltd.

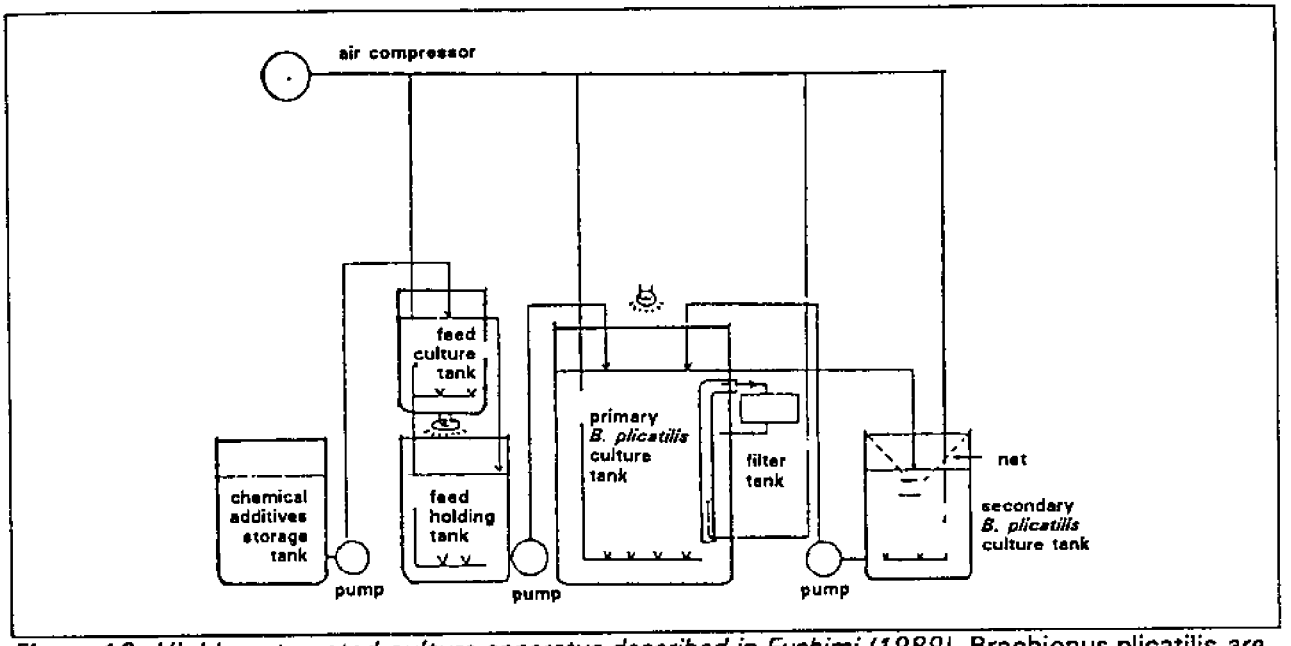

Figure 10. Highly automated culture apparatus described in Fushimi (1989). Brachionus plicatilis *are cultured in .5-m^3 tanks.*
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m^3 of *N. oculata* and 0.9 kg of baker's yeast in the process.

Finally, an example of large-scale batch culture is described in Fushimi (1989). This system is used at the Hiroshima Prefecture Fish Farming Center. *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. *Nannochloropsis oculata* and ω-yeast were used as feed. Tanks averaged 18.25 billion and 24.1 billion *B. plicatilis*/day for L- and S-type rotifers, respectively.

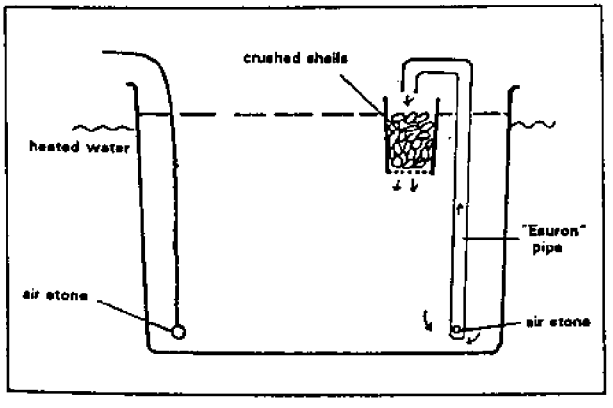

Figure 11. Diagram of the .5-m^3 tank used for batch culture at the Miyazaki Prefecture Marine Production site (from Yoshida 1989). Reprinted with permission of Koseisha-Koseikaku, Tokyo.

3.6.2 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 (*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

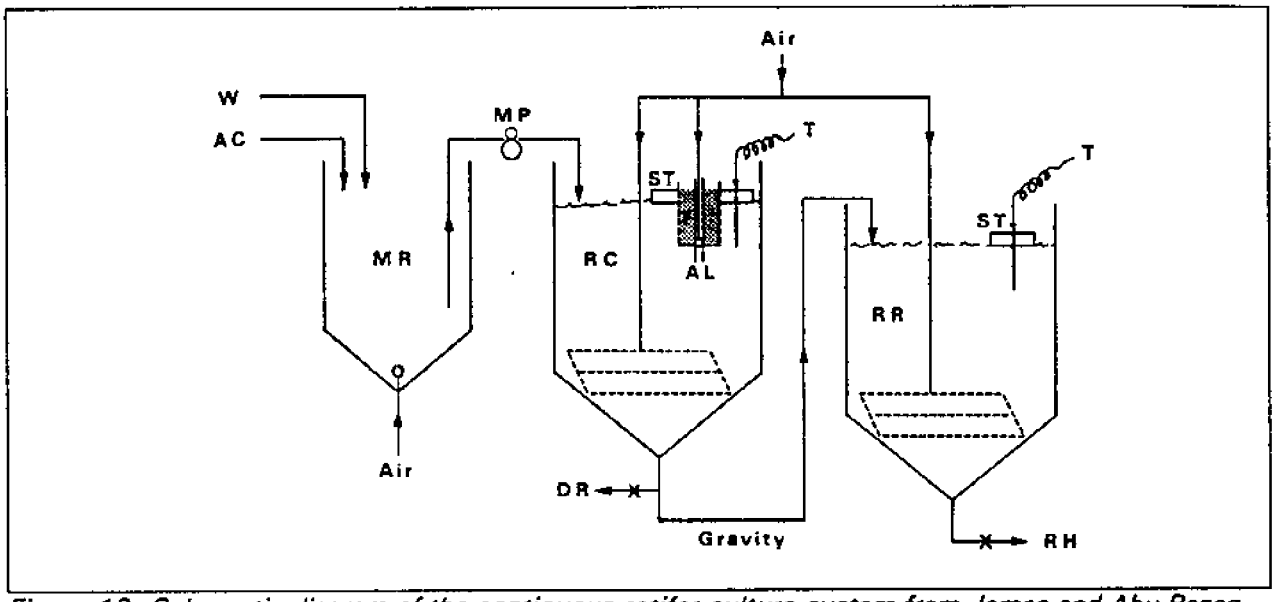

Figure 12. Schematic diagram of the continuous rotifer culture system from James and Abu Rezeq (1989b). W = 30 ppt water inlet; AC = inlet from stage-1 algal chemostat; MR = mixing reactor; MP = piston metering pump; RC = rotifer chemostat; ST = styrofoam float; T = temperature controller; AL = air lift; DR = drainage outlet; RR = rotifer reservoir; RH = outlet for rotfier harvest. Reprinted with permission from Elsevier Science Publishers B.V. and the author.

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.

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Part II

Rotifer and Microalgae Culture Systems. Proceedings of a U.S. – Asia Workshop. Honolulu, HI, 1991.

Review of the Research Status of Zooplankton Production In Japan

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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, *Tigriopus 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 ω 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-m^3 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, Shirota 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; Iwasaki and Kamiya 1977).

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

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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-m^3 outdoor tanks and baker's yeast was the feed.

Table 1. Copepods and cladocerans considered to be promising species for mass culture in Japan (Anraku 1979).

Copepods	*Acartia clausi* *A. longiremis* *Eurytemora pacifica* *Euterpina acutifrons* *Oithona brevicornis* *O. similis* *Pseudodiaptomus inopinus* *P. marinus* *Microsetella norvegica* *Sinocalanus tenellus* *Tigriopus japonicus*
Cladocerans	*Evandne tergestina* *Penilia avirostris* *Podon polyphemoides*

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.

The two Societies mentioned above held a symposium, "Aquaculture and Plankton" in 1985. Six papers were presented and a brief proceedings was published in the Bull. Plankton Soc. Japan, Vol. 32, No. 1, pp. 65-66, 1985.

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 m^3) 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-

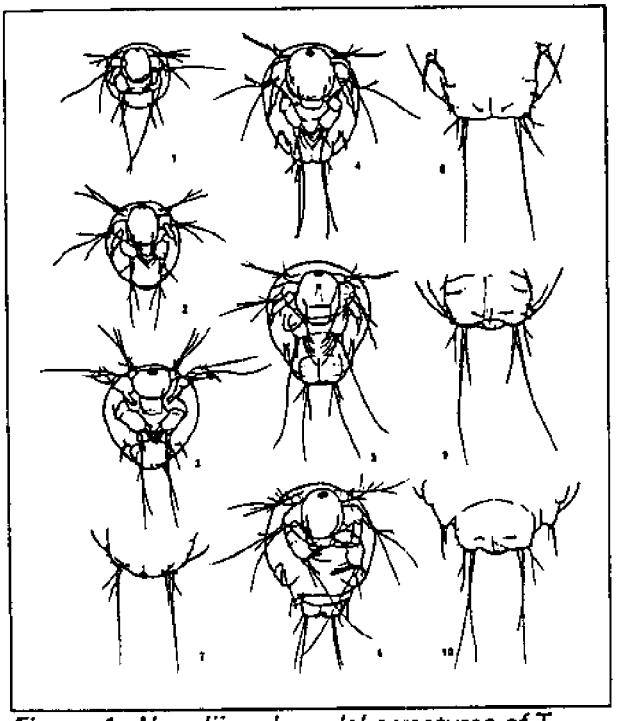

Figure 1. Nauplii and caudal armatures of T. japonicus *in ventral view. 1-6; stage I-IV (x 120). 7-10; caudal armatures of stage III-VI (x 240). (Koga 1970).*

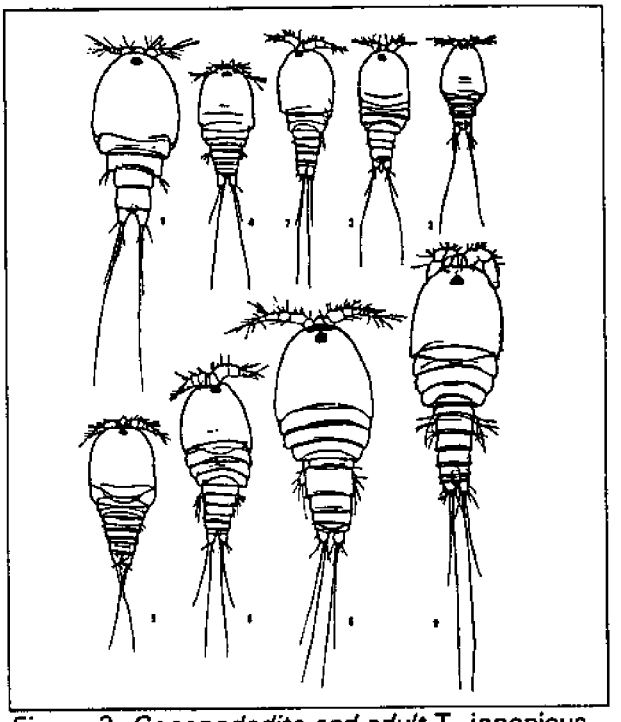

Figure 2. Copepododite and adult T. japonicus *in dorsal view (x 60, but 1- x120). 1-3; stage I-III. 4-6; stage IV, V and adult (female). 7-9; stage IV, V and adult (male). (Koga 1970).*

ever, 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 microparticulated 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 (Taniguchi 1978). To date, tintinnids are not, however, used intensively at mass production facilities in Japan.

ROTIFERS, *BRACHIONUS PLICATILIS*

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 tetrathele*, 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 Hirayama 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 microfauna 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.

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Rotifer and Microalgae Culture Systems. Proceedings of a U.S. - Asia Workshop. Honolulu, HI, 1991.

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

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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

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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

describes a typical Japanese hatchery using 100-m^3 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-m^3 tanks hold a standing crop of rotifers from which 1 - 2 billion can be harvested daily. Using this system, 600 m^3 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 m^3. 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-m^3 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-m^3 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-m^3 chemostats with the yields cited above. This means that 6 - 7 m^3 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 rotifers can be more tightly controlled. James and Abu-Rezeq (1989a) reported that

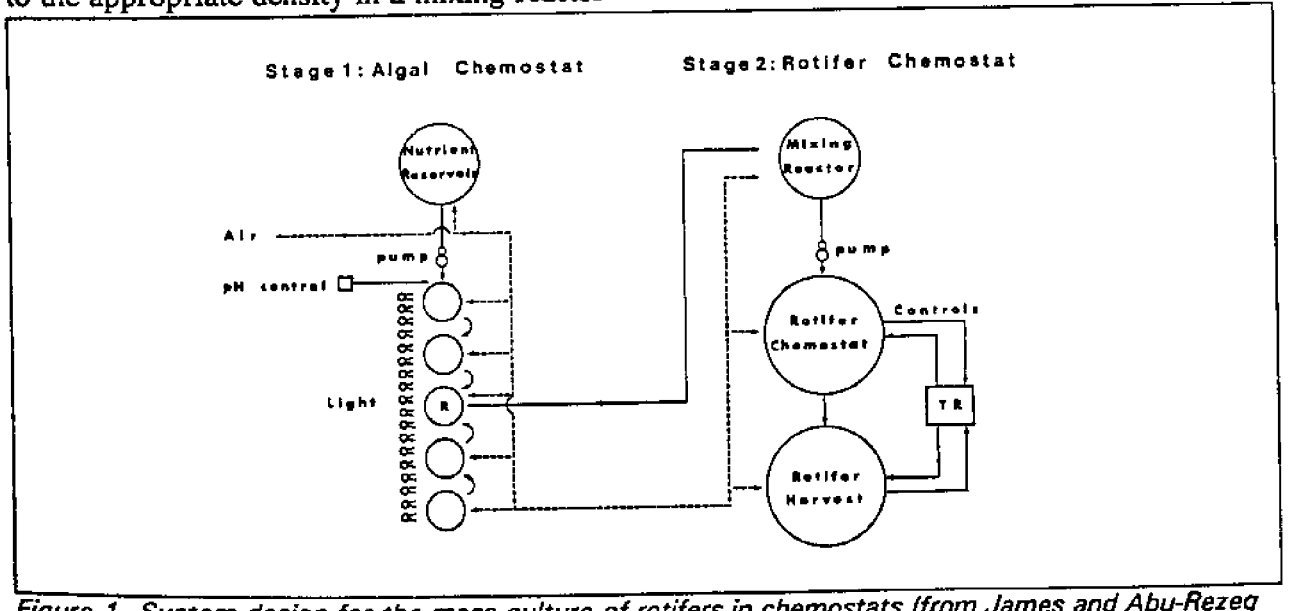

Figure 1. System design for the mass culture of rotifers in chemostats (from James and Abu-Rezeq 1989b).

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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-m^3 tanks. To meet the rotifer requirements of very large hatcheries, 10-m^3 chemostats would be preferable so that 12 - 14 could yield 20 billion rotifers/day. Ten-m^3 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 m^3. 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 (NH_3) (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 (NH_4^+) (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 (R_o). Un-ionized ammonia significantly depressed r and R_o 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 (NO_2-) 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, Groeneweg 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 technician 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 (Calipatria, 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 x 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

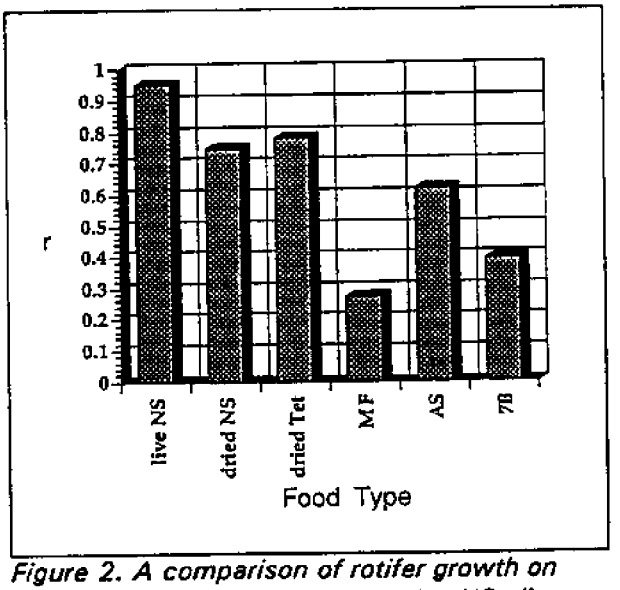

Figure 2. A comparison of rotifer growth on diets of dried algae and yeast. Live NS- live Nannochloropsis salina *cells, dried NS- spray dried* N. salina *cells, dried Tet- dried* Tetraselmis suecica *cells, MF- Microfeast L-10, AS- Culture Selco, 7B- Fleischmann's yeast. r is population growth rate in offspring/female/day.*

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.

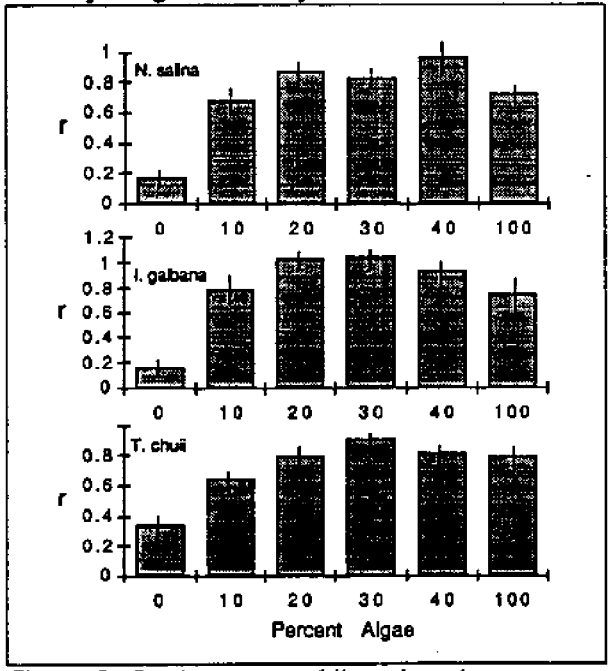

Figure 3. Replacement of live algae by yeast. The X-axis is the percent algae of a 100 µg dry weight/ml diet. The remaining portion of the diet is Microfeast L-10 yeast. r is population growth rate in offspring/female/day. Vertical lines indicate standard error.

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-mercaptoenthanol 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.

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 B_{12}-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

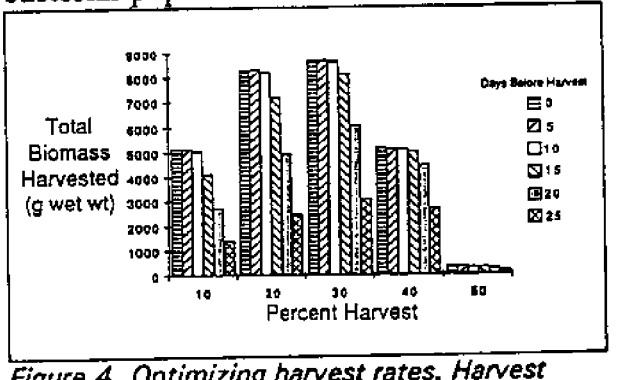

Figure 4. Optimizing harvest rates. Harvest begins on day 0-25 and continues daily until day 30 when the culture is terminated. Percent harvest is the portion of the population removed each day. The total biomass harvested is the sum of the rotifers removed each day times 3 µg/rotifer.

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.

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

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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 x 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-μm pore size). Water temperatures in the rotifer cultures fluctuated from 23 to 28°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-μm aperture was used and the instrument was adjusted to count particles with volumes equivalent to spherical particles with diameters 100 - 200 μm.

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 0.5×10^6, 10.0×10^6 and 1.0×10^6 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 x 61 x 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

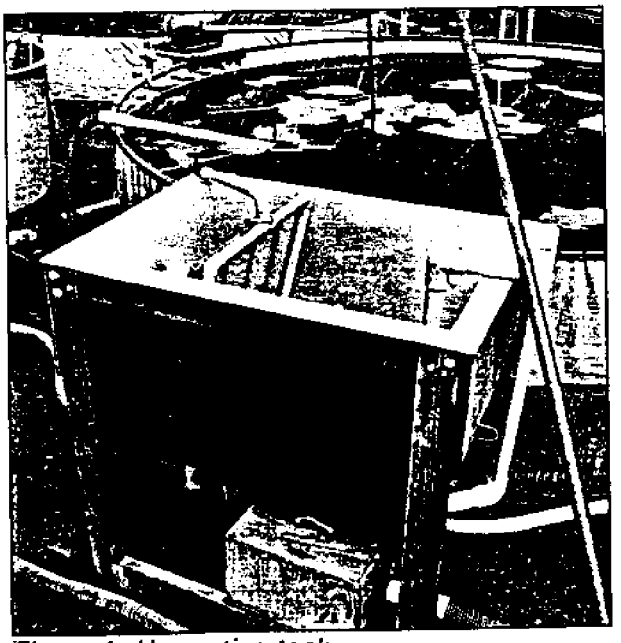

Figure 1. Harvesting tank.

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%).

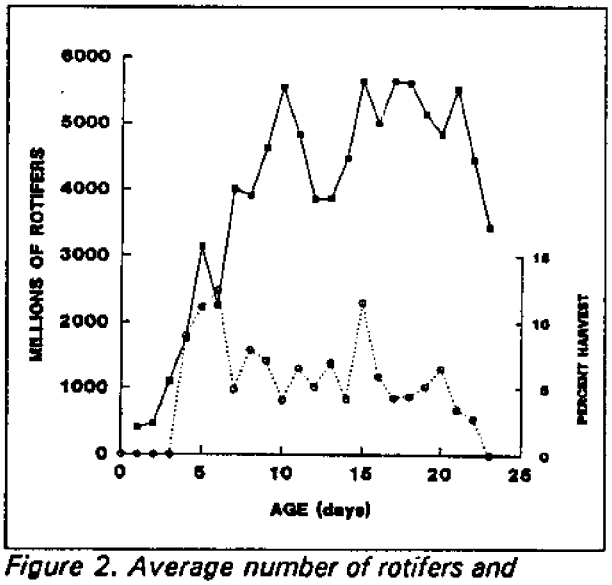

Figure 2. Average number of rotifers and average daily percent harvest of Pool 13.

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.

Pool No.	Mean rotifer density ± SD (#/ml)	Initial rotifer density (#/ml)	Culture duration /No. of harvests (days)	Average percent daily harvest	Average daily harvest (x 10^6)	Average rotifer production (#/day/ml)	Percentage of egg carrying females (%)	Average amount of algae fed (liters/day)
1	542 ± 275	39	56/26	4.4	217	29.5	39.6	404
2	399 ± 199	105	29/5	1.5	59	13.4	34.7	321
3	640 ± 241	344	44/27	4.9	219	32.0	36.5	376
4	596 ± 204	61	60/36	4.9	247	37.2	34.9	342
5	635 ± 208	372	25/21	7.0	383	47.1	37.8	324
6	596 ± 168	490	58/48	4.0	190	21.8	34.8	312
7	470 ± 146	551	38/30	4.8	184	18.7	29.9	267
8	591 ± 162	271	15/12	1.8	98	22.9	30.3	338
9	632 ± 117	547	13/11	2.6	149	30.3	32.3	375
10	564 ± 112	718	16/7	3.7	177	15.5	26.8	380
11	644 ± 152	560	8/5	5.3	279	23.7	29.5	414
12	391 ± 79	460	20/17	10.2	324	42.8	31.4	289
13	461 ± 186	79	24/19	5.1	214	41.7	34.0	211
14	520 ± 100	591	22/14	2.8	124	12.0	27.8	248
15	418 ± 134	496	12/6	1.7	71	-6.0[1]	24.9	414
16	588 ± 300	241	29/27	5.8	288	31.1	30.8	200
17	490 ± 242	237	44/39	8.1	215	23.2	33.6	258
$\bar{X}$	540 ± 87	363	30/21	4.6	202	25.7	32.3	322
Σ			513/350					163,096[2]

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

sion 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 hardiness of the rotifer and its ability to withstand the physical stress of water velocities at pumping rates of 5 - 8 m^3/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.

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A Review of Automation and Mechanization Used In the Production of Rotifers in Japan

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ABSTRACT

Recent annual fish fry and rotifer production statistics are given for several hatcheries in Japan. The status of microalgae culture, including problems and countermeasures for the different types, is presented. Three methods used for rotifer production are also discussed and the problems are mentioned. Rotifer harvesting machines and some automatic, continuous culture equipment are described, as are techniques for storing concentrated microalgae. Finally, the author introduces you to new facilities in Ehime Prefecture constructed with several innovative mechanization and automation features.

THE STATUS OF FISH FRY AND ROTIFER PRODUCTION

In Japan, we produce many juvenile aquatic animals for release and consumption. In most cases, organisms must be cultured to feed these animals. A large quantity of live feeds is required for the large-scale rearing of larval fish, shrimp and crabs. Both zooplankton and phytoplankton culture are essential. The former consists mainly of rotifer, *Brachionus plicatilis*, culture, and the latter of green microalgae culture (mainly *Nannochloropsis* sp.).

Microalgae are not only fed to rotifers, they are also added to fish rearing tanks to improve water quality, promote calmness due to low illumination and opacity, and to prevent starvation of the rotifers, especially in the early stages of fish culture.

Recent annual fry production, rotifer production, and rotifer consumption for several representative stations in Japan are shown in Table 1. At the Ehime Station, we produce six species: red sea bream (*Pagrus major*), Japanese flounder (*Paralichthys olivaceus*), Japanese sweet fish, Ayu (*Plecoglossus altivelis*), Japanese striped knifejaw (*Oplegnathus faciatus*), kuruma prawn (*Penaeus japonicus*) and Japanese black abalone (*Haliotis discus*). We do not feed rotifers to abalone, but the four species of fish are fed live rotifers, and the shrimp are fed frozen rotifers. This is because the supply of live rotifers is limited because fish and shrimp are reared at the same time.

Table 1. Recent annual production of rotifer-fed fry and rotifer production in several stations in Japan[a] (fiscal years).

Japan Fish Farming Association, Hakatajima Station (J.F.F.A., 1985 - 1990)

	Red sea bream			Japanese flounder			Rotifer production[c]
	Length (mm)	Number[b]	Rotifers fed[c]	Length (mm)	Number	Rotifers fed	
1984	19	2,855	207.4	17	1,706	49.8	611.6
1985	26	478	94.5	16	1,362	138.4	408.3
1986	23	2,582	—[d]	21	2,555	151.1	881.7
1987	26	2,628	—	21	3,109	—	665.0
1988	25	2,351	—	18	3,006	—	500.6

Mie Prefectural Fish Farming Center (M.P.F.F.C., 1988 - 1990)

	Red sea bream			Japanese flounder			Tiger puffer			Rotifer production
	Length (mm)	Number	Rotifers fed	Length (mm)	Number	Rotifers fed	Length (mm)	Number	Rotifers fed	
1987				49	130	41.3				397.3
1988	38	253	31.9	43	287	56.0	23	287	58.0	417.8
1989	28	408	21.2	40	509	19.5	21	186	18.0	325.5

Hiroshima Prefectural Fish Farming Association (H.P.F.F.A., 1985 - 1990)

	Red sea bream			Black sea bream			Japanese flounder			Japanese sweet fish, Ayu			Rotifer production
	Length (mm)	Number	Rotifers fed	Length (mm)	Number	Rotifers fed	Length (mm)	Number	Rotifers fed	Length (mm)	Number	Rotifers fed	
1984	13	2,845	192.5	14	3,866	277.7	15	517	25.2	57	1,947	97.7	1,214.1
1985	13	2,131	128.6	12	6,759	106.5				56	4,585	82.4	842.3
1986	16	2,617	125.0	12	4,573	131.5				—	2,608	208.0	752.6
1987	18	3,012	121.2	14	6,049	131.6				56	2,350	345.3	803.7
1988	16	2,158	79.1	12	5,262	113.6	27	276	11.8	55	2,511	395.0	834.7
1989	18	2,143	78.3	13	6,423	139.3	26	143	12.1	49	2,416	271.0	696.6

a) Mass production of rotifers is carried on only during the rearing seasons. In other seasons, rotifers are cultured in small volumes only to feed broodstock.

b) Units in all "number" columns: thousands

c) Units in all "rotifers fed" and "rotifer production" columns: billions

Table 1. Continued.

Ehime Prefectural Fish Farming Center (E.P.F.F.C., 1985 - 1990)

	Red sea bream			Japanese flounder			Japanese sweet fish, Ayu			Rotifer production[c]
	Length (mm)	Number[b]	Rotifers fed[c]	Length (mm)	Number	Rotifers fed	Length (mm)	Number	Rotifers fed	
1984	—	891	177.1				51	945	276.0	456.4
1985	12	1,176	191.5				51	651	166.5	336.7
	—	630								
1986	14	1,218	151.8	—	96	—	56	541	155.1	333.7
	52	873								
1987	15	1,500	257.4	31	218	37.6	57	610	153.3	548.2
	55	1,099								
1988	15	2,000	400.9	43	188	39.7	53	497	163.6	710.6
	48	1,134								
1989	14	2,000	409.5	35	450	48.2	60	640	226.7	726.0
	46	1,039								

b) Units in all "number" columns: thousands

c) Units in all "rotifers fed" and "rotifer production" columns: billions

d) No data

MICROALGAE CULTURE

Nannochloropsis sp. (formerly referred to as "marine *Chlorella*," Maruyama et al. 1986) and *Tetraselmis* sp. are essential as feed for rotifer cultures. In hatcheries, algae is cultured in 0.5 - 300-m^3 tanks. The total culture volume ranges from several hundred to 3,000 m^3. Ordinarily the density of microalgae is 10 - 30 million cells/ml and density is lowest in the warm seasons. It is possible to grow dense, pure stock cultures, but mass culture is extensive and somewhat mixed. Sometimes serious contamination occurs in outdoor cultures. Ammonium sulfate, calcium superphosphate, urea and Clewat 32 are used as fertilizer. The amount added differs slightly among stations, but is approximately 100, 30, 10 and 5 g per m^3, respectively (see Okauchi, this volume).

When microalgae is fed to rotifers it is transferred utilizing both pumps and pipes. In many hatcheries, including our station, algae and rotifer production tanks are connected with pipes. To transfer microalgae to rotifer production tanks, we just turn on a switch after the valves of both tanks have been opened. We use a 2.2-kw pump (about 27 m^3/hour).

We maintain a stock of tens of billions of rotifers during the growing season. In order to maintain them stably, 40 - 60 m^3 of microalgae must be supplied daily. We have four 150-m^3 concrete tanks (although a tank has a capacity of 300 m^3, we only fill it half-way because it would be too deep otherwise) and two 100-m^3 fabricated nylon tanks framed with FRP. Figure 1 shows the annual process of algal (*Nannochloropsis* sp.) culture at our station in 1987-1988.

Problems and Countermeasures

The problems associated with microalgae culture are as follows:

- A great deal of land is required. Large-scale culture tanks must be located at a sunny site and the cost of land is very high in Japan.
- Influence of weather: sometimes culture crashes occur during the rainy and summer seasons. This is believed to be caused by a lack of solar rays and contamination.

Alternatively, freshwater *Chlorella* which has been condensed and enriched with vitamin B_{12} is sold commercially (Maruyama et al. 1989, Yu et al. 1989). There are also a few private hatcheries which use freshwater *Chlorella* instead of marine microalgae to feed

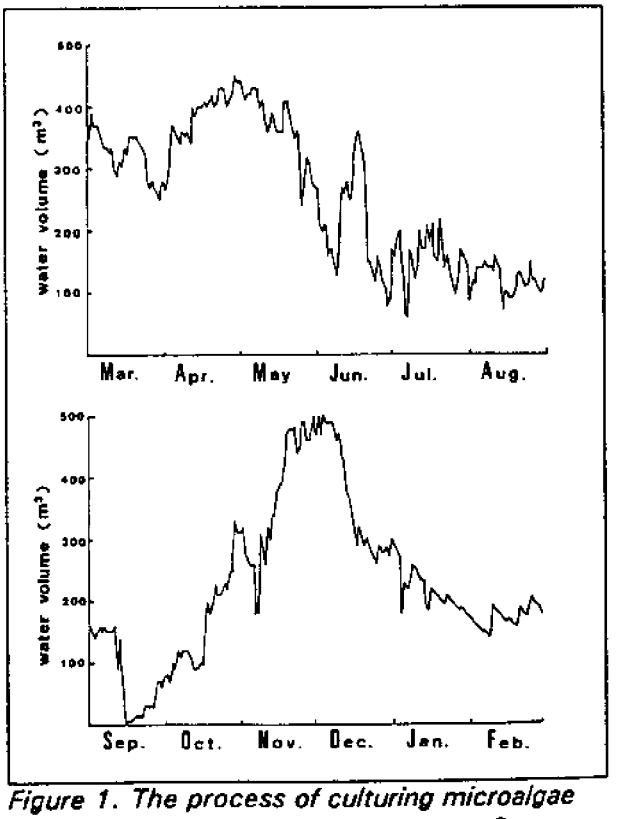

Figure 1. The process of culturing microalgae at the Ehime Prefectural Fish Farming Center from Mar. 1987 - Feb. 1988.

rotifers. This is because they consider it important to reduce labor, increase the reliability of rotifer culture, save land and use tanks efficiently (Mizuguchi, personal communication).

ROTIFER CULTURE

There are two types of systems: batch and semi-continuous. According to an operator's preference and the facilities available, different systems (culture methods) are adopted.

In general, batch culture is done in small tanks. Although the yield is high, more labor is needed. The density of rotifers is about 300 - 500 ind./ml at harvest. For these systems, equipment for filtering suspended substances and excrement is essential. Thus, artificial seaweed, commercial plastic filter mats, oyster shells, etc. are used as filter material. The culture period lasts two to four days. Initially green microalgae is fed, but thereafter baker's yeast or ω yeast (Imada et al. 1979) is fed. The amount of yeast fed is often 1 g/million individuals, though it varies depending on the rotifer strain (S, L) and water temperature.

On the other hand, semi-continuous culture is usually performed in large, 50 - 200-m^3 tanks. In contrast to batch systems, the yield is lower, but the amount of labor is also lower. The density of rotifers is maintained at about 100 ind./ml, so rotifers are harvested at a constant rate. The culture period is approximately 20 to 60 days, however, Okada and Hirano (1990) reported that they could continue rotifer culture without changing tanks, harvesting many rotifers, for an entire year. The amount of yeast fed to semi-continuous cultures is often less than in batch systems because of the potential for water pollution due to overfeeding.

We at the Ehime Prefectural Fish Farming Center have used a semi-continuous system since our establishment in 1980. We have ten 60-m^3 tanks, hence reducing working hours and conserving microalgae are priorities.

Figure 2 shows the annual process and rotifer harvests in 1987-1988. Heating was used from Nov. to Apr.; water temperature was maintained at 22°C. The rotifers were fed 2,024 kg of ω yeast, 293 kg of baker's yeast and 6,419 m^3 of phytoplankton, and we harvested 663 billion rotifers (both L- and S-type).

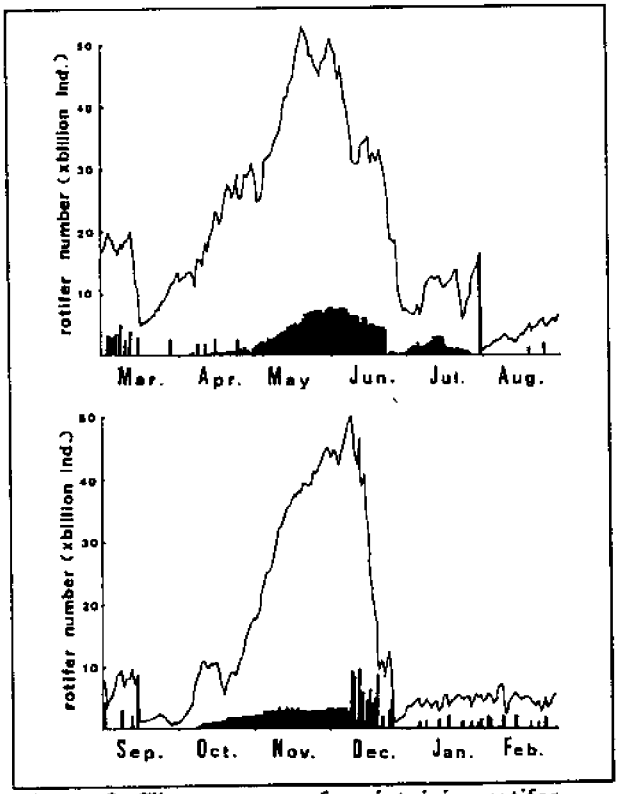

Figure 2. The process of maintaining rotifer stocks and harvesting rotifers from Mar. 1987 to Feb. 1988. Solid line shows rotifer stocks, shaded portions represent harvested rotifers.

Problems

The problems are as follows:

- Decrease in rotifer density (culture crashes).

The following explanations for this phenomenon have been put forth: Changes in water temperature, water pollution, propagation of microorganisms, diseases, a lack of microalgae, and poor quality microalgae (Sugimoto 1989).

- Labor.

Much labor is needed to culture and harvest rotifers—observing and measuring them, feeding them, washing filters and production tanks, and harvesting, transferring and feeding them to larval fishes.

MECHANIZATION AND AUTOMATION

To decrease labor, systematization and various apparatuses have been designed, studied and put into practice (Fujita et al. 1982).

Fushimi (1989) describes the "software" of a system as including variables such as selection of a rotifer strain, water temperature, food, culture method, rotifer density, propagation rate and harvest rate. Alternatively, the "hardware" of a system includes tanks, heating, measuring rotifers, removing suspended substances, transferring, harvest method, cleaning tanks, aeration and secondary culture procedures.

A great deal of labor and time are needed to harvest rotifers, therefore, operators have been developing different harvesting apparatuses for ten years. Seikai (1979) reported on one that moved repeatedly, thereby preventing clogging. It could filter 10 m^3 of culture medium per day automatically (Fig. 3).

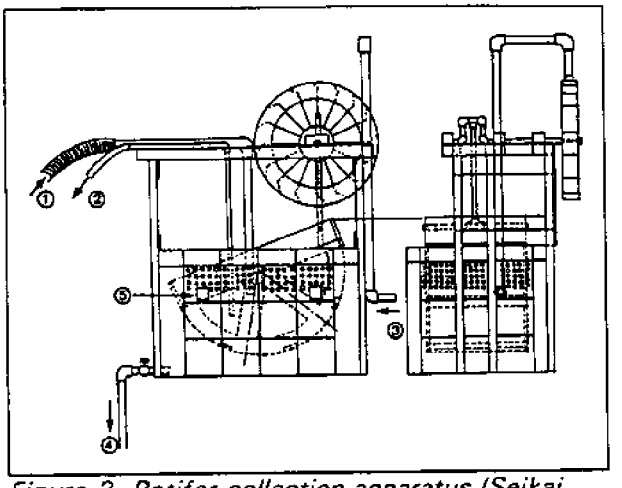

Figure 3. Rotifer collection apparatus (Seikai 1979). 1: input by pump; 2: harvesting by siphon; 3: seawater for propulsion; 4: drain; 5: outlet for overflow.

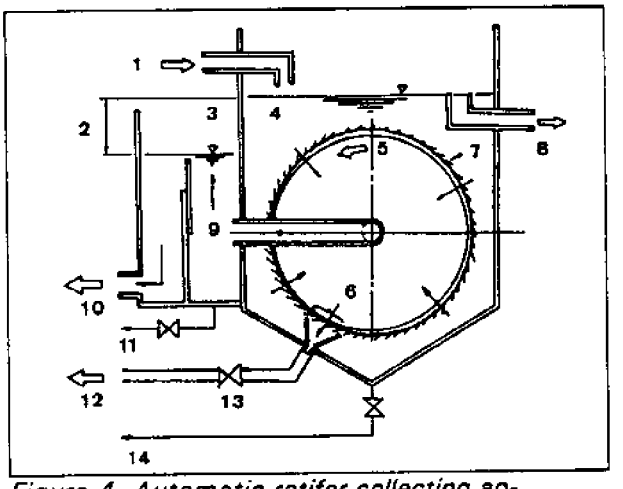

Figure 4. Automatic rotifer collecting apparatus (Hiramoto et al. 1984). 1: rotifer culture water; 2: pressure filtration; 3: accessory tank; 4: main tank; 5: rotating drum; 6: rotifer collection; 7: filtering mat with long bristles; 8: overflow outlet; 9: filtered water; 10: filtered water; 11: drain; 12: water with concentrated rotifers; 13: valve; 14: drain.

Hiramoto et al. (1984, 1985, 1986) reported on the use of an automatic continuous

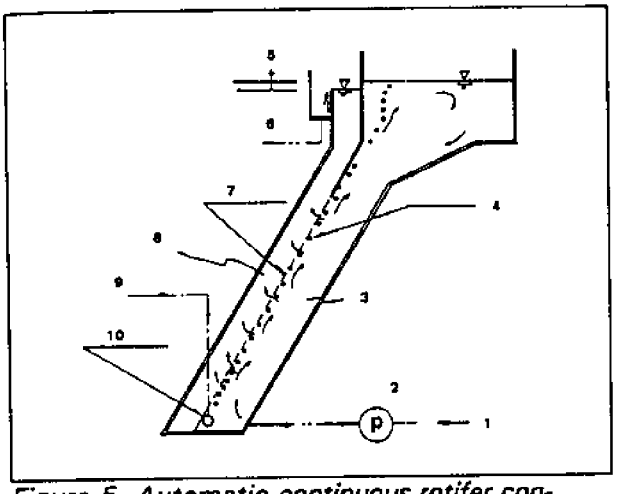

Figure 5. Automatic continuous rotifer concentrator. The principle of the apparatus (Jap. Fish Farming Assoc. 1984). 1: original water; 2: pump; 3: condensing space; 4: air bubbles; 5: differential pressure of the filter; 6: outlet for filtered water; 7: filter; 8: water filtration space; 9: compressed air from blower; 10: air pipe (produces bubbles).

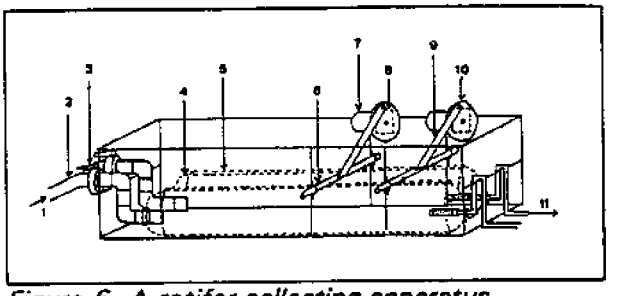

Figure 6. A rotifer collecting apparatus (Mekuchi et al. 1988). 1: culture water; 2: 100-mm pipe; 3: butterfly valve; 4: plankton net; 5: 34 x 32 mesh screen; 6: pvc shaft; 7: induction motor; 8: gear head; 9: acrylic crank; 10: pvc pulley; 11: condensed rotifers, to pump.

harvesting apparatus with a rotary drum which washed, filtered and removed algae, bacteria, etc., from polluted water. The apparatus was capable of treating 900 m^3/day, which it concentrated by a factor of 112 (Fig. 4).

The Japan Fish Farming Association (1984) reported that J.F.F.A. and Mitsui Kaiyo Kaihatsu Ltd. had developed an automatic, continuous rotifer concentrator which was capable of 100X concentration and filtering 10 m^3/hour (Fig. 5). Furthermore, Mekuchi et al. (1988) produced a machine with the ability to shake collecting nets to prevent clogging. It is capable of filtering 1 m^3/min. The maximum harvest density was 30,000 individuals and this apparatus is being used now (Fig. 6).

A few automatic continuous culture apparatuses have also been reported. Mochizuki et al. (1978) developed a system consisting of a 5-m^3 rotifer production tank and a feed production tank, which produced from 130 million to 550 million ind./day for thirty days (beginning from the day density reached 100/ml). However, this system didn't spread to hatcheries because it was difficult to separate the rotifers from suspended solids and the cost of construction was high (Fushimi 1989).

Marinoforum 21 (1988, 1989) is trying to develop automatic continuous rotifer culture equipment which has the following features: intensive production, reliability, low cost, reduced labor and smaller size. The equipment they investigated yielded satisfactory results with small, 100-liter vessels and research is presently being directed toward larger scale and low cost.

On the other hand, it has been proposed that concentrated microalgae be kept in storage because microalgal production is unreliable and difficult in rainy and hot seasons (Yoneda 1983). There are two ways to concentrate microalgae, by centrifugation, and by means of an ultra-filter membrane. The reserves are stored in a freezer if the microalgae are to be used directly as feed for rotifers and in a refrigerator if they are to be re-cultured

(Yoneda 1983). Sugiyama and Kinjo (1988) reported that marine microalgae stored in a -70°C freezer grew again. Several stations recently reported that they intend to introduce microalgal concentration with ultra-filters.

MECHANIZATION AND AUTOMATION AT NEW FACILITIES

The Ehime Prefectural Government decided to construct new facilities in order to expand the number of fish species released in 1988. When the new facilities were designed, we intended to introduce an automatic system to reduce manpower and improve reliability, because we are required to produce fry with very few staff. The new facilities were completed in December 1990.

The systematization of facilities we had planned were as follows:

- Related tanks would be connected with pipes, and be close together,
- All seawater supplied would be filtered by sand. Moreover, seawater supplied for microalgae culture would be sterilized if possible,
- Storage of microalgae and rotifers,
- Automation of feeding rotifers, harvesting rotifers and feeding larval fish,
- Microalgae culture process from stock culture to production tanks,
- Heated rotifer and larval fish tanks, and
- Automatic generator.

The newly constructed rotifer production facilities are as follows:

- Microalgae production
  a) Stock culture building with temperature control and illumination; 100 m^2
  b) Extensive culture tanks; six 10-m^3 capacity
  c) Production tanks; six 100-m^3 capacity,
- Rotifer culture tanks; six 50-m^3, with heating, and
- Laboratory; 80 m^2.

In addition, the following specialized equipment has been installed:

- 0.1-μm ultra-filtering apparatus (mainly used for production of molluscs and sea urchins); 4 m^3/hour,
- 1-μm cartilage filtering apparatus (mainly used for production of molluscs and sea urchins); 22-m^3/hour capacity,
- Ultraviolet sterilizer; 3- and 32-m^3/hour capacities,
- Microalgae ultra-filter membrane concentrator; 40 m^3/18 hours, 500X concentration,
- Refrigerator and freezer for microalgae storage,
- Automatic rotifer harvesting apparatus; 10 m^3/hour, and
- Automatic rotifer feeding apparatus in larval rearing tanks.

Figure 7 charts the production process at the new facility. While the facility is not fully operational yet, we believe the equipment can function successfully. However, some time is necessary for adjustment, and to become skilled in operating the equipment. At present, we believe that stored microalgae should be fed in urgent situations. However, since there are times during which we can't culture microalgae successfully, we plan to produce and store a great deal of microalgae during the off-season and when culture is easy.

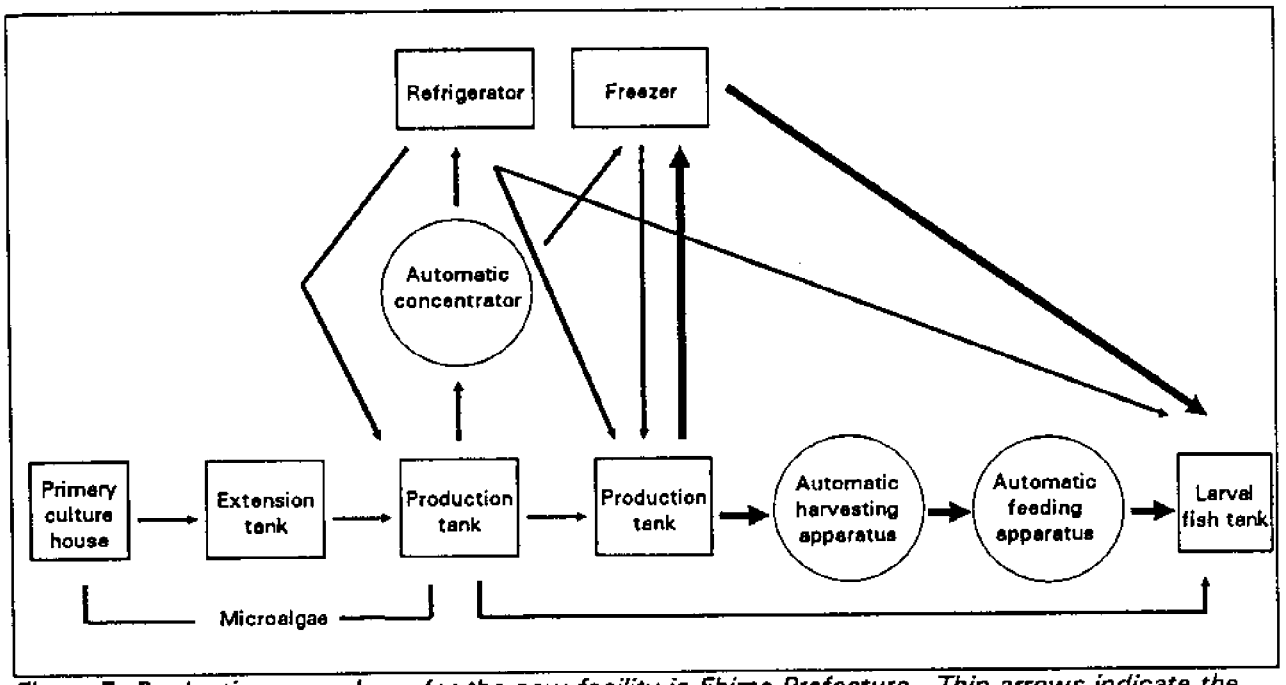

Figure 7. Production procedures for the new facility in Ehime Prefecture. Thin arrows indicate the flow of microalgae, thick arrows represent flow of rotifers.

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Improving the Larval Rearing of Striped Mullet (*Mugil cephalus*) by Manipulating Quantity and Quality of the Rotifer, *Brachionus plicatilis*

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ABSTRACT

Larval rearing technology is the last remaining barrier to the development of an artificial propagation program for striped mullet. This report presents the results from a series of experiments focused on defining the optimal rotifer quantities and quality during the first 15 days post-hatching. The results have been incorporated into a feeding regimen that has resulted in larval survival and growth that at one time was inconceivable for this species.

INTRODUCTION

Culture of striped mullet (*Mugil cephalus*) has been practiced for centuries in many parts of the world. Their ability to tolerate wide ranges in salinity and the fact that they feed at the lowest trophic levels make this species ideal for culture. Because of their economic value, research on the artificial propagation of striped mullet has been ongoing for more than two decades (Lee and Tamaru 1988). Despite this, stocking depends exclusively on fry caught from the wild.

One peculiarity of the striped mullet is that it does not normally spawn in captivity. Induction of spawning, however, has been

accomplished using a variety of hormonal preparations (Tang 1964, Shehadeh et al. 1973a, Kuo et al. 1974a, Liao 1975, Lee et. al. 1987). Control of maturation in both males and females has also been investigated and has resulted in a variety of treatments in which the state of reproductive maturity of either sex can be manipulated (Shehadeh et al. 1973b, Weber and Lee 1985, Kuo et al. 1974b, Kelley et al. 1987). In summary, major strides have been made toward controlling maturation and spawning of this species.

The major remaining obstacle to the artificial propagation of striped mullet is the development of effective larval rearing procedures. It is generally accepted that rotifers play a pivotal role in the successful rearing of marine fish larvae (Lubzens et al. 1989). For this reason, larval rearing research at our Institute has focused on identifying the optimal rotifer and larval densities for first feeding and characterizing the temporal changes in food preference. Recognizing that the rotifers' nutritional quality cannot only vary depending on what they are fed, but that it can be manipulated to ensure a nutritionally adequate rotifer, was a major breakthrough in the culture of marine fish larvae (Kitajima and Koda 1976, Fukusho 1989, Watanabe et al. 1983, Kitajima 1983). Evaluating the nutritional quality of the mullet larvae's first food (i.e., rotifers) was also a major focus of our research.

The results have been incorporated into a larval rearing strategy which has resulted in larval survival and growth during the first 50 days post-hatching that is unprecedented for this species. In this report, the results of our experiments are summarized and the most effective larval rearing procedure developed for the mass rearing of mullet larvae is presented.

Method of Culturing Rotifers

Rotifer culture at The Oceanic Institute can be characterized as a batch system (Lubzens 1987). First, 600 liters of phytoplankton (10 - 20 million cells/ml) are inoculated with 100 rotifers/ml. Twenty four hours later, the volume is increased to 1,200 liters with algae. After another 24 hours, the entire tank is harvested and the rotifers are fed to fish larvae immediately or are used to stock additional rotifer culture tanks. On average, an 88% increase in the number of rotifers is achieved over the 48-hour growing period in this system. Average daily production is 160,000 rotifers/liter, which is relatively high in comparison with other reports. This is largely due to our high initial stocking density (Lubzens 1987). The high density allows us to maintain a smaller rotifer production facility. A major drawback to this method, however, is the long start up time required in the event of a crash.

Food for Rotifers

Several different feeds have been employed for growing rotifers using the same protocol. For example, two types of phytoplankton, *Nannochloropsis oculata* and *Tetraselmis tetrathele* have been used to culture rotifers in our laboratory. The phytoplankton culture methods are presented elsewhere (Eda et al. 1991). Use of *T. tetrathele* has been reported to result in a higher rate of *B. plicatilis* production compared to other algal species (Okauchi and Fukusho 1984), and our results are consistent with those reported (Table 1). At present however, we culture rotifers with *N. oculata* because it produces rotifers with higher protein and lipid contents (Tables 2 and 3). Data to be discussed in another section reveal that this species of algae

Table 1. Average daily rotifer production from 1,200-liter rotifer tanks during the 1990 mullet season.

Food type	Average daily production (rotifers/liter)
T. tetrathele	240,000
N. oculata	160,000
Baker's yeast/*N. oculata*	153,000
Baker's yeast	143,000

possesses more than adequate amounts of protein and fatty acids for mullet larvae.

Baker's yeast, used either alone or in combination with phytoplankton, has also been reported to be an adequate feed for rotifers (Hirayama and Funamoto 1983, James et al. 1987). We have also used baker's yeast, and a summary of the average daily production obtained during this year's trials with the various feeds is presented in Table 1. With the exception of *T. tetrathele*, the average daily production values do not differ very much for the different feeds. However, it should be noted that rotifer production varied considerably when yeast was used exclusively as a food source. Such variation in production has also been reported in the literature (Hirayama 1987).

Nutritional Quality of Rotifers

Protein

The amino acid profiles of rotifers cultured on a variety of feeds were determined after hydrolysis with a Dionex D-300 amino acid analyzer using a sulfonated polystyrene column. The range in total protein content was 38 - 42% depending on the food given to the rotifers. We detected no differences between the protein levels of rotifers cultured with *N. oculata*, baker's yeast, or a combination of both. Our results were consistent with those reported in the literature (Lubzens et al. 1989).

Table 2. Amino acid profile of rotifers cultured on a variety of different feeds (mg/100 mg dry weight).

Essential amino acid	*N. oculata*	*T. tetrathele*	Baker's yeast	Yeast + *N. oculata*	Mullet eggs
Thr	2.18	1.93	2.26	2.37	2.90
Val	2.04	1.82	2.19	2.38	3.09
Met	1.08	0.87	1.12	1.16	1.14
Ile	1.93	1.58	1.93	2.07	2.42
Leu	3.65	3.29	3.74	3.90	4.45
Phe	2.33	2.13	2.19	2.18	2.59
His	0.87	0.81	0.81	0.90	1.36
Lys	3.80	3.59	3.83	3.89	3.87
Arg	2.58	2.55	2.65	2.73	3.24
Nonessential	20.50	18.57	21.26	20.34	23.65
Total	40.96	37.14	41.98	41.92	48.71

Table 3. Fatty acid profile of rotifers cultured on a variety of different feeds (mg/100 mg dry weight).

Fatty acid	*N. oculata*	*T. tetrathele*	Baker's yeast	Yeast + *N. oculata*	Mullet eggs
Myristate	0.34	0.17	0.17	0.23	0.23
Palmitate	1.33	0.93	0.49	1.15	1.72
Palmitoleate	1.73	0.31	1.38	1.30	3.61
Stearate	0.39	0.22	0.30	0.29	0.41
Oleate	0.42	0.39	1.20	0.73	3.70
Linoleate	0.31	0.76	0.59	0.38	1.91
Linolenate	0.01	0.51	0.05	0.03	0.15
Octadecatetraenoate	0.01	0.08	0.03	0.03	0.05
Eicosenoate	0.21	0.51	0.39	0.29	0.13
Arachidonate	0.43	0.16	0.11	0.30	0.44
Eicosapentaenoate	1.25	0.52	0.09	0.68	0.67
Erucate	0.04	0.20	0.08	0.05	0.09
Docosahexaenoate	0.51	0.08	0.11	0.45	1.19
Total Fatty acids	6.98	4.84	4.99	5.91	14.30

Rotifers grown on *T. tetrathele* in our laboratory had much lower levels of threonine, methionine, and isoleucine, compared to those grown on *N. oculata* and yeast (Table 2). For comparison, the amino acid profile of fertilized mullet eggs is also presented. The profile most similar to mullet eggs was obtained from rotifers fed half yeast and half *N. oculata*.

Fatty Acids

The fatty acid profiles of the rotifers grown on a variety of foodstuffs were also determined (Table 3). Rotifers were freeze dried and lipids were extracted as described by Folch et al. (1956) and methylated using the method described by Klopfenstein (1971). Methylated fatty acids were quantified on a HP 5840A gas chromatograph. As has been reported elsewhere, significant differences were seen between groups. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (highly unsaturated fatty acids, or HUFAs) have been reported to be essential for early larval fish survival and growth (Watanabe et al. 1983). The amounts of EPA and DHA in the rotifers was inversely proportional to the amount of yeast in their diet. Further, the relative amounts of these two HUFAs observed are consistent with past reports on the effect of rotifer feeds on their fatty acid profiles (Watanabe et al. 1983, James et al. 1987, Lubzens et al. 1989). A question not usually addressed however, concerns the appropriate levels of HUFAs required for the fish larvae in question. Hence, we compared the fatty acid profiles of the rotifers to that of fertilized mullet eggs (Table 3). In another section of this report we address the question of defining the amount of fatty acid required by striped mullet larvae.

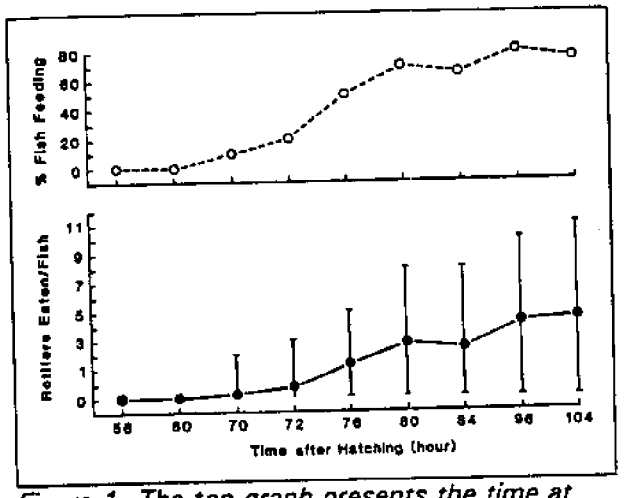

Figure 1. The top graph presents the time at which striped mullet larvae begin to feed when presented with rotifers. Each point represents the percentage of feeding larvae from 20 individuals sampled. The lower graph presents the average number of rotifers eaten per larvae during the first 104 hours post-hatch. Each point represents the average from 20 individuals and the bars represent the observed ranges.

Initial Feeding of Striped Mullet Larvae

Rotifers are the primary component of the initial feeding regimen for a variety of marine fish species (Watanabe et al. 1983, Lubzens 1987). One of the principal concerns in the mass culture of larval fish is the appropriate time at which to introduce rotifers. Traditionally this is done somewhere between when the mouths of the larvae first open and when the yolk sacs are completely absorbed. This time period can be comparatively long (e.g., weeks, as observed in some freshwater species such as catfish and the salmonids), or short (e.g., a few days, as observed for most marine species). The mouths of mullet larvae are open and yolk sac absorption is reportedly complete by the second and fifth day after hatching, respectively (Kuo et al. 1973, Nash et al. 1974).

The time of first feeding and the early effects of starvation were determined by sampling individuals from a 5,000-liter rearing tank and a 500-liter fiberglass tank stocked with larvae from the same spawning. Initial stocking densities in both tanks were 20 larvae/liter. The 5,000-liter tank was stocked with rotifer and algae at densities of 10 - 20 ind. and 500,000 cells/ml, respectively. The 500-liter tank was not supplied with phytoplankton or rotifers. Groups of 20 larvae were collected from both tanks at four-hour intervals during the first three days after hatching, and at eight-hour intervals the following seven days. Total length, gut content, and percentage of fish feeding were recorded at each interval.

In this study, a few larvae began feeding on S-type rotifers at 70 hours (Fig. 1). By 80 hours the majority of larvae possessed rotifers in their gut. Apparently, active feeding begins before completion of yolk sac absorption. Also, a difference in the total length of fed and unfed larvae can be detected as early as three and a half days after hatching (Fig. 2). This further implies that larvae may gain nutritional benefits from rotifers prior to completion of yolk sac absorption. The effects of withholding food from larvae were seen much earlier than previously reported (Kuo et al. 1973, Nash et al. 1974). We concluded, therefore, that food should be presented to the mullet larvae by 36 hours after hatching, when the mouth is first open, a day before the larvae actively begin to feed. This feeding strategy, currently used in all rearing trials, is designed to ensure the availability of food at the earliest time. In this manner, the effects of early starvation observed in mullet larvae and other species (Lasker et al. 1970) can be avoided.

Initial Larval Mullet and Rotifer Density

The growth and survival of marine fish larvae are also significantly affected by the ratio of predators to prey (Houde 1977, Werner and Blaxter 1980), hence culturists try to provide fish larvae with a suitable number of food organisms, usually deriving the amounts empirically. Because of its size and capacity to be cultured in very large numbers, the S-type rotifer *Brachionus plicatilis* is the most popular organism for initial larval feeding (Lubzens 1987). One of the first steps toward improving and stabilizing overall larval survival under hatchery conditions is identifying optimum initial densities of rotifers and larvae.

We designed a series of experiments to: 1) ascertain the effect of rotifer density on the incidence of first feeding in mullet larvae and 2) determine the growth and survival of mullet larvae in response to various rotifer and larval densities during the first eight days post-hatching. This was accomplished by using a 2 x 3 factorial design which tested the effects of two rotifer and three mullet densities. Rotifer densities were 1 and 10 rotifers/ml whereas larval densities were 25, 50, and 100 larvae/liter. The experiment was carried out from hatching to eight days post-hatching, at which time larval survival and dry weights were determined.

Results from the experiments are summarized in Figures 3 and 4. It is apparent that the incidence of first feeding mullet larvae is significantly affected by rotifer density. This indicates that at first feeding, the mullet larvae strike randomly at whatever prey swims into their path. This is consistent with the observations made when mullet larvae are given a variety of food organisms to choose from (Eda et al. 1991). On the other hand, the significant increase in the percentage of larvae feeding by the fourth day post-hatching indicates that by this time, the larvae are actively pursuing their prey. Providing a "high" density of rotifers at initial feeding should ensure that a high percentage of mullet larvae begin to feed. The upper limit however, was not determined in this investigation.

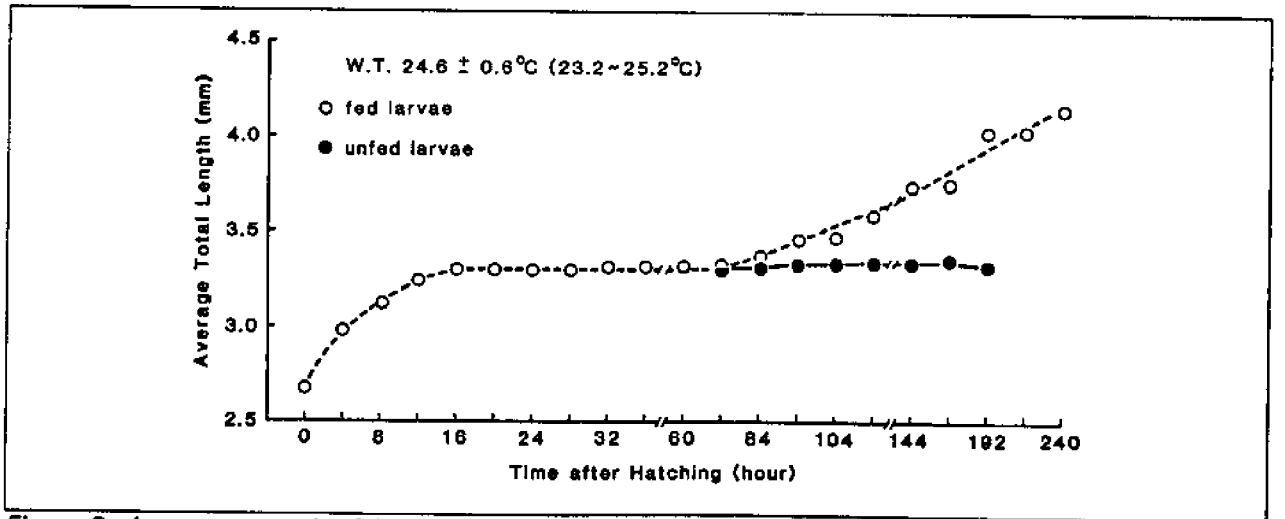

Figure 2. Average growth of fed and unfed larvae during the first 240 hours post-hatch. Each point is the average total length obtained from 20 individuals. No larvae from the unfed group survived after 192 hours.

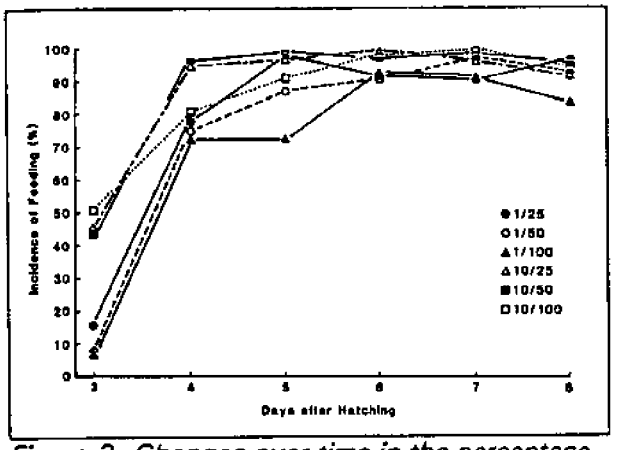

Figure 3. Changes over time in the percentage of mullet larvae feeding during the first eight days post-hatching, with the larvae stocked at various rotifer/larval ratios. The numbers represent: (rotifers/ml)/(larvae/liter).

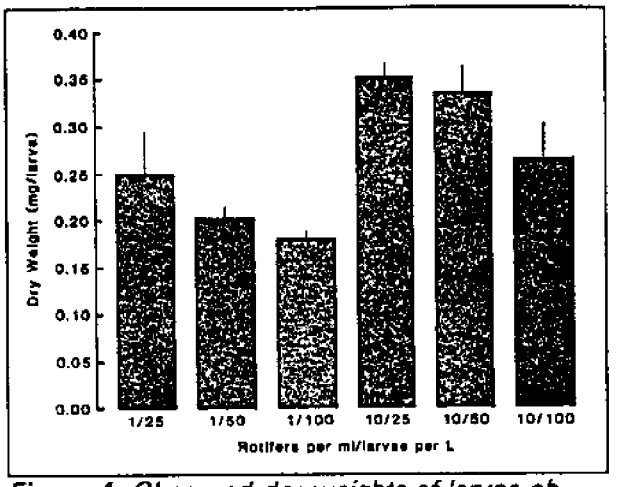

Figure 4. Observed dry weights of larvae obtained from the various rotifer/larvae stocking densities.

The fact that there was no difference in survival between treatments was unexpected, because the densities tested represent an order of magnitude difference in the amount of food available per larva. However, survival alone is not an adequate index of the effects of food densities. The dry weights obtained from each treatment indicate that the most appropriate food density is 10 rotifers/ml for densities of 25 and 50 larvae/liter. Larvae in the other treatments were merely surviving and would most likely not have survived a complete larval rearing trial of 50 to 55 days.

Temporal Changes In Food Preferences Exhibited by Mullet Larvae

Both the size and shape of prey organisms have been reported to influence growth and survival of cultured fishes (Blaxter 1963). This implies that first feeding larvae exhibit preferences for certain feeds. To gain insight into the temporal changes in food preference exhibited by striped mullet larvae, newly-hatched larvae were stocked in three 500-liter fiberglass tanks at a density of 20/liter. Three types of live feed: S-type (110 - 230 μm lorica length) and L-type (130 - 340 μm) rotifers, and newly hatched nauplii of *Artemia salina* (450 - 500 μm total length) were stocked at densities of 3, 3 and 0.3 ind./ml, respectively (Fig. 5). Food densities in the water column were monitored daily and maintained at the designated densities. Larvae were sampled on Day

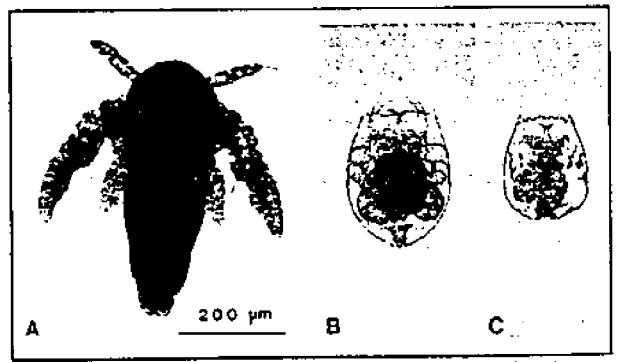

Figure 5. The live food organisms used in the food selectivity experiment. A = freshly hatched Artemia *nauplii, B = L-type rotifer, C = S-type rotifer. All photographs were taken at the same magnification, 40X.*

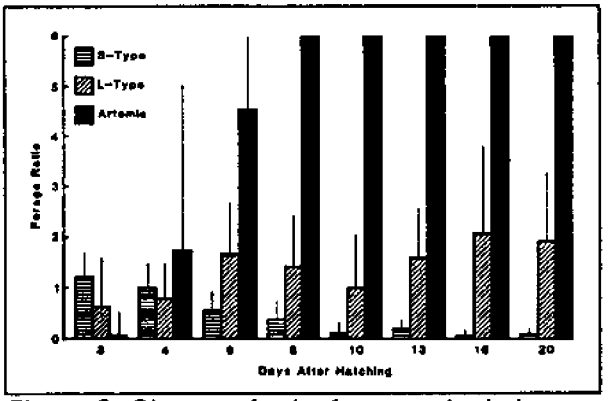

Figure 6. Changes in the forage ratio during the first 20 days after hatching for mullet larvae presented three feeds. The bars represent the average ratio obtained from three experimental tanks. Lines represent standard error.

3, 4, 6, 8, 10, 13, 16 and 20 post-hatch, and again approximately one hour after the addition of *Artemia* nauplii. A random sample of 20 larvae was then subjected to gut content analysis with a compound microscope. The L- and S-type rotifers were distinguished by size, shape, and shape of their anterior spines, as described by Ito et al. (1981).

Initially, the gut content compositions mirrored what was present in the water column: S-type rotifers were the most abundant, followed by L-type rotifers and *Artemia*. Between Days 6 and 10 gut content changed and after Day 10, *Artemia* nauplii predominated. If the relative proportion of food organisms in the water column is accounted for (Ivlev's forage ratio: % in gut/% in water column), larger food types predominated from Day 4 onward (Fig. 6). From Day 6 the forage ratio is highest to lowest for *A. salina* nauplii, L-type rotifers and S-type rotifers, respectively. The S-type rotifers employed in our study may be suitable as an initial food organism, but the food selectivity experiment demonstrates that feeding preferences shift quickly. Mullet survival may be further enhanced with the introduction of a more suitable food organism. *Artemia* nauplii however, are the only live food organism that can be supplied consistently and at appropriate densities.

Nutritional Quality of Rotifers

It has been well established that baker's yeast can be used as an algal substitute in rotifer production. There are obvious benefits to this practice, such as the reduction or elimination of algal production facilities. However, there is a price to pay in terms of the nutritional quality of the resulting rotifers. The suitability of yeast-fed rotifers for mullet larvae was addressed during the 1989-90 mullet season. Larval rearing trials were carried out in 30-liter polycarbonate tanks in which spawned eggs were distributed to achieve an initial stocking density of 20 - 25 larvae/liter. Newly hatched mullet larvae were provided rotifers grown exclusively on *N. oculata*, baker's yeast, and a combination of half algae and half yeast. The rotifers were provided beginning on the second day post-hatching and densities were maintained at 10 - 20 rotifers /ml. No background algae was added to the rearing tanks. The experiment was carried out until 15 days post-hatching, at which time survival and total length of the surviving larvae were obtained. The fatty acid and amino acid profiles of the resulting rotifers are given in Tables 2 and 3, respectively.

The resulting percent survival, total length and percentage of larvae possessing flexion for the various groups are presented in Figures 7a, 7b and 7c, respectively. Mullet larvae reared on yeast-fed rotifers were significantly smaller ($p < 0.01$) 15 days after hatching compared to other treatments. No statistical difference in survival was detected

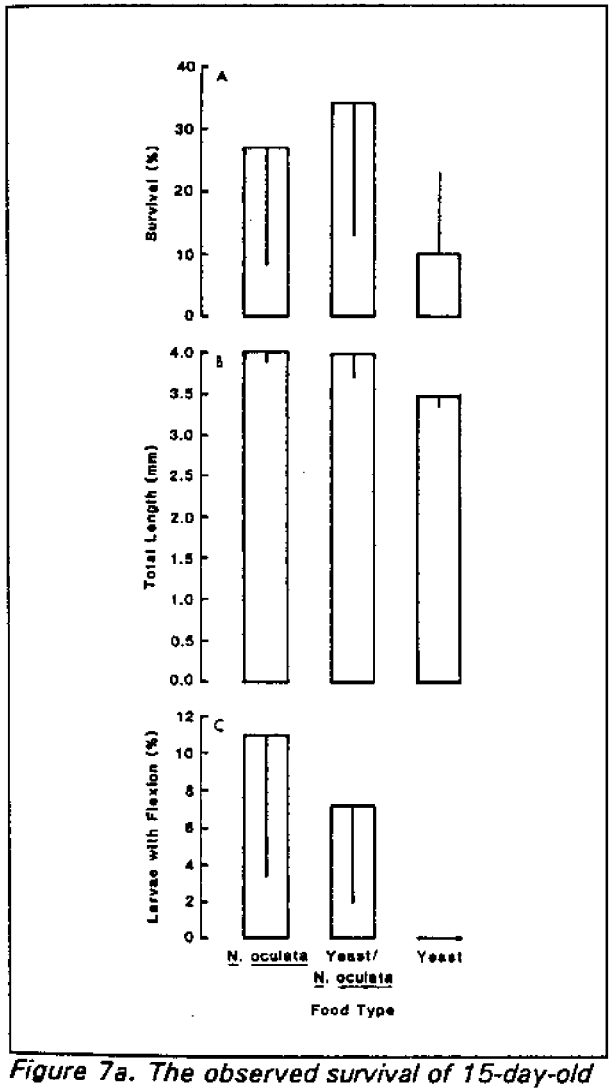

Figure 7a. The observed survival of 15-day-old mullet larvae given rotifers fed Nannochloropsis oculata, *half baker's yeast and half algae, and yeast alone. Average from triplicate tanks, bars represent standard errors.*

Figure 7b. Observed total length of 15-day-old mullet larvae (n = 60) given rotifers fed Nannochloropsis oculata, *half baker's yeast and half algae, and yeast alone. Bars represent standard errors.*

Figure 7c. The observed percentage of 15-day-old larvae (n = 60) given rotifers fed Nannochloropsis oculata, *half baker's yeast and half algae, and yeast alone, observed with flexion. Average was obtained from triplicate tanks, bars represent standard errors.*

between mullet raised on *N. oculata*-fed rotifers vs. those given half algae and half yeast. Survival was, however, significantly lower ($p < 0.01$) for the larvae provided rotifers grown exclusively on yeast.

The same graded response in the percentage of 15-day-old mullet larvae possessing flexion was also observed for the different groups. Because there is no significant difference in the amount of protein found in the different rotifer treatments, we conclude that the fatty acid composition of the rotifers is a major contributing factor to the larval growth, development and survival obtained in these experiments.

Feeding rotifers *N. oculata* results in a nutritionally adequate rotifer both in terms of protein and fatty acids. Producing enough algae to mass culture rotifers, however, requires immense tank space and can be a limiting factor in hatchery productivity. Substituting half of the phytoplankton with baker's yeast results in a rotifer with equivalent protein levels which are deficient in some fatty acids, particularly, C 20:5 n-3. No significant differences in larval survival and growth however, were detected between the *N. oculata*- and *N. oculata* + yeast-fed rotifers. This implies that the required amounts of HUFAs are approximated by the rotifers fed the "half and half" combination.

Interestingly, the ω3 HUFAs found in the "half and half" rotifers mirrored those in fertilized mullet eggs. In practical terms, this means that the phytoplankton base of the culturist's food web can be doubled without increasing tank space or compromising the nutritional requirements of mullet larvae.

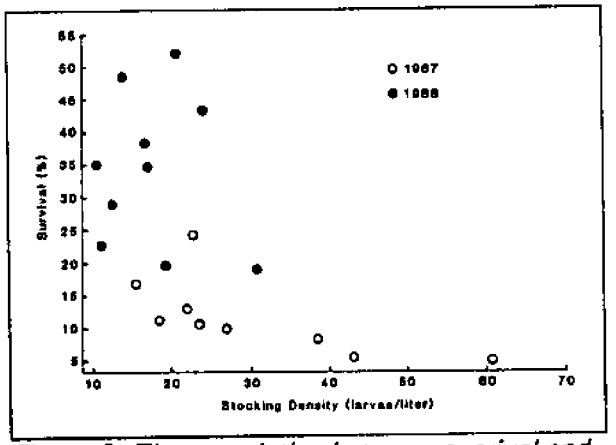

Figure 8. The correlation between survival and initial stocking density obtained during the 1987 and 1988 mullet larval rearing seasons.

Large-Scale Larval Rearing Trials

Initial feeding and larval stocking densities resulting in optimal survival were 10 rotifers/ml and 25 and 50 larvae/liter. To verify this result, large-scale rearing trials in which we varied the initial stocking densities of mullet larvae (range: 11.1 - 60.8/liter) were conducted between 1988 and 1989. Food densities did not vary (10 - 20 rotifers/ml and 500,000 cells/ml).

A significant, inverse relationship was found between initial stocking density and percent survival ($p < 0.01$, $r = -0.58$) (Fig. 8). The results confirm the optimal initial feeding and stocking densities defined in the small scale experiments described earlier. The relationship between survival and stocking density has been reported in previous studies of mullet and other cultured teleosts (Blaxter 1968, Okamoto 1969, Kuo et al. 1973, Nash and Kuo 1975). Interestingly, similar larval densities (in the range of 25 - 50/liter) were reported to be optimal for red sea bream, *Pagrus major* (Yamaguchi 1978), redlip mullet, *Liza haematocheila* (Fujita 1979), and rabbitfish, *Siganus guttatus* (Hara et al. 1986).

Rotifers and background algae are provided to larvae beginning on the second day post-hatching. Although the S-type rotifers employed in our study may be suitable as an initial food organism, mortality continued to occur between Day 10 and 15. The food selectivity experiment demonstrated that the feeding preference shifts during this time, suggesting that survival and/or growth may be further improved with the introduction of a more suitable feed. The L-type rotifer grows best under temperate conditions and low salinity (Hagiwara et al. 1989) and has proven more difficult to mass culture than the S-type rotifer in Hawaii. *Artemia* nauplii are the only other organism that can be supplied consistently and at appropriate densities.

Although initial stocking densities in 1988 were lower than in 1987, resulting harvest densities were at least two times greater. Growth was also significantly improved in 1988 (Fig. 9). The faster growth could not be attributed to temperature, since average water temperatures were equivalent in both years. The two seasons can be distinguished primarily by the time at which *Artemia* nauplii were introduced, Day 25 - 26 in 1987, Day 20 in 1988. This probably contributed to the increased growth and survival observed in 1988.

Whether the current practice of providing rotifers until Day 40, or the previous practice of weaning larvae onto *Artemia* nauplii between Days 12 and 15 post-hatching (Nash et al. 1974) is better remains to be answered. Although the proportions of the different food organisms found in the gut changes as development proceeds, both rotifers and *Artemia* are found in the majority of larvae. We believe the continued addition of rotifers after Day 15 alleviates competition

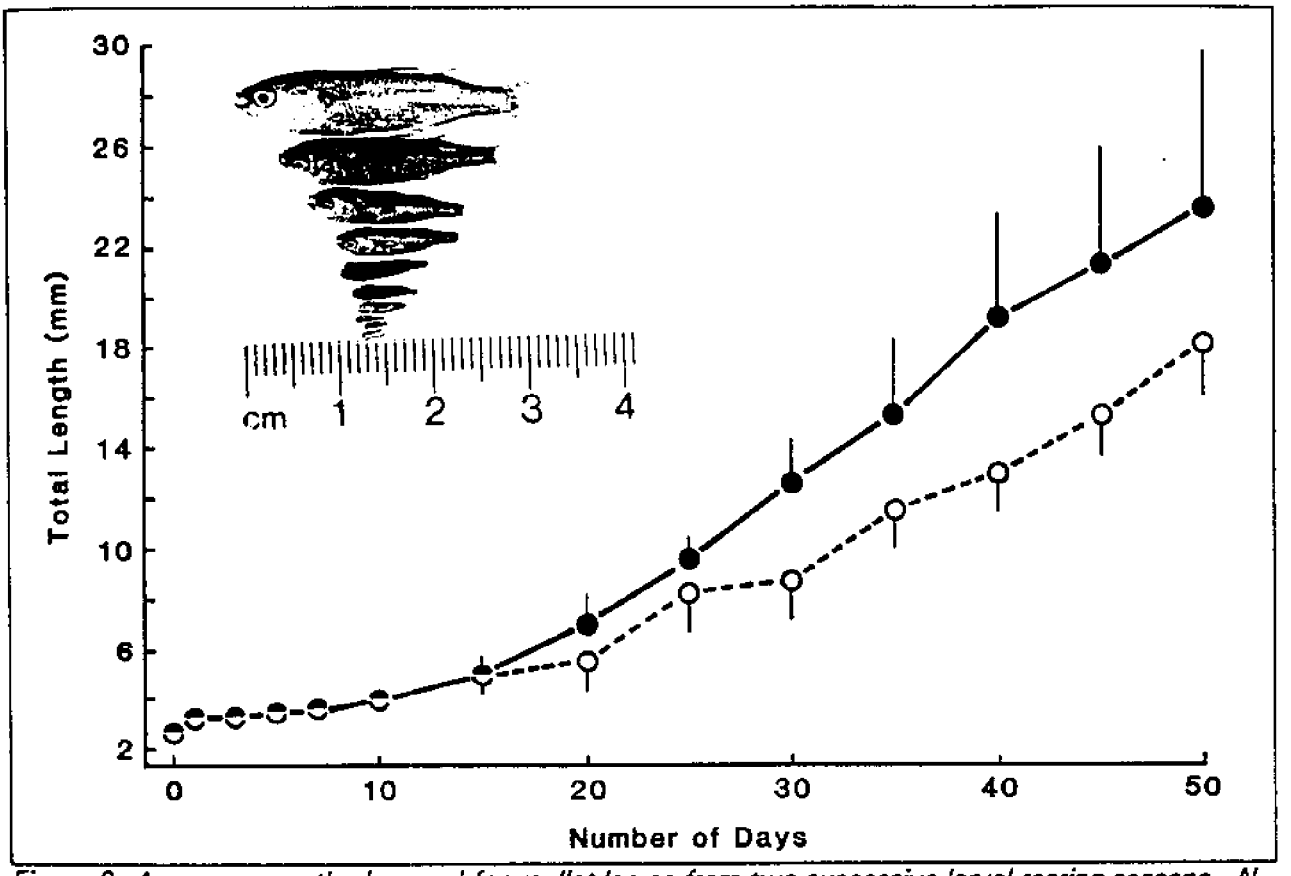

Figure 9. Average growth observed for mullet larvae from two successive larval-rearing seasons. N = 9 and N = 11 trials for the 1987 and 1988 seasons, respectively. Bars represent standard errors. Open circles = 1987 and closed circles = 1988.

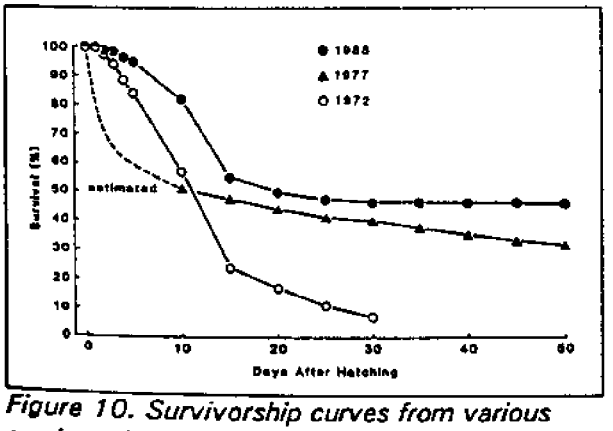

Figure 10. Survivorship curves from various rearing trials with striped mullet larvae reported in the literature. Also included are the results of Trials #3 and #4 from the 1988 season at The Oceanic Institute. 1972 = Kuo et al. 1973; 1977 = Nash et al. 1977.

for *Artemia* nauplii and increases survival of the smaller larvae. However, a comparison of the survivorship curves available in the literature (Fig. 10) indicates that most of the mortality experienced in rearing trials of mullet larvae occurs during the initial 15 days post-hatching, independent of the feeding regimen (Liao et al. 1972, Nash et al. 1977, Eda et al. 1991). This comparison also demonstrates that the major improvements in overall survival obtained in our current rearing trials is primarily due to the improved survival during the early stages.

The aforementioned findings have been incorporated into a larval rearing protocol for

striped mullet that has produced the most promising results to date for this species. A schematic representation of the feeding regimen used in the rearing of mullet larvae is presented in Figure 11. The major components (i.e., use of rotifers, *Artemia*, and prepared feed) do not differ greatly from feeding regimens used for other marine fish larvae. However, the quantities, quality and timing of their presentation, as well as larval stocking densities, represent a composite of the results that were investigated separately.

Our experimental results were confirmed in large-scale trials. Table 4 summarizes the rearing trials conducted in 5,000-liter tanks during the 1987, 1988 and 1990 mullet larval rearing seasons at The Oceanic Institute. Only trials in which either *N. oculata* alone or half yeast and half *N. oculata* were used for feeding are presented.

In summary, steady improvement has been made in larval rearing over the last three seasons. This can be measured by the increases in the total number of juveniles produced per season and in survival rates. These dramatically increased our harvest densities. Lastly, the improved quality of the juveniles decreased the time larvae must spend in the hatchery. We believe that the current feeding regimen, as well as the quality of feed is the most effective strategy devised for mass producing striped mullet larvae.

Acknowledgements

Support for this research was provided by The United States Agency for International Development, contract #DAN-4161-A-00-4055-00 and DAN-A-4161-00-0009-00. The authors wish to recognize the technical assistance of Jessie Banno, Paul Bass, Christopher Demarke, John Ginoza, Ryan Murashige, Vernon Sato, and Chris Webb.

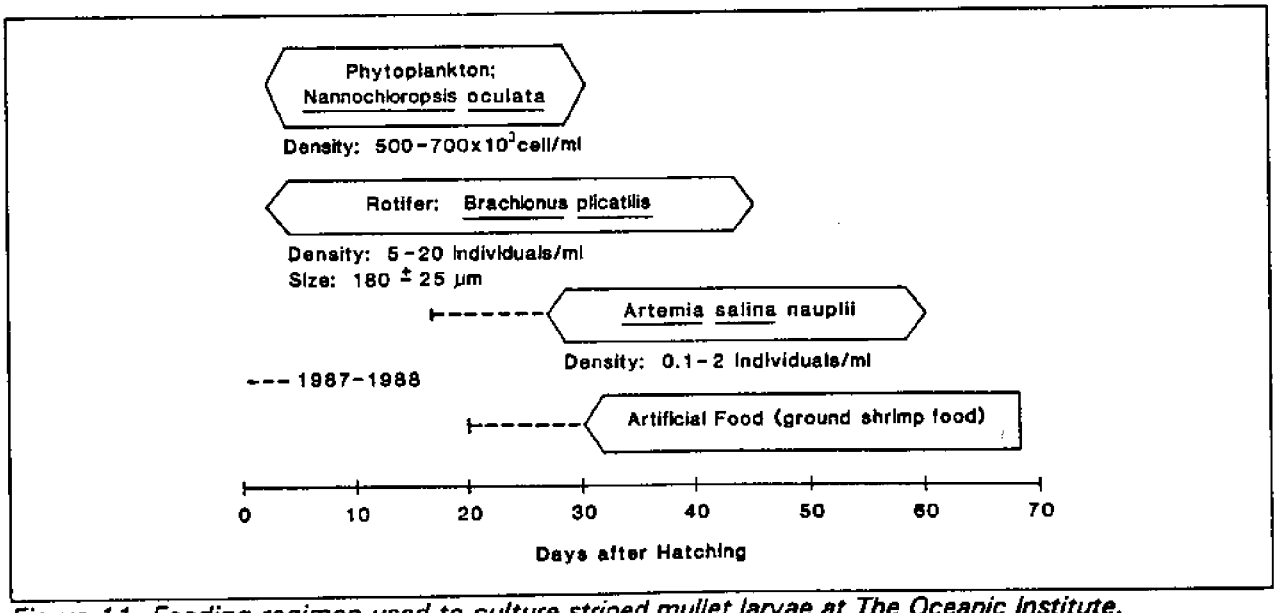

Figure 11. Feeding regimen used to culture striped mullet larvae at The Oceanic Institute.

Table 4. Summary of large-scale (5,000-liter) striped mullet larval rearing trials during the 1987, 1988 and 1990 seasons.

1987 Trials	Stocking density (no. ind./liter)	Harvest density (no. ind./liter)	Survival (%)	Total length (mm, 60 days)
1	15.5	2.6	16.9	23.0
2	18.4	2.1	11.3	21.1
3	38.4	3.3	8.5	18.2
4	43.1	2.4	5.6	19.8
5	23.4	2.5	10.7	23.4
6	22.0	2.9	13.1	30.8
7	26.8	2.7	10.0	34.0
8	22.7	5.6	24.5	29.0
9	60.8	3.4	5.1	26.2
108,500 juvenile mullet produced				
1988 Trials	**Stocking density (no. ind./liter)**	**Harvest density (no. ind./liter)**	**Survival (%)**	**Total length (mm, 50 days)**
1	13.7	4.6	33.7	19.6
2	20.7	10.8	52.2	16.6
3	24.0	10.5	43.5	20.3
4	13.9	6.8	48.6	24.4*
5	15.3	3.7	24.2	15.6*
6	12.5	3.6	29.1	21.8*
7	30.6	5.9	19.1	31.2
8	11.1	2.6	23.4	31.3
9	17.1	6.0	34.9	17.4
10	16.7	6.4	38.5	26.7
11	19.3	3.8	19.7	24.3
* = total length determined at day 45.				
258,400 juvenile mullet produced				
1990 Trials	**Stocking density (no. ind./liter)**	**Harvest density (no. ind./liter)**	**Survival (%)**	**Total length (mm, 42 days)**
1	20.9	9.0	43.1	17.7
2	26.5	12.3	46.6	15.5
3	22.8	14.1	61.9	13.9
4	22.2	14.7	66.6	16.3
5	18.6	11.6	62.5	18.5
6	22.3	13.2	59.3	16.1
7	18.9	12.2	64.9	15.3
8	21.1	14.6	69.2	15.0
408,000 juvenile mullet produced				

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Commercial Production of Microalgae and Rotifers in China

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ABSTRACT

Techniques for the commercial production of microalgae as feed for the larvae of Chinese shrimp (*Penaeus chinensis*) and scallops (*Chlamys farreri* and *Argopecten irradians*) are summarized, and the microalgal production facilities used in the People's Republic of China, including cement ponds, fiberglass tanks and transparent polyethylene bags, are described. This paper also presents methods used in the mass production of rotifers in earthen ponds in North China to feed *P. chinensis* and finfish larvae.

INTRODUCTION

With the development of marine animal farming in China, commercial production of larvae has become a key problem. Live feeds are the basal diets for marine larvae; their quantity and quality affect survival and growth rates. This paper summarizes the present state of commercial production of microalgae and rotifers in China.

COMMERCIAL PRODUCTION OF MICROALGAE

Studies of microalgae culture in China began in the 1940s. In order to supply live feeds to the larvae of marine animals, Guo et al. (1959) isolated two unicellular green algae, *Tetraselmis* (*Platymonas*) sp. and *Dunaliella* sp. and published their culture methods. Jin et al. (1965) studied optimal growth conditions for three cultured diatoms. Before 1980, *Phaeodactylum tricornutum* and *Tetraselmis subcordiformis* were the only species cultured for shrimp. The former, however, was grown at temperatures below 25°C, and the latter was too large and therefore unsuitable as feed for newly hatched larvae. Furthermore, many technical problems remained unsolved in those open cement pond cultures. For example, the seawater was inadequately treated, thus zooplankton propagated quickly, resulting in culture failure and an insufficient supply of live feeds. This restricted the development of marine animal culture.

After 1980, many species were isolated from our coastal region. In addition to *Gymnodinium* sp. (Guan et al. 1980), others such as *Chaetoceros muelleri* (Chen et al. 1982, Ma

Table 1. Analysis of nutrient components of six species of microalgae (percentage of dry weight of cells).

Species	Protein (Nitrogen x 6.25)	Fat	Carbohydrate	Ash
Chaetoceros muelleri	34.75 - 38.50	33.15	19.40	14.7
Dicrateria sp.	38.06	29.09	22.45	10.4
Isochrysis galbana 3011	41.53 - 46.81	22.54	22.54	8.4
Pavlova viridis	58.51 - 62.25	15.31	15.04	7.4
Tetraselmis sp.	30.06	5.16	26.68	38.1
Tetraselmis subcordiformis	46.38	5.09	27.43	21.1

1986), *Dicrateria* sp. (Chen et al. 1978*), *Tetraselmis* sp. (Zhang and Li 1983), *Isochrysis galbana* 3011 (a warm water strain) (Chen and Pan 1987) and *Pavlova viridis* (Chen et al. 1985, Tseng et al. 1990) were isolated. All of these species have been cultured commercially as feed for the larvae of marine animals or zooplankton.

In 1985, we introduced *Isochrysis galbana* (an excellent feed for the larvae of scallops) as well as the other species listed above and their culture techniques to farmers along the coast of China. All of these algae can be cultured at 25°C. Recently, we analyzed the nutrient composition of the six microalgae and found that *Pavlova viridis* has the highest protein content (up to 62.25%) (Table 1). The reproductive rate and fecundity of *P. viridis*-fed rotifers were also highest.

Species

The species which are now used extensively as feed for marine animals are *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Chaetoceros muelleri*, *Dicrateria* sp., *Tetraselmis subcordiformis* and *Tetraselmis* sp. The optimum growth conditions for these algae and the recently introduced *Pavlova viridis* are summarized in Table 2. *Phaeodactylum tricornutum* is suitable as feed for scallop broodstock because it grows faster than the other species at the low temperatures which prevail during early spring. *Isochrysis galbana* and *P. viridis* are better for the scallop larvae. The later larval stages are also fed with some *T. subcordiformis*. In the culture of shrimp, *I. galbana*, *C. muelleri* and *D.* sp. are all good larval feeds.

Inoculation Density of Algae

A dense inoculation is necessary if one is to achieve success in open cultures. Ordinarily, the inoculation density for *T. subcordiformis* is 8 - 10 x 10^4 cells/ml, whereas 30 - 50 x 10^4 cells/ml is used for the other species. Under optimal conditions, all species will propagate rapidly, reaching population peaks in four to five days. The maximum cell density reached in cement ponds and tanks is 70 - 100 x 10^4 cells/ml for *T. subcordiformis*, 200 - 250 x 10^4 cells/ml for *C. muelleri*, 180 - 200 x 10^4 cells/ml for *I. galbana*, 150 - 180 x 10^4 cells/ml for *D.* sp. and 250 - 350 x 10^4 cells/ml for *P. viridis*. Dense cultures are pumped into the animal culturing ponds to feed the larvae. With cement ponds and fiberglass tanks, 10 -

*This species was reported as *Dicrateria zhanjiangensis* Hu sp. nov., but no description of the new species is available.

Table 2. Optimum growth conditions for six microalgae.

Species	Temperature (°C)	Light intensity (lux)	Salinity (ppt)
Chaeotoceros muelleri	25 - 35	8,000 - 10,000	20 - 35
Phaeodactylum tricornutum	18 - 22	3,000 - 5,000	25 - 32
Dicrateria sp.*	25 - 32	3,000 - 10,000	15 - 30
Isochrysis galbana 3011	25 - 30	10,000	10 - 30
Pavlova viridis	15 - 30	4,000 - 8,000	10 - 40
Tetraselmis subcordiformis	20 - 28	5,000 - 10,000	30 - 40

*Based on data of Chen et al. 1978

20 tons of algae can be produced per 100 - 250 m^3 of culture. One 28-m^3 polyethylene bag, by contrast, can yield 3.5 - 5 tons of concentrated algae.

Algae Culture Facilities

Algae culture takes place in two rooms, a stock room and a production room. The roof of the stock room is covered with ordinary tiles, and culture vessels are exposed to either natural light or light from fluorescent lamps. The roof of the production room, by contrast, is covered with transparent fiberglass reinforced plastic (FRP) tiles. The windows around the room provide sufficient illumination. A number of cement or fiberglass tanks are built. The ratio of water volume used for animal culture to that used for algae culture should be 2 : 1 for scallops and 5 : 1 for shrimp. Thus, the tanks for animal breeding are generally 500 m^3 and those for algae culture 100 - 250 m^3.

Culture Vessels of Stock Room

Thirty 3-liter flasks, fifty 2-liter slender-mouth carboys and thirty 0.1-ton fiberglass containers are needed to culture scallops. All culture vessels are inoculated continually to supply algae to the open, cement ponds or tanks.

Production Facilities

Three kinds of enclosures are used: cement ponds, fiberglass tanks, and polyethylene bags.

Cement ponds are usually rectangular and measure approximately 4 m in length, 3 m in width and 0.8 m in height, depending on the requirement. When the culture medium is 0.6 m^3 deep, the total capacity of a pond is approximately 7.2 tons. The ponds are arranged in two rows running south-north in rooms with sufficient sunlight. Each row has seven to 18 ponds. An inlet pipe is installed at one end of each pond and an outlet is placed at the other end. Four openings to which air stones can be fitted are available on each side of a pond. Cement ponds are the most common type of culture enclosure for commercially produced algae to be used as feed for the larvae of marine animals, especially scallops (*Chlamys farreri, Argopecten irradians*) in China. Their advantages include low cost and easy management, but they are fixed facilities which require a great deal of land. They are

also easily contaminated by protozoa and other airborne microorganisms.

Fiberglass tanks, adopted by the Binhai Shrimp Farm in Tianjin (Fig. 1), are cylindrical containers with white interiors. Each tank is 3 m in diameter and 1.2 m tall. A 6.5-cm dia. outlet is located in the middle of each tank's base. A rocker arm made of plastic pipe is connected to the outlet hole; water flows when the rocker arm is put down. The fiberglass tank is placed on a 25-cm high transparent fiberglass tray filled with fine sand or cement. This platform supports the tank's bottom. A length of plastic flexible tubing is fixed inside the tank with iron wire. Eight pipes with airstones are attached to the tubing for ventilation and to prevent dead space. These culture containers can be moved freely.

The use of transparent polyethylene bags for semi-closed culture (Fig. 2) was first adopted by Lin and Chen (1987). The bags are hung under a simple outdoor shelter, which is 52.5 m long, 2.3 m wide and 2.5 m high. Its roof is constructed of white FRP tiles to prevent exposure to direct sun. There are two cement platforms, 49.0 m long, 0.5 m wide and 0.55 m high, under the shelter. Up to 140 bags can be placed on the platforms. The total volume of water in each bag is 28 m^3. The polyethylene is 0.12 mm thick, and each bag measures 1 m in circumference and 3.7 m long. Each bag has a capacity of 0.2 tons. Two plastic tubes, 5 cm in diameter and 20 cm in length, are placed in each of the bag's openings and fastened with a rope. The bag is folded into a "V" shape and placed on the cement platform. The plastic tubes are fixed to the shelter and airstones are suspended from each bag's bottom for continuous aeration. Algae and culture medium are poured into the bag through the plastic tube and harvested with a siphon.

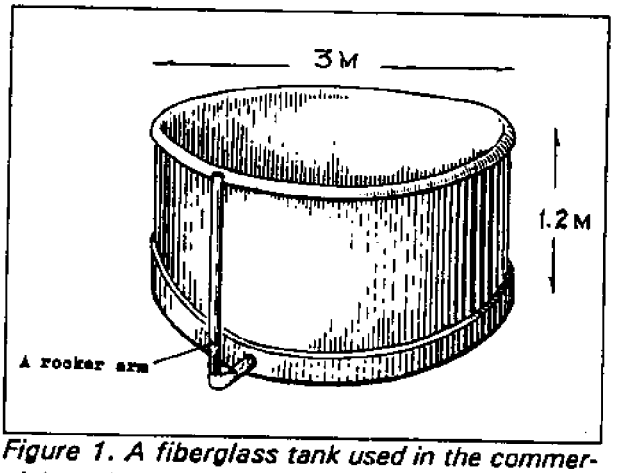

Figure 1. A fiberglass tank used in the commercial production of microalgae.

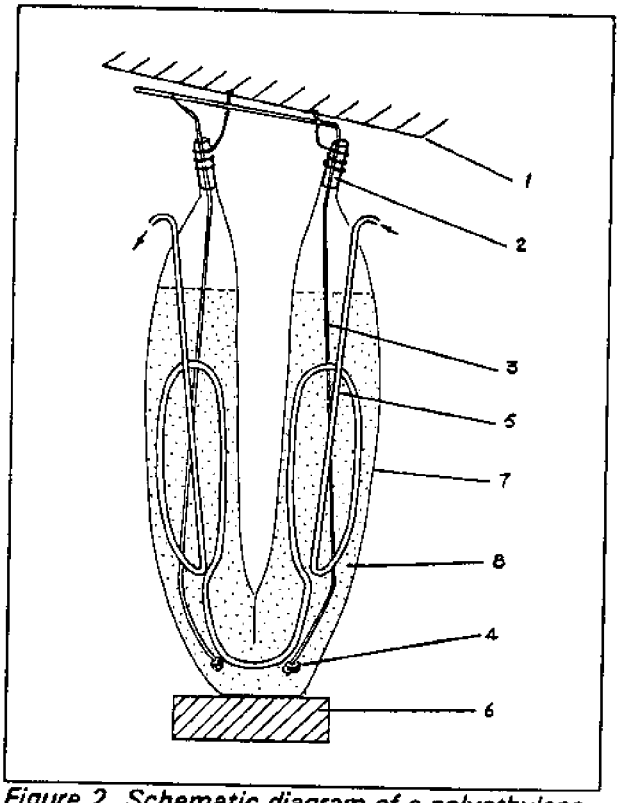

Figure 2. Schematic diagram of a polyethylene bag used for semi-closed culture. (1) simple shelter; (2) plastic tube; (3) flexible pipe for ventilation; (4) air stone; (5) soft pipe used to regulate the culture temperature; (6) cement platform; (7) polyethylene bag; (8) algae and medium.

Depending on the environmental conditions and the requirements of the algae, a curved, flexible plastic tube, 2.5 cm in diameter and 10 m long, may be placed in the bag, with an inlet hole on one side and an outlet hole on the other. This is to regulate the temperature. Water having a higher or lower temperature can be pumped in, and bags can also be positioned flat on the platforms or on land (Fig. 3) and their net volume can exceed 4 m^3 (Miao et al. 1989).

Polyethylene bag culture has the advantages of simplicity, low cost, a large surface area exposed to light, utilization of three dimensional space, little contamination, high density (592 - 640 x 10^4 cells/ml for *D.* sp. in four to five days) and the possibility of continuous culture. It has been shown to be more efficient than open culture in cement ponds or tanks.

Treatment of Seawater

The seawater used for mariculture is pumped from the nearshore coast. The water passes through a sand filter and is settled for 48 hours before it is used in the ponds. If the seawater contains a great deal of organic material and is turbid, it first must be sedimented by addition of 40 to 60 ppm alum, followed by sand filtration or adsorption by activated carbon. Five to 10 ppm of sodium hypochlorite containing 5 - 8% effective chlorine is added to the seawater in the algae pond to kill zooplankton and other microorganisms. After one hour, 7 ppm sodium thiosulfate is added to neutralize the residual chlorine in the water before use.

Culture Conditions

We primarily use natural sunlight, together with one or two 500-watt iodine tungsten lamps placed above the ponds. These provide supplemental light on cloudy or rainy days. Ambient temperatures are usually sufficient, but a plastic, 1,000-watt electric heater is installed in the ponds for cool days. All species cultured are euryhaline, but if necessary, the salinity can be lowered by dilution with fresh water, or increased by adding salt or brine.

Nutrient Medium

For each ton of seawater, 60 g $NaNO_3$ and 4 g KH_2PO_4 are added to all cultures. For cultures of Chlorophytes such as *T. subcordiformis* and *T.* sp., 18 g $NH_2CO_2NH_2$ is added, while Chrysophytes such as *I. galbana*, *D.* sp. and *P. viridis* receive an additional 100 mg of vitamin B_1 and 0.5 mg of vitamin B_{12}. Only agricultural-grade fertilizers and vitamins are used.

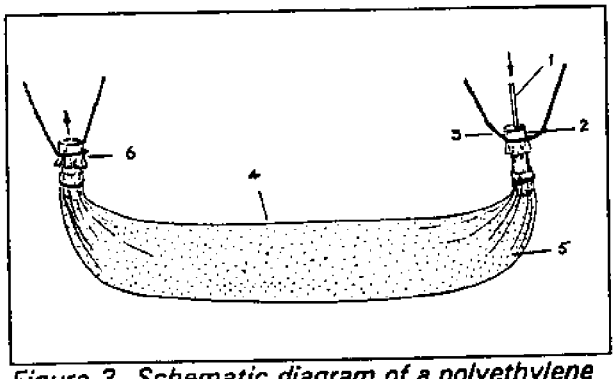

Figure 3. Schematic diagram of a polyethylene microalgae culture bag laid flat. (1) influent gas tube; (2) polyethylene film seal; (3) rope sling; (4) polyethylene bag; (5) algae medium; (6) gauze stopper at the gas outlet.

COMMERCIAL CULTIVATION OF ROTIFERS

Rotifers were originally cultured as feed for the larvae and juveniles of fish when scientists were studying their reproduction and life history. Because of the recent progress made in the commercial cultivation of marine animals, most rotifer culture research has been done since 1980. For example, Wang and Liang (1980) studied the effect of temperature, salinity, feed and microalgal density on the growth and hatching of the rotifer *Brachionus plicatilis*. They showed that the optimal temperature range for hatching was 20 - 25°C, and the optimum salinity was 17.53 ppt. They also found that *Tetraselmis subcordiformis*, *Chlorella* sp., *Chlamydomonas* sp. and *Nitzschia closterium* f. *minutissima* were all excellent feeds for rotifers, although the green algae *Tetraselmis* was better than the others in mass culture. The most suitable density for the *Tetraselmis* feed was approximately 2.5 - 5 x 10^4 cells/ml.

Zhang (1983) carried out a *B. plicatilis* culture experiment in which he used the live beer yeast, *Saccharomyces cerevisiae*, as feed. He found that the optimum inoculation density is 14 - 17 ind./ml at 25°C. When the temperature is 30°C, the optimum feeding quantity of yeast is 3 g/g rotifer. Zhang also added approximately 80 ppm emulsified fish liver oil to the yeast, thereby improving the rotifers' quality as feed. These studies provided the basic information for rotifer mass culture. However, naturally occurring *B. plicatilis* are about 270 μm long and 190 μm wide. These are good feed for *Paralichthys olivaceus* and *Mugil so-iuy* but are too large for the newly hatched larvae of some fish, including *Sparus macrocephalus* and *Pagrosomus major*. For this reason, smaller *Ostrea* juveniles often replace *B. plicatilis* in fish breeding.

Recently, workers from the Institute of Oceanology, Academia Sinica and the Department of Biology of Ocean University of Qingdao have collaborated on studies of *B. plicatilis* mutations and have selected a micromutant, BFI-21. This rotifer is only 185 μm long and has been stabilized genetically over 50 - 60 generations. This achievement will significantly promote the culture of economically valuable fishes.

Rotifer Culture Techniques

The rotifer culture facility at the Binhai Shrimp Farm has twelve fiberglass tanks. Each has a capacity of 2 tons and is divided into an upper cylindrical body and a lower conical bottom (Fig. 4). The transparent fiberglass body is 2 m in diameter, 1.2 m high and about 2 mm thick. A 20-cm long rim

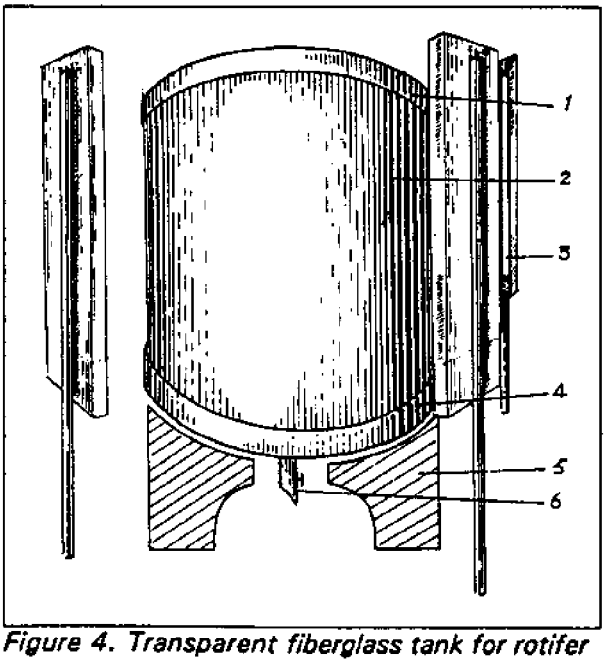

Figure 4. Transparent fiberglass tank for rotifer culture. (1) tank rim; (2) cylindrical body; (3) conical bottom; (4) cement platform; (5) lamp shelf; (6) valve.

constructed of similar materials is glued on for reinforcement. Beneath the body is a conical bottom made of a thick plastic plate equipped with a drain. The tank rests on a 50-cm high cement platform. There are four light shelves around each tank, each with two fluorescent tubes, one red and one blue. These offer supplemental light on cloudy days.

With the development of the shrimp culture industry, the demand for *Artemia* cysts to feed the mysis stage of *Penaeus chinensis* increased dramatically. As a result, *Artemia* cysts were over-harvested, reducing natural production and causing prices to rise sharply. Therefore, scientific and fisheries workers turned to rotifer culture to replace *Artemia* and reduce the cost of producing *P. chinensis*.

Workers at the Institute of Oceanology of Academia Sinica in cooperation with the workers at the Mariculture Farm of Jiaozhou county successfully grew high density rotifer cultures in earthen ponds in 1987-1988. Only five to seven 1-mu (15 mu = 1 ha) earthen ponds are needed to feed 100 million shrimp fry. Basic manure and organic fertilizers are added and the salinity is monitored and adjusted with fresh water (optimum salinity: 18%) in the spring. To supplement the natural food, some soybean milk and bean dregs are added. Rotifers reproduce rapidly in the highly enriched water, without competition from other organisms.

The rotifer ponds can be harvested alternately every day during the shrimp hatchery season. Two-thirds of a pond's volume is pumped through a large net and the remaining one-third is then diluted with clean seawater and left to produce more rotifers. Many rotifers can be harvested every day. The highest density reported was 34.5 rotifers/ml. The survival rate of *P. chinensis* larvae can reach 80% when rotifers are used as feed.

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The Design and Operation of a Large-Scale Rotifer Culture System at a Sung-Ji Industry Farm, South Korea

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ABSTRACT

The facilities and techniques used to mass culture *Nannochloropsis oculata* and *Brachionus plicatilis* at Sung-Ji Industry are described.

INTRODUCTION

Recently, fish culture has become popular in Korea, and many production centers have been established. Because of a shortage of wild larvae, however, it is necessary to produce large quantities of various species in hatcheries (Table 1).

Sung-Ji Industry was established in 1989 in Chung-Mu in the southernmost part of Korea for the primary purpose of fish larval production. Our goal in 1990 was to produce one million larvae: 200,000 red sea bream (*Pagrus major*), 500,000 flatfish (*Paralichthys olivaceus*) and 300,000 rockfish (*Sebastes schlegeli*). Sung-Ji Industry is also operating 3 ha of fish culture cages for growout and 0.6 ha of larviculture tanks in Cheju Island.

Sung-Ji Industry has also released a study on the live feeds used in fish larviculture. The study reports on various kinds of microalgae and rotifer culture along with their nutritional composition and was conducted in collaboration with the Korean Ocean Research and Development Institute (Korean Ocean Res. and Develop. Inst. and Sung-Ji Industries 1990).

The primary feed of cultured rotifers used to be called *Chlorella*. In accordance with Fukusho and Hirayama (1989), however, this species will be called *Nannochloropsis oculata* here.

System Design

The facility used for larval production is 25 m above the mean water level. This allows us to use gravity to transfer the microalgae and rotifers, saving energy and labor.

Table 1. Annual fish seedling demand and estimated production in 1990[1].

Species	Demand	Production (x 1,000)	
		Natural	Cultured
Flatfish	7,121	300	7,672
Yellowtail	13,100	130	—
Rockfish	2,470	300	2,274
Sea bream	—	300	267
Total	22,691	1,030	10,213

[1]Anon. 1990.

Reservoir tank

There is one 200-m^3 capacity reservoir tank which is constantly resupplied with seawater by means of four 100-hp pumps and one 75-hp emergency pump. This tank is located at the highest point, making it possible to use gravity to propel seawater to the microalgae, rotifer, and fish larvae tanks (Fig. 1).

Phytoplankton culture tanks

These are used primarily to produce *N. oculata* as feed for rotifers. Altogether, there are eighteen 4 x 4 x 2.2-m concrete tanks, each coated with fiberglass reinforced plastic (FRP). Each tank is supplied with seawater through a main pipe (200 mm dia.) and salinity is adjusted with well water. Air bubbles are discharged through holes (1 mm dia.) in three

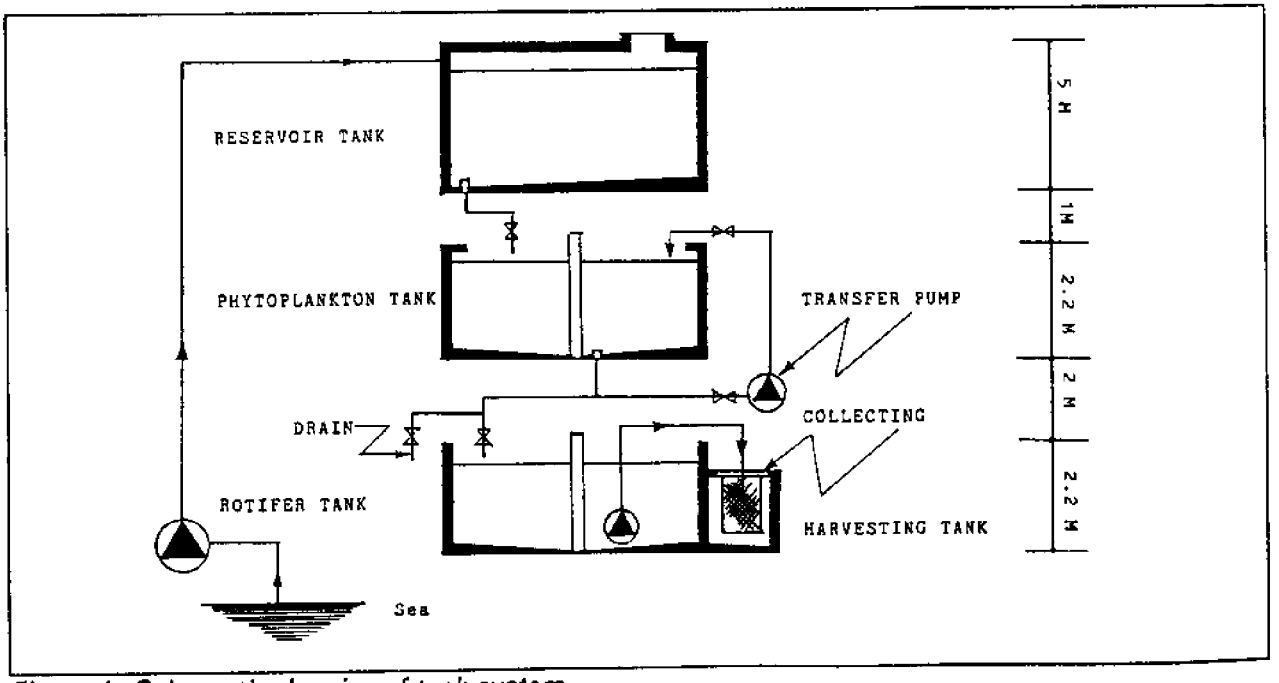

Figure 1. Schematic drawing of tank system.

16-mm dia. PVC pipes which are attached to the bottom of each tank; a roots blower (7.3 m^3 air/min. capacity) provides the air.

For clean-up and drainage, a 100-mm dia. PVC standpipe is used. Next to the standpipe is a 150-mm dia. transfer pipe which is used either for inoculation or to transfer chlorine-treated seawater. *Nannochloropsis oculata* can also be transferred through this pipe to the rotifer culture tanks (Fig. 2). The difference in height between these tanks and the rotifer culture tanks is 2 m.

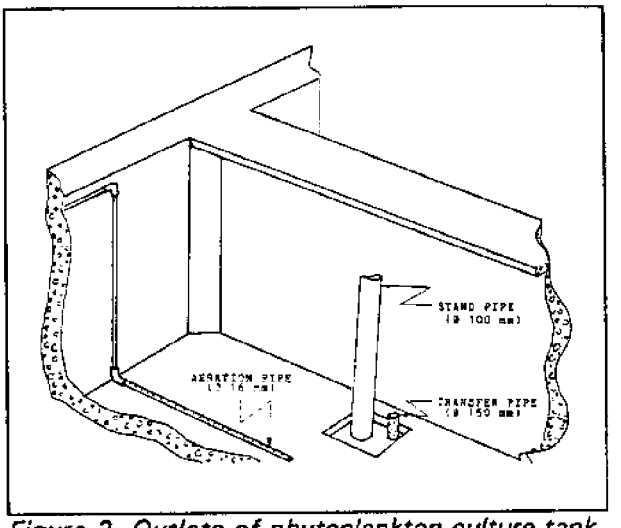

Figure 2. Outlets of phytoplankton culture tank (inside).

Rotifer culture tanks

There are five 6 x 3 x 1.8-m FRP-coated rotifer culture tanks. The water depth is 2 m and about 32 tons of water is used. In winter, when the water temperature is low, a 300,000-kcal boiler is used to heat the water that is then circulated through pipelines. The temperature is maintained between 23 and 25°C. For filtration, three plastic cages filled with oyster shells and three pieces of folded nylon window screening (40 x 70 m) are suspended 20 cm from the bottom of each tank, trapping rotifer excrement and food particles. Using a submersible pump, rotifers are transferred to a 1 x 1 x 1.5-m harvesting tank which is attached to the outside of the rotifer culture tank (Fig. 1).

At harvesting time, in order to reduce rotifer damage, the water level is manipulated with the outside standpipe and a 50-μm collecting net (70 cm long, 50 cm dia.) is immersed in the tank. The net is gradually raised, and the concentrated rotifers are transferred to a secondary culture tank.

Rotifer secondary culture tanks (for nutritional supplementation)

This tank (dimensions: 2.5 x 2.5 x 1.5 m) is separate from the gravity flow system. Presently, there are four tanks used for rotifer enrichment (Fig. 3) and one storage tank, which holds heated water until it is needed. The heating system is similar to that used in the regular culture tanks, but a separate, 15,000-kcal boiler is used.

The heating pipe is equipped with a thermal sensor and solenoid valve which enables very accurate adjustment of the water temperature. The bottom of this tank is sloped (5/100) and the bottom pipe runs to the outside of the tank, making it easy to collect and harvest the

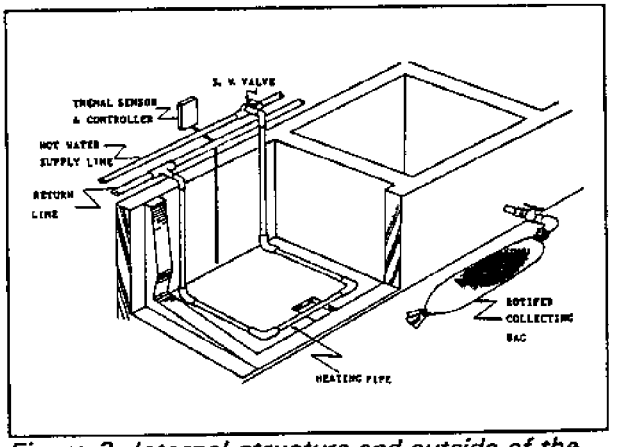

Figure 3. Internal structure and outside of the rotifer collecting bag.

Brachionus plicatilis with a bag (10 cm dia., 150 cm long).

Culture Process

Nannochloropsis oculata *culture*

Our phytoplankton stock culture is axenic and maintained in test tubes at 22 ± 1°C. The photoperiod is 18:6 hours light :dark.

Nannochloropsis oculata culture begins in test tubes. The culture then proceeds to the starter culture stage in 200-ml flasks and is gradually expanded to the carboy culture stage. Water, which has been pasteurized for 30 min. at 60°C, is used during this stage and Guillard's F/2 medium (Guillard 1975) is used.

The carboy culture is gradually expanded to 100-, 500-, and 1,000-liter transparent plastic tanks before it is finally transferred to a 35-m^3 tank.

At this stage, one tank is used as a water sterilizer, holding seawater that is treated with calcium hypochloride (60% Cl). In the summer 10 ppm is used, but in the winter only 5 ppm is necessary. Immediately after sterilization, for 24 hours, free chlorine is dispelled by strong aeration. O-toluidine is used to test for residual chlorine before the water is used for culture.

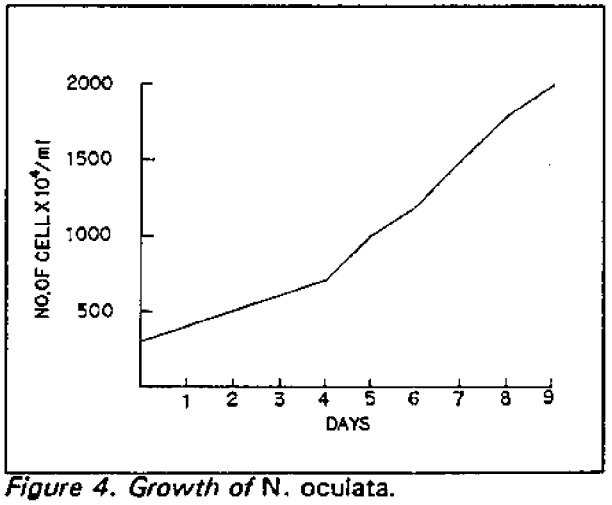

Figure 4. Growth of N. oculata.

The treated seawater is transferred to a culture tank where its salinity is adjusted to 27 ppt by addition of well water. About 4,000 liters of dense *N. oculata* culture is placed in the sterile seawater. After inoculation, the culture depth is approximately 1 m.

The following agriculture-grade fertilizers are added per 1,000 liters of culture water:

- 100 g ammonium sulfate,
- 5 g urea,
- and 50 g superphosphate.

Superphosphate is autoclaved before use.

The rate of microalgal growth is observed and sterile seawater and fertilizer are added as needed. If there is a shortage of trace metals an agricultural-grade trace metal mix is added.

As shown in Figure 4, the average density of *N. oculata* immediately after inoculation is about 300 x 10^4 cells/ml. On the second day it climbs to approximately 500 x 10^4 cells/ml, and on the ninth day reaches about 2,000 x 10^4 cells/ml. When the growth rate reaches its maximum, the algae is transferred by gravity to rotifer or fish larvae tanks.

Rotifer culture

The rotifer population is a mixture of S- and L-types. They are present in different frequencies depending on the season.

Rotifers to be used for inoculation purposes ("seed rotifers") are grown solely on *N. oculata*. During the spawning season, the rotifer stock culture is gradually upscaled into a 32-m^3 tank. Then, when the density reaches about 150 ind./ml, half of the rotifers are

transferred to another tank by means of a water pump or a 50-μm plankton net.

As shown in Figure 5, the initial rotifer density is 70 - 100 ind./ml. On the fifth day that number increases to 200 ind./ml, and every five to seven days, a portion of the rotifers (density: 100 - 200 ind./ml) is transferred to secondary culture tanks. The remaining *B. plicatilis* are harvested and used to inoculate another tank. Sometimes the cultures are thinned with a net in the middle of the culture period.

Nannochloropsis oculata is fed to the rotifers at the initial density of 2,000 x 10^4 cells/ml. When the water loses its green color, approximately 0.5 - 1.2 g of baker's yeast or ω yeast/million rotifers is added twice daily. Baker's yeast is stored in a refrigerator and ω yeast is stored at temperatures below -25°C to prevent spoilage.

The oyster shells and folded nylon window screens are removed once a day and cleaned with pressurized water. Waste, dead rotifers, and air bubbles formed by *N. oculata* are removed from the surface of the culture water every day with a net. The pH is approximately 8.4 immediately following inoculation; on the second day it is 7.9, and 7.6 on the third day.

Secondary rotifer culture

To produce healthy larvae and decrease the incidence of abnormally pigmented flatfish, we need to produce rotifers which are high in ω3 HUFAs. To achieve this, it is necessary to supplement the diet of the rotifers with other nutrients.

In secondary culture, the following are added after inoculation with 2,000 x 10^4 *N. oculata* cells/ml:

- fish oil emulsion (10 ml/m^3 of culture water),

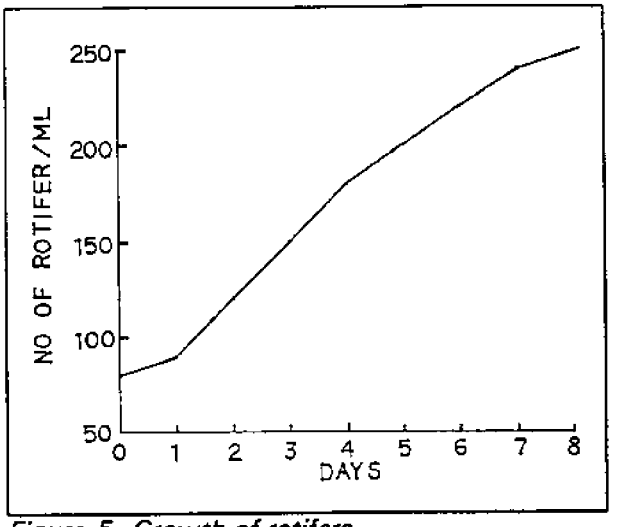

Figure 5. Growth of rotifers.

- vitamin mix (containing A, D and E, 100 ml/m^3 of culture water),
- and Frippak booster (a product of SANOFI France Aquaculture) (0.1 g/30 x 10^4 rotifers).

Secondary culture lasts approximately 20 hours. Then the rotifers are collected by draining the tank slowly through a 50-μm nylon bag (20 cm dia., 150 cm long) which is attached to an outlet pipe at the bottom of the tank (Fig. 3). The concentrated rotifers are then washed with clean water and fed to fish larvae.

Problems

For successful mass production of high quality rotifers, the present microalgal production system must be improved. Less than 2,000 x 10^4 cells/ml is not enough for primary and secondary culturing of rotifers. Therefore, the following solutions are proposed for consideration:

- For effective illumination and aeration, reduce the culture depth to 1.5 m;
- Develop a method for concentrating *N. oculata*;

- Select appropriate trace metal fertilizers; and
- Substitute *Tetraselmis* for *N. oculata.*

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Various Methods for the Culture of the Rotifer, *Brachionus plicatilis*, in Texas

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ABSTRACT

Various methods for culturing rotifers in both indoor culture tanks and outdoor culture systems are discussed. The use of algae in combination with yeast and emulsified oil is discussed, and advantages of the various culture methods are also included.

INTRODUCTION

The rotifer, *Brachionus plicatilis*, is an important food organism for the first feeding stages of larval marine animals around the world. It is a cosmopolitan euryhaline species, thus it is very versatile in marine culture. Pertinent literature citations are covered in Wohlschlag et al. (1990). The rotifer varies in size depending on strain and culture conditions, with adult size ranging from 123 to 315 μm in length. This allows strains to be cultured for a specific size (Yufera 1982, Snell and Carrillo 1984).

Rotifers can be grown in seawater in a wide range of salinities. Our studies indicate 18 ppt is the optimum salinity for the strain we use. The initial culture was obtained from the National Marine Fisheries Service Laboratories in La Jolla, California. Feed types vary, but the most common are single-celled algae such as *Tetraselmis*, *Chlorella*, and *Isochrysis*. Others include baker's and torula yeast, emulsified oil enrichment, and artificial diets. There are advantages and disadvantages to each type of culture. An algae diet helps preserve good water quality in the rotifer culture system, but at the added expense of maintaining algae cultures. Yeast is a simple and inexpensive source of food, but the nutritional quality of yeast-fed rotifers is deficient for many marine organisms. To eliminate this problem, enrichment of yeast-fed rotifers has become popular (Watanabe et al. 1983a).

METHODS

Stock cultures for inoculant or starter cultures are maintained in 1 - 2-liter flasks.

These should be kept in an area separate from the mass culture tanks if possible, to prevent contamination. Stock cultures can be maintained on an algal diet of *Isochrysis galbana* at 24 - 25°C and a light cycle of 12 light:12 dark. Cultures should be restarted periodically, at least every month or more, depending on environmental factors.

Culture Methods

The following methods have been used by the University of Texas Mariculture Program over the past ten years with varying degrees of success. Outdoor culture is limited to the warmer months of the year.

Rotifers have been cultured in 1.8-m dia., round, flat-bottomed tanks that hold up to 1,800 liters of water; 140-liter conical tanks; 160-liter clear, cylindrical fiberglass (reinforced polymer sheet) tanks; 50-liter polyethylene bags; and 1.8-m dia., 3,200-liter round outdoor tanks. Tanks are sterilized before use by addition of 2.5 ppm bleach for 12 - 24 hours, rinsed and cleaned. They are then filled with filtered seawater and the salinity is adjusted with dechlorinated fresh water. Temperature is maintained at 24 - 26°C except in the outside tanks. All except the 140-liter conical and outside tanks have continuous light supplied from light banks with 40-watt florescent bulbs above or beside the tanks. The conical tanks have no light other than room ceiling lights, and only sunlight is used for the outside tanks.

Method using baker's yeast and emulsified oil

Set up 1,800-liter round tank or conical tanks as described above. On Day 1, yeast is fed at 0.6 - 0.8 g/liter, emulsified oil at 1.0 ml/10 liters and rotifers are inoculated at 10 - 40/ml after being rinsed three times through a 60-μm filtering cloth, to rid the culture of most contaminants. Beginning on Day 2, yeast is fed at 1.5 g/10^6 rotifers and emulsified oil at 3 ml/10^6 rotifers daily until rotifer density reaches 50/ml. Then add 1 - 1.3 g of yeast/10^6 rotifers and 2 - 3 ml of emulsified oil/10^6 rotifers daily until a density of 100/ml is attained. Then feed yeast at 0.6 - 1.0 g/10^6 rotifers and emulsified oil at 2 ml/10^6 rotifers until the density reaches 150 - 200/ml, at which time harvesting may begin (Wohlschlag et al. 1990) (Fig. 1).

To harvest, drain 20 - 25% of the tank volume through a 48-μm filtering cloth to

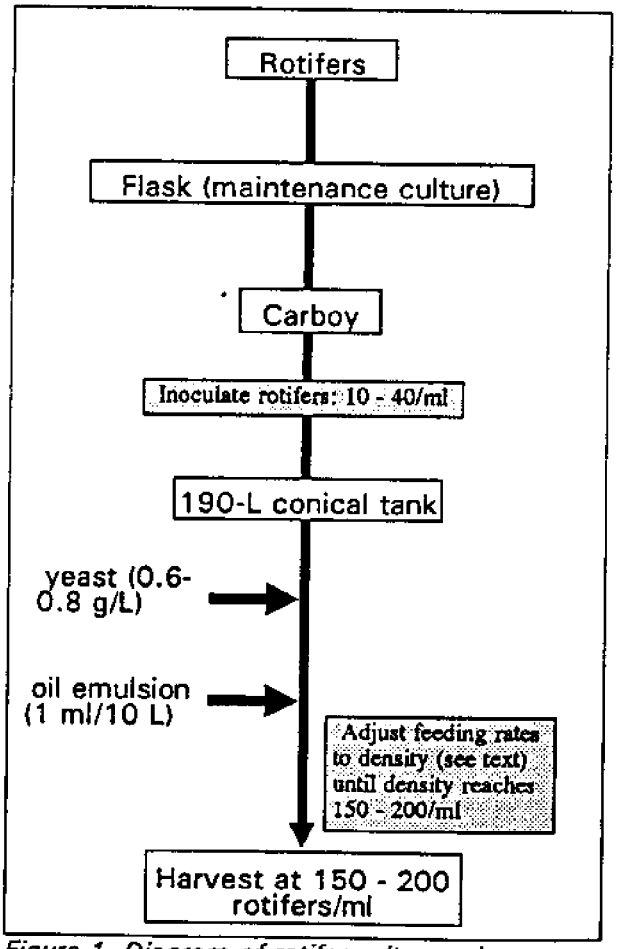

Figure 1. Diagram of rotifer culture using baker's yeast and emulsified fish oil.

collect rotifers. Refill tanks with filtered seawater adjusted to a salinity of 18 ppt. Add yeast at 0.6 - 0.8 g/10^6 rotifers and emulsified oil at 2 - 3 ml/10^6 rotifers. Repeat daily until the rotifer population declines.

Method using algae (Isochrysis galbana), *yeast and emulsified oil*

Set up one 1,800-liter tank as described above and inoculate with a 12-liter carboy of *Isochrysis galbana* (132,000 cells/ml) and medium (0.2 ml/liter of F/2 medium). Methods for the carboy culture of *I. galbana* and F/2 medium formulation are discussed by Treece and Wohlschlag (1990). On the second and third days add 0.1 ml/liter of F/2 medium, and when the algae density reaches 132,000 cells/ml, inoculate with 1 - 10 rotifers/ml. When the concentration of algae decreases, begin adding yeast at 50 g/tank and emulsified oil at 1 - 2 ml/10 liters each day. When rotifer density reaches 100/ml or more, increase the daily ration of yeast and emulsified oil to 0.7 - 1.0 g/10^6 rotifers and 2 - 3 ml/10^6 rotifers, respectively. Harvesting rotifers can begin when the density reaches 200 rotifers/ml (see Fig. 2). Drain 15 - 25% of the tank/day. Repeat until the rotifer density drops. This culture method should maintain rotifer densities at 150 - 200/ml for about 30 days (Wohlschlag et al. 1990).

Methods using algae as the sole nutrient source

When using algae, two methods can be used. The algae can either be cultured separately from the rotifers or both may be grown in the same container.

Algae and rotifers cultured separately

Algae (*Isochrysis galbana* or *Tetraselmis chuii*) is cultured in an 1,800-liter tank using

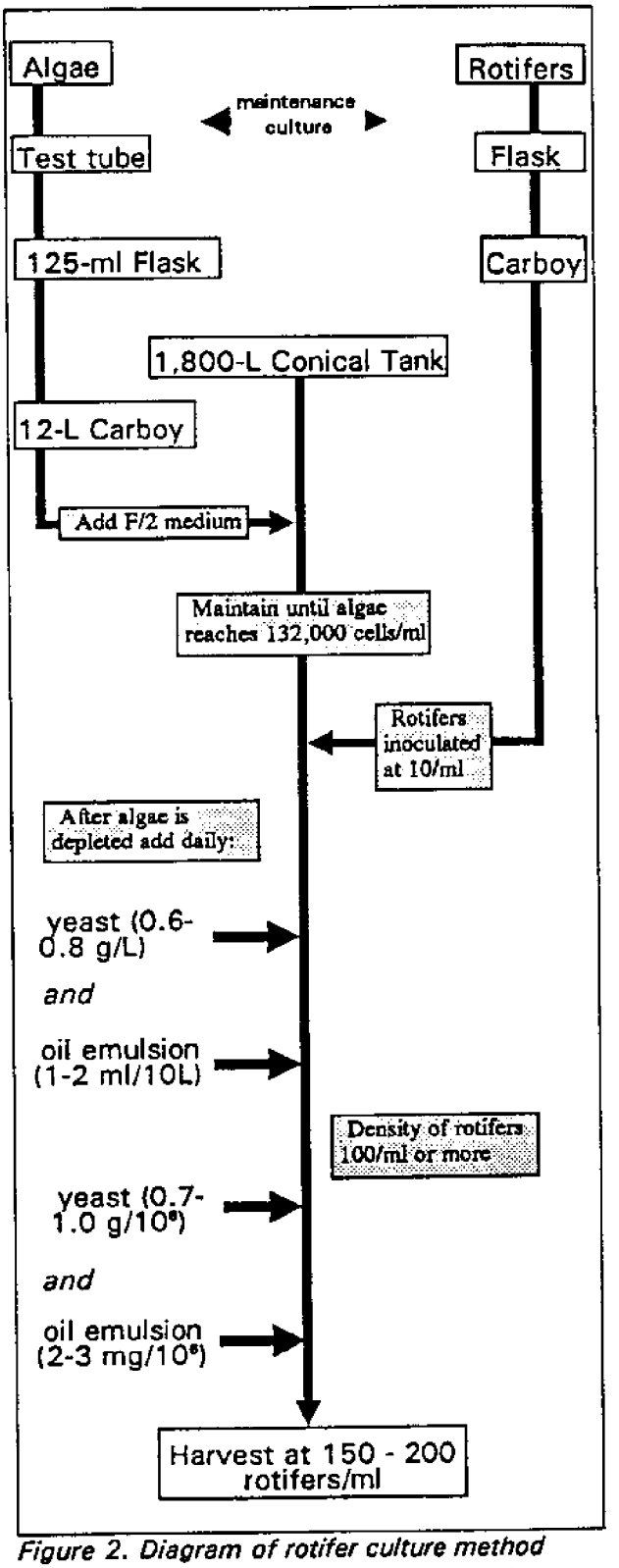

Figure 2. Diagram of rotifer culture method using algae, yeast and emulsified fish oil.

"fish emulsion", an organic fertilizer available in liquid form (Treece and Wohlschlag 1990). Rotifers are cultured in separate 1,800-liter tanks. All tanks receive continuous illumination from overhead light banks and are well aerated. The culture room is kept at 24 ± 2°C.

Algae tanks are filled with seawater which has been filtered through a 1-μm filter. The salinity depends on the species of algae and the requirement of the rotifer strain, but generally is in the range of 16 - 30 ppt. The tank is then inoculated with 100 liters of algae stock (130,000 cells/ml). If this much algae is not available, lower the volume of seawater in tank. It should take three to four days to reach an algal density of 132,000 cells/ml. If a smaller volume is used, double the volume with filtered seawater and fertilizer daily until a volume of 1,800 liters is reached. This culture can now be used to feed rotifers by draining 50 - 60% of the tank volume daily and refilling with filtered seawater and adding fertilizer. This should be done daily even if algae is not needed.

The rotifer culture tank is filled with 900 liters of filtered seawater and 900 liters of algal culture water. Rotifers are added, at least 1/ml, more if available. When algae has been consumed and the culture water becomes clear, rotifers may be harvested. This is accomplished by draining 30 - 50% of the tank volume. The density should be 100 - 150/ml. To maintain this rotifer count, refill the tank with algal culture water and repeat daily until rotifer population declines (Arnold et al. 1976).

A modification of this method is to start with 300 - 400 liters of seawater and add 100 liters of algae. Inoculate with rotifers at 12 - 15/ml. Add 100 liters of algae daily until the rotifer count is constant for two days. It is necessary to harvest at least 40% of the tank to restart an increase in the daily count. Harvest as needed on a daily basis, but the culture must be harvested as above if the daily count is the same for two consecutive days.

Algae and rotifers cultured together

Fifty-liter polyethylene bags are used as culture containers. They are clamped and attached to a support frame. Illumination is supplied by 40-watt fluorescent lamps (wall light banks). Aeration is supplemented with CO_2 (approximately 50 standard cubic feet per hour), injected every hour for 20 seconds to promote algae growth. A valve is attached to the top for aeration and seawater addition.

Bags are filled with filtered seawater, nutrient medium is added (F/2) and algae is inoculated at 500 cells/ml. Algae is grown for three days, and then rotifers are inoculated at 10/ml. It should take approximately four days to reach maximum rotifer density. Densities as high as 400 rotifers/ml have been achieved with this method. Rotifers are harvested by draining the entire bag (batch culture) and bags are discarded after use (Trotta 1981, Trotta 1983).

Outside culture

The culture of rotifers outdoors occurs between March and November in south Texas. The tanks are covered with a 60% shade cloth during the hottest months of the summer to maintain the temperature below 30°C. The 3,200-liter tanks are filled with unfiltered seawater and provided with aeration; the tank is allowed to sit for two to three days until the algae bloom. It is then inoculated with rotifers at 10/ml. Sixty grams of torula yeast is added daily, beginning on the third day until harvest begins. There is daily monitoring of the rotifer population, and once the concentration reaches 40/ml, harvest begins. At that time, 60 g of

yeast is added twice daily. Harvest up to 40% of the tank volume daily and refill the tank with seawater. The tank bottoms need to be vacuumed weekly to prevent the buildup of sludge which can become anaerobic and cause the culture to crash.

Feeding Rotifers to Larval Fish

Larval red drum begin feeding approximately three days after hatching, when their mouth parts develop (earlier in high temperatures, later in low temperatures). Rotifers are fed at this time at a rate of 3 - 5 rotifers/ml until larger feed can be consumed (Holt et al. 1981).

Due to the loss in nutritional value a few hours after harvest, it is best to feed rotifers to fish at least twice a day or whenever rotifer density drops below 3/ml (Gatesoupe and Robin 1981). Another method of keeping or increasing the nutritional value of the rotifers is by enriching them. There are many methods of doing this. We have evaluated algae, baker's yeast and fish oil emulsion enrichment. The best growth was with algae or baker's yeast plus an emulsion of menhaden fish oil. The lowest growth rates occurred when larvae were fed rotifers which had been cultured only on baker's yeast (Holt, in press). Rotifers grown solely on yeast are of low nutritional value because they have low levels of $\omega 3$ HUFAs, compared to algae-enriched rotifers (Watanabe et al. 1983b, Craig et al., submitted).

CONCLUSIONS

There are many ways to culture rotifers, but there does not seem to be a sure way to mass produce them without periodic culture failures. However, there are certain procedures which appear to help maintain a semi-continuous culture for an indefinite period. The more important ones are:

- keep culture containers and water clean,
- control contaminants such as ciliates and bacteria,
- harvest daily to maintain the culture in growth phase — this can be accomplished by daily counts and noting the number of egg carrying females,
- and add some algae daily — even a small amount seems to help.

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Environmental Management for Mass Culture of the Rotifer, *Brachionus plicatilis*

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ABSTRACT

Bacteria and protozoa are important biotic factors affecting the growth of cultured rotifers. The bacterial and protozoan flora of rotifer cultures and their effect on rotifer growth were investigated. The bacterial flora changed very rapidly, but an equilibrium was struck between those microbes which were beneficial to rotifers and those which were deleterious to rotifer growth.

The ciliated protozoan flora was composed mainly of *Uronema* sp. and *Euplotes* sp. When *Euplotes* sp. was dominant, *Uronema* sp. disappeared due to competition for bacterial food. Furthermore, the bacteria which coexisted with *Euplotes* sp. strongly inhibited rotifer growth. We also found that ciliates could be removed effectively with a coarse vinyl filter or addition of the predator *Artemia salina*.

A brief summary of important abiotic factors affecting cultured rotifers is also given.

INTRODUCTION

The culture environment is composed of two types of factors, abiotic and biotic. Abiotic factors, temperature, pH, dissolved oxygen, ammonium ion concentration, chemical oxygen demand, etc. are generally regulated by exchanging water, aeration and other means. On the other hand, microorganisms, a biotic factor, tend to be neglected because of the difficulty in handling them. Also, in aquaculture, quite a few people are only interested in high production of fishes, despite the significant role of live feeds in aquaculture production.

Even though the cultivation of rotifers seems to work well, the nutritional quality of rotifers used as feed varies depending on their

diet. This seriously affects the survival and growth of fishes. Also, rotifers transmit pathogenic bacteria, mainly *Vibrio* spp., to fish culture water (Suzuki et al. 1990). In fact, people have been realizing that microbes in rotifer rearing water affect not only the growth of rotifers, but also the physiological and hygienic conditions of cultured fishes. A rotifer culture program should be established with these points in mind. In this paper the means by which bacteria and protozoa affect rotifer growth and possible methods to protect rotifers from the deleterious activities of microorganisms are described. Also, a brief summary of important abiotic factors affecting rotifer culture water is given.

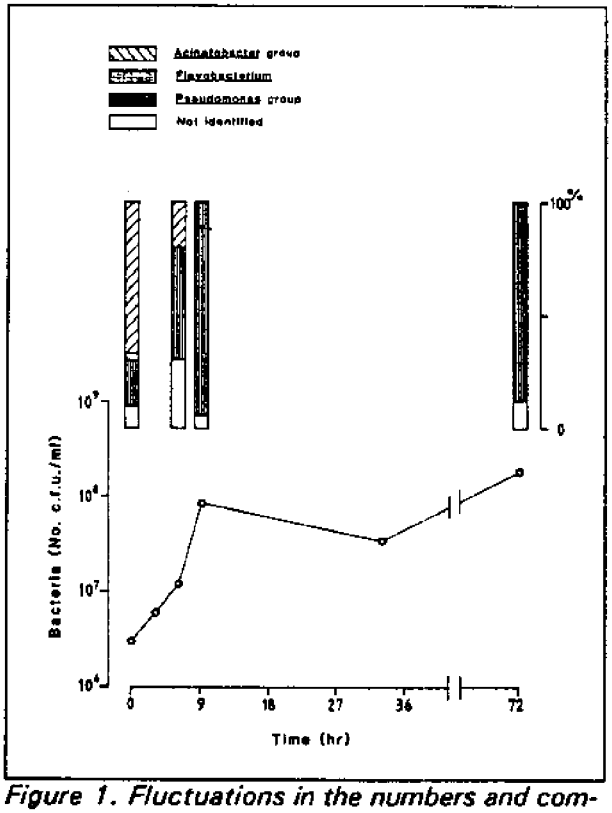

Figure 1. Fluctuations in the numbers and composition of bacterial flora during the culture of rotifers.

MICROBIAL AND PROTOZOAN SUCCESSION IN ROTIFER REARING WATER

When rotifers are cultured in an open system, quite a few bacterial species are usually present. Some of them promote the growth of rotifers but others, if present in high frequencies, might repress growth. Figure 1 shows the succession of bacterial flora during the cultivation of rotifers in a 500-liter container. Rotifers and *Nannochloropsis oculata* were inoculated at 200 ind./ml and 5×10^7 cells/ml, respectively, and baker's yeast was added regularly and maintained at 2×10^7 cells/ml. The number of rotifers increased to 1,000 ind./ml after three days, whereas bacterial numbers climbed to 10^8 colony forming units/ml (Figs. 1 and 2).

At the beginning of the experiment, *Acinetobacter* sp. dominated the bacterial population and *Flavobacterium* and *Pseudomonas* spp. appeared together. In time, however, the composition of flora changed. The proportion of *Pseudomonas* increased, becoming dominant after nine hours (Fig. 1). Thus, the bacterial flora changed very quickly. In Figure 2, ratios of *Pseudomonas* species are shown, from 24.1 to 93.0%. Among *Pseudomonas* groups, the strains NT-9, NT-10 and NT-11 dominated. After nine hours, only two strains, NT-10 and NT-11, could be isolated. NT-10 inhibited the growth of rotifers, but NT-11 promoted their growth (Table 1).

Although these data are not shown here, NT-10 supported the growth of the ciliated protozoa *Euplotes* sp. This may explain why large numbers of *Euplotes* sp. suddenly appeared in the aforementioned experiment, reaching 5,000 cells/liter after 72 hours (Fig. 2). These data suggest that the production of rotifers is assured if an equilibrium is estab-

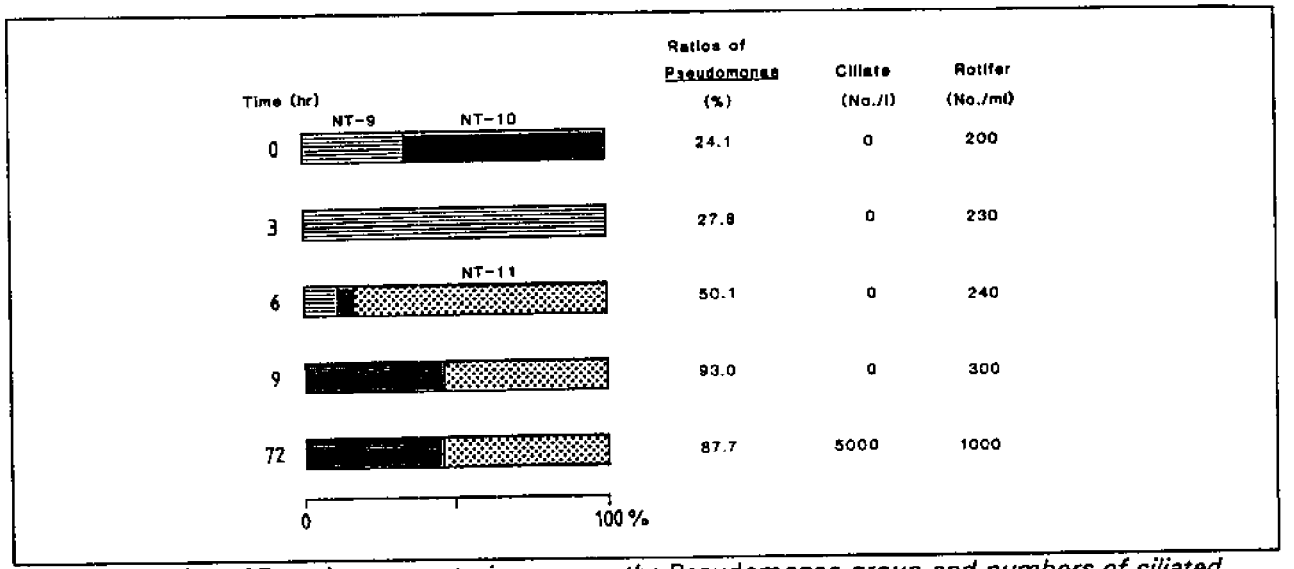

Figure 2. Ratios of Pseudomonas *strains among the* Pseudomonas *group and numbers of ciliated protozoa and rotifers present in the experiment shown in Figure 1.*

lished between the beneficial and deleterious bacteria in rearing water.

In Figure 1 it can be seen that *Flavobacterium* sp. appeared only on the first day. Table 2 shows how four genera of marine bacteria affected the growth of rotifers. Although most, including *Pseudomonas, Acinetobacter,* and *Vibrio* spp. seemed to support growth, *Flavobacterium* shows an inhibitory effect. Many *Flavobacterium* were isolated from *Nannochloropsis* rearing water (Table 1). If rotifers do not grow well using *Nannochloropsis* sp. as feed, it may be due to the presence of *Flavobacterium.*

It is known that animals or plants can promote the growth of certain beneficial bacteria. This seems to be occurring between *Pseudomonas* and rotifers. That is, bacteria which support the growth of rotifers eventually dominate the bacterial population in culture water.

In rotifer rearing water, mainly two species of ciliates were observed, *Uronema* and *Euplotes* (Figs. 3 and 4). At first, *Uronema* appeared on and around dying rotifers, trying to invade the bodies of weakened individuals. In fact, approximately ten *Uronema* packed inside each dead rotifer. After multiplying inside these corpses, they exited and eventually became dominant over the attached forms (Fig. 5). Immediately after the *Euplotes* sp. population started increasing, the *Uronema* population rapidly decreased, falling to zero when *Euplotes* numbered about 100 cells/ml (Fig. 5). This is because *Uronema* sp. was outcompeted for food (mainly bacteria) (Table 3). Finally, although neither ciliate feeds on *Nannochloropsis*, they do compete with rotifers for yeast.

CHARACTERISTIC FEATURES OF BACTERIAL FLORA IN PROTOZOA AND ROTIFER CULTURE

Table 4 shows the relative abundance of bacterial strains isolated from rotifer and protozoan (*Euplotes* sp.) cultures in which an

Table 1. Growth of rotifers cultured in the presence of various bacteria collected from *Nannochloropsis* and rotifer rearing water.

Strain	Taxonomic group	Place collected	Number of rotifers present after seven days*
1	Fla.	Nanno. pond	11
2	Fla.	Nanno. pond	10
3	Aci.	Nanno. pond	39
4	Pse.	Nanno. pond	89
5	Aci.	Rotifer tank	27
6	Pse.	Rotifer tank	139
7	Aci.	Rotifer tank	113
8	Aci.	Rotifer tank	38
9	Pse.	Rotifer tank	28
10	Pse.	Rotifer tank	10
11	Pse.	Rotifer tank	35
12	Vib.	Rotifer tank	18
13	Pse.	Rotifer tank	12
14	Pse.	Rotifer tank	10
15	Pse.	Rotifer tank	39
16	Pse.	Rotifer tank	10
17	Fla.	Nanno. pond	10
18	Aci.	Nanno. pond	56
19	Pse.	Nanno. pond	58
20	Aci.	Nanno. pond	15
Control			18

Fla.: *Flavobacterium*, Aci.: *Acinetobacter-Moraxella* group, Pse.: *Pseudomonas-Alteromonas-Alcaligenes* group, Vib.: *Vibrio*

*Ten rotifers were added to 10 ml of seawater containing 200 μg baker's yeast/ml with a bacterial strain (10^9 cells/ml), and cultured for seven days in the dark at 25°C. Bacteria were not added in the control experiment.

equilibrium between *B. plicatilis* and *Euplotes* sp. was not established. In Culture I, densities of rotifers and *Euplotes* sp. were 240 ind./ml and 20 cells/ml, respectively. In Culture II they were zero and 3×10^4 cells/ml, respectively. *Euplotes* outcompeted the rotifers in Culture II. The strain R-1, which was dominant in Culture I, effectively supported the growth of rotifers (Table 5). On the other hand, the dominant strains E-1 and E-2 in Culture II strongly inhibited the growth of rotifers (Table 5). Since supernatant seawater from both cultures did not affect the growth of rotifers, the deleterious or beneficial effects

Table 2. Average growth values of rotifers in the presence of four bacterial genera.*

	No. strains tested	Rotifers (number /5 ml)
Pseudomonas	10	47
Acinetobacter	6	53
Vibrio	2	31
Flavobacter	4	10
Control	—	11

*Experimental conditions shown in Table 1.

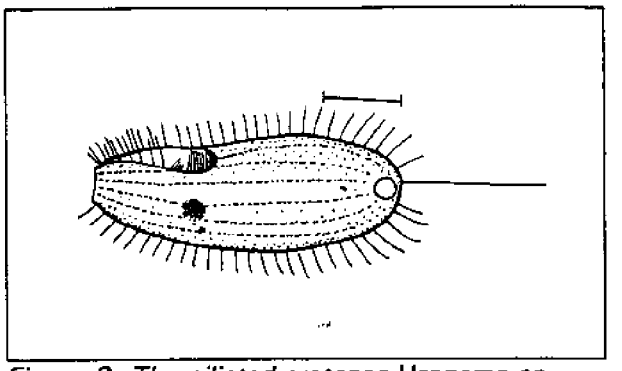

Figure 3. The ciliated protozoa Uronema *sp. (Bar indicates 10 μm).*

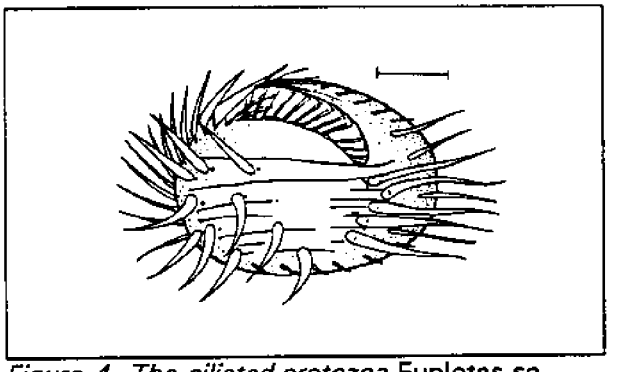

Figure 4. The ciliated protozoa Euplotes *sp. (bar indicates 10 μm).*

were attributed to the bacterial strains themselves. If, in situ, *Euplotes* numbers increase in rotifer rearing water, these inhibitory bacterial strains might appear and repress rotifer growth.

REMOVAL OF PROTOZOA FROM ROTIFER CULTURES

Free-swimming protozoa are divided into two groups, planktonic and psammophilic. Although the psammophilic protozoa, which include *Euplotes* and *Uronema*, can swim, they tend to crawl or slide along the surfaces of substrata. This characteristic behavior might aid in the removal of these protozoa.

Table 3. Live feeds of the ciliated protozoa, *Uronema* sp. and *Euplotes* sp., in rotifer culture water.

Protozoa	Bacteria	Yeast	Bacteria + Yeast	*Nannochloropsis*	*Uronema*
Euplotes	+	+	+	—	±*
Uronema	+	+	+	—	

+ : utilized as feeds.

- : not utilized.

*: some larger *Euplotes* sp. feed on *Uronema* sp.

Table 4. Proportions of the different bacterial strains isolated from the rotifer-dominant culture (Culture I) and *Euplotes* sp.-dominant culture (Culture II).

Culture		Number/ml	Bacterial flora (%)			
I	Rotifers	240	Strain R-1	Strain R-2	Strain R-3	
	Euplotes	20	80	13	7	
II	Rotifers	0	Strain E-1	Strain E-2	Strain E-3	Strain E-4
	Euplotes	3×10^4	43	26	20	11

A 10-liter tank containing rotifers and *Euplotes* sp. was supplied with a 100-cm^3 container filled with thick, coarse filter material (pore size about 5 mm). Rotifer culture water containing *Euplotes* sp. was air lifted, poured into the filter, and returned to the rotifer tank by an overflowing process. In this circulating system, the density of rotifers was maintained at about 100 ind./ml. As shown in Figure 6, with increasing retention time, the number of protozoa decreased from 500 cells/ml to less than 100 cells/ml. Although protozoan numbers decreased, the maximum rotifer density was 100 ind./ml, the disadvantage of this method.

We found that *Artemia salina* feeds on *Euplotes* and *Uronema*. This fact should be

Table 5 . Growth of rotifers in the presence of bacterial strains in Table 4.*

Strains	Rotifers (no./5 ml)
R-1	105
R-2	19
R-3	31
E-1	10
E-2	12
E-3	30
E-4	26
Seawater	25
Supernatants	
Rotifers	23
Euplotes	20

*Experimental conditions shown in Table 1.

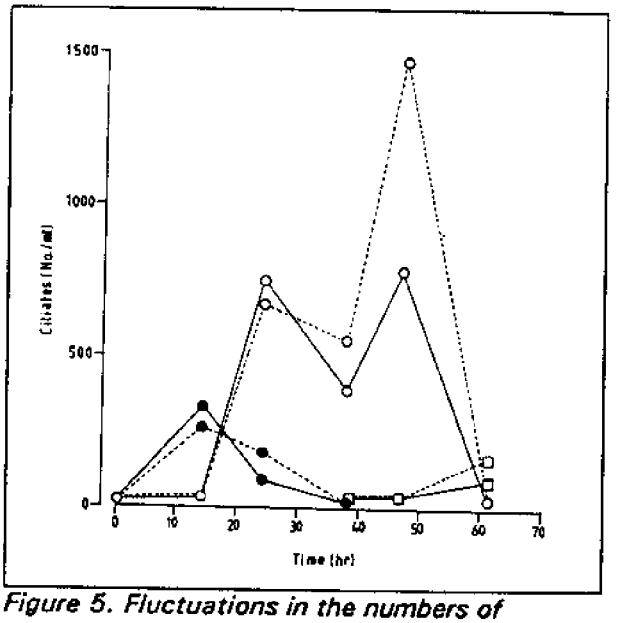

Figure 5. Fluctuations in the numbers of Uronema *sp. and* Euplotes *sp. in rotifer culture water.*
( ):Attached form of Uronema *sp.*
( ):Planktonic form of Uronema *sp.*
( ):Euplotes sp.
—:Experiment I.
—·:Experiment II.

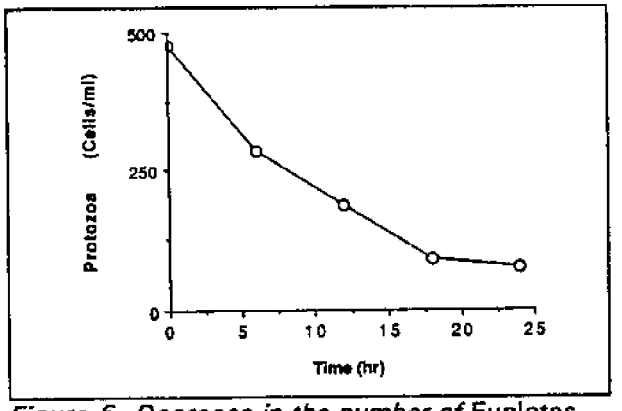

Figure 6. Decrease in the number of Euplotes *sp. resulting from the use of a coarse vinyl filter.*

employed for the efficient removal of protozoa in water. The size of *Artemia* used in this work ranged from 1.2 - 7.0 mm in length (Fig. 7) and the concentration of *Artemia* was 1 ind./ml; those of *Euplotes* and *Uronema* approximately 10^4 cells/ml. As shown in Figures 8 and 9, *Uronema* sp. and *Euplotes* sp. were consumed very quickly. Feeding rates for large *Artemia* were higher than those of smaller sizes. When rotifers, *Artemia* and *Euplotes* sp. were cultured together, the number of *Euplotes* dropped quickly, but rotifers were able to grow in this mixed culture (Fig. 10).

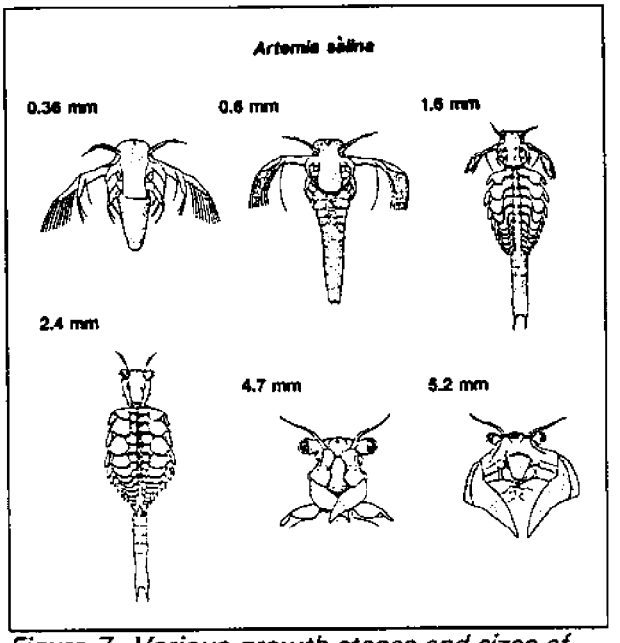

Figure 7. Various growth stages and sizes of Artemia salina *(from Heath 1924).*

Nannochloropsis oculata is eaten by *Paraphysomonas* sp., a flagellated protozoan which can reduce a population of *N. oculata* from 10^7 cells/ml to 10^6 cells/ml within a day. *Paraphysomonas* sp. grows well at low salt concentrations, i.e. less than 30 ppt salt concentration. To prevent rapid growth of this protozoan, salinity should be checked carefully and maintained higher than 30 ppt at all

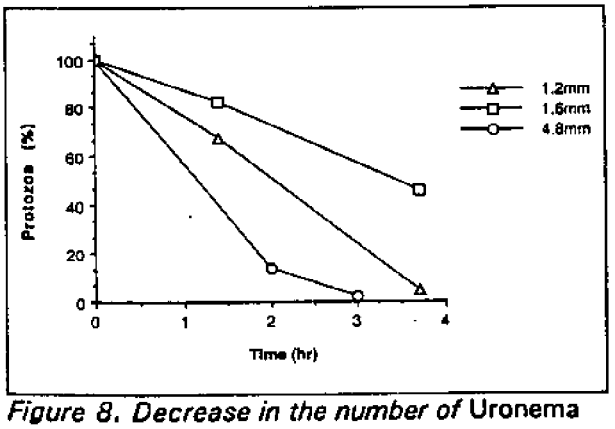

Figure 8. Decrease in the number of Uronema *sp. when cultured with* Artemia salina.

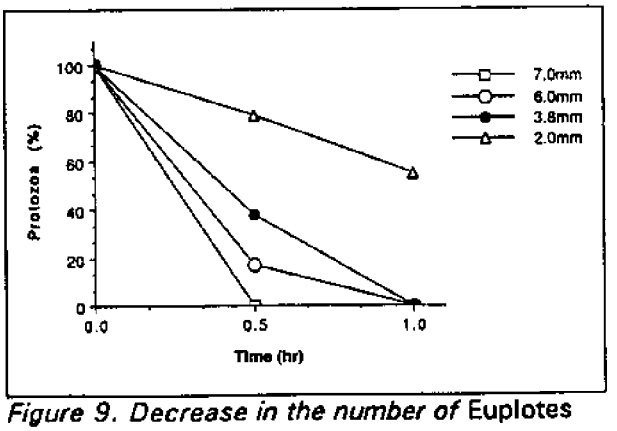

Figure 9. Decrease in the number of Euplotes *sp. when cultured with* Artemia salina.

times. This flagellate could also be reduced by means of physical stimulation, such as adding a waterfall to *N. oculata* rearing tanks. Details are reported in M. Kanematsu et al. (1989).

ABIOTIC FACTORS FOR ENVIRONMENTAL MANAGEMENT

Abiotic factors, including water temperature, dissolved oxygen, pH, NH_4^+ concentrations and chemical oxygen demand (COD), have been summarized thoroughly by Oka (1989) and Sugimoto (1989).

Growth rates of L- and S-type rotifers vary at different temperatures. S-type grow faster than L-type rotifers above 25°C. On the other hand, below 25°C, the growth rates of L-type rotifers are higher. In fact, optimum temperatures for the cultures of L- and S-type rotifers are approximately 25 and 30°C, respectively. Rotifer growth rate decreases greatly below 15°C, however, and once rotifers are exposed to such low temperatures, it may be difficult for them to resume normal growth even if the temperature is again raised above 25°C.

The optimum chlorine concentration for *B. plicatilis* is 6 - 10 ppt. Fish, however, are normally reared at 18 ppt chlorinity (normal seawater), too high for rotifers grown at 6 - 10 ppt. For this reason, rotifers are generally reared in normal seawater.

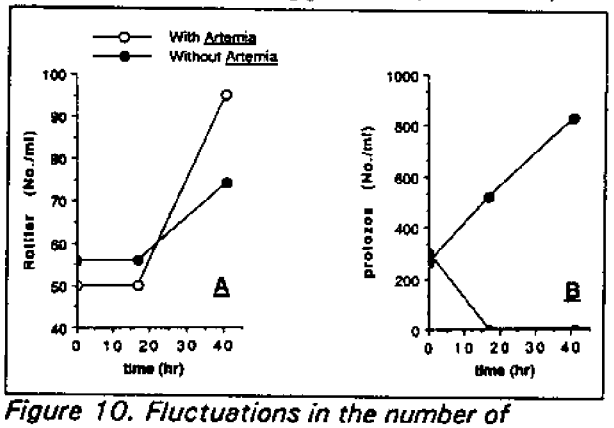

Figure 10. Fluctuations in the number of rotifers and Euplotes *sp. cultured with and without* Artemia salina. *A: Number of rotifers.; B: Number of* Euplotes *sp.*

Rotifers are very tolerant of dissolved oxygen (DO) deficiencies. Under anoxic conditions, 50% of a test population of rotifers survived for six hours. All died after 12 hours. Oxygen consumption rates for rotifers are approximately 4 - 7 x 10^{-5} ml DO/ind./day at 20 - 25°C. Since large populations of bacteria and protozoa consume more oxygen than rotifers, aeration is necessary for rotifer cultivation.

Rotifers grow within a pH range of 5 - 10, but stable growth and high feeding rates on *Nannochloropsis* or baker's yeast can be obtained at pH 7 - 8.

According to Yu and Hirayama (1986), an acute toxicity test of NH_3-N to rotifers showed that the 24-hour LC_{50} was 17.0 ppm at 23°C. Above 2 ppm, however, the physiological and reproductive states of rotifers were affected.

Optimum COD for rotifer production is generally within the range of 20 - 100 ppm.

Sudden decreases in the growth rate of rotifer populations are frequently observed in hatcheries. This might be caused by temperature fluctuations, a feed deficiency, low quality feeds, and/or increased NH_4^+ concentrations and COD. In addition to these abiotic factors, the species composition of the bacterial and protozoan flora can also seriously affect the growth of rotifers (as mentioned above).

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An Overview of Live Feeds Production System Design in Taiwan

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ABSTRACT

Over five billion prawn and clam larvae and 150 million fish larvae were produced commercially in 1990 in Taiwan. The main live feeds used are microalgae (*Skeletonema costatum*, *Isochrysis* aff. *galbana*, *Nannochloropsis oculata*, *Tetraselmis chui*, and *Chlorella* sp.), rotifers and natural plankton. In this paper, research efforts on the production and use of these live feeds in Taiwan are discussed and production facility designs and operating procedures are described.

INTRODUCTION

Commercial production of crustacean, molluscan and finfish larvae in 1990 in Taiwan was about 5.85 billion, 5 billion and 152 million, respectively (Table 1). This production was mainly from *Metapenaeus ensis*, the sand shrimp (3 billion); *Penaeus japonicus*, the kuruma prawn (2 billion); *Meretrix lusoria*, the hard clam (5 billion); and *Chanos chanos*, the milkfish (130 million). The rest of the production was from three other penaeid species and 10 finfish species. Prawn larvae are produced mainly in southern Taiwan, particularly in Kaohsiung and Pingtung Counties, except for *P. japonicus* larvae, one-fourth of which are produced in northern Taiwan, in Ilan County. Hard clam larvae are produced exclusively in central Taiwan, in Yunlin County. Finfish larvae are produced mainly in southern Taiwan, in Kaohsiung and Pingtung Counties, except for *Plecoglossus altivelis*, the

Table 1. Commercial production of crustacean, molluscan and finfish larvae in 1990 in Taiwan (units: individuals).

Species	Estimated production
Crustaceans	5,850,000,000
Metapenaeus ensis	3,000,000,000
Penaeus chinensis	50,000,000
Penaeus japonicus	2,000,000,000
Penaeus monodon	300,000,000
Penaeus penicillatus	500,000,000
Mollusc	
Meretrix lusoria	5,000,000,000
Finfishes	152,000,000
Acanthopagrus latus	3,000,000
Acanthopagrus schlegeli	5,000,000
Chanos chanos	130,000,000
Epinephelus malabaricus	2,000,000
Lates calcarifer	1,000,000
Lateolabrax japonicus	3,000,000
Lutjanus argentimaculatus	2,000,000
Pagrus major	2,000,000
Plecoglossus altivelis	500,000
Sparus sarba	3,000,000
Trachinotus blochii	900,000

ayu, which is produced in northern Taiwan (Fig. 1).

All prawn larvae are produced indoors and fed with microalgae at the zoeal stage. Mollusc larvae are also produced indoors and fed only microalgae before they settle. Most of the finfish larvae are produced outdoors and fed with natural plankton.

This paper summarizes research efforts on both rotifer and microalgal production and use in Taiwan. Live feeds production facility designs and operating procedures are also discussed.

CRUSTACEAN LARVAE

Live Feeds Used and Their Biological Characteristics

The first success in the artificial propagation of *Penaeus monodon* larvae was obtained using *Skeletonema costatum* as feed at the zoeal stage (Liao et al. 1969a). *Skeletonema costatum* was collected from Kaohsiung Harbor using 100-mesh plankton nets and then cultured using the separate tank method (Liao et al. 1983; Liao 1984). Since then, larvae of several penaeid species have been propagated artificially using the same feeding regime (Liao 1970). *Spirulina platensis* combined

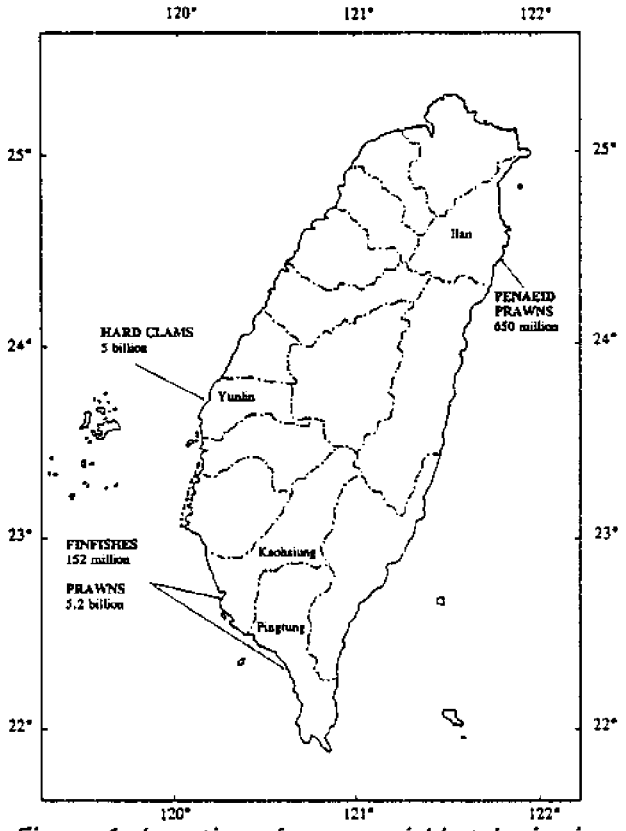

Figure 1. Location of commercial hatcheries in Taiwan and total production of prawn, clam, and finfish larvae (1990).

with *Skeletonema costatum* was found suitable to feed the mysis stage of *P. japonicus* (Tang 1977). *Spirulina platensis* was also found suitable as feed for the mysis stage of *P. monodon* (Tsai 1980). Later, commercially-produced *S. platensis* powder was used as supplemental feed by commercial hatcheries.

The effects of selected live feeds on the growth and survival rate of *P. monodon* larvae was tested by Lei and Su (1985). The results showed *Skeletonema costatum* and rotifers to be the most nutritious feeds. *Isochrysis* aff. *galbana*, *Chaetoceros gracilis* and *Tetraselmis chui* were found to be inferior to *S. costatum* and rotifers, while *Dunaliella* sp., *Chlorella* sp., and *Spirulina platensis* were unsuitable for the zoeal stage. When *Tetraselmis* sp., *Skeletonema costatum* and *Artemia* nauplii were fed to *P. chinensis* from Z_1 to P_5, survival rates of 13.7 - 64.0% were obtained (Tzeng et al. 1990). Among these suitable microalgae, *S. costatum* was preferred because it has more advantages. *S. costatum* is eurythermal and euryhaline, and its optimum temperature and salinity are between 20 and 30°C and 15 and 30 ppt, respectively (Su et al. 1990). It grows very fast and can be concentrated in a net. Furthermore, its EPA (eicosapentaenoic acid) content is higher (35 - 43%) than that of *C. gracilis (19 - 28%) and T. chui* (4 - 8%) (Su et al. 1988). Although its stock culture maintenance is difficult (Su et al. 1990), inocula can be easily collected from Kaohsiung Harbor. All these advantages have resulted in the use of *S. costatum* in hatcheries in Taiwan almost exclusively.

Skeletonema costatum Production

Stock cultures

Stock cultures for laboratory studies are grown in 250-ml flasks containing 100 ml F/2 medium (McLachlan 1973) and placed in a growth chamber at 20°C and 500 lux (12L: 12D). Subcultures are transferred every one to two weeks. Mass cultures are developed stepwise in the following order: 250-ml flask ⇒ 1-liter flat flask ⇒ 15-liter circular glass beaker ⇒ 500-liter circular FRP tank ⇒ 10-ton concrete pond. These cultures are agitated with an aerator with the exception of the 250-ml flask culture, which is manually shaken occasionally.

Stock cultures for commercial hatcheries are bought from suppliers which collect *S. costatum* by boat with 100-mesh nets in Kaohsiung Harbor. The microalgae are immediately packed in plastic bags (25 kg/bag) or

Table 2. Enriched seawater medium for *Skeletonema costatum* in commercial hatcheries (unit: g/ton).

Additive	Hatchery				
	I	II	III	IV	V
KNO_3	2	10 - 50			
Urea*	20		30	12	4
Ammonium sulfate*			20	4	4
$Na_2HPO_4 \cdot 12H_2O$	3	1			
Calcium superphosphate*			15	4	4
Potassium chloride*			15	4**	2
Na_2SiO_3		2 - 5			
$Fe_2SO_4 \cdot 7H_2O$				0.25	
$FeCl_3 \cdot 6H_2O$		0.25			
EDTA				5 - 10**	

*Agricultural fertilizer; **Used occasionally.

packed after subculture within 24 hours. The density of *S. costatum* in the plastic bags is such that two bags are enough to inoculate one 10-ton concrete pond. The suppliers began this specialized business in 1985, and the highest demand on record was between 1986 and 1987 at 300 bags per day with an average of 200 bags per day.

Skeletonema costatum is distributed in all coastal waters around Taiwan (Huang et al. 1986). Before 1988, *S. costatum* could be collected year-round in Kaohsiung Harbor. Its density was high during the rainy (May to June) and fall (October to December) seasons, but low prior to the rainy season (February to March). This corroborated the findings of Liao (1970). However, after 1988, *S. costatum* collection in Kaohsiung Harbor became difficult because of pollution. Likewise, the mass culture of *S. costatum* encountered difficulties.

Fertilizers

The media available for *S. costatum* culture have been described by Liao et al. (1983). At present, F/2 medium is being used for stock culture at the Tungkang Marine Laboratory (TML), Taiwan Fisheries Research Institute (TFRI). The reagents used for commercial hatcheries are listed in Table 2. Fertilizers such as urea, ammonium sulfate, calcium superphosphate, and potassium chloride are major sources because they are cheap and suitable for *S. costatum* growth. As shown in Table 2, the quantity used varies greatly and is dependent on who is preparing them. This shows that both the quantity and quality of fertilizers are not very critical in the mass culture of *S. costatum*.

Culture techniques

Generally, *S. costatum* is cultured outdoors in rectangular concrete ponds with 10 -

Table 3. Schedule of inoculation (▽) and harvest (♦) for *Skeletonema costatum*.

Pond No.	Date of inoculation and harvest									
	1	2	3	4	5	6	7	8	9	10
I	▽		♦		▽		♦			
II		▽		♦		▽		♦		
III			▽		♦		▽		♦	
IV				▽		♦		▽		♦

40 ton water volume. Water depth in the pond is 1.5 - 2.0 m. Higher illuminance, over 5,000 lux (saturated illuminance), was found to decrease the growth rate and shorten the exponential period (Su et al. 1990). Increasing the culture water depth results in decreased light intensity, thus benefitting the culture by slowing the growth rate and extending the exponential period.

To begin with, two bags of stock culture from Kaohsiung Harbor or another hatchery are inoculated in one 10 - 20 ton pond. On the second or third day, *S. costatum* is collected with 100 mesh nets or drained into cloth bags. The collected microalgae may be used as an inoculum for a new pond. The ratio of inoculum to culture volume ranges from 1:20 to 1:100, depending on the concentration of the inoculum, the period of harvest, light and temperature, and on who is preparing the inoculum. The concentration of the culture at the start is about 100 - 250 chains/ml or 1,500 - 4,000 cells/ml. Time of inoculation is 0800 - 0900 h or 1500 - 1600 h. After 30 hours (at least 12 hours), one-fourth or one-fifth is harvested to feed prawn larvae by draining the culture into cloth bags for one or two days.

The optimum number of ponds is four. Table 3 lists the culture schedule. Every day, one of the four ponds is either ready for culture, under culture, ready for harvest or being sun-dried. Each pond is cultured for three days. One run, from start to finish, is about 10 days, comparable to the period from hatching to zoeal stage of prawn larvae, which is also consistent with the change in the size of *S. costatum*.

In culture, the diameter of *S. costatum* decreases after cell division, thus, the *S. costatum* chains become thinner and thinner. As the diameter becomes smaller, the cell division rate increases, while the concentrated biomass and the duration of the exponential period decrease. At this point, a culture is considered unsuitable as an inoculum. Therefore, new stocks are collected from Kaohsiung Harbor or nearby hatcheries for the next larval rearing run.

The quality of *S. costatum* is very important, and can be determined by its color and smell. Light brown and briny-smelling cells often appear in the exponential phase. These cells have a particularly high EPA content (Su et al. 1988).

It is estimated that about one ton of microalgal culture is needed for one 10 - 15-ton larval rearing pond. For example, two 50-ton *S. costatum* cultures are sufficient to supply eight 130-ton larval ponds in which 160 million *P. japonicus* larvae are reared; and three 20-ton *S. costatum* cultures will supply

340-ton larval ponds in which 2 - 3 million/30 tons *P. monodon* larvae are reared.

Problems and prospects

Heavy metals like copper and cadmium, which are found in seawater and absorbed by *S. costatum* in high concentrations, are suspected to be one of the factors causing the mortality of *P. monodon* larvae in 1986. Thus, formulated feeds were developed to replace *S. costatum*. At present, *S. costatum* is used in combination with formulated feeds for prawn larvae at the zoeal stage. This has resulted in the decreased use of *S. costatum*. However, the nutritional and operational advantages of this live feed make it superior to other feeds, including formulated feeds (Liao et al. 1988).

A major problem in *S. costatum* culture is the short exponential growth phase and its tendency to perish after only a short culture period. Frequent subcultures are thus necessary to maintain the microalgae in good condition. A stock culture supply center is, therefore, necessary since Kaohsiung Harbor has become so polluted, allowing other microalgae to gradually displace *S. costatum*.

MOLLUSCAN LARVAE

Live Feeds Used and Their Biological Characteristics

More than 13 molluscan species have been successfully cultured in tidal lands, estuaries, and shallow seas along the west coast of Taiwan. Artificial propagation has been successful for *Meretrix lusoria* (Chen 1984); *Hiatula diphos*, the purple clam (Lai 1984); *Tapes variegata*, the Manila clam (Yen 1985); and *Anadara granosa*, the bloom clam (Tsai 1986). All the larvae were fed with microalgae. The best microalgal species for the swimming larvae is *I.* aff. *galbana*, with *Tetraselmis* sp. next; but species with thick cell walls are not recommended (Chen 1984). On the other hand, Yang and Ting (1985) found *I.* aff. *galbana* and *Chlorella* sp. to be the best feeds for the growth of purple clam larvae, with *Tetraselmis* sp. and *Spirulina platensis* coming in next. They found *Chaetoceros gracilis* unsuitable. *Isochrysis* aff. *galbana* has been used solely for the mass production of the swimming larvae of hard clam, but its nutritive value as feed for juveniles was inferior to prepared feed (Hon 1990).

Isochrysis aff. *galbana* was introduced to Taiwan from the Tahiti-based AQUACOP in 1980. Its stock cultures are maintained in TML, and distributed to hatcheries in Taiwan. A study by Kao (1986) on the influence of nutrients, salinity and pH on the growth of *I.* aff. *galbana* showed that it was euryhaline with an optimum salinity of 10 - 25 ppt.

Isochrysis aff. *galbana* Production

The following production system is being adopted in TFRI's Taishi Branch.

Medium and water filtration

The medium used for *I.* aff. *galbana* culture is Walne medium (Walne 1966) (Table 4). Reagent-grade ingredients are used for stock and indoor mass culture while industrial chemicals are used for outdoor mass culture. Vitamins are not added to outdoor cultures.

Seawater is treated as follows:

- Ceramic filter ⇒ autoclave ⇒ **stock culture**
- Storage tower ⇒ filtration tank (60 cm dia. x 200 cm, 25-μm net filter) ⇒ 2-μm filter cartridge ⇒ ceramic filter ⇒ **indoor culture**

Table 4. Formula of Walne medium (Walne 1966).

Seawater			1,000 ml
SOLUTION I			1 ml
$NaNO_3$		100.00 g	
$NaH_2PO_4 \cdot H_2O$		20.00 g	
Na_2EDTA		45.00 g	
H_3BO_3		33.60 g	
$MnCl_2 \cdot 4H_2O$		0.36 g	
$FeCl_3 \cdot 6H_2O$		1.30 g	
SOLUTION II*		1.00 ml	
$ZnCl_2$	2.1 g		
$CoCl_2 \cdot 6H_2O$	2.0 g		
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.9 g		
$CuSO_4 \cdot 5H_2O$	2.0 g		
Distilled water to	100 ml		
Distilled water to		1,000 ml	
SOLUTION III**			0.1 ml
Vitamin B_{12} (Cyanocobalamin)		10 mg	
Vitamin B_1 (Thiamin)		200 mg	
Distilled water to		100 ml	

*Acidify with sufficient concentrated HCl to get a clear solution; **Acidify to pH 4.5 before autoclaving.

- Local seawater ⇒ settling pond ⇒ first filtration pond (10 x 12 x 2.5 m^3) ⇒ reservoir (6 x 200 tons) ⇒ second filtration pond ⇒ storage tower ⇒ **outdoor culture.**

Culture facilities

Culture rooms

Two separate 40-m^2 (12.3 x 3.4 m^2) rooms (Fig. 2) located on the first and second floor of the algal culture center of the branch are used for indoor culture. Installed on the first floor are 22 350-liter rectangular glass tanks (120 *l* x 45 *w* x 75 *d* cm^3, 0.8-cm thick glass). Its southern wall is fitted with glass, while its northern wall has a mirror to increase illumination.

Stock cultures of 2.5-liter plate flasks are kept in a 14°C water bath-type rectangular glass tank (1,200 x 45 x 30 cm^3) on the second floor. The glass tank is placed on top of a wooden desk and located at the south end of the room. Fourteen 60-liter rectangular glass tanks (75 *l* x 30 *w* x 30 *d* cm^3, 0.5-cm thick glass) are situated at the north end of the room.

The temperature for stock culture is regulated at 14°C using cool water. The culture room temperature is maintained at 25°C with an air-conditioner.

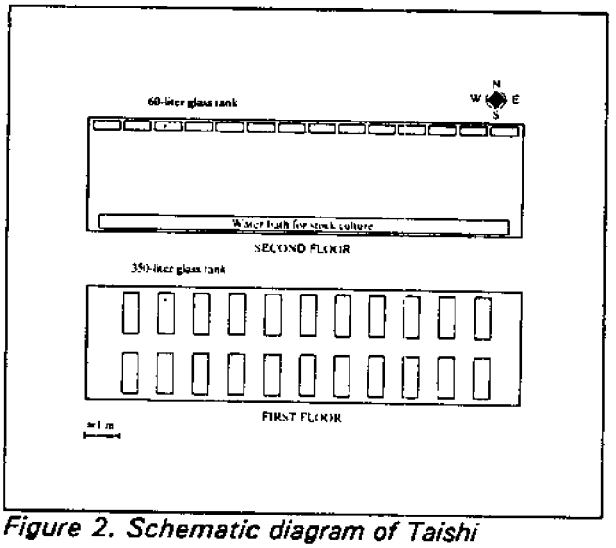

Figure 2. Schematic diagram of Taishi Branch's culture rooms.

Natural and artificial lights provide illumination. Artificial light is provided by 40-watt fluorescent lamps installed on the ceiling or wall. The lamps are controlled automatically during the light period, that is, the lamps turn on if the light is inadequate and off if there is enough natural light. The light period lasts 12 hours, and the light intensity for stock cultures, 60-liter tank and 350-liter tank cultures are 1,000 - 1,500, 5,000, and 6,000 - 7,000 lux, respectively.

Culture ponds

Outdoor culture tanks and ponds are located on the southern side of the culture rooms. Twenty-two 700-liter conical fiberglass tanks, 20 2.5-ton, 18 5-ton and 14 10-ton concrete ponds are used either for the mass culture of *I.* aff. *galbana* or *Nannochloropsis oculata*, or both.

Culture techniques

Microalgal culture is routinely conducted using the batch technique. One 60-liter microalgal tank is inoculated with four to six 2.5-liter stock cultures, and cultured for four to seven days. The tank is then drained by gravity and used as the inoculum for one 350-liter tank. The 350-liter tanks are the first in the culture procedure to be provided with aeration. One 700-liter outdoor conical tank is inoculated from a four to seven-day-old 350-liter culture. Afterwards, every four to seven days, a larger pond is inoculated from a smaller pond in the following order: 700 liters ⇒ 2.5 tons ⇒ 5 tons ⇒ 10 tons.

Each time, 3 - 5 tons of *I.* aff. *galbana* culture is pumped to one 400-ton indoor clam larvae pond to maintain the microalgal density at 1,000 cells/ml. Too high a density inhibits larval growth. Larval density is 2 - 10 ind./ml.

Problems and prospects

Few problems are encountered in indoor culture. On the other hand, outdoor cultures experience frequent problems in the summer due to contamination with protozoa or too high temperatures. These problems also occur during the rainy season or when the change in water temperature exceeds 5°C.

Outdoor commercial pond culture, particularly *I.* aff. *galbana* culture, encounters problems frequently. Thus, naturally-occurring microalgae in growout ponds are instead used to feed the swimming larvae. The method used is as follows: first, prawn or fish pond water and brown or green water from nearby ponds are filtered through a 25-μm net. They are then either transported by motor vehicle or through a pipeline, and used directly or indirectly to feed clam larvae. If indirectly fed, filtrates are provided as inoculum for outdoor pond culture, which are enriched with chemical and organic fertilizers. Organic fertilizers include fish meal, fish soluble and prepared food. The unstable supply of suitable natural phytoplankton, however, is the main problem

when using this method. Selection of more environmentally resistant and nutritious microalgae like *S. costatum* in local water would be a solution. Also, formulated feeds are currently being developed to replace live feeds.

FINFISH LARVAE

Live Feeds Used and Their Biological Characteristics

Since 1968, when the first success in the artificial propagation of grey mullet (*Mugil cephalus*) fry was achieved by Liao et al. (1969b, Liao et al. 1972), many finfish fry have been produced commercially (Table 1). The feeding regimes used are almost the same. Routinely, as the larvae grow, artificially-fertilized oyster eggs, rotifers, copepods, *Artemia* nauplii, and prepared food are given successively. Green water is also used to feed the zooplankton and to stabilize the water quality.

Most strains of *Brachionus plicatilis* found in aquaculture ponds in Taiwan are of the S-type and range from 100 - 305 μm. Temperature, salinity and feed concentration all affect the growth rate of the L- and S-type strains. Of these factors, temperature is the most critical. The most suitable temperatures for both strains are between 28 and 32°C. Above 28°C, the salinity and size of the strain are not very critical, but the density of feed is very important. However, below 28°C, the bigger strains (183 - 233 μm in length, mean = 210 $\pm$3 μm) grow faster than the smaller ones (126 - 172 μm, mean = 143 $\pm$3 μm). Decreasing salinity (10 - 30 ppt) increases the growth rate of both strains. Therefore, enough food must be provided during the warm seasons, and enough heat during the winter and spring seasons in Taiwan. Otherwise, bigger strains and low salinity are the best choices if heaters are not used during the cold season. Recently, TML has collected more strains from natural waters and ponds, and the selection of strains with higher growth rates is in progress. This study hopes to obtain suitable strains with optimum sizes.

Microalgae, baker's yeast, yeast powder, prepared feeds, and chicken droppings are used to feed rotifers, but the best growth is obtained with microalgae. Microalgae (*Chlorella* sp., *Tetraselmis chui*, *N. oculata*, and *I.* aff. *galbana*) are all suitable to feed rotifers. The rotifers grow best, however, when fed *T. chui*. The HUFA (highly unsaturated fatty acid) content of rotifers varies a great deal and is related to its feed. The EPA content of rotifers fed *N. oculata* is the highest, about 15 - 20% of total fatty acid; with *I.* aff. *galbana* or *T. chui*, EPA content is low, about 5 - 10%; and with *Chlorella* sp. or yeast, very low, nearly 0% (H.M. Su, unpublished data).

Nannochloropsis oculata was provided by the National Research Institute of Aquaculture (NRIA), Japan and introduced to Taiwan in 1987. At present, most hatcheries use this species and consider it to be the best nutritive feed for rotifers. However, it is very difficult to culture in southern Taiwan, where most hatcheries are found, during summer due to high temperatures and contamination with protozoa. Contaminated cultures turn brown and then perish. Treatments, such as adding sodium hypochlorite or increasing salinity as reported by Kanematsu et al. (1989) did not help. Techniques for repressing or diminishing protozoan contamination must be developed to stabilize production of *N. oculata*. Aside from these, culture temperature must not exceed 30°C. *Chlorella* sp. occurs often

Table 5. Ponds' capacity, initial and final larval density, and survival rate of finfish larvae reared indoors* and outdoors.**

Species	Water volume (tons)	Eggs stocked		Initial		Final		Survival rate (%)
		No. (x1,000)	Density (No./ton)	No. (x1,000)	Density (No./ton)	No. (x1,000)	Density (No./ton)	
Chanos chanos	300**	600	2,000			180	600	30
Epinephelus malabaricus	14*			50	3,500	16	1,143	30
	15*	190	12,666	180	12,000	10	666	5.7
	300**	1,200	4,000	720	2,400	20	67	2.8
	475**			600	1,263	40	84	6.7
	840**	3,600	4,285	2,160	2,571	40	48	2.2
Acanthopagrus schlegeli	15*	150	10,000			17	1,113	11.1
	25*	370	15,000			120	5,000	30
	1,000**	3,000	3,000			50	50	1.7
Acanthopagrus latus	25*	370	15,000			40	1,600	10
	45*	2,190	48,666	1,660	36,888	20	444	1.4
	400**	1,100	2,750			250	625	22
	475**	2,000	4,210			50	105	2.5

in brackishwater ponds in Taiwan. However, the six strains isolated have a very low EPA content (almost 0%), and their nutritional value for rotifers is inferior to *N. oculata*'s. Therefore, *Chlorella* sp. culture is now rare.

Tetraselmis chui was obtained in 1983 from the microalgae section of the Philippine-based Southeast Asian Fisheries Development Center (SEAFDEC). Although its EPA content (4 - 8%) is also lower than that of *N. oculata*, its nutritive value for rotifers is higher. Mass cultures of *T. chui* are used as feed for rotifer cultures.

Zooplankton Production

Various designs and procedures have been developed for producing rotifers and other zooplankton in Taiwan based on the larval rearing methods used. There are two basic methods. With the intensive method, high densities of larvae (10 - 50 ind./liter) are reared indoors in concrete ponds; microalgae, rotifers, and *Artemia* nauplii are provided as feeds. The other is the extensive method, that is, low densities of larvae (1 - 4 ind./liter) are reared outdoors in earth-bottom ponds; natural plankton supplemented with rotifers and copepods are the main live feeds. The ponds' capacity for larval rearing, the initial and final larval density, and survival rate achieved with these two methods are summarized in Table 5. To satisfy the heavy demand for live feeds, intensive mass production of microalgae and rotifers are performed. To simulate and maintain the ecosystem, natural plankton are raised

Table 6. Six different systems used for the culture of microalgae, rotifers and fish larvae in some indoor and outdoor larval rearing ponds (unit: tons).

	Indoor pond				Outdoor pond	
	I	II	III	IV	V	VI
Microalgae	0.5 x 15 40 x 10 60 x 2	20 x 6 80 x 2	0.2 x 10 1 x 14	0.4 x 24 5 x 12 15 x 6	100,000	5,200 6,000
Total	530	280	16	160	100,000	11,200
Rotifer		80 x 6	2 x 4	10 x 8	150 x 2 300 x 1	
Total	0	480	8	80	600	0
Fish larvae	15 x 6	30 x 3 45 x 4	4	40	475 x 9 1,160 x 1	300 840
Total	90	270	4	40	5,435	1,140
A:R:F*	6:0:1	1:1.7:1	4:2:1	4:2:1	18:0.1:1	10:0:1

*Capacity ratio of microalgae:rotifers:fish larval culture ponds.

to provide diversified natural live feeds for larvae reared outdoors.

Culture systems

Examples of six different systems used to culture microalgae, rotifers and fish larvae in some indoor and outdoor larval rearing ponds are listed in Table 6. In Case I, there are no exclusive rotifer ponds. Instead, microalgal ponds contaminated with rotifers are used for rotifer culture. Otherwise, naturally occurring rotifers are collected for culture in microalgal ponds and harvested as feed after two or three days. On the other hand, microalgal water is added daily to larval ponds to feed the rotifers and stabilize water quality. Therefore, more microalgal ponds are needed. In Case II, although larvae are reared in clear water and no microalgal water is added for larval ponds, the rotifer yields are often deficient due to the smaller capacity of the microalgal ponds. In Case III, experiments and trials were designed to determine the optimum design system for rotifer production. Case IV is a model system designed to be tested in the future. The best set for microalgal culture is six ponds in a group; for rotifer culture, four ponds in a group. In Cases V and VI, microalgae are provided from prawn and finfish growout ponds. Only green pond water is used. Thus, many ponds are needed. Blooms leading to green water can easily occur and be sustained in prawn ponds. The main species found in green water include *Synechocystis pevalekii*, *Chroococcus cohaerens*, *Chlorella vulgaris*, *Oocystis borgei* and *Ankistrodesmus convolutus*. Besides these, flagellates, diatoms, protozoa, rotifers and copepods also occur and could be used as live feeds for larvae. Facilities for rotifer culture are either very limited or not

Table 7. Fertilizers for microalgal culture (unit: g/ton).

	Hatchery					
	I	II	III	IV	V	VI
Ammonium sulfate	50 - 100	90	100	100	50	10
Urea	5 - 10	5	10	10	5	5
Calcium superphosphate	20 - 30	20	30		20 - 30	2
Trash fish						20
Microalgae species*	C,N,T	N	N	N	C	Green

*C: *Chlorella* sp.; N: *Nannochloropsis oculata*; T: *Tetraselmis chui*

available. In extreme cases, surface water containing a natural plankton bloom is directly introduced into larval ponds, and copepods are provided at the late stage of larval rearing.

Culture techniques for microalgae

Table 7 shows the fertilizers used in microalgal culture and the species cultivated. They are almost the same in quality but vary in quantity due to local water and weather conditions. Microalgal culture is routinely performed according to the batch technique, and fertilizers are provided at the beginning of the culture. In TML, stock cultures in 250-ml flasks, 1,000-ml flat flask cultures, and 15-liter glass beaker cultures are performed indoors. Fifteen-liter subcultures are transferred every four to five days, and 15-liter cultures are provided as inoculum for the 200-liter outdoor culture tanks. From 200 liters, the cultures are expanded stepwise from 200 liters ⇒700 liters ⇒ 3,000 liters ⇒ 10,000 liters. The inoculated ratio is 1:2 - 1:5. Culture periods for each step last three to five days according to requirements and culture conditions.

Seawater for indoor culture is sterilized by autoclave, while seawater for outdoor culture is treated by settling, addition of 10 ppm sodium hypochloride, and neutralized with sodium thiosulphate. Light for indoor culture is provided by fluorescent lamps (4,000 - 5,000 lux); natural light is used for outdoor culture. Temperature is maintained at 25 - 30°C for indoor culture. For outdoor culture, temperature changes naturally. In other research facilities and commercial hatcheries, the same outdoor procedures are used.

There are no definite methods to follow for exploiting green water naturally occurring in prawn or finfish ponds. By experience, good quality green water often occurs during the middle part of the prawn growout cycle. On the other hand, raw feeds seem to be superior to prepared feed in promoting blooms in the finfish ponds. Furthermore, the culture of high-priced finfishes is more lucrative. Therefore, 1,000 - 5,000 carnivorous fishes like grouper, sea perch and porgy are cultivated in one 0.2-ha pond to provide the green water. Then, this green water is pumped via a pipe to larval rearing ponds to stabilize the water quality and to feed zooplankton. Some of these pipelines are more than 2 km long.

Culture techniques for rotifers

For small ponds (below 20 tons), batch techniques are adopted. At the start, three parts of 20-ppt seawater are added to one part

Table 8. Feeds for *Brachionus plicatilis*.

	I	II	III	IV	V	VI
Nannochloropsis oculata	5×10^6 cells/ml					
Tetraselmis chui		10^5 cells/ml				
Green water			+	+		
Baker's yeast	1 g/10^6 rotifers	1 g/10^6 rotifers				
Yeast powder			2 - 3 kg/1,000 tons			
Chicken droppings				100 kg/ 400 tons	60 kg/50 tons	
Boiled fish meat					10 kg/50 tons	
Tilapia feed (soaked)						45 kg/30 tons

+Concentration unknown.

microalgal culture and inoculated with rotifers having a high growth rate. The initial rotifer density is 30 - 50 ind./ml. One-third to one-fifth part of microalgal water combined with baker's yeast (Table 8, columns I and II) is provided daily from the second or third day to the end. After five to seven days, rotifers are harvested and used as feed or to inoculate a new culture. The operation is then repeated. The harvest densities exceed 100 ind./ml. Salinity is adjusted at 25 ppt, and heaters are used in winter to maintain water temperature at 26 - 28°C. Light and pH are not controlled.

For bigger ponds (more than 20 tons) semi-continuous techniques are adopted. At the beginning, microalgae is grown in a rotifer pond, then the rotifers are inoculated when microalgal cell density reaches 10^7 cells/ml (*N. oculata* or *Chlorella* sp.) or 10^5 cells/ml (*T. chui*). Baker's yeast is provided as supplemental feed. One-third to one-fifth of the rotifer culture is harvested and an equal volume of microalgal water is added daily or once every two to three days. The harvest density is usually 50 - 60 ind./ml. Each pond is used for one month or more.

The organic wastes used to feed rotifers are listed in Table 8 (columns III, IV, V, VI). Generally, naturally-occurring green water or brackish water is introduced into rotifer ponds. Chicken droppings, fish meat, powdered yeast, or prepared *Tilapia* feed are provided, as described in Table 8, to supply organic detritus, bacteria and microalgae to rotifers. Usually, rotifers are inoculated, but in some hatcheries no inoculations are used. After four to 15 days, rotifers are harvested, with densities below 50 ind./ml. Yields are hard to predict and usually vary. The operation, however, is easy. Natural plankton and artificial feeds are used to reduce the need for rotifers. Therefore, this production technique is practiced by most commercial hatcheries in Taiwan.

Problems and prospects

The critical problems encountered in intensive rotifer culture are the difficulty of maintaining a continuous supply of microalgae and low temperature during winter when heaters are not used. Microalgal shortages occurred often in summer due to high temperatures and protozoan contamination. Therefore, temperature-controlled culture systems for microalgae in summer and rotifers in winter must be designed. To decrease contamination, the water used for mass microalgal culture must be sterilized more thoroughly, for example, by UV light or ozone after filtration in series with 25-, 5-, 1-, and 0.45-μm filters. Inocula for outdoor cultures must also be restarted from the indoor culture once every one or two weeks.

In addition, *Nannochloropsis* sp. must be cultured in greenhouses where temperature is kept below 30°C. *Nannochloropsis* sp. is therefore used only for secondary enrichment of rotifers and maintenance of the microalgal density in larval rearing ponds. The selection of high temperature-tolerant species may help abate this problem. *Tetraselmis* sp. can be cultured as feed for rotifers because it is less sensitive to environmental stress. However, the rotifers must be secondarily enriched with *Nannochloropsis* sp. due to the low EPA content of *Tetraselmis* sp.

Furthermore, the labor-intensive work of inoculation, culturing, harvest, and washing must be decreased by adopting automated systems. To stabilize the water parameters and production of rotifers, a continuous culture system may be an alternative. It has more advantages than the batch culture system and can be automated, thereby greatly decreasing labor. However, the initial investment and operating costs are higher and more specialized skills are needed. The design of a prototype continuous mass culture system with a capacity of more than one ton must be undertaken to determine its technical and economic feasibility in Taiwan.

Taiwan has several indigenous microalgal species and rotifer strains. Likewise, several exotic species and strains have also been introduced. A one-stop culture collection and research center must, therefore, be established.

On the other hand, in extensive larval rearing systems, natural blooms of green algae and plankton are difficult to control and very unstable. Based on the commercial hatcheries' experience, a total of 10 ha of ponds are needed to supply sufficient microalgae for 0.5-ha fish larval ponds. If optimum conditions could be maintained, the pond area could be greatly reduced. Therefore, ways to manage the growout pond water to stabilize the supply of live feeds for fish larvae must be investigated in the future.

CONCLUSIONS

Most production systems for live feeds developed in Taiwan are extensive, and the operations simple. There are several reasons for these, namely:

- culture species often vary a great deal, thus, construction costs of specialized facilities for live feeds are expensive;
- manpower is limited. Likewise, specialists on live feeds production are few. The operation of extensive systems is simple and easy to handle. It is also available anytime. Furthermore, 20 ha can be handled by one person;
- most owners of commercial hatcheries have been practicing the growout of prawns, finfishes, and molluscs. They

are familiar with the traditional techniques, and thus, know how to enrich pond water with natural plankton;

- the weather in Taiwan is suitable for the diverse assemblage of plankton which occur naturally in ponds. Aquaculture ponds are distributed almost everywhere, thus, selected plankton are easily obtained;
- providing several species of plankton might be more beneficial to the larvae than some monospecific diets, such as green algae, rotifers, and *Artemia* nauplii. The first success in the mass production of milkfish larvae and the larval production of over 150 million for the past several years attests to this fact;
- mass production of larvae is easily established in some species like milkfish and sea breams (Table 1). Thus, using the intensive system would only add to overproduction.

However, there are some disadvantages. First, the extensive system is easily affected by natural phenomena such as typhoons and long periods of rain or low temperatures. Second, many finfish or prawn ponds are needed to provide green or brown water. Third, the volume of the larval pond must be large enough to stabilize water quality and temperature. Fourth, there are frequent disease outbreaks. There is a need, therefore, to refine the production systems of live feeds and larvae.

On the other hand, several successes obtained with indoor larval rearing trials for grouper in 1990 increased the estimated figures for the intensive production of live feeds for some hatcheries. Therefore, commercial-scale intensive culture systems are expected to be developed and adopted in the future. How to bring the success of laboratory studies to the commercial scale will be the main topic to be studied.

The automated and mechanized production of rotifers in Japan is intended to stabilize the rotifer supply and replace repetitious tasks. This system is expensive, and is desirable only where there is limited space for aquaculture and the weather is unfavorable for naturally-occurring plankton. Introducing this intensive system wholly into Taiwan might not be practical and economical. On the other hand, research efforts on formulated diets to replace live feeds for prawns, molluscs and finfish larvae are ongoing, and already, several successes have been reported for diets for some penaeid and finfish species. We hope a complete formulated diet will be developed so that only the simple and cheaper production systems for live feeds will need to be used.

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The Nutritional Improvement of Baker's Yeast for the Growth of the Rotifer, *Brachionus plicatilis*

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ABSTRACT

Baker's yeast is deficient in several nutrients essential to the growth of the rotifer (*Brachionus plicatilis* Müller). The bacterial flora present in a culture tank are an important food source for rotifers in mass cultures. The nutritional value of baker's yeast may be improved by rearing yeast in a medium rich in organic nutrients and incorporating essential lipids into the cells.

INTRODUCTION

Mass cultures of the rotifer, *Brachionus plicatilis*, sometimes suffer from sudden population decreases or suppressed growth, especially when baker's yeast is used as feed. This phenomenon makes larval production of economically valuable fish unstable. To avoid this obstacle, research should focus on the nutritional and environmental requirements of rotifers. In this review, we summarize research in these two areas.

NUTRITIONAL REQUIREMENTS OF ROTIFERS

Materials and Methods

The dietary value of baker's yeast and the supplementary effect of added nutrients on the population growth of rotifers was evaluated. First laid eggs of *B. plicatilis* were sterilized and cultured in basic (control) and experimental food suspensions under bacteria-free conditions. Rotifer population growth in the two

groups were compared. The rotifers used in the experiments were amictic females derived from the same strain (large-type) studied in a previous experiment (Hirayama and Funamoto 1983). During the experiments, temperature was 23°C and no mictic females or males were observed.

The growth indices used to determine dietary value were obtained by two culture methods; batch culture and individual culture. In the first instance, rotifers were batch cultured in the test food suspensions for several days. The increase in the number of rotifers was used as an index. In individual culture, about 20 eggs were placed into test tubes, two eggs/tube. Two indices were used to evaluate population growth — the intrinsic rate of population increase (r) and the net reproductive rate (R_o). These were calculated from the daily survival rates and fecundities obtained by daily observation. The test suspensions were continually renewed during the lifespan of each rotifer. These two culture methods are explained in more detail elsewhere (Hirayama 1985).

The test tubes were stirred with a Circle Shaker set at 130 rotations/min. for 15 minutes, six times per day to keep the yeast cells suspended throughout the investigation (Satuito and Hirayama 1990).

Results

The nutritional deficiency of baker's yeast and the supplementary effect of vitamin B_{12} were examined by Hirayama and Funamoto (1983). However, those results were reconfirmed with agitated cultures because the previous experiment neglected the problem of yeast settling to the bottom of culture vessels (prior experiments were conducted under static conditions). Table 1 shows the rotifer growth indices obtained with the two culture methods for suspensions with and without vitamin B_{12} supplementation. The indices confirmed previous findings that 1) baker's yeast has low nutritional value for rotifers and 2) supplementation of yeast with vitamin B_{12} improves *B. plicatilis* growth.

The fat soluble vitamin requirements of rotifers were determined by observing the effects of addition of these vitamins to a baker's yeast suspension enriched with vitamin B_{12} (Satuito and Hirayama 1986). In Figure 1, the supplementary effect of each

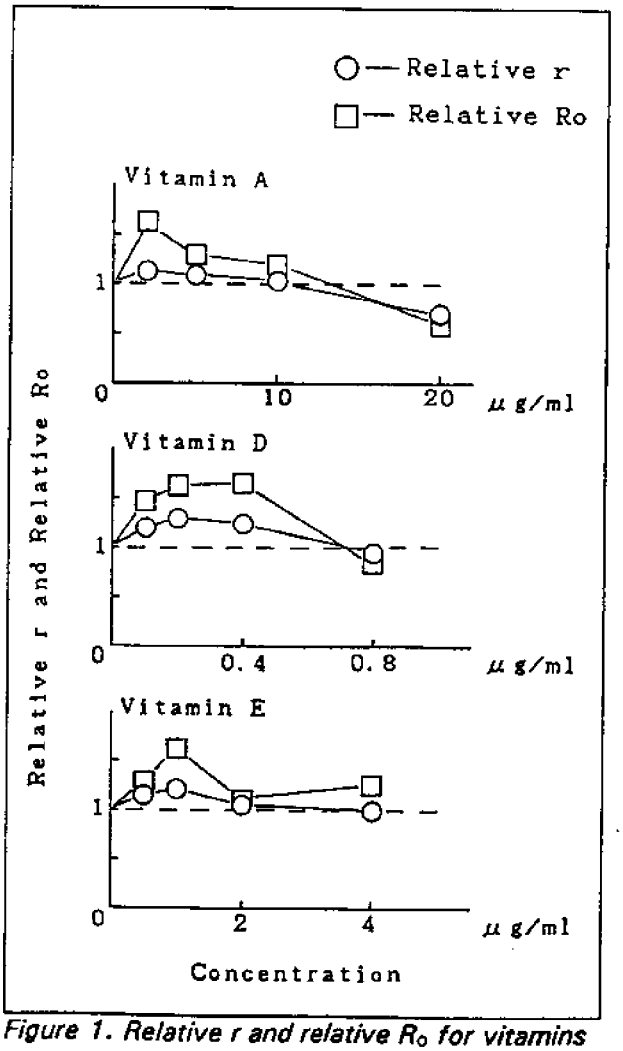

Figure 1. Relative r and relative R_o for vitamins A, D and E at different concentrations.

Table 1. Effect of vitamin B_{12} on the growth of the rotifer using individual and batch culture methods.

Nutrients added to basic food suspension* (μg/ml)	Population growth indices from individual culture				
	r	R_o			
None	0.23	1.93			
Vitamin B_{12} (1.4)	0.39	3.20			
	No. inoculated ind.	Average number after three days of batch culture**			
		Alive	Dead	Eggs	Total
None	20	19.0	6.5	9.0	33.0
Vitamin B_{12} (1.4)	20	24.5	4.0	15.5	44.0
		Average number after six days of batch culture**			
		Alive	Dead	Eggs	Total
None	20	22.0	5.5	5.5	33.0
Vitamin B_{12} (1.4)	20	35.0	10.0	9.5	54.5

*The basic food suspension was prepared by suspending 200 μg/ml of baker's yeast in 2/3 diluted seawater.
**Average values from two replicate experiments.

Table 2. Indices r and R_o obtained from individual culture method for squid liver oil and vitamins A, D and E.

Nutrients added to basic food suspension*	Concentration in μg/ml	Experiment 1	
		r	R_o
Squid liver oil	0	0.34	4.20
	2.0	0.34	4.72
	4.0	0.51	9.57
	8.0	0.44	6.86
		Experiment 2	
		r	R_o
None	—	0.46	5.65
Squid liver oil	4.0	0.56	10.84
Vitamins A, D and E	2.0, 0.2, 1.0	0.52	7.85

*The nutrients were added to the basic food suspension consisting of 200 μg/ml of baker's yeast and 1.4 μg/ml of vitamin B_{12}.

vitamin added is evaluated by a comparison of growth indices obtained from individuals cultured in the test suspensions with those obtained with control suspensions. These are expressed as relative indices obtained for each vitamin. Vitamins A, D and E each had a positive effect on growth. However, supplementation at high concentrations produced

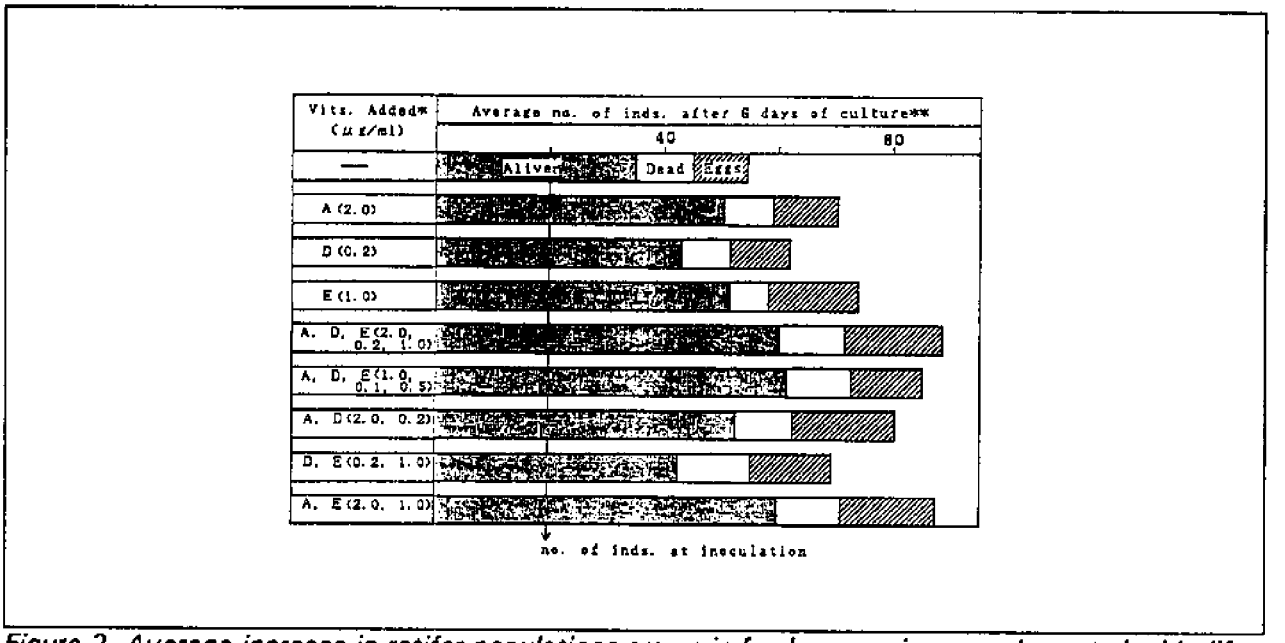

Figure 2. Average increase in rotifer populations grown in food suspensions supplemented with different combinations of vitamins using batch the culture method.

* *The vitamins added to the basic food suspension consisted of 200 μg/ml of baker's yeast and 1.4 μg/ml of vitamin B_{12}.*

** *Average values of two replicate experiments.*

a negative effect. Figure 2 shows the results from batch cultures. The separate and combined effects of the three vitamins added to a baker's yeast suspension were tested. Each vitamin enhanced the nutritional value of the baker's yeast suspension. Furthermore, addition of the three vitamins together was improved rotifer population growth.

Squid liver oil (Riken Vitamin Co., Ltd.) also improved rotifer growth when it was added to a baker's yeast suspension (Satuito and Hirayama 1991a). Table 2 shows that squid liver oil enhanced rotifer growth and resulted in higher population growth than cultures supplemented with fat soluble vitamins. In actual mass culture, squid liver oil is usually offered to increase the fatty acid content of rotifers which are to be used as feed for the larvae of marine species. Our results indicate that rotifers also incorporate squid liver oil, using it as an exogenous source of fatty acids for their own use.

Figures 3 and 4 show the effect of addition of vitamin C to the enriched yeast suspension (Satuito and Hirayama 1991a). Addition of vitamin C to the food suspensions promoted rotifer growth, implying that the vitamin is also required by the rotifer.

Baker's yeast itself has a low nutritional value for rotifers, since it is deficient in nutrients required by the rotifer for growth. The enhancing effect of tested nutrients on the growth of rotifers indicates that these nutrients are essential for rotifer growth.

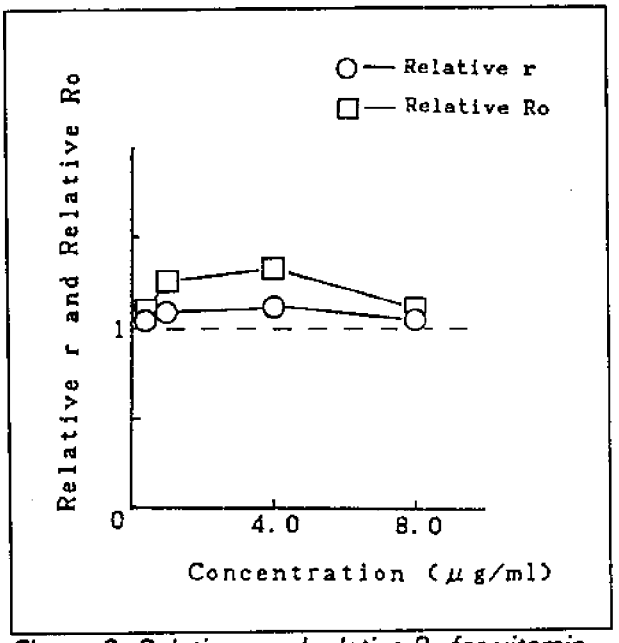

Figure 3. Relative r and relative R_o for vitamin C at different concentrations.

IMPROVING THE NUTRITIONAL VALUE OF YEAST

One way to improve the nutritional value of baker's yeast is to add required nutrients directly to yeast suspensions as solutes or in emulsified form. However, without modification this method may lead to unfavorable results. For instance, direct addition of a vitamin B_{12} solution to *B. plicatilis* culture tanks may stimulate growth of vitamin-consuming bacteria and result in the complete depletion of dissolved vitamin B_{12}. On the other hand, fat soluble nutrients added directly to culture water in emulsified form may lead to pollution of the culture water if they are not completely consumed. *Brachionus plicatilis* is known to display high selectivity for various foods (Chotiyaputta and Hirayama 1978, Funamoto and Hirayama 1982).

A better understanding of the different relationships existing in rotifer culture tanks should, therefore, be considered as another means of improving the nutritional value of baker's yeast.

Control of the Bacterial Flora

Hirayama (1987) suggested that vitamin B_{12}-producing bacteria propagating in rotifer mass culture tanks play an important role in supplying this vitamin to rotifers. Yu et al. (1988) provided direct evidence to support this theory. They isolated many bacterial strains

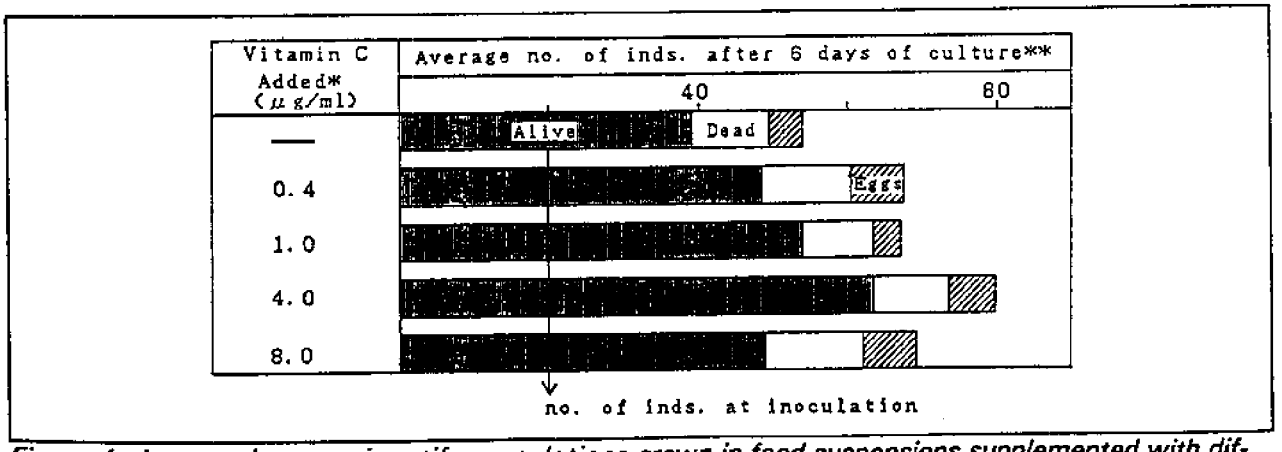

Figure 4. Average increase in rotifer populations grown in food suspensions supplemented with different concentrations of vitamin C using the batch culture method.

**Vitamin C was added to the basic food suspension, which consisted of 200 μg/ml of baker's yeast and of vitamins B_{12}, A, D and E at 1.4, 2.0, 0.2 and 1.0 μg/ml, respectively.*

Table 3. Preliminary testing of all isolated bacterial strains. Number of rotifers produced in a 5-ml batch culture after seven days (Yu et al. 1988).

Strains*	Number of rotifers	Vitamin B_{12} productivity	Strains*	Number of rotifers	Vitamin B_{12} productivity
A1	256	+	D7	40	-
A2	25	-	D8	166	+
A3	8	-	D9	22	-
A4	10	-	E1	141	+
A5	8	-	E2	23	-
B1	31	-	E3	63	-
B2	26	-	E4	24	+
B3	33	-	E5	9	-
B4	31	-	E6	27	-
B5	43	-	E7	23	-
B6	8	-	E8	28	-
C1	38	-	E9	35	-
C3	97	+	E10	35	-
C5	16	-	E11	30	-
C6	208	+	E12	14	-
D1	29	-	E13	40	-
D2	101	+	F1	15	-
D3	74	+	F2	11	-
D4	113	+	Control	26	-
D5	10	-			
D6	9	-			

Note: The initial number of rotifers was 10.
*Strains marked with the same capital letter were collected from the same tanks.

from *B. plicatilis* mass culture tanks. All the strains were tested for their effect on rotifers grown in "germ-free" batch cultures. The bacteria were added to a baker's yeast suspension which was fed to *B. plicatilis*. The results (Table 3) indicate that bacterial strains with the ability to produce vitamin B_{12} promoted rotifer growth, with the exception of one strain. Bacterial strains which did not produce vitamin B_{12} did not support rotifer growth.

Yu et al. (1989) calculated the balance of vitamin B_{12} in two rotifer mass culture tanks — one batch culture tank (Table 4) and one semi-continuous culture tank (Table 5). The total output of the vitamin was much higher than the total input. This suggests that the increase in vitamin B_{12} must have been caused by bacterial production. Further evidence may be found in Yu (1989). He cultured rotifers in 2 liters of sterilized seawater and fed them baker's yeast. Periodic addition of mass

Table 4. Balance of vitamin B_{12} in batch culture in Tamano (Yu et al. 1989).

Input	Vitamin B_{12} (μg)	Output	Vitamin B_{12} (μg)
Inoculated rotifers	2,486	Harvested rotifers	1,639
Suspended particles*	359	Suspended particles*	1,255
Water	217	Water	2,152
ω yeast	42		
Total	3,104		5,046

*Including *Nannochloropsis*.

Table 5. Balance of vitamin B_{12} in semi-continuous culture in Kanagawa (Yu et al. 1989).

Input	Vitamin B_{12} (μg)	Output	Vitamin B_{12} (μg)
Inoculated rotifers	13,960	Harvested rotifers	36,676
Suspended particles*	5,300	Suspended particles*	12,836
Water	1,200	Water	7,300
*Nannochloropsis**	4,833		
ω yeast	3		
Total	25,296		56,812

*Fluid and cells.

Table 6. Nitrogen budgets in rotifer culture tanks throughout the whole culture period (Ushiro et al. 1990).

	Tank number			
INPUT	E1	E2	E3	E4
Nannochloropsis (g)	2,528	1,196	1,806	3,160
Culture water (g)	5,577	2,638	3,985	6,971
ω yeast (g)	658	568	499	431
Rotifers (g)	134	105	147	*
Total (g)	8,897	4,507	6,437	10,562
OUTPUT				
Rotifers (g)	1,874	1,249	1,415	2,337
Culture water (g)	8,857	5,348	6,729	13,059
Deposits (g)	460	232	372	411
Total (g)	11,191	6,829	8,516	15,807
Food conversion rate (%)	58.8	70.8	61.4	65.1

*Not recorded

amounts of vitamin B_{12}-producing bacteria resulted in a very dense rotifer harvest, 5,000/ml.

Ushiro et al. (1990) conducted semi-continuous rotifer cultures in four outdoor tanks for 30 days and calculated food conversion rates ranging from 58.8 to 70.8% in the four tanks (Table 6). They pointed out that the rates they observed were much higher than those usually observed during the culture of animals. These studies indicate that the bacterial flora present in culture tanks play an important role as food for rotifers.

The above results suggest that regulation of the bacterial flora is important in mass culture of *B. plicatilis*, not only for the maintenance of water quality but also as a food source for the rotifers.

ARTIFICIAL CONTROL OF THE NUTRITIONAL VALUE OF YEAST

Methods for manipulating the fatty acid content of baker's yeast cells to meet the requirements of marine finfish larvae have long been established (Imada et al. 1979). So-called "ω yeast" is produced commercially and is made by culturing yeast in a medium containing fish oil or cuttlefish liver oil. Hence, the nutritional value of baker's yeast cells may be regulated by the type of yeast growth medium employed.

Satuito and Hirayama (1991b) attempted to regulate the amino acid contents of baker's yeast by rearing them in different types of growth media. Table 7 shows the composition of Mayer's medium and the amino acids added to the medium to prepare yeasts M1 and M3. Table 8 shows the composition of Wickerham's medium, used to prepare yeast W1. The amino acid content of the yeasts reared in each growth medium are shown in Table 9. Although addition of amino acids did not increase the concentration of specific amino acids, the type of growth medium was found to somehow regulate the total amino acid content of the yeast cells. Yeast reared in a medium that was rich in organic nutrients had a higher total amino acid content than the other prepared yeasts. Figures 5 and 6 show the nutritional value of these yeasts when fed to *B. plicatilis*. Yeast, which had a higher amino acid content, supported better growth than the other yeasts.

Several reports have documented that the fatty acid composition of baker's yeast cells depends on the fatty acid composition of the growth medium (Imada 1983, Dendrinos and Thorpe 1987). Table 10 shows the fatty acid

Table 7. Composition of 1/2 diluted Mayer's medium (basic medium) and amino acid enrichments added to yeast growth media M1 and M3.

Formula of basic medium (medium M1)	
Saccharose	75.0 g
K_3PO_4	2.5 g
$MgSO_4 \cdot 7H_20$	0.5 g
$Ca_2HPO_4 \cdot 2H_2O$	0.4 g
Ammonium tartarate	5.0 g
Distilled water	1 liter
pH	5.4
Amino acid added as enrichment to the basic medium (medium M3)	**mg/liter medium**
Phenylalanine	20
Leucine	20
Methionine	20
Valine	10
Lysine	10

Table 8. Composition of Wickerham's medium.*

Formula	Amount
Nitrogen sources	
Ammonium sulfate	5 g
Amino acids	
L-histidine hydrochloride	10 mg
LD-methionine	20 mg
LD-tryptophan	20 mg
Vitamins	
Biotin	2 μg
Calcium pantothenate	400 μg
Folic acid	2 μg
Inositol	2000 μg
Niacin	400 μg
p-Aminobenzoic acid, Difco	200 μg
Pyridoxine hydrochloride	400 μg
Riboflavin	200 μg
Thiamine hydrochloride	400 μg
Compounds supplying trace elements	
Boric acid	500 μg
Copper sulfate	40 μg
Potassium iodide	100 μg
Ferric chloride	200 μg
Manganese sulfate	400 μg
Sodium molybdate	200 μg
Zinc sulfate	400 μg
Salts	
Potassium phosphate, monobasic	1.0 g
Magnesium sulfate	0.5 g
Sodium chloride	0.1 g
Calcium chloride	0.1 g
Amount of final medium from 100 g dehydrated medium	14.9 L
Final pH ± 0.2 at 25°C	5.4

*Wickerham's medium is commercially available as Bacto yeast nitrogen base (Difco). 6.7 g yeast nitrogen base and 5 g of glucose was suspended in 1 liter of distilled water. This solution was autoclaved prior to use.

Table 9. Amino acid compostions of differently prepared yeasts.

Amino acids (%)	Yeasts		
	M1	M3	W1
Aspartic a.	2.4	2.6	4.1
Threonine	1.3	1.6	2.1
Serine	1.3	1.6	2.0
Glutamic a.	3.3	3.6	5.2
Proline	1.2	1.2	1.4
Glycine	1.1	1.3	2.0
Alanine	2.3	2.2	2.4
Cystine	0.6	0.4	0.6
Valine	1.4	1.5	2.7
Methionine	0.3	0.4	0.7
Isoleucine	1.2	1.4	2.2
Leucine	1.6	2.1	3.1
Tyrosine	1.0	1.1	1.6
Phenylalanine	0.8	1.2	2.1
Histidine	0.9	0.7	1.0
Lysine	2.1	2.3	3.6
Arginine	3.1	2.0	2.7
Tryptophan	not detected	not detected	not detected
Cytrulline	0.8	1.0	0.6
Ornithine	0.2	0.1	0.1
Ammonia	0.3	0.3	0.4
Total (%)	27.2	28.6	40.6

composition of yeasts prepared by several authors. Compared to the enriched yeasts prepared by other authors, Satuito and Hirayama (1991b) were able to incorporate only a small amount of ω3 HUFAs. However, the fatty acid enriched yeast they produced improved the growth of rotifers in the same way as when squid liver oil was directly added to the food suspension (Table 11).

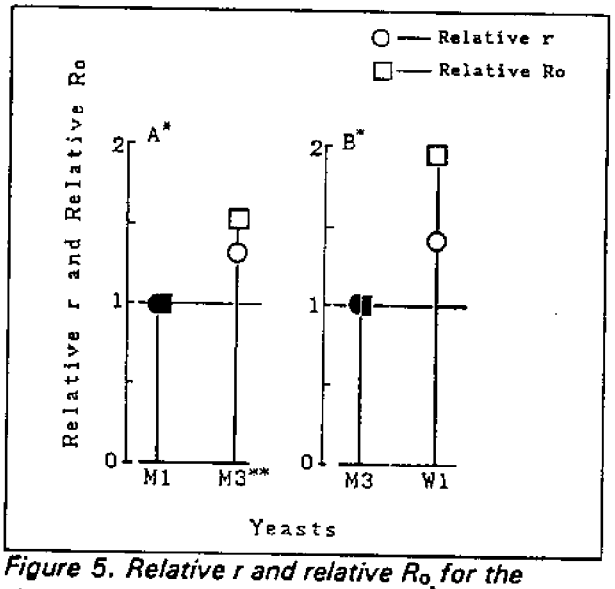

Figure 5. Relative r and relative R_o for the three differently prepared yeasts. *Values shown are average values from two experiments.*

**Relative values taken in comparison to indices obtained for yeast M3.*

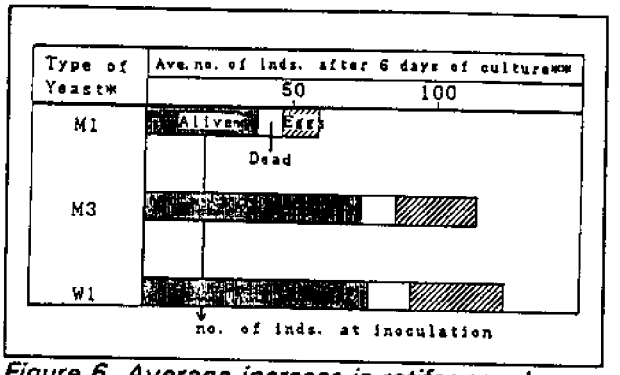

Figure 6. Average increase in rotifer populations cultured in the three differently prepared yeasts using the batch culture method.

Yeast was suspended at 200 µg/ml in the culture water containing vitamins B_{12}, C, A, D and E at 1.4, 4.0, 2.0, 0.2 and 1.0 µg/ml respectively.

**Average values of two replicate experiments.*

CONCLUSION

Baker's yeast is deficient in nutrients which are required by *B. plicatilis* for good growth. The nutritional value of baker's yeast can be improved by 1) regulating the bacterial flora which serve as food for the rotifers in the culture tank and 2) manipulating the nutritional value of yeast.

Table 10. Fatty acid composition of non-enriched and enriched baker's yeasts.

Fatty acid	Satuito and Hirayama (1991b)		Imada (1983)		Dendrinos and Thorpe (1987)	
	W1	EnW1	Yeast	ω yeast	ScL4	ScL5
14:0	1.7	5.4	0.3	4.1	3.4	2.1
14:1	0.3	0.2				
15:0	—	0.6				
16:0	10.6	19.1	8.3	13.4	27.4	21.4
16:1	40.8	25.5	38.2	6.6	24.7	26.4
17:0	0.2	0.5				
18:0	6.2	4.5	4.1	2.4	trace	1.4
18:1	37.9	31.5	45.9	16.4	9.3	5.2
18:2	—	0.4	2.8	1.1	1.7	5.3
18:3			0.5	0.8		
20:1	0.2	4.7	0.2	9.1	0.9	trace
20:2					1.5	2.1
18:4	0.2	—				
20:4ω3#			—	1.1		
20:3ω3#				3.0		
20:4ω6			—		2.9	1.6
20:5ω3#			—	17.7	7.7	4.7
22:1			—	2.1	trace	trace
22:5ω3#				1.0	3.2	4.7
22:6ω3#	—	0.4		12.8	2.5	3.1
24:1	1.0	0.5		1.3	2.9	2.9
Unknown	0.9	6.7				
HUFA $\sum$ ω3 (#)	—	0.4	—	35.6	13.4	12.5
Total	2.4	2.1	1.1	12.7	9.4	8.1

Table 11. Indices r and R_o obtained from non-enriched and enriched yeast suspensions by individual culture method.

Type of food suspension*			r	R_o
Type of yeast	Food density (μg/ml)	Squid liver oil (μg/ml)		
Non-enriched yeast (W1)	200	—	0.35	4.70
W1	200	4.0	0.48	7.57
Enriched yeast (EnW1)	200	—	0.45	6.71

*Food suspensions were supplemented with 1.4 μg/ml of vitamin B_{12}.

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The Selection of Optimum Phytoplankton Species for Rotifer Culture During Cold and Warm Seasons and Their Nutritional Value for Marine Finfish Larvae

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ABSTRACT

Chlorella is the most common type of microalgae used to feed rotifers cultured on a mass scale. *Chlorella*, however, requires a high light intensity and does not grow well at high and low temperatures. Therefore, the growth rates of 34 different species of phytoplankton were examined in a search for species more amenable to culture under these conditions. *Nannochloris oculata* and *Phaeodactylum tricornutum* were chosen to substitute for *Chlorella* in the warm and cold seasons, respectively. The growth of rotifers (*Brachionus plicatilis*) cultured with *N. oculata* was higher than when *Chlorella ellipsoidea* was used as feed. However, the nutritional value of *P. tricornutum* for rotifers appears to be inadequate. Finally, to determine the dietary value of *N. oculata*, the survival and growth rates of Japanese flounder larvae reared on *C. ellipsoidea*-fed rotifers were compared to those obtained for *N. oculata*-fed rotifers. Based on survival rate alone, *N. oculata* appears to be the better rotifer feed. However, the effect of *N. oculata*-fed rotifers on larval growth rate should be reinvestigated.

INTRODUCTION

A series of experiments were performed on potential feed organisms for rotifers (*Brachionus plicatilis*) which were being mass cultured for marine finfish fry. Phytoplankton and yeast (Hirayama and Watanabe 1973, Hirata and Mori 1967, Kitajima et al. 1979) are the primary feeds for mass cultured rotifers. *Chlorella** is the most common type of phytoplankton used to feed rotifers (Fukusho et al. 1976, Hirayama and Nakumura 1976, Fontaine and Revera 1980), but *Tetraselmis tetrathele*, (Okauchi and Fukusho 1984, Fukusho et al. 1985) which is eurythermal and euryhaline, is also widely used. In addition to these algae, *Tetraselmis suecica* (Laing and Helm 1981, Trotta 1983), *Nannochloris* sp. (Witt et al. 1981, Ben-Amotz and Fishler 1982), *Dunaliella tertiolecta*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, etc. (Scott and Baynes 1978), have also been used to mass culture rotifers.

*Recently identified as *Nannochloropsis* in Japan

Chlorella ellipsoidea requires a high light intensity and does not grow well on a large scale at high and low temperatures (above ca. 28°C and below ca. 10°C) such as those experienced during the summer and winter in Korea. It is particularly difficult to produce rotifers in warm, cloudy regions in the summer (e.g., Jeju island and the southern coast where the main culture grounds for marine finfish in Korea are located). Hence, heating and cooling facilities and strong halogen lamps must be used in the summer and winter. These are too costly for fish farmers.

Consequently, this study was carried out to find substitutes for *Chlorella* that would grow well during the seasons in which it is difficult to culture *Chlorella*. The growth rates of 34 different species of phytoplankton cultured in 100-ml flasks at low and high temperatures were compared. Suitable species were then selected and the growth rates of rotifers fed the test species and those fed *Chlorella ellipsoidea* (the control group) were compared. Fatty acid compositions of the selected species of phytoplankton and the rotifers were also examined. Finally, the test and control rotifers were fed to Japanese flounder (*Paralichthys olivaceus*) larvae in a comparison of their dietary values.

COMPARISON OF GROWTH RATES OF PHYTOPLANKTON

To select more optimal microalgae, that is, species which grow well in the summer and/or winter, the growth rates of 34 isolates (14 diatoms, 12 Chlorophytes, 8 other species) were analyzed. The culture medium was F/2 for the 32 marine species and Complesal (commercial liquid fertilizer for plants) for the two freshwater species. Cultures were conducted in 100-ml flasks at 5 and 10°C and at 25 and 30°C to simulate winter and summer temperatures, respectively. Salinity was 33 ppt for the marine species and 0 ppt for freshwater species. Light intensities were 2,500 and 5,000 lux and light was provided continuously.

In the high-temperauure experiments, the growth rates of *Nannochloris oculata*, *Heterosigma* sp., *Palmella mucosa* and *Microcystis aeruginosa* were better than that of *Chlorella ellipsoidea* (Table 1), which is widely used for rotifer culture in Korea. From the viewpoint of light intensity and temperature, *Nannochloris oculata* and *Palmella mucosa*, which showed better growth at low light intensities, seemed to be suitable substitutes for *Chlorella* during the summer.

The growth of the tested microalgae was generally poor at temperatures below 10°C. However, two diatoms, *Phaeodactylum tricornutum* and *Skeletonema costatum*, showed better growth rates than *C. ellipsoidea*. Furthermore, *Dunaliella tertiolecta* grew better than *C. ellipsoidea* at 5°C. Comparing these diatoms, *P. tricornutum* appears to be better than *S. costatum* because of its high growth rate and the fact that *S. costatum* is a long-chained diatom.

OPTIMUM ENVIRONMENT FOR *N. OCULATA* AND *P. MUCOSA*

The optimum conditions (temperature, salinity and light) for culturing *N. oculata* and *P. mucosa* were investigated using *C. ellipsoidea* as a control.

Tables 2, 3 and 4 show the growth of the three species at different temperatures (27, 28, 30 and 32°C) and salinities (27, 30, 33 and 36 ppt) with continuous light of 2,500 and 5,000

Table 1. Specific growth rates† of algae cultured at low and high temperatures (Marine strain: 33 ppt, LD 24:0, F/2 medium; Freshwater strain: 0 ppt, LD 24:0, Complesal medium).

	Low temperature (°C)				High temperature (°C)			
	5		10		25		30	
Light intensity (lux)	5,000	2,500	5,000	2,500	5,000	2,500	5,000	2,500
Caloneis schroderi	-0.01	-0.50	-0.10	0.25	0.28	0.30	0.38	0.29
Chaetoceros gracilis	-0.01	-0.05	0.16	0.07	0.52	0.39	0.62	0.73
Chaetoceros simplex	-0.06	-0.01	0.27	0.55	0.47	0.52	0.54	0.63
Cyclotella sp.[1]	0.16	0.08	0.00	-0.10	0.00	0.00	-0.18	-0.19
Cyclotella sp.[2]	-0.06	-0.20	0.12	0.16	0.57	0.58	0.42	0.26
Hanzchia marina	-0.36	-0.07	-0.68	-0.21	0.21	0.15	0.11	0.06
Navicula incerta	0.16	0.20	0.37	0.22	0.39	0.37	0.48	0.35
Navicula sp.	0.08	0.02	0.31	-0.01	0.29	0.26	0.30	0.43
Nitzschia sp.	-0.10	0.12	0.20	0.23	0.23	0.29	0.46	0.40
Phaeodactylum tricornutum[3]	0.37	0.39	0.71	0.63	0.85	0.84	-0.53	-0.53
Phaeodactylum tricornutum[4]	0.22	0.26	0.66	0.51	-0.03	-0.43	-0.45	-0.46
Skeletonema costatum	0.29	0.34	0.66	0.61	0.60	0.67	0.48	0.43
Thalassiosira fluviatilis	0.03	0.06	0.10	0.11	-0.03	0.10	0.28	0.23
Thalassiosira sp.	-0.29	-0.17	-0.04	-0.12	0.13	0.15	0.40	0.41
Boekelovia sp.	0.04	0.01	0.18	0.27	0.67	0.62	0.01	0.27
Isochrysis galbana	-0.57	-0.07	0.42	0.25	0.55	0.55	0.52	0.51
Isochrysis aff. *galbana*	-0.62	-0.67	0.06	-0.14	0.78	0.81	0.76	0.74
Nannochloris oculata	0.01	-0.03	0.01	0.04	0.85	0.92	1.09	1.14
Nannochloropsis salina	0.06	0.07	0.04	0.54	-0.32	-0.34	0.46	0.06
Chlorella ellipsoidea	0.03	0.02	0.58	0.53	0.88	0.85	0.98	0.94
Chlorella stigmatophora	0.01	0.24	0.36	0.24	0.78	0.56	0.66	0.39
*Chlorella vulgaris**	-0.25	0.00	0.21	0.17	0.68	0.67	-0.29	-0.20
Dunaliella terriolecta	0.28	0.17	0.43	0.42	0.60	0.64	0.60	0.45
Eudorina elegans	-0.24	-0.23	0.15	0.13	0.28	0.35	0.05	0.05
Gloeocystis sp.	-0.20	0.01	0.14	0.25	-0.19	0.09	0.03	0.20
Heterosigma sp.	0.02	0.00	0.43	0.38	0.92	0.78	1.02	0.60
Oocystis pusilla	0.14	0.14	0.21	0.19	0.16	0.15	0.17	0.27
Palmella mucosa	-0.01	0.02	0.76	0.57	0.93	1.10	0.93	1.05
Scenedesmus sp.*	-0.18	-0.45	-0.26	-0.36	-0.25	-0.18	-0.16	-0.13
Tetraselmis suecica	0.04	-0.02	0.42	0.28	0.46	0.44	0.55	0.51
Platymonas subcordiformis	0.04	0.11	0.38	0.39	0.30	0.30	0.23	0.28
Microcystis aeruginosa	0.08	0.04	0.10	0.03	0.94	0.87	0.91	0.59
Protogonyaulax sp.	-0.03	0.07	0.37	0.39	0.74	0.57	0.70	0.78
Euglena sp.	-0.24	-0.61	0.51	0.38	0.65	0.51	0.52	0.55

† $k\ (\text{divisions/day}) = 3.322 \dfrac{\log \frac{N_1}{N_0}}{t_2 - t_1}$ (Guillard 1973)

*Freshwater strain, [1]NFUP-9, [2]NFUP-13, [3]NFUP-2, [4]NFUP-10

Table 2. Cell number and specific growth rate (s.g.r.) of *Chlorella ellipsoidea* under high temperatures with different conditions of salinity and light intensity (LD: 24:0)(cell numbers reported in units of 10^4 cells/ml).

Temperature (°C)	Salinity (ppt)	27		30		33		36	
	Light intensity (lux)	5,000	2,500	5,000	2,500	5,000	2,500	5,000	2,500
26	Initial cell no.	13	15	13	14	11	14	14	13
	Maximum cell no.	2,641	2,080	2,686	1,893	2,218	1,771	2,256	1,338
	s.g.r.	1.09	1.01	1.09	1.01	1.09	0.99	1.04	0.95
28	Initial cell no.	13	10	11	9	7	8	8	7
	Maximum cell no.	2,706	1.725	2,877	2,459	2,663	2,334	2,326	1,368
	s.g.r.	1.10	1.06	1.14	1.15	1.22	1.17	1.16	1.08
30	Initial cell no.	11	10	9	9	10	11	10	8
	Maximum cell no.	2,366	1,556	2,360	1,305	2,234	1,575	2,374	1,418
	s.g.r.	1.10	1.04	1.14	1.02	1.11	1.02	1.12	1.06
32	Initial cell no.	9	9	9	9	8	11	8	10
	Maximum cell no.	61	935	59	911	182	1,305	113	1,112
	s.g.r.	0.39	0.95	0.38	0.95	0.64	0.98	0.54	0.97

Table 3. Cell number and specific growth rate (s.g.r.) of *Nannochloris oculata* under high temperatures with different conditions of salinity and light intensity (LD: 24:0)(cell numbers reported in units of 10^4 cells/ml).

Temperature (°C)	Salinity (ppt)	27		30		33		36	
	Light intensity (lux)	5,000	2,500	5,000	2,500	5,000	2,500	5,000	2,500
26	Initial cell no.	14	10	12	13	10	11	11	13
	Maximum cell no.	2,173	1,975	2,245	1,559	2,471	1,684	2,559	1,553
	s.g.r.	1.04	1.08	1.07	0.98	1.13	1.03	1.12	0.98
28	Initial cell no.	12	14	9	11	11	9	14	9
	Maximum cell no.	2,599	2,435	2,550	2,945	3,176	2,214	2,912	1,957
	s.g.r.	1.10	1.06	1.16	1.15	1.16	1.13	1.10	1.10
30	Initial cell no.	7	10	8	9	9	9	9	9
	Maximum cell no.	2,057	1,654	2,373	1,872	2,898	1,638	2,562	1,299
	s.g.r.	1.17	1.05	1.17	1.10	1.19	1.07	1.16	1.02
32	Initial cell no.	6	6	6	6	5	9	5	5
	Maximum cell no.	1,397	884	1,760	718	1,084	1,042	697	496
	s.g.r.	1.12	1.02	1.17	0.98	1.10	0.97	1.01	0.94

Table 4. Cell number and specific growth rate (s.g.r.) of *Palmella mucosa* under high temperatures with different conditions of salinity and light intensity (LD: 24:0)(cell numbers reported in units of 10^4 cells/ml).

Light intensity (lux)	Culture days								s.g.r. for 7 days
	0	1	2	3	4	5	6	7	
8,000	9	11	41	134	399	800	1,384	2,082	1.12
6,000	10	11	37	154	469	971	1,811	2,607	1.14
5,000	8	11	37	150	520	1,026	1,965	2,917	1.21
4,000	11	12	42	155	502	1,129	1,571	2,366	1.10
2,000	10	11	18	31	65	126	266	527	0.81

Table 5. Cell number and specific growth rate (s.g.r.) of *Nannochloris oculata* under different light intensities (28.5 °C, 33 ppt, LD: 24:0)(cell numbers reported in units of 10^4 cells/ml).

Temperature (°C)	Salinity (ppt)	27		30		33		36	
	Light intensity (lux)	5,000	2,500	5,000	2,500	5,000	2,500	5,000	2,500
26	Initial cell no.	8	9	9	9	10	9	11	9
	Maximum cell no.	1,148	811	1,414	1,037	1,058	760	961	567
	s.g.r.	1.02	0.92	1.04	0.97	0.96	0.91	0.92	0.85
28	Initial cell no.	10	7	6	7	10	8	8	7
	Maximum cell no.	2,482	1,223	2,837	1,306	2,596	821	2,012	756
	s.g.r.	1.13	1.06	1.26	1.07	1.14	0.95	1.13	0.96
30	Initial cell no.	1	9	12	9	13	12	12	10
	Maximum cell no.	1,570	1,470	2,258	1.529	2,400	909	1,977	700
	s.g.r.	1.02	1.05	1.07	1.05	1.07	0.89	1.05	0.87
32	Initial cell no.	9	9	10	9	10	10	9	10
	Maximum cell no.	1,014	1,026	1,291	1,203	1,549	1,272	1,433	1,336
	s.g.r.	0.97	0.97	1.00	1.00	1.03	0.99	1.04	1.00

lux. The growth of *C. ellipsoidea* increased with increasing temperature up to 28°C, but growth decreased sharply beginning at 30°C. The growth of *N. oculata* and *P. mucosa* also dropped at 30°C, however the decline was less severe. Salinity had no observed effect on growth. The specific growth rate and maximum culture density of *N. oculata* were higher than those of *P. mucosa*. Consequently, *N. oculata* was chosen as a substitute for *Chlorella* in the warm season.

To discover the optimum light intensity for *N. oculata*, I cultured this species with constant illumination of 2,000, 4,000, 5,000, 6,000 and 8,000 lux. Best growth was obtained at 5,000 lux (Table 5). Hence, I con-

Table 6. Cell number and specific growth rate (s.g.r.) of *Chlorella ellipsoidea* and *Phaeodactylum tricornutum* under high temperatures with different conditions of salinity and light intensity (LD: 24:0)(cell numbers reported in units of 10^4 cells/ml).

Temp. (°C)	Species	*C. ellipsoidea*		*P. tricornutum*							
	Salinity (ppt)	33		27		30		33		36	
	Light intensity (lux)	5,000	2,500	5,000	2,500	5,000	2,500	5,000	2,500	5,000	2,500
4	Initial cell no.	9	8	8	9	9	10	10	10	9	10
	Maximum cell no.	7	6	51	38	44	39	43	34	36	31
	s.g.r.	-0.05	-0.05	0.38	0.29	0.32	0.28	0.30	0.25	0.28	0.23
6	Initial cell no.	10	9	10	11	9	10	11	9	10	11
	Maximum cell no.	73	42	344	207	372	223	369	211	342	216
	s.g.r.	0.40	0.31	0.72	0.60	0.76	0.64	0.72	0.65	0.72	0.61
8	Initial cell no.	11	10	10	9	10	9	9	10	10	11
	Maximum cell no.	90	55	704	389	738	411	682	348	638	337
	s.g.r.	0.43	0.35	0.87	0.77	0.88	0.78	0.89	0.73	0.85	0.70
10	Initial cell no.	11	10	8	8	8	8	8	8	8	8
	Maximum cell no.	221	158	889	490	955	544	828	477	764	443
	s.g.r.	0.61	0.56	0.97	0.84	0.98	0.86	0.95	0.84	0.93	0.82

cluded that the optimum growth conditions for *N. oculata* are 28°C, 33 ppt and 5,000 lux of continuous illumination.

OPTIMUM ENVIRONMENT FOR *P. TRICORNUTUM*

In a previous experiment, the diatom *P. tricornutum* appeared to be a suitable species to culture for rotifers in the winter. Its growth at a variety of temperatures (4, 6, 8 and 10°C), salinities (27, 30, 33 and 36 ppt) and light intensities (2,500 and 5,000 lux) was tested in 100-ml flasks to determine the optimum culture parameters. *C. ellipsoidea* was again used as the control.

The best growth was obtained at the highest temperature, 10°C, and at 30 ppt and 5,000 lux. *P. tricornutum* had consistently higher growth rates than *C. ellipsoidea* (Table 6). A wider range of light intensities was also tested (Table 7); better growth occurred at higher intensities. Hence, during the winter when it is 10°C, the optimum culture parameters for *P. tricornutum* are 33 ppt and 8,000 lux of continuous light.

GROWTH RATE OF *N. OCULATA* AND *P. TRICORNUTUM* IN MASS CULTURE

Large-scale cultures of some species of microalgae yield different growth rates than laboratory-scale cultures of the same species. This may be due to differences in illumination or because aeration is usually provided outdoors. Therefore, a mass culture experiment with *N. oculata* and *C. ellipsoidea* at a high

Table 7. Cell number and specific growth rate (s.g.r.) of *Phaeodactylum tricornutum* under different light intensities (10°C, 33 ppt, LD: 24:0)(cell numbers reported in units of 10^4 cells/ml).

Light intensity (lux)	Culture days								s.g.r. for 7 days
	0	1	2	3	4	5	6	7	
8,000	9	12	59	227	588	950	1,139	1,182	1.01
6,000	8	11	27	40	124	305	498	879	0.96
5,000	9	9	19	36	79	251	400	781	0.92
4,000	9	9	19	33	71	184	307	566	0.85
2,000	9	9	14	25	50	113	198	330	0.74

Table 8. Mass culture data for *Chlorella ellipsoidea* and *Nannochloris oculata* at 25°C, 4,000 lux and LD: 24:0 (cell numbers reported in units of 10^4 cells/ml).

Species	Culture days											s.g.r. for 10 days	Salinity	Container size (liters)
	0	1	2	3	4	5	6	7	8	9	10			
C. ellipsoidea	218	249	357	692	1,032	1,308	1,716	2,026	2,194	2,249	2,206	0.33	33	100
N. oculata	189	224	408	811	1,609	2,481	3,022	3,864	4,305	4,762	4,808	0.46	33	100

Table 9. Mass culture data for *Chlorella ellipsoidea* and *Phaeodactylum tricornutum* at 9°C, 3,000 lux and LD: 24:0 (cell numbers reported in units of 10^4 cells/ml).

Species	Culture days											s.g.r. for 10 days	Salinity	Container size (liters)
	0	1	2	3	4	5	6	7	8	9	10			
C. ellipsoidea	212	220	241	278	339	438	568	687	803	940	1,091	0.23	33	20
P. tricornutum	184	209	288	381	475	610	752	876	1,087	1,188	1,294	0.28	30	20

temperature was conducted in a 100-liter FRP vessel and another experiment was conducted with *P. tricornutum* and *C. ellipsoidea* at a low temperature in a 20-liter vinyl bag. Results conformed to those found at the laboratory scale in the absence of aeration. The growth of *N. oculata* in warm conditions and *P. tricornutum* in cold conditions was superior to that of *C. ellipsoidea* cultured at the same temperatures (Tables 8 and 9).

GROWTH OF ROTIFERS FED *N. OCULATA* AND *P. TRICORNUTUM*

Rotifers (*B. plicatilis*) cultured with *N. oculata* and *P. tricornutum* were compared to those fed *C. ellipsoidea*. *Nannochloris oculata*-fed rotifers had a higher final yield and a higher growth rate than *C. ellipsoidea*-fed rotifers. On the ninth day of culture, the former had a density of 240 ind./ml whereas the latter was only 171 ind./ml (Fig. 1). Growth of rotifers fed *P. tricornutum*, on the other hand, was inferior to the growth of those given *C. ellipsoidea*. Although the growth rate of *P. tricornutum* was higher at low temperatures, its nutritional value for rotifers appears to be inadequate (Fig. 2).

FATTY ACID COMPOSITION

The fatty acid composition of selected phytoplankton species and rotifers fed on those species were examined (Table 10). *Nannochloris oculata* was found to have the highest linolenic acid (18:3ω–3, ω–6) content, but *P. tricornutum* had the highest percentage of eicosapentaenoic acid (20:5ω–3). The fatty acid compositions of the rotifers were similar to that of their feed.

SURVIVAL AND GROWTH RATE OF JAPANESE FLOUNDER LARVAE

To determine the dietary value of *N. oculata*, the survival and growth rates of Japanese flounder (*Paralichthys olivaceus*) larvae reared on *C. ellipsoidea*-fed rotifers (Group I) were compared to those obtained for *N. oculata*-fed rotifers (Group II). Larval culture was conducted in 100-liter FRP vessels. In the first experiment (Table 11), survival 20 days after hatching was 8.3% for Group I and 13.5% for Group II. In the second experiment, 10,000 fertilized eggs were reared in 500-liter FRP containers for ten days with only rotifers and for twenty days with rotifers mixed with *Artemia* nauplii (San Francisco Bay strain). The *N. oculata*-fed rotifers yielded higher larval survival rates (Table 12) but lower growth rates. Finally, when the larvae were

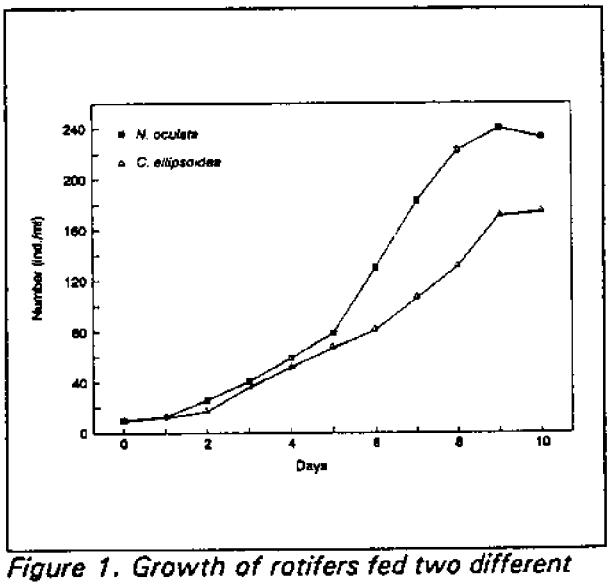

Figure 1. Growth of rotifers fed two different algal diets for 10 days in 5-liter bags (temperature: 23 - 25°C, light intensity: 2,000 lux).

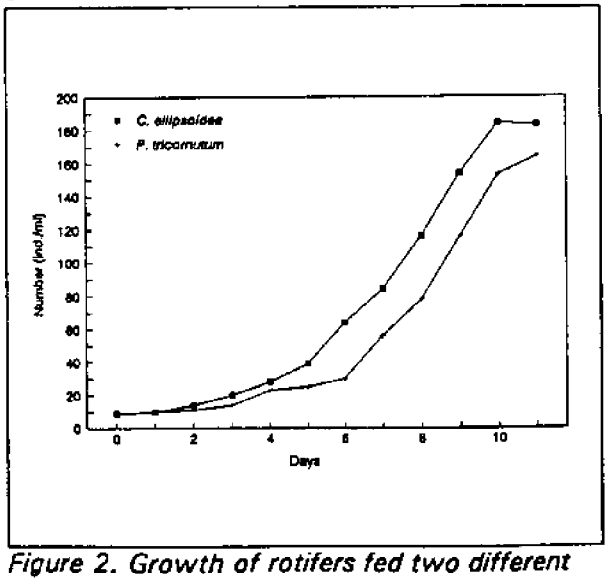

Figure 2. Growth of rotifers fed two different algal diets for 11 days in 5-liter bags (temperature: 21 - 23°C, light intensity: 2,000 lux).

Table 10. Fatty acid composition for the three kinds of phytoplankton feed species and rotifers fed these species (percent).

Fatty acid	*Chlorella ellipsoidea*	*Nannochloris oculata*	*Phaeodactylum tricornutum*	Rotifers fed		
				C. ellipsoidea	*N. oculata*	*P. tricornutum*
12:0	0.4	0.2	0.1	0.3	0.2	0.2
14:0	5.7	1.0	5.3	5.1	2.0	4.5
15:0	0.7	0.2	0.5	1.0	1.4	0.6
16:0	29.9	22.7	16.9	24.3	17.6	18.9
17:0	0.8	7.3	1.6	4.1	3.8	0.6
18:0	1.5	1.3	2.3	15.5	3.7	5.0
20:0	0.2	—	0.7	—	—	0.4
22:0	—	0.2	0.5	0.7	3.4	1.8
saturated	39.2	32.9	27.9	51.0	32.1	32.0
16:1ω-7	29.2	5.4	32.2	19.4	3.4	25.3
18:1ω-7, ω-9	11.0	13.0	8.6	5.6	7.9	9.4
20:1ω-9, ω-11	0.4	0.4	0.9	1.0	1.8	0.6
monoene	40.6	18.8	41.7	26.0	13.1	35.3
18:2ω-6	3.9	24.0	3.6	3.8	25.8	7.4
18:3ω-3, ω-6	0.2	22.7	0.7	1.1	23.0	5.9
18:4	0.1	0.1	0.5	4.0	2.0	1.4
20:4ω-3	2.7	0.7	0.7	0.3	2.8	2.2
20:5ω-3	13.3	0.8	24.9	13.8	1.2	15.8
polyene	20.2	48.3	30.4	23.0	54.8	32.7
Total	100.0	100.0	100.0	100.0	100.0	100.0

given *N. oculata*- or *C. ellipsoidea*-fed rotifers for twenty days after hatching followed by *Artemia* nauplii for ten days in 100-liter FRP vessels, survival was again higher for the *N. oculata*-fed rotifer group (94% vs. 82%) but the growth rate was lower (Table 13). The effect of rotifer diet on the survival and growth rates of Japanese flounder larvae was found to be significant (*t*-test, $p = 0.01$).

An investigation of *N. oculata*'s docosapentaenoic acid (22:6ω-3) content may help explain the discrepancy between survival and growth rates of the larvae raised on *N. oculata*-fed rotifers. *Nannochloris oculata*'s small size and its digestibility are other factors which should be considered.

In conclusion, based on survival rate alone, *N. oculata* appears to be the better rotifer feed. However, the effect of *N. oculata*-fed rotifers on larval growth rate should be investigated further.

Table 11. Growth and survival of Japanese flounder larvae (*Paralichthys olivaceus*) fed on rotifers cultured with *Chlorella ellipsoidea* and *Nannochloris oculata*.

Phytoplankton		Stocking density (no. of inds.)	Total body length (mm ± SD)			Final survival 20 days after hatching (No. of inds.)
			Initial (No. 30)	10 days after hatching (No. 30)	20 days after hatching (No. 30)	
I	1	1,000	2.44 ± 0.131	5.63 ± 0.481	9.93 ± 0.378	84
	2	1,000	2.44 ± 0.131	5.46 ± 0.553	9.90 ± 0.468	82
	mean	1,000	2.44 ± 0.131	5.55 ± 0.528	9.91 ± 0.425	83
II	1	1,000	2.44 ± 0.131	4.97 ± 0.504	9.56 ± 0.552	137
	2	1,000	2.44 ± 0.131	4.81 ± 0.409	9.49 ± 0.769	133
	mean	1,000	2.44 ± 0.131	4.89 ± 0.465	9.53 ± 0.671	135

I: Rotifers cultured with *Chlorella ellipsoidea*
II: Rotifers cultured with *Nannochloris oculata*

Table 12. Growth and survival of Japanese flounder larvae (*Paralichthys olivaceus*) fed on rotifers cultured with *Chlorella ellipsoidea* and *Nannochloris oculata*.

Phyto-plankton	Stocking density (no. of eggs)	Total body length (mm ± SD)		Total body weight (g ± SD)		Final survival 30 days after hatching (No. of inds.)
		15 days after hatching (No. 30)	30 days after hatching (No. 30)	15 days after hatching (No. 30)	30 days after hatching (No. 30)	
I	10,000	9.14 ± 1.104	22.44 ± 0.847	0.015 ± 0.004	0.122 ± 0.021	409
II	10,000	7.75 ± 0.957	17.72 ± 0.854	0.009 ± 0.003	0.067 ± 0.023	726

I: Rotifers cultured with *Chlorella ellipsoidea*
II: Rotifers cultured with *Nannochloris oculata*

Table 13. Growth and survival of Japanese flounder larvae (*Paralichthys olivaceus*) fed *Artemia* (San Francisco Bay strain) nauplii.

Larvae group		Stocking density (no. of inds.)	Total body length (mm ± SD)		Total body weight (g ± SD)		Final survival (No. of inds.)
			Initial (20 days after hatching)	Final (30 days after hatching	Initial	Final	
I	1	100	9.91 ± 0.425	20.88 ± 3.227	0.028 ± 0.005	0.106 ± 0.038	84
	2	100	9.32 ± 0.857	19.16 ± 2.062	0.022 ± 0.004	0.087 ± 0.022	79
	mean	100	9.62 ± 0.653	20.02 ± 2.840	0.025 ± 0.005	0.097 ± 0.033	82
II	1	100	9.53 ± 0.671	18.96 ± 3.642	0.025 ± 0.004	0.091 ± 0.043	93
	2	100	9.53 ± 0.425	15.93 ± 1.960	0.023 ± 0.003	0.056 ± 0.020	94
	mean	100	9.53 ± 0.531	17.44 ± 3.294	0.024 ± 0.004	0.074 ± 0.038	94

I: Larvae fed rotifers cultured with *Chlorella ellipsoidea* for 20 days prior to the experiment.

II: Larvae fed rotifers cultured with *Nannochloris oculata* for 20 days prior to the experiment.

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An Overview of Live Feeds Production System Design In Thailand

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ABSTRACT

In Thailand, the culture of *Chaetoceros calcitrans*, *Skeletonema costatum* and rotifers is commonly practiced in commercial hatcheries. Starter cultures of microalgae are provided by government institutes free of charge to encourage development of the industry. To minimize production costs and labor, it is not necessary for small- and medium-scale hatcheries to maintain algae laboratories. Methods of mass culture have also been simplified so that the small-scale farmers can apply them. Culture practices for a variety of important feeds species are described.

INTRODUCTION

Thailand's principal coastal aquaculture products are penaeid shrimp, finfish and molluscs. The development of shrimp farming, for example, has increased tremendously, from 16,000 MT in 1986 to 90,000 MT in 1989 (Kongkeo 1990). Thailand is ranked second among shrimp producing countries of the world, following the People's Republic of China, with a total area of 75,000 ha under cultivation.

Ninety percent of the cultured shrimp is *Penaeus monodon* reared in semi-intensive, intensive and super-intensive systems. The remaining 10% are *P. merguiensis* and *Metapenaeus ensis* which are reared extensively. Success in the rapid development of *P. monodon* farming in Thailand is based on the expansion of small backyard hatcheries that were originally built for larval production of giant freshwater prawns, *Macrobrachium rosenbergii*.

These low investment and simple technology hatcheries are managed completely by the local farmers and comprise 90% of the almost 2,000 hatcheries in the country. The average production of each hatchery is one million PL15 per month. These backyard hatcheries mass culture their own microalgae, employing starters supplied by government institutes (such as the Department of Fisheries, universities and marine research institutes) and large-scale private hatcheries. Government brackishwater and marine fisheries stations throughout the country have microalgae lab-

oratories to provide starters to private hatchery operators. The list of stocks provided free of charge by the Department of Fisheries is shown in Table 1.

Seabass, *Lates calcarifer*, is the second most successful cultured species. It is commercially grown in both cages and earthen ponds, with an annual production of approximately 1,200 MT. The larvae are produced in large private hatcheries and moved to small nursery ponds for fingerling production. Rotifers, *Brachionus plicatilis* (S-type), are commonly used for larval feeding to reduce *Artemia* costs. The same concept also applies to the production of penaeid shrimp and *Macrobrachium* larvae. Approximate annual production of the latter is nearly 12,000 MT (New 1990).

The following live feeds production systems are those generally used by commercial hatcheries. Experimental or research-scale systems have not been included.

Table 1. Stocks available free of charge from the Department of Fisheries in Thailand.

Species	Source
Chlorella sp.	Thailand, Japan, Australia
Chaetoceros calcitrans	Thailand and Japan
Chaetoceros ceratosporum	Japan
Chaetoceros simplex	Japan
Chaetoceros sp.	Thailand
Chlamydomonas sp.	Thailand
Dunaliella tertiolecta	Thailand
Skeletonema costatum	Thailand, Japan and Taiwan
Tetraselmis sp.	Thailand
Tetraselmis suecica	Hawaii
Tetraselmis chuii	Philippines
Isochrysis galbana	Thailand
Isochrysis sp.	Hawaii
Thalassiosira sp.	Thailand
Thalassiosira pseudonana	Hawaii
Brachionus sp.	Thailand
Microcyclops sp.	Thailand
Schizopera subterranea	Thailand
Diaphanosoma sp.	Thailand
Cyclotella nana	Thailand

MICROALGAE

In Thailand many isolation methods are commonly used to obtain single species from natural plankton and from contaminated cultures. Nutrient media used in the pure culture of microalgae include Sato and Serikawa, Conwy, Modified F, and TMRL media (details of preparation are shown in Table 2) depending on the species cultured. Absolute care must be taken to ensure that seawater, glassware, air tubes, cotton plugs, etc., are clean and sterilized in autoclaves prior to use (Table 3). Room temperature is maintained between 20 and 25°C by air conditioning, and four, 32-watt fluorescent lights are provided for each m^2 of white shelving. Culture vessels (flasks or test tubes) are placed 10 - 20 cm away from the lamps to avoid overheating and to ensure optimum light intensity.

The stock for isolation may be obtained either by towing a plankton net through the surface of a body of seawater, or from contaminated cultures. Water obtained by towing is filtered first through a 250-μm plankton net to remove large zooplankton, such as copepods and jellyfish larvae. Then the water, enriched with one of the media listed in Table

Table 2. Culture media used for laboratory pure culture.

1. SATO AND SERIKAWA (1968)		
a) Solution A		g
$NaNO_3$	10.0	g
$Na_2HPO_4 \cdot 12H_20$	1.0	g
$NaHCO_3$	16.8	g
$Na_2SiO_3 \cdot 9H_20$	0.4	g
Distilled water	900	ml
Sterilized at 15 lbs/inch^2 for 15 minutes		
b) Solution B		
Na_2 EDTA	3.0	g
$CuSO_4 \cdot 5H_2O$	0.0004	g
$CoCl_2 \cdot 6H_20$	0.0008	g
$MnCl_2 \cdot 4H_20$	0.27	g
$FeCl_3 \cdot 6H_2O$	0.24	g
$ZnCl_2$	0.03	g
H_3BO_3	3.44	g
Distilled water	1,000	ml
Sterilized at 15 lbs/inch^2 for 15 minutes		
-Utilization: 9 ml of solution A and 1 ml of solution B/liter of seawater		
-For cultivation of: *Chaetoceros*, *Skeletonema*, *Tetraselmis* and *Chlorella*		

2. CONWY MEDIUM (Walne 1974)		
$NaNO_3$	100.0	g
Na2EDTA	45.0	g
H_3BO_3	33.6	g
$NaH_2PO_4 \cdot 2H_20$	20.0	g
$FeCl_3 \cdot 6H_2O$	1.30	g
$MnCl_2 \cdot 4H_20$	0.36	g
Trace Metal Solution*	1	ml
Vitamin Mix**	100	ml
Distilled water (to make)	1,000	ml
*Trace Metal Solution		
$ZnCl_2$	2.1	g
$CoCl_2 \cdot 6H_20$	2.1	g
$(NH_4)_6 Mo_7O_{24} \cdot 4H_20$	2.1	g
$CuSO_4 \cdot 5H_2O$	2.0	g
Distilled water	100	ml
(acidify with 1N HCl until solution is clear)		
**Vitamin Mix		
Vitamin B_1	20	mg
Vitamin B_{12}	10	mg
Distilled water	200	ml
-Utilization: 1 ml Conwy medium/liter of seawater		
-For cultivation of: *Chlorella*, *Tetraselmis* and *Isochrysis*		

2, is cultured in 5-liter cylindrical glass containers, with aeration. After seven to ten days, plankton are ready for isolation. In Thailand the methods of isolation are generally as follows:

Isolation of Microalgae

Step 1

Plankton water is diluted to several concentrations with sterile seawater. A drop from each concentration is placed on separate agar plates (details of preparation are in Table 4), and spread over the surface with a glass rod.

Table 2. Continued.

3. MODIFIED F MEDIUM (Guillard & Ryther 1962)

a)	N-P Stock (500x)			*TM Primary Stock A		
	$NaNO_3$	42.07	g	$CuSO4 \cdot 5H_2O$	1.96	g
	$NaH_2PO_4 \cdot H_2O$	5.0	g	$ZnSO_4 \cdot 7H_2O$	4.4	g
	Distilled water	1,000	ml	Distilled water	100	ml
b)	Sodium Metasilicate Stock (500x)			**TM Primary Stock B		
	$Na_2SiO_3 \cdot 9H_2O$	15.0	g	$Na_2MoO_4 \cdot 2H_2O$	1.26	g
	Distilled water	1,000	ml	$(NH_4)_6 Mo_7O_{24} \cdot 2H_2O$	0.9	g
c)	Ferric Chloride Stock (500x)			Distilled water	100	ml
	$FeCl_3 \cdot 6H_2O$	1.45	g	***TM Primary Stock C		
	Distilled water	1,000	ml	$MnCl_2 \cdot 4H_2O$	36.0	g
d)	EDTA Stock (1,000x)			Distilled water	100	ml
	Na_2 EDTA	10.0	g	****TM Primary Stock D		
	Distilled water	1,000	ml	$CoCl_2 \cdot 6H_2O$	2.0	g
e)	Vitamin Stock			Distilled water	100	ml
	B_1	0.2	g			
	B_{12} primary stock (0.1 g/liter)	10	ml			
	Biotin primary stock (0.1 g/liter)	10	ml			
	Distilled water (make to)	1,000	ml			
f)	Trace Metal Stock (1,000x)					
	TM primary stock A*	1	ml			
	TM primary stock B**	1	ml			
	TM primary stock C***	1	ml			
	TM primary stock D****	1	ml			
	Distilled water (to make)	1,000	ml			

-Utilization: 2 ml each of solutions a, b, and c plus 1 ml each of solutions d and e per liter of seawater

-For cultivation of: *Skeletonema*, *Chaetoceros* and *Isochrysis*

Then the agar plates are incubated (upside down) at 20 - 25°C, 2 ft. beneath fluorescent lamps. After seven days, colonies of microalgae and bacteria appear on the surface of each plate. A monospecific colony of the desired species is selected with the aid of a 10X microscope.

Another common method is to isolate a single cell of the desired species from a drop of plankton water on a microscope slide. A specially designed capillary pipette and a microscope are used. Selected cells are placed on a second slide with a few drops of sterile seawater for cleaning purposes. Approx-

Table 2. Continued.

4. TMRL Enrichment (Liao & Huang 1970)		
KNO_3	100.0	g
$Na_2HPO_4 \cdot 12H_2O$	10.0	g
$FeCl_3 \cdot 6H_2O$	3.0	g
$Na_2SiO_3 \cdot 9H_2O$	1.0	g
Distilled water	1,000	ml
-Utilization: 1 ml TMRL/liter of seawater		
-For cultivation of: *Skeletonema* and *Chaetoceros*		

imately 50 cells of isolated algae are used to inoculate a 5-ml test tube in Step 2. This method can be difficult due to unsteadiness of the hand, but practice can improve one's skill.

Step 2

Selected microalgae from Step 1 are inoculated in a 5- or 20-ml test tube filled with nutrient medium and incubated at 20 - 25°C for seven days. Then a loopful of culture is streaked onto the surface of an agar plate. If the culture is too concentrated, it should be diluted with sterile seawater before streaking. The plate is then incubated at 20 - 25°C (upside down) for seven days. The whole process is repeated several times until a pure strain is obtained. Repeated subcultures on agar plates alone for a long period of time causes cell death due to the long exposure to air.

Pure Culture of Microalgae in Algae Room

One loopful of microalgae from the best colony obtained above is inoculated in a sterile 20-ml test tube with enriched medium. The tube is stoppered with cotton and kept on a lighted shelf in a 20 - 25°C algae laboratory for three to four days. The test tube is agitated twice a day. Then the culture is expanded into 250- and 500-ml flasks, respectively, using the same procedure.

After three days, 200 ml of algae from the 500-ml flask is used to inoculate a 1-liter flask filled with 800 ml of enriched seawater. Then it is incubated for two to three days. Aeration is provided beginning at this stage. Aeration is not provided earlier because it is very important to keep the early stages completely free of contamination. At every step, the cultures are examined under a microscope for contamination. The 1-liter flask is used as the starter for outdoor mass culture. Also, at least 20% of the 500-ml flask is set aside to prepare a duplicate batch in case of emergency.

Following the isolation process described above, the best colony is also selected for inoculation on an agar slant (Table 4) and incubated at 20 - 25°C for three to four days under fluorescent lamps. This culture can then be kept in a refrigerator for several months as pure stock to be used for further expansion in the laboratory.

It should be noted that the incubation period for *Skeletonema costatum* may be shorter than for other algae. When population growth peaks, however, it is necessary to expand immediately to avoid rapid collapse.

Algae for Bivalve Culture

Although there are many species of molluscs cultured commercially in Thailand, i.e., cockle (*Anadara granosa*), giant oyster (*Crassostrea belcherli*), green mussel (*Mytilus smaragdimus*) and horse mussel (*Modiola senhonsenil*), larval production is unnecessary due to an adequate supply of wild spat. The research-scale oyster hatcheries run by gov-

Table 3. Methods of disinfection.

1.	All glassware such as pipettes, test tubes, petri dishes, flasks, bottles, etc., used in the laboratory must be sterilized in an oven at 180°C for two hours.
2.	Air tubes and cotton plugs must be sterilized in an oven at 80°C for three hours.
3.	There are many methods used for disinfection of seawater including the following: ■ 23-ppt seawater in small vessels (such as flasks stoppered with cotton plugs and aluminum foil) is sterilized at 15 lbs/inch^2 for 15 minutes (final salinity will be 25 ppt). ■ 23-ppt seawater in larger vessels (such as 800 - 1,000-cc bottles) is disinfected in an oven at 90°C for two hours (final salinity is 25 ppt). ■ Large volumes of seawater (1 - 20 liters) are prepared by application of 20 ppm calcium hypochloride with heavy aeration for two to three days. Farmers usually smell the water to check for residual chlorine instead of using a potassium iodide test as the government and large-scale hatcheries do. ■ For outdoor culture, seawater is filtered through anthracite and sand before final filtration through a 30-μm net. In areas which are always affected by luminescent bacteria, seawater is usually treated with 1 ppm iodine with heavy aeration for three hours. ■ In the case of small-scale backyard hatcheries which are usually located far away from the sea, hypersaline (150 - 200 ppt) water from salt farm evaporation ponds is used. The water is free of contaminants and is diluted with disinfected fresh water to 25 - 30 ppt for use in larval rearing and diatom culture. Fresh water is treated with 20 ppm calcium hypochloride with heavy aeration for two to three days before use.

ernment institutes, though, use primarily *Isochrysis galbana* for larval feeding.

Algae for Penaeid Shrimp Culture

The popular phytoplankton species found to be suitable as feed for the early stages of penaeid shrimp, particularly *Penaeus monodon*, are *Chaetoceros calcitrans, Skeletonema costatum* and *Tetraselmis* sp. These tiny oceanic plants require certain environmental conditions for growth. In the wild, phytoplankton abundance is affected by fluctuations in temperature, daylight, the presence of grazers, the availability of nutrients, water depth, and turbidity as well as the seasons of the year.

The commercial shrimp hatcheries in Thailand prefer to use *C. calcitrans* rather than *S. costatum* because its nutrient value and digestibility are better for shrimp. Some of the advantages and disadvantages of producing *C. calcitrans* and *S. costatum* are listed in Table 5. The latter, which can generally tolerate lower light intensities and shorter photoperiods better than *C. calcitrans*, is commonly used only during the rainy season. The local strain of *S. costatum* always causes high mortality of shrimp larvae due to its poor digestibility, so most of the *S. costatum* used in Thailand is imported from Japan or Taiwan.

Tetraselmis sp. is a mobile organism which is not suitable for larvae during the early larval stage (zoea) but can replace *C. calci-*

Table 4. Preparation of an agar plate and slant.

1.	Six grams of Bacto agar is dissolved in 300 ml of warm 25-ppt seawater and poured into a 500-ml flask. It is sterilized in an autoclave at 15 lbs/inch^2 for 15 minutes. When the agar cools (about 45 - 50°C), 0.3 ml of Sato's medium is added and well mixed. Twenty ml of mixed agar is then poured into a sterile petri dish using the aseptic technique. After the agar becomes solid, it is inverted to avoid condensation on the surface of the agar. After incubation at room temperature for one day, the plate is checked for contamination. The plate can then be used or stored in a refrigerator.
2.	To prepare an agar slant, 10 ml of warm enriched agar prepared as above is pipetted into a 20-ml sterile test tube using the aseptic technique. The test tube is then stoppered with a sterile cotton plug. The tube is leaned until the agar solidifies. After one day, it is checked for contamination.

Table 5. Comparison of *S. costatum* and *C. calcitrans* in shrimp larval rearing.

		Skeletonema	*Chaetoceros*
1.	Larval survival	60%	70%
2.	Nutritional value	good	best
3.	Digestibility	poor (long-chained, thick cell wall)	good (mainly single-celled, thin cell wall)
4.	Availability of natural stock	throughout the year	some seasons, low quantity
5.	Contamination	little	easy
6.	Mixed culture technique	possible	nearly impossible
7.	Optimum temperature	26 - 28°C	28 - 30°C
8.	Minimum temperature	20°C	24°C
9.	Optimum salinity	27 - 30 ppt	22 - 28 ppt
10.	Light intensity requirement	< 10,000 lux	> 10,000 lux
11.	Peak of growth	within 6 - 10 hours	within 12 - 24 hours
12.	Harvesting	easy (by filtration)	difficult (only transfer with culture water)
13.	Cell degradation	within 3 - 4 generations	after more than 10 generations

Table 6. Fertilizers for mass culturing algae.

1. *Chaetoceros calcitrans*		
KNO_3	300.0	g
$Na_2HPO_4 \cdot 12H_2O$	30.0	g
$FeCl_3 \cdot 6H_2O$	5.0	g
$Na_2SiO_3 \cdot 9H_2O$	1.5	g
EDTA (if available)	20.0 - 40.0	g
Seawater	1.0	ton
2. *Skeletonema costatum* and *Chaetoceros calcitrans* (Modified TMRL medium)		
Urea	200	g
$Na_2HPO_4 \cdot 12H_2O$	20	g
$FeCl_3 \cdot 6H_2O$	6	g
$Na_2SiO_3 \cdot 9H_2O$	4	g
Seawater	1	ton
3. *Tetraselmis* sp.		
3.1 Use the same formula as modified TMRL medium without $Na_2SiO_3 \cdot 9H_2O$.		
3.2 KNO_3	100 - 200	g
NaH_2PO_4	10 - 20	g
Seawater (22 ppt)	1	ton
4. *Chlorella* sp.		
4.1 Use the same formula as modified TMRL medium without $Na_2SiO_3 \cdot 9H2O$.		
4.2 Ammonium sulfate	100 - 200	g
Super phosphate	10 - 20	g
Urea	10 - 20	g
Seawater	1	ton

trans during the mysis stage in the rainy season.

Chaetoceros calcitrans

Outdoor mass culture

Three 1-liter flasks of the starter culture from the algae laboratory are transferred to a closed transparent plastic bag containing 20 liters of fertilized seawater (Table 6). The stocking density is approximately 5,000 - 10,000 cells/ml. Each bag is provided with aeration and incubated for two to three days until the density peaks at approximately 50,000 cells/ml. Then three plastic bags containing a total of 60 liters are used to inoculate a 500-liter transparent fiberglass tank, filled with 350 liters of fertilized seawater.

After one to two days, the culture is ready for further expansion into a 1-ton transparent fiberglass tank, and then a 10-ton concrete tank. Each has an incubation period of one to two days. Unless shaded with a transparent roof, the tanks need to be partially covered with roofing tiles in the afternoon to moderate the light intensity, reduce temperature and shield the culture from rain. The temperature in Thailand is usually 20 - 35°C throughout the year, which is optimal for microalgae culture.

For larval feeding, culture water is transferred directly to larval rearing tanks by siphoning or with submersible pumps. The water is filtered through a 40-μm plankton net to remove clumped cells and debris. Twenty percent of the culture is left as an inoculum for a second subculture in a new tank (1-ton fiberglass or 10-ton concrete tank). Each batch of *C. calcitrans* will be repeatedly subcultured for two to four weeks until contamination occurs. It is then replenished using a new starter provided from government institutes or large-scale private hatcheries.

During the rainy season, when *C. calcitrans* cultures usually collapse, artificial

plankton is supplemented or *S. costatum* or *Tetraselmis* sp. is substituted (see Table 7).

Skeletonema costatum

Skeletonema costatum is a chain-forming diatom in which the cell size ranges from 15 - 25 μm in length and 10 - 15 μm in width. The size always gradually decreases under culture conditions. After three to four generations, the size is reduced to 7 - 15 μm in length and 7 - 10 μm in width, therefore it is necessary to replenish the culture regularly with new, fresh stock every week. This is another reason this species is not widely used in Thailand.

Because of the low density of *S. costatum* in Thai waters, the method using direct fertilization of seawater (generally practiced in other countries like Singapore, Philippines, Vietnam, etc.), is impossible. Only the use of monospecific starters from government institutes and large-scale hatcheries is feasible for outdoor mass culture at small-scale hatcheries.

Outdoor Mass Culture

The method used for outdoor mass culture of *S. costatum* is similar to that for *C. calcitrans*, except *S. costatum*'s incubation period is shorter. Within six hours after constant exposure to strong sunlight, density will peak at 50,000 cells/ml at which time the culture is ready for further subculture and harvesting. On a rainy day, ten to twelve hours may be necessary. Any delay in harvesting or transfer will result in over-blooming and subsequent culture collapse within four to six hours. *Skeletonema costatum* culture collapses are characterized by clumps of plasmolyzed cells, turbidity and a milky coloring of the culture water. The fertilizer employed in mass culture is primarily modified TMRL medium.

Unlike other unicellular algae in which the culture water must be directly transferred to shrimp larval tanks, the chain-forming *S. costatum* can be harvested with a 40-μm net. This minimizes the risk of larval mortality from polluted algae culture water. For harvesting, the culture water is siphoned through a bag net tied to the discharge end of a siphon hose. Once the net clogs, siphoning is stopped and the algae are transferred to the larval tank.

Ten percent of the algae in the tank, particularly that from the top layer, is used for subculturing. The quality of algae is poor at the tank bottom due to the clumps of dead cells, debris and other contaminants.

Tetraselmis sp.

The outdoor mass culture of *Tetraselmis* sp. (using the fertilizer shown in Table 6) is similar to *S. costatum*, but requires a longer incubation period (one to two days).

ROTIFERS (*Brachionus plicatilis*)

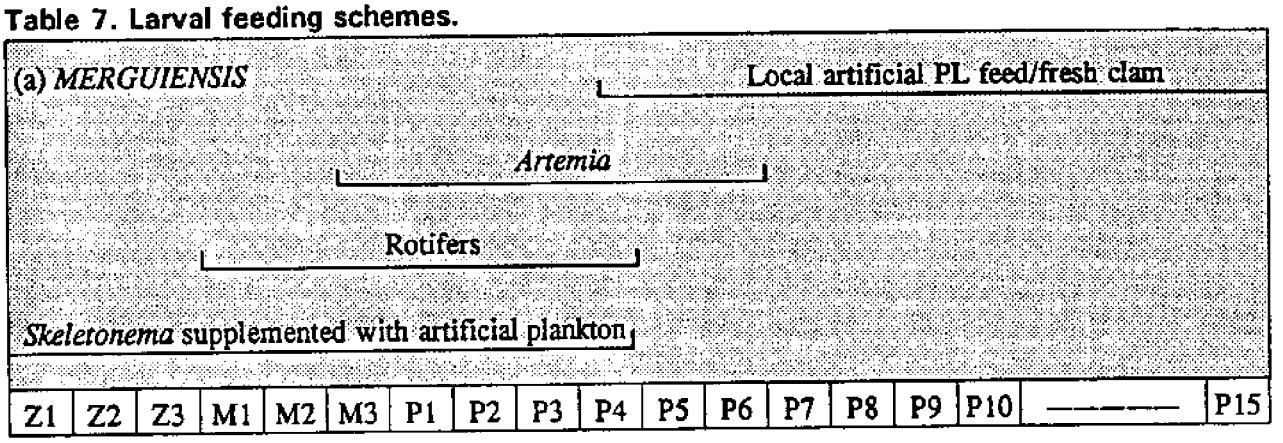
The advantages of using rotifers as larval feed are as follows: they

- tolerate alkaline, acid, hard, soft and polluted water conditions,
- live in both fresh- and seawater,
- survive in every depth of water such as bottom, middle and surface water,
- supplement expensive *Artemia* nauplii and artificial plankton,
- minimize the use of phytoplankton, particularly *C. calcitrans* which always collapses during the rainy season,

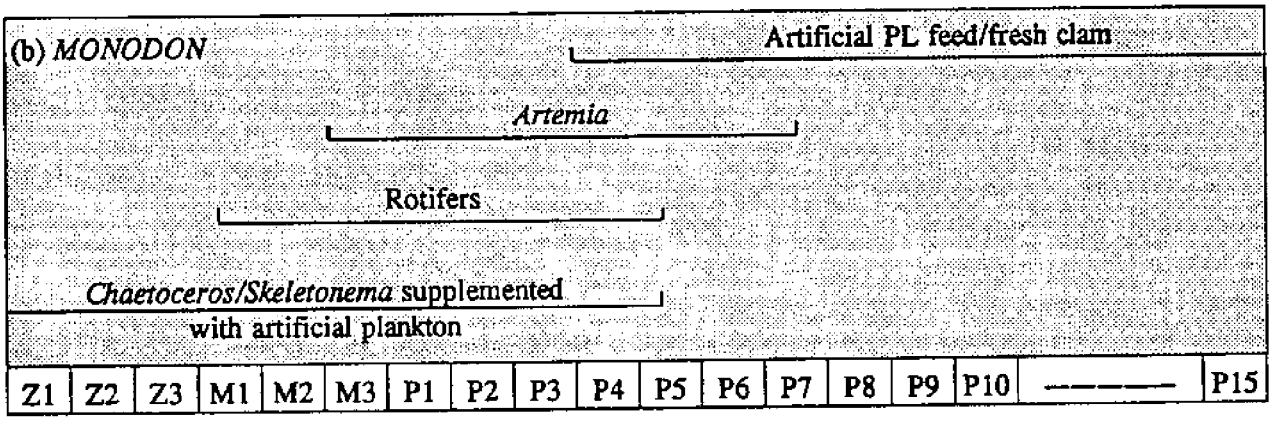
and

- the cost of the fertilizers used in rotifer culture is cheaper than those used for *C. calcitrans* and *S. costatum*.

Table 7. Larval feeding schemes.



AMOUNT OF DAILY FEED			
Feed	Zoea	Mysis	Postlarvae
Skeletonema	50,000/ml	50,000/ml	40,000/ml
Rotifers	—	200/larva	200/larva
Artemia nauplii	—	20/larva	50 - 100/larva
Artificial PL feed	—		20 - 50 g/100,000
or fresh clam	—		50 - 200 g/100,000



AMOUNT OF DAILY FEED			
Feed	Zoea	Mysis	Postlarvae
Skeletonema	40,000/ml	50,000/ml	50,000/ml
or *Chaetoceros*	50,000/ml	60,000/ml	50,000/ml
Rotifers		100/larva	100/larva
Artemia nauplii	—	40/larva	100 - 200/larva
Artificial PL feed	—	—	20 - 50 g/100,000
or fresh clam	—	—	50 - 200 g/100,000

Because they are smaller than *Artemia* nauplii, rotifers can be used as a supplementary feed for penaeid Mysis I up to PL4. The feeding rate is 100 - 200 ind./larva/day. For seabass larvae, the concentration of rotifers in the tank is maintained at 10 - 20 ind./ml.

Although there are many advantages to using rotifers as both shrimp and finfish feed, the major drawback of rotifer culture is that it is a very time consuming process. It is necessary to initiate rotifer culture at least two weeks in advance of the hatching of shrimp or fish larvae, but usually the spawning of fish cannot be predicted that accurately.

The feeds used for rotifer culture in Thailand are mainly *Nannochloropsis oculata* (usually referred to as "*Chlorella* sp." in Thailand), *Tetraselmis* sp., baker's or marine yeast and *Spirulina* powder. Although mass production of the above phytoplankton is less complicated than *C. calcitrans* or *S. costatum* production, it is labor intensive. Therefore, a combination of phytoplankton and dry feed (yeast or dried algae) is usually used in commercial operations.

Nannochloropsis oculata starter may be collected from freshwater or brackishwater ponds. The nutrient medium is shown in Table 6. If *N. oculata* is unavailable, *Tetraselmis* sp. stock from government institutes or large-scale hatcheries can be used.

Preparation of Live Feed

To begin with, 20 liters of the phytoplankton stock (*N. oculata* or *Tetraselmis* sp.) is added to a 1-ton transparent fiberglass tank with 15-ppt enriched seawater. After three days, the culture is expanded to a 10-ton concrete tank for another three days (salinity 22 ppt). Every two to three days, fertilizer is added to prolong the stationary phase of growth. Ten percent of the culture is then added to another 10-ton concrete tank and used to mass culture rotifers.

Preparation of the Rotifer Starter

Rotifers collected from the sea or brackish water or from pure laboratory cultures are initially grown in 1-liter flasks using a stocking density of 10 - 30 ind./ml and a salinity of 22 ppt. *Nannochloropsis oculata*, 1 - 2 x 10^6 cells/ml, or *Tetraselmis* sp., 2 - 4 x 10^4 cells/ml, are fed daily. This indoor culture is maintained at 25 - 28°C. After the rotifer density reaches 100 - 200 ind./ml, the culture is expanded to 20-liter containers and then 1-ton fiberglass tanks. These are then used as starters for outdoor mass culture in larger tanks.

Mass Culture of Rotifers

When the density of *N. oculata* or *Tetraselmis* sp. in the 10-ton outdoor tanks is optimal, rotifers are inoculated at 10 - 50 ind./ml. Baker's yeast (1 g/million rotifers /day) or marine yeast (1 - 5 x 10^6 cells/ml/day) is supplemented, beginning on the third day. When the density reaches 100 - 200 rotifers/ml (within five to seven days), 25% of the culture is utilized to start another tank. The remaining 75% is harvested for larval feed, but rotifer quality is better if they are given phytoplankton twelve hours prior to harvesting. Harvesting is accomplished by siphoning the culture water through a 60-μm net.

The optimal temperature for rotifer growth is 27°C, and although strong light intensity and outdoor conditions are not required by rotifers, these will promote phytoplankton growth in the tank.

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Research and Production of Live Feeds in China

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ABSTRACT

Live feeds production is the key to success or failure of the larviculture of marine animals. This paper is a summary of the research, culture and application of live feeds production to the aquaculture industry in China.

INTRODUCTION

Feed is one of the keys to aquatic animal culture. Its value will directly affect survival and development of larvae. Because live feeds have the advantages of 1) not contaminating the culture water, 2) easy digestion and assimilation, 3) promoting high growth rates and 4) having a higher nutritional value than other feeds, their culture is the principal means of providing food for larvae. Furthermore, as mariculture has progressed, the culture of marine finfish, crustacean and mollusc larvae and their feeds have expanded and become more important. Research into the development and culture of food organisms must be expanded rapidly to satisfy the growing demands of marine larviculture.

The artificial culture of live feeds in China did not begin until the second half of this century. Experiments on the large-scale culture of unicellular algae as feed were launched in the late 1950s (Hua 1959). Then, with the development of economically valuable marine finfish, crustacean and mollusc larviculture, mass production experiments for various feed species were conducted in coastal provinces. In recent years, great progress has been made and much experience has been acquired. This paper is a systematic summary of live feeds research in China and its application to the larviculture of marine animals.

MICROALGAE PRODUCTION

General Research Conditions

Research into the isolation and culture of marine unicellular algae in China was begun

Table 1. Unicellular algal species isolated and cultured for feed in China.

Planktonic species					Benthic Species
Chrysophyta	Xanthophyta	Cryptophyta	Bacillariophyta	Chlorophyta	Bacillariophyta
-*Dicrateria zhanjiangensis* -*Isochrysis galbana*	-*Heterogloea* sp.	-*Cryptomonas* sp. -*Rhodomonas* sp.	-*Phaeodactylum tricornutum* -*Nitzschia closterium* -*Skeletonema costatum* -*Chaetoceros muelleri* -*Chaetoceros simplex* -*Chaetoceros* sp. -*Cyclotella* sp. -*Amphiprora* sp.	-*Tetraselmis subcordiformis* -*Tetraselmis helgolandica* var. *tsingtaoensis* -*Dunaliella* spp. -*Chlamydomonas* sp. -*Carteria* sp. -*Chlorella* sp. -*Nannochloris oculata*	-*Navicula latissima* -Navicula spp. -*Amphora* sp. -*Achnanthes orientalis* -*Cocconeis* sp.

by Chu (1942, 1949). Subsequently, a few species of green algae were isolated and cultured by Guo et al. (1959), three diatoms were isolated by Chin et al. (1965) and one species of Xanthophyta was isolated by Chen (1979). Now more than twenty species of microalgae used as feed are regularly isolated and cultured in China. They include: seven species of Chlorophyta, two species of Chrysophyta, one species of Xanthophyta, two species of Cryptophyta and thirteen species of Bacillariophyta (Table 1). Some of these species can be mass produced (Zhanjiang Fisheries School 1980).

Different species have different nutrient, illumination, temperature, salinity and pH requirements for optimum growth. Chu et al. (1964) and Chen et al. (1978) studied the effects of different nutrients on algal growth and reproduction. Additionally, Chin et al. (1965) and Chen et al. (1982) investigated the effects of various illumination, temperature and salinity regimes on the growth of cultured algae. Table 2 shows the growth conditions for algae which are often used as feed in China.

Chen et al. (1977) and Chen (1979) reported on the polyculture of three benthic diatoms and the monoculture of a Xanthophyta species. Moreover, Xiong (1964) and Xiang et al. (1986, 1989) studied the role of plant hormones in accelerating the growth and reproduction of microalgae. Zhao et al. (1984) and Wang et al. (1989) researched the mechanism by which magnetized water promotes the cultivation of *Tetraselmis* (*Platymonas*) *subcordiformis*, *Dicrateria zhanjiangensis* Hu and *Nitzschia closterium*. Finally, work has been done in the area of nutrient analysis (Wang 1984, Chen and Pan 1987, Yang 1988) and the results of feeding trials have been reported (Chen et al. 1977, Chen et al. 1978, Zhang 1982, Chen et al. 1985).

Table 2. Suitable growth conditions for the microalgae commonly used as feed in China.

	Illumination (lux)	Temperature (°C)	Salinity (ppt)	pH	Nutrients (ppm)
Tetraselmis subcordiformis	1,000 - 20,000* (5,000 - 10,000)†	7 - 30 (20 - 28)	8 - 80 (30 - 40)	6 - 9 (7.5 - 8.5)	N : P : Fe = 100 : 10 : 1 NH_4^+, NO_3^-
Dunaliella spp.	2,000 - 6,000	20 - 30 (25 - 30)	>30 (60 - 70)	7 - 10 (7.5 - 8.5)	N : P : Fe = 100 : 10 : 1 NO_3^-
Nannochloris oculata	1,000	10 - 36 (25 - 30)	4 - 36	7.5 - 8.5	N : P : Fe = 100 : 10 : 1 NH_4^+, NO_3^-
Chlorella sp.	1,000	10 - 36 (25)		6 - 8	N : P : Fe = 100 : 10 : 1 NH_4^+, NO_3^-
Heterogloea sp.	1,000 - 8,000	8 - 35 (15 - 35)	19 - 35		N : P : Fe = 100 : 10 : 1 NH_4^+, 0.2% urine
Isochrysis galbana	1,000 - 6,000	10 - 35 (25 - 30)	10 - 30 (28)	7.8 - 8.75 (8)	N : P : Fe : Si = 120 : 8 : 1 : 10 NO_3^-, 100 μg/liter vit. B_1, 0.5 μg/liter vit. B_2
Dicrateria zhanjiangensis Hu.	3,000 - 8,000	9 - 33.5 (18 - 28)	(20 - 36)		N : P : Fe = 100 : 10 : 1 NH_4^+, NO_3^-
Chaetoceros simplex		10 - 39 (25 - 30)	13 - 18		N : P : Fe = 10 : 1 : 1 NH_4^+, NO_3^-, 0.2% urine
Chaetoceros sp.	6,000 - 8,000	10 - 40 (25 - 35)	18 - 22	8 - 9	N : P : Fe : Si = 120 : 12 : 1 : 10 NO_3^-
Skeletonema costatum		8 - 32 (20 - 25)	7 - 50 (25 - 30)	7.5 - 8.5	N : P : Fe : Si = 100 : 10 : 1 : 5 NO_3^-, 5 - 50 μg/liter vit. B_{12}, 0.1% sea mud extract
Phaeodactylum tricornutum	1,000 - 8,000 (3,000 - 5,000)	5 - 25 (10 - 15)	9 - 92 (25 - 32)	7 - 10 (7.5 - 8.5)	N : P : Fe = 80 : 8 : 1 NO_3^-
Nitzschia closterium	3,000 - 8,000	5 - 28 (15 - 20)	18 - 61.5 (25 - 32)	(7.5 - 8.5)	N : P : Fe = 80 : 8 : 1 NO_3^-

*Suitable range for growth

†Numbers in parentheses indicate range for optimal growth.

Small-Scale Culture

Pure stocks are a necessity and their culture always takes place indoors in 500 - 5,000-ml Erlenmeyer flasks and 10 - 20-liter bottles. Vessels and media must be sterilized before use. Small volumes of water (less than 5,000 ml) are often boiled, whereas larger amounts are sterilized with UV lamps after sand or bolting silk filtration. Stocks are scaled up gradually to meet demand.

Recently, a stock culture and upscaling experiment was performed on *Tetraselmis subcordiformis* and *Isochrysis galbana* by Yu et al. (1990) and Miao et al. (1989) with closed polyethylene film bags (0.08 and 0.2 - 0.4 m^3, respectively). The results indicated that this method has advantages over traditional algae culture in bottles and concrete tanks due to the large area exposed to light, high algal densities obtained, lack of contamination and short growth cycle. Furthermore, algae culture in plastic bags is less expensive than the closed column culture method invented by Wang et al. (unpublished). These results have popularized the aforementioned method, and in 1988 good results were obtained culturing microalgae in plastic bags to feed *Argopecten irradians* at several hatcheries in Weihai, Shandong province.

Mass Production

Mass production of algae in China, however, is still based on the traditional open concrete tank culture method. In northern China, production usually takes place indoors in concrete tanks which are heated either by natural sunlight in greenhouses or with a heating system (electric heaters or boilers). In southern China, however, where it is warmer, culture is conducted in outdoor concrete tanks equipped with emergency heating facilities.

There are two types of mass algal production:

1) Fertilized water method. First seawater (containing microalgae) must be filtered through 150-mesh bolting silk and fertilized according to the nutritional requirements of the desired species. When algae reach a density of approximately 10^5 cells/ml the culture can be transferred to larval rearing tanks or inoculated with zooplankton. This method is used by individuals, small farms, and also some shrimp hatcheries. It has the following advantages:

- a relatively small amount of water is needed,
- there is no need for the numerous tanks required for gradual upscaling, and
- feeding such mixed cultures may prevent nutritional deficiencies which can result from feeding only one species of algae.

2) Single species culture. The volume of water required varies from several m^3 to more than 100 m^3 depending on the scale of production. Tanks are sterilized with potassium permanganate or chlorine. All water is filtered through sand or 250-mesh bolting silk. This method requires large volumes of stock culture. We have found that it is best to inoculate the tanks and fill them only about 1/5 full to begin with. Filtered seawater is added gradually and the tank is fertilized every one to two days until the desired volume is reached. The density of the algae should be maintained near 10^5 cells/ml and tanks should be stirred or aerated 3 - 4 times/day. When the density reaches 10^6 cells/ml, the algae is ready to be used.

There are two advantages to this method:

- it conserves algae stocks and

- adding media in batches can accelerate growth and propagation of the algae and prevent "aging" of the culture.

In 1987 and 1988 we combined the two methods for large scale larval production of *Penaeus chinensis* and *P. merguiensis* and succeeded in providing sufficient amounts of feed. Mollusc hatcheries in China commonly use the single species method to provide monospecific algae or various mixtures of desired species (He et al. 1981, Liu et al. 1988, Cai et al. 1989).

Nutrients

Chemical fertilizers or manure are usually used as a source of nutrients in mass algal cultures. The nitrogen source is usually $CO(NH_2)_2$, NH_4NO_3, $(NH_4)_2SO_4$ or $(NH_4)_2CO_3$ and phosphorus is usually added in the form of calcium superphosphate. The ratio of N:P varies with the species cultured. Finally, addition of a small amount of soil extract or human urine can accelerate growth and reproduction of algae.

ANIMAL FEEDS PRODUCTION

Live animal feeds are needed in the larval production of fish and crustaceans. There are many types of such feeds. Bivalve and polychaete larvae, rotifers, *Artemia* and *Artemia* nauplii, copepods, cladocerans, and crustacean mysis are all commonly used. These animals differ with respect to size and activity, hence their use will depend on the predatory ability of the larvae. Figure 1 shows the various feeds used in all stages of marine finfish culture.

Rotifers

Research situation

As Figure 1 indicates, rotifers are fed throughout the majority of the larval period. The success or failure of finfish larviculture can be determined by a hatchery's ability to produce rotifers. *Brachionus plicatilis* Müller is the species widely used in China.

Rotifers are an ideal early food for fish and shrimp larvae because of their hardiness, rapid reproduction, suitable size, digestibility, ease of culture and especially because their nutritional value can be supplemented by addition of certain feeds. In China, rotifer culture experiments were conducted at the end of the 1950s (Fu and Chen 1962). These were followed by studies on the reproduction and culture of rotifers in 1979 and the early 1980s (Zheng et al. 1979, Wang and Liang 1980, He et al. 1981). The effects of temperature and food on rotifer growth have also been investigated (Huang 1985, 1989).

Rotifer feed

Rotifers eat bacteria, planktonic algae and small protozoa. Their nutritive value

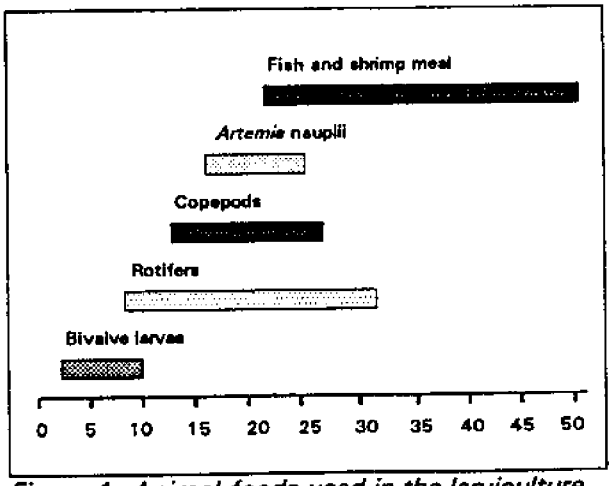

Figure 1. Animal feeds used in the larviculture of marine fish in China.

varies with their diet. Lin (1980) studied the application of marine yeast as feed, but its use is unpopular in China, however, because its culture techniques have not matured. Baker's yeast, on the other hand, is relatively expensive and only used when the supply of microalgae is insufficient. Soybean milk has also been used to culture rotifers for shrimp (Zhejiang Marine Fisheries Institute 1981), but the milk fouls the culture water and decreases rotifer reproduction when it is supplied in improper quantities.

For mass culture, unicellular algae such as *Chlorella* sp., *Tetraselmis* sp., *Chlamydomonas* sp., *Dunaliella* sp., *Nannochloris oculata*, *Phaeodactylum tricornutum* and *Nitzschia* sp. are often used as feed. Of these, *Tetraselmis* sp. is the most popular due to its ease of culture.

Rotifer mass production

There are two primary methods used to mass culture rotifers in China:

1) Fertilizing seawater. Microalgal growth is promoted by fertilizing seawater which has been filtered through 150-mesh bolting silk. Rotifers are inoculated when the algal density reaches approximately 10^5 cells/ml. In this way rotifers are fed a mixture of algae, the species composition differing depending on the fertilizer used and the season.

2) Monospecific microalgal culture. Single species microalgal cultures are inoculated with rotifers when the density reaches 10^5 cells/ml.

In our trials with the larviculture of marine finfish in recent years, we have used two 145-m^3 outdoor concrete tanks (16 x 7 x 1.4 m) for rotifer culture. The tank bottoms are sloped and the seawater and fresh water intake valves are located on the shallow side allowing for salinity adjustment. There are three outlet taps on the deep side. Two of these can be open or closed freely from outside the tank. All tanks are equipped with a good aeration system.

The protocol includes upscaling algal stocks from laboratory bottles to 0.1 - 0.2-m^3 aquaria. The cultures are then expanded to 3-m^3 cement tanks paved with ceramic tile. All vessels are sterilized with potassium permanganate and filtered seawater is used (small volumes of water are sterilized with UV lamps). When the density exceeds 10^6 cells/ml, these cultures may be introduced into 145-m^3 concrete tanks. Only 20 - 30 cm of treated seawater is added at first to avoid diluting the culture too much. Later, more seawater and fertilizer are added every one to two days to maintain the algal density at approximately 10^5 cells/ml.

When the tank is full, rotifers are inoculated at a density of 1 - 5/liter depending on the timing of harvest. Rotifers are stocked at a high density if they are to be harvested soon and at a low density if the harvest date is more distant. Rotifer stock cultures are maintained in aquaria. After inoculation, the 145-m^3 tanks are fertilized every three to five days to guarantee good algal growth. Some spare tanks (totalling approximately 300 m^3) are used to culture algae to supplement that growing in the rotifer tank. When the density reaches 350 - 500 ind./ml, the rotifers are harvested by draining the culture through a 300-mesh bolting silk filter (constructed at the lab). At this stage, concentrated algae from the spare tanks is added daily. If they are not to be used immediately, the rotifers are either

- fed concentrated algae or
- diluted and fed 0.5 - 1 g bread yeast/10^6 rotifers seven to eight times/day and enriched with concentrated algae before use.

In general, rotifers are introduced into the two tanks at three to five day intervals, depending on the production schedule and the length of harvest. When the supply of rotifers in the two large tanks is exhausted, the supplemental tanks are inoculated with rotifers to ensure a continuous supply and to provide rotifers during transitional feeding periods (when the larvae are being weaned onto a different type of food).

The following are essential for good rotifer production: exchanging water frequently, keeping the water clean, providing sufficient feeds and aeration. With the method described above, we are able to fully utilize our tanks and precisely adjust the volumes of algae, rotifer and finfish larvae culture water. In general, the feeds requirements are met when the ratio of the culture water for algae, rotifers and finfish larvae is 4:4:3, respectively.

Artemia

Artemia are widely used as larval feeds in mariculture because their resting eggs may be stored for long periods of time and hatched when needed. There are abundant and widespread *Artemia* resources in China. Since they have begun to be exploited, reports have been published concerning their hatching characteristics (Chen et al. 1975, Zhao 1980, Li et al. 1982, Ye and Zhang 1986, Yang et al. 1989) and application to aquaculture (Li et al. 1982). However, adult *Artemia* are seldom cultured as feeds because their nutritional value is lower than that of nauplii and they are easily substituted with trash fish or shrimp meal. For this reason only experimental reports have been published (Li 1982).

Presently, *Artemia* nauplii are used widely in the culture of finfish and crustacean larvae. Only clones from Hebei, Shandong, and Liaoning provinces are used, however. These cysts have yielded good results, whereas the nutritional value of *Artemia* in the other provinces has not been investigated.

Others

Other animals such as copepods and cladocera are important feed for some finfish and crustacean larval stages but they are difficult to mass produce. In China, studies on the biology of copepods have been conducted since the 1960s (Chen 1964, Li and Fang 1983a, 1983b, 1984; Lin and Li 1984). Techniques for the culture of the copepod *Schmackeria dubia* (Kiefer) are most advanced.

There are no reports on the mass production of cladocera; only a few freshwater culture experiments have been conducted (Shen 1962, Sung 1962, He et al. 1986). The efficacy of using some new types of animal feeds has also been explored. *Nereis* larvae (Lian et al. 1990) and *Mytilus* trochophores (Niu et al. 1982) have been fed to shrimp larvae and finfish larvae have been given *Balanus* nauplii (Yang et al. 1982).

THE APPLICATION OF LIVE FEEDS CULTURE

Feeds species must have the following characteristics: 1) their size should correspond with the capacity of the target species — the various developmental stages require different sizes; 2) their swimming speed and buoyancy should allow them to be easily captured and ingested by the target species; 3) they should have a high nutritional value and be easily digested and assimilated; 4) they should have non-toxic metabolites and yield high survival

rates in the target species; and 5) they should be easy to mass produce, and adaptable to various environments, displaying high growth and reproductive rates.

Many types of feeds are used in China, depending on the location, season, and target species. The climate in the north is much cooler than in the south, hence different species are cultured in the two regions. Table 3 shows the types of microalgal feeds cultured in the different provinces and the periods of culture (Jiang and Wang 1986). Finally, there have been many studies on the suitability of different feeds for the larvae of economically valuable marine animals (Table 4).

CONCLUSIONS

In recent years the problem of producing live larval feeds in China has become important due to the rapid expansion of hatchery operations for finfish, crustaceans and molluscs. The supply of live feeds will remain key to the success or failure of hatcheries until the use of microencapsulated diets becomes a viable alternative. Although the techniques used for the mass culture of live feeds have matured, some questions remain to be resolved:

- only a few species of microalgae can be mass produced successfully,
- the open culture methods are susceptible to collapse because of changing environmental conditions,
- unstable production results from contamination of cultures, preventing the establishment of a reliable continuous supply of feed for hatcheries,
- yields of animals cultured as feeds are often affected by the lack of microalgae and cannot be maintained stably and at high densities, and
- mass production of economical and effective feeds for first-feeding fish is even more difficult and incapable of meeting the demands of fish larviculture.

In view of these questions, future research should focus on improving the present culture techniques for microalgae, preventing and treating contamination problems, isolating and selecting feeds species more suited to open culture and designing culture systems which grow organisms more quickly and which are more simple and economically feasible.

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Table 3. Suitable months for the culture of microalgae along the coast of China.

Place	*Tetraselmis subcordiformis*	*Dunaliella* spp.	*Chlorella* sp.	*Nannochloris oculata*	*Heterogloea* sp.	*Phaeodactylum tricornutum*	*Nitzschia closterium*	*Chaetoceros calcitrans*	*Skeletonema costatum*	*Dicrateria zhanjiangensis* Hu.	*Isochrysis galbana*
Dandong (Liaoning)	7 - 9	8	8	8	6 - 10	4 - 5 10 - 11	6 - 7 9 - 10	8	7 - 9	6 - 9	7 - 9
Dalian (Liaoning)	7 - 9	7 - 8	7 - 8	7 - 8	5 - 9	5, 10	6, 9	7 - 8	7 - 9	6 - 9	7 - 9
Boyuquan (Liaoning)	6 - 9	7 - 8	7	7 - 8	5 - 9	4, 10	5, 9	7 - 8	6 - 9	5 - 9	6 - 9
Qinhuangdao (Hebei)	6 - 9	7 - 8	7 - 8	7 - 8	5 - 9	4 - 5, 10	5, 9	7 - 8	6 - 9	5 - 9	6 - 9
Tanggu (Tianjin)	5 - 9	7 - 8	6, 8	7 - 8	4 - 10	4, 10	5, 9	7 - 8	5 - 6, 8 - 9	5 - 9	5 - 6, 8 - 9
Yantai (Shandong)	6 - 9	7 - 8	6, 9	7 - 8	5 - 10	4, 10	5, 9 - 10	7 - 8	6 - 9	5 - 9	6 - 9
Qingdao (Shandong)	6 - 9	6 - 8	6, 8	6 - 8	5 - 10	4, 10	5, 9 - 10	6 - 8	6, 8 - 9	6 - 9	6, 8 - 9
Lianyungang (Jiangsu)	5 - 9	6 - 8	6, 8	6 - 8	4 - 10	4, 10	4 - 5, 9 - 10	6 - 8	5 - 6, 9	5 - 9	5 - 6, 9
Mouth of Changjiang River	6 - 9	7 - 8	6, 8	7 - 8	5 - 10	3 - 4, 10 - 11	4 - 5, 10	7 - 8	6, 9	5 - 10	6, 9
Zhoushan (Zhejiang)	6 - 9	7 - 8	7 - 8	7 - 8	5 - 10	3 - 4, 11	4 - 5, 10	7 - 8	6, 9	5 - 10	6, 9
Wenzhou (Zhejiang)	6 - 10	7 - 8	6, 9	7 - 8	4 - 11	3 - 4, 11	4 - 5, 10	7 - 8	5 - 6, 9	5 - 10	5 - 6, 9
Sansha (Fujian)	5 - 9	6 - 8	6, 9	6 - 8	4 - 11	2 - 3, 11	4, 10 - 11	6 - 8	5 - 6, 9	5 - 10	5 - 6, 9
Pingtan (Fujian)	5 - 10	7 - 9	6, 9	7 - 9	4 - 11	12 - 3NY	4, 11	7 - 9	5 - 6, 9 - 10	4 - 10	5 - 6, 9 - 10

Table 3. Continued.

Place	*Tetraselmis subcordiformis*	*Dunaliella* spp.	*Chlorella* sp.	*Nannochloris oculata*	*Heterogloea* sp.	*Phaeodactylum tricornutum*	*Nitzschia closterium*	*Chaetoceros calcitrans*	*Skeletonema costatum*	*Dicrateria zhanjiangensis* Hu.	*Isochrysis galbana*
Xiamen (Fujian)	4 - 10	6 - 9	6, 9	6 - 9	3 - 11	12 - 3NY	3, 11	6 - 9	4 - 5, 9 - 10	4 - 10	4 - 5, 9 - 11
Shanwei (Guangdong)	3 - 10	5 - 9	5, 10	5 - 9	2 - 12	12 - 2NY	2, 11 - 12	5 - 9	3 - 4, 10 - 11	3 - 11	3 - 4, 10 - 11
Naozhou (Guangdong)	2 - 6, 9 - 11	4 - 10	4, 10	4 - 10	1 - 12		12 - 2NY	4 - 10	2 - 3, 10 - 11	1 - 6, 9 - 12	2 - 3, 10 - 11
Hainan (Hainan)	1 - 5, 9 - 11	4 - 10	4, 10	4 - 10	1 - 12		12	4 - 10	10 - 3NY	1 - 5, 9 - 12	10 - 3NY
Beihai (Guangxi)	3 - 6, 9 - 11	4 - 9	4, 9	4 - 9	1 - 12		11 - 2NY	4 - 9	3, 10	3 - 5, 9 - 11	3 - 10
Hualian (Taiwan)	3 - 11	5 - 8	5, 9	5 - 8	1 - 12		11 - 3NY	5 - 8	4 - 5, 9 - 11	3 - 11	4 - 5, 9 - 11
Xiangang	3 - 5, 8 - 11	5 - 9	5, 10	5 - 9	1 - 12	12, 1	2, 11	5 - 9	3 - 4, 10 - 11	3 - 5, 8 - 11	3 - 4, 10 - 11

NY = Next year

Table 4. Suitable feeds used in the larviculture of economically valuable marine animals in China.

	Target species	Microalgal feeds (fed singly or in combination)	Animal feeds
Crustaceans	*Penaeus chinensis*	*Phaeodactylum tricornutum*, *Chaetoceros muelleri*, *Tetraselmis* spp. (Feng 1980, Zhang 1982)	*Artemia* nauplii
	P. japonicus	*Skeletonema costatum*, *Nitzschia closterium*, *Phaeodactylum tricornutum*, *Chaetoceros muelleri*, *Tetraselmis* spp.	*Artemia* nauplii
	P. penicillatus	*Skeletonema costatum*, *Nitzschia closterium*, *Chaetoceros muelleri*, *Tetraselmis* sp.	*Artemia* nauplii
	P. merguiensis	*Skeletonema costatum*, *Tetraselmis* spp., *Phaeodactylum tricornutum*	*Artemia* nauplii
	P. monodon	*Skeletonema costatum*, *Nitzschia closterium*, *Tetraselmis* spp.	*Artemia* nauplii
	Charybdis japonica	*Pyramimonas* sp., *Isochrysis galbana* (Sun et al. 1989)	Rotifers , *Artemia* nauplii
	Portunus (Neptunus) trituberculatus	*Phaeodactylum tricornutum* (Liu and Liu 1990)	Rotifers , *Artemia* nauplii
	Scylla serrata	*Phaeodactylum tricornutum*, *Nitzschia closterium*, *Chaeotoceros* sp., *Chlorella* sp. (Lai 1990)	Rotifers , *Artemia* nauplii

Table 4. Continued.

Target species		Microalgal feeds (fed singly or in combination)	Animal feeds
Molluscs	*Chlamys farreri*	*Phaeodactylum tricornutum*, *Tetraselmis* spp. *Chaetoceros muelleri*, *Dicrateria zhanjiangensis* (Wang and Kou 1986)	
	Argopecten irradians	*Isochrysis galbana*, *Phaeodactylum tricornutum*, *Pyramimonas* sp., *Chlorella* sp. (Yan et al. 1990)	
	Pecten yesoensis	*Nitzschia closterium* (Liu et al. 1988)	
	Pinctada martensii	*Dicrateria zhanjiangensis*, *Tetraselmis* spp. (Chen et al. 1978, Jin et al. 1982)	
	Pinctada maxima	*Dicrateria inornata*, *Chlorella* sp., *Tetraselmis* spp., Bacillariophyta (Xie et al. 1990)	
	Mytilus viridis	*Tetraselmis* spp. (Wei et al. 1982)	
	Meretrix meretrix	*Heterogloea* sp., *Isochrysis galbana*, *Chaetoceros muelleri*, *Tetraselmis* spp. (Wang 1980, Cui et al. 1982)	
	Ruditapes philippinarum	*Dicrateria zhanjiangensis*, *Chaetoceros* sp., *Phaeodactylum tricornutum* (He et al. 1981, Qiu et al. 1983)	
	Ruditapes variegata	*Chaetoceros* sp., *Dicrateria inornata*	
	Cardium multicum	*Chaetoceros* sp., *Chlorella* sp. (Sun 1990)	
	Sinonovacula constricta	*Phaeodactylum tricornutum*, *Dicrateria inornata*, *Chaetoceros simplex* (He et al. 1986)	
	Ostrea rivularis	*Dicrateria zhanjiangensis* Hu., *Gymnodinium* sp., *Chaetoceros* sp., *Tetraselmis subcordiformis*, *Chaetoceros* sp. (Cai et al. 1989)	
	Crassostrea gigas	*Chaetoceros* sp., *Tetraselmis* spp., *Gymnodinium* sp. (Xu and Gao 1986)	
	Haliotis	*Navicula* spp., *Amphora* sp., *Navicula latissima*, *Achnanthes orientalis* (Chen et al. 1977)	

Table 4. Continued.

Target species		Microalgal feeds	Animal feeds
Fish	*Bostrichthys sinensis*		Bivalve larvae, rotifers, *Artemia* nauplii, copepods
	Sparus latus		Rotifers, *Artemia* nauplii, copepod nauplii (Zheng et al. 1986)
	Pagrosomus major		Bivalve larvae, rotifers, *Artemia* nauplii
	Epinephelus		Bivalve larvae, rotifers, *Artemia* nauplii
	Lates calcarifer		Bivalve larvae, rotifers, *Artemia* nauplii

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An Overview of Live Feeds Production Systems in Singapore

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ABSTRACT

This paper describes the live feeds production systems used at shrimp and fish hatcheries in Singapore. Systems include those for the production of 1) eggs and larvae of the green mussel, *Perna viridis*, and 2) the rotifer, *Brachionus plicatilis*, to feed fish larvae, and 3) microalgae, *Nannochloropsis oculata*, *Tetraselmis tetrathele* and *Skeletonema costatum*, to feed rotifers and shrimp larvae.

Mature mussels are induced to spawn using the "temperature jump" technique. *Skeletonema costatum* is cultured in 5 - 12-m^3 fiberglass tanks using stock collected from the sea. Rotifers are produced in 5 - 10-m^3 concrete tanks by either the semi-continuous culture method using *N. oculata* or *T. tetrathele* as feed, or the batch culture method using microalgae and baker's yeast. The culture of green microalgae is upscaled from laboratory culture in 125-ml flasks to mass culture in 5 - 20-m^3 concrete or fiberglass tanks. The live feeds production system designs used to support the production of five million banana shrimp, *Penaeus merguiensis*, fry or two million tiger shrimp, *P. monodon*, fry as well as one million Asian seabass, *Lates calcarifer*, fry are discussed.

Recent research includes the development of an intensive microalgal culture system, the use of dried microalgal feed for rotifer culture, an HUFA enrichment strategy for rotifers and development of a small S-type rotifer strain for marine finfish larviculture.

INTRODUCTION

The Primary Production Department of the Ministry of National Development is responsible for the research and development of marine aquaculture in Singapore. The Marine Aquaculture Section (MAS) of the Department is engaged in the development of shrimp and fish hatchery technology. In order to support its work on fish breeding, the Section has set up live feeds production systems for shrimp and fish larviculture. There are also six commercial shrimp and fish hatcheries in Singapore equipped with similar production systems.

Live feeds production is one of the most important operations of shrimp and fish hatcheries. It is crucial that suitable feeds be produced in sufficient quantities for the different larval stages. The feed organisms must be of the appropriate size, motile, palatable, digestible and of high nutritional quality. In

addition, the feed species must be hardy, able to reproduce rapidly and amenable to mass production under controlled conditions. Organisms mass produced at the MAS include green mussel, *Perna viridis*, eggs and larvae and rotifers, *Brachionus plicatilis*, to feed finfish larvae; and microalgae, *Nannochloropsis oculata*, *Tetraselmis tetrathele* and *Skeletonema costatum*, to feed rotifers and shrimp larvae. This paper describes the techniques used to produce these organisms in Singapore and the designs of their production systems.

GENERAL FACILITIES

Seawater Supply System

The seawater supply system consists of three components: the seawater intake system, the filtration and chlorination facilities and the water storage tank.

Seawater intake system

The water used for live feeds production and other hatchery activities in Singapore is drawn directly from the sea. The use of underground seawater is not feasible in Singapore because in most areas it contains high levels of ferrous iron, which oxidizes into ferric iron upon exposure to air. Removal of the precipitate is complicated and tedious.

The intake point is located well beyond the lowest low water line to ensure continuous pumping at all tidal levels. The minimum depth there is 5 m. The intake point is positioned at least 2 m below the surface of the water and 3 m above the sea floor. The position and depth of the intake point is adjusted by means of floats and anchors. Seawater obtained from the system is generally of good quality, with suspended solids less than 10 mg/liter.

The seawater intake lines are made of PVC and laid directly on the sea floor. Two intake lines, each connected to a centrifugal pump, are used. The two pumps run alternately at 24-hour intervals. When one line or pump is not functional, the other is used. This arrangement ensures a continuous supply of seawater to the hatchery. The seawater is delivered through PVC pipes to various points for treatment before use.

Seawater filtration facilities

At the MAS, seawater for live feeds production is filtered through a pair of multi-layer depth filters which have a particle retention size of 5 μm. The commercial hatcheries in Singapore use cheaper single-layer filters. Up to three such filters are used. They are arranged serially and employ different grades of sand, ranging from coarse to fine. The particle retention size of the filters is 30 μm or more. The seawater is then treated by chlorination before use.

Water storage tank

Storage tanks are used to store filtered seawater for chlorination. Only chlorinated seawater is used in green microalgal culture because using filtered, non-chlorinated seawater usually leads to ciliate infestation.

Aeration System

All the commercial hatcheries use high volume, low pressure air blowers. Each hatchery normally has two blowers which run alternately at 12-hour intervals. This arrangement ensures a continuous air supply should either of the blowers become non-functional.

The PVC air delivery lines run downhill and are provided with several condensation traps.

Laboratory Facilities

Most commercial hatcheries are equipped with a laboratory for examination of cultures and other analyses. Laboratory facilities for live feeds culture include a microscope (up to 1,500X magnification) for culture monitoring, a dissecting microscope (up to 60X magnification) for enumeration of rotifer density, a micro-balance and laboratory glassware for preparation of culture media, a culture bench provided with artificial illumination for laboratory culture, and a refrigerator for storage of pure microalgal stocks and culture media stock solutions. Isolation and inoculation of laboratory cultures are performed in the laboratory.

Outdoor Facilities

Outdoor facilities include concrete or fiberglass tanks for intermediate and mass cultures of microalgae and rotifers. The MAS's facilities also include a shed provided with artificial illumination for starter culture of green microalgae. The details of these facilities are described in their respective sections later in this paper.

Drainage System

Adequate drainage is provided throughout the production facility for wastewater discharge. The water is first drained into an underground sump for sedimentation and then discharged into the sea.

PRODUCTION OF MUSSEL EGGS AND LARVAE

Fish larvae must be given feeds smaller than their mouths. The mouths of some species of marine fish, such as the greasy grouper (*Epinephelus tauvina*) and the golden snapper (*Lutjanus johnii*), are smaller than 100 μm at the time of first feeding, hence they can not eat the conventional type of live feed, rotifers, which are more than 100 μm wide. Fertilized green mussel eggs are about 55 - 60 μm in diameter, while the larvae, up to the 24-hour veliger stage, are 60 - 80 μm (Lim et al. 1982). Thus they can be used as initial feeds for fish larvae with particularly small mouths. Mussel eggs and larvae are produced when broodstock are induced to spawn with the "temperature jump" technique reported by Lim et al. (1982).

Collection of Green Mussels

Mussels used for spawning are collected from mussel culture rafts. Only groups with a high percentage of mature mussels are chosen. They are collected in clumps and stocked in 2.5-m^3 fiberglass tanks (2.25 m dia. x 0.6 m depth) at a density of less than 1,000 mussels/m^3 of water, or 2,000 mussels per tank. These tanks are equipped with flow-through raw seawater flowing at a rate of about 40 liters/min. The mussels are conditioned in the tanks for at least one day before spawning. It has been shown that conditioned mussels perform better in terms of egg production and spawning response than freshly collected mussels (Lim et al. 1982). The mussels can be kept in tanks for up to two weeks without losing their spawning ability.

Induced Spawning

Before spawning, the mussel clumps are declustered into individuals and their byssal threads are removed. Barnacles, algae and other benthic organisms attached to the shells are scraped off. After cleaning, the mussels are washed thoroughly to minimize the risk of contaminating the spawning water. They are then subjected to temperature jump treatments in 80-liter plastic buckets (28.5 cm dia. x 42 cm depth) which are filled halfway with seawater. The mussel density is 200 mussels per bucket.

Mussels are first immersed in warm seawater, 33 - 35°C, for half an hour followed by a cold seawater treatment at 23 - 25°C for another 30 min. Thereafter, they are placed in three 125-liter spawning tanks (100 cm x 50 cm x 25 cm depth), each containing 40 liters of filtered seawater. Spawning usually occurs within 0.5 to 1 hour. Male mussels are removed immediately after they begin milting and replaced with female mussels. This prevents the water from being polluted with the milt. The final stocking density is less than or equal to 80 female spawners per tank. Spawning is complete in two to three hours.

Harvesting Mussel Eggs and Larvae

The eggs sink and are removed with a siphon. They are collected in a 20 μm mesh cylindrical plankton net (30 cm dia. x 30 cm depth) and washed thoroughly to remove the milt. The fertilized eggs develop into trochophores in seven to eight hours and are either fed directly to the fish larvae or stored in a bucket of seawater provided with weak aeration in a refrigerator for a day before feeding. The number of eggs produced depends largely on the maturity of the mussels and varies widely, from one to 10 million per female.

The Mussel Egg and Larvae Production System at the MAS

The mussel egg and larvae production system at the MAS is designed to support larviculture studies of greasy grouper and golden snapper, which require up to one million larvae for each experiment. At the stocking density of 30,000 fish larvae/m^3, the culture volume is about 35 m^3. Mussel larvae are used to feed the larvae of both fish species for six days only, from Day 3 through Day 8. The density of eggs and larvae provided is 10/ml, and a total of 350 - 400 million mussel eggs and larvae are required. Based on the mean production of one million larvae per female spawner and a sex ratio of 1:1, approximately 700 - 800 mussels per day, or 4,200 mussels per run, are needed to produce the required number of mussel larvae. The facilities used to produce mussel eggs and larvae are as follows:

- three 2.5-m^3 tanks for the conditioning of 5,000 - 6,000 mussels at 1,500 - 2,000 per tank,
- eight 80-liter buckets for cold and warm treatment of 800 mussels — 200 mussels per bucket, and
- five 125-liter spawning tanks to hold 200 female spawners — 80 per tank.

DIATOM CULTURE

Diatoms are widely cultured in Singapore's hatcheries to support the fry production of two penaeid shrimp, the banana shrimp, *Penaeus merguiensis*, and the tiger shrimp, *P. monodon*. Of the four microalgal species

which have been tested by the MAS and found suitable for shrimp larviculture, *T. tetrathele*, *Isochrysis galbana*, *Chaetoceros calcitrans* and *S. costatum*, *S. costatum* is the most popular for the following reasons:

- *Skeletonema costatum* can be readily collected from Singapore's coastal waters throughout almost the entire year,
- under local conditions, *S. costatum* culture proceeds easily with no serious contamination problems, and
- this long-chained diatom can be concentrated and harvested with a plankton net.

The size of *S. costatum* ranges from 15 - 25 μm in length and 10 - 15 μm in width in nature, to 7 - 15 μm in length and 7 - 10 μm in width after two to three culture cycles. Hence, stocks must be replenished once every two to three cycles.

As the stock used in *S. costatum* production is collected directly from the sea, it is actually a mixed diatom culture consisting of more than 80% *S. costatum* cells. Other diatoms commonly found in the culture are *Chaetoceros*, *Leptocylindrus*, *Melosira*, *Rhizosolenia* and *Thalassiosira*. The diatom culture technique used in Singapore has been described by Lim et al. (1987). It consists of two stages only, starter stock culture and mass culture.

Starter Stock Culture

Collection of stock

Diatom culture stock is collected from the coastal waters of Singapore by towing a plankton net (mouth 35 cm dia. and length 72 cm) of 110 μm mesh size through the top 1 m of the sea.

Culture procedure

Starter stock culture is performed outdoors in 0.5 to 1-m^3 fiberglass tanks. In most commercial hatcheries, this type of culture is conducted in shrimp spawning tanks. Before inoculation, the diatoms are filtered through a 250-μm mesh plankton net to remove large zooplankton and jellyfish. Initial cell density is 5,000 - 10,000 cells/ml. Nutrient mix is then added to the culture, one part per m^3. "One part" consists of 300 g potassium nitrate (KNO_3), 30 g disodium hydrogen phosphate (Na_2HPO_4), 5 g ferric chloride ($FeCl_3$) and 15 g sodium silicate (Na_2SiO_3). The culture is provided with strong aeration, 20 liters/min., and a peak density of 40,000 - 60,000 cells/ml is attained on the first or second day (Fig. 1). At this time, the culture is brown and ready to be transferred to larger tanks for mass culture.

Mass Culture

Mass culture in Singapore is conducted in 5 - 12-m^3 fiberglass or concrete tanks. To start a mass culture in an 8-m^3 tank, the tank is first filled with 3 m^3 of filtered seawater and inoculated with cells from a starter stock culture or another mass culture. The initial density is 5,000 - 10,000 *S. costatum* cells/ml. Each tank is provided with strong aeration through four lines: 20 liters air/min./line. Then three parts of nutrient mix are added. When the density increases to 40,000 or more cells/ml, the volume is increased to 6 m^3 and another three parts of nutrient mix are added. The density will reach 30,000 - 40,000 cells/ml by the next day (Fig. 1), at which time the diatoms will be ready to feed to shrimp larvae. It is important to harvest the diatoms when they reach their peak density and the water is brown. Otherwise, the culture may

collapse within four to six hours of exposure to strong solar radiation.

Harvesting

The long-chain *S. costatum* are harvested with a 40-μm mesh plankton net shaped like a pillowcase. The culture is siphoned into the harvesting net with a 25-cm dia. hose and the net is tied to the discharge end of the hose. Then the collected diatoms are washed into a container used for feeding shrimp larvae. This harvesting method has the advantage of minimizing water pollution which may arise if the diatom culture water is added to the shrimp tanks.

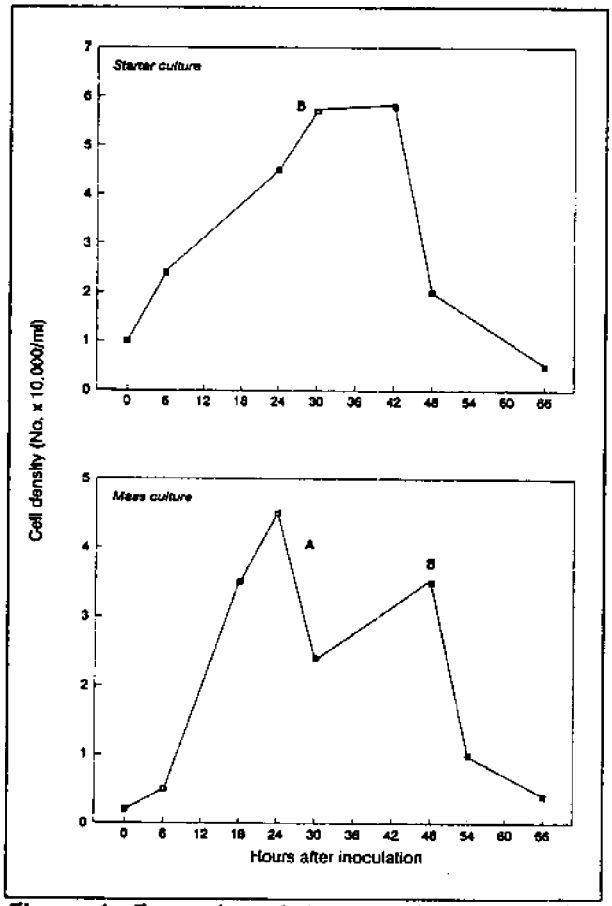

Figure 1. Examples of the growth patterns of S. costatum *in starter stock culture (above) and mass culture (below). (A): Lowering of cell density due to 100% dilution of the culture; (B): Recommended harvest time.*

GREEN MICROALGAL CULTURE

Green microalgae are cultured as feed for rotifers and as "water conditioners" during fish larviculture. Adding algae to the fish culture water sustains the rotifers and improves the water quality. *Nannochloropsis oculata* and *T. tetrathele* have been selected for culture in Singapore because of their relatively high production rate and their suitability for rotifer culture. *Nannochloropsis oculata* cells are 4.5 - 5.0 μm long and 3.5 - 4.0 μm wide. *Tetraselmis tetrathele*, by comparison, is 15.5 - 21.0 μm long and 10.5 - 15.5 μm wide.

Cultures of the two microalgae begin with laboratory flask cultures and are upscaled gradually to outdoor mass culture in large tanks. At the MAS, the process consists of the following stages:

- **laboratory culture**, consisting of maintenance culture and stock culture, in 125-ml flasks,
- **outdoor starter culture** in 3- and 20-liter polyethylene bags,
- **outdoor intermediate culture** in 1.3-m^3 tanks,
- and **outdoor mass culture** in 8-m^3 tanks.

At present, only the MAS performs laboratory and starter cultures of *N. oculata* and *T. tetrathele* in Singapore. Commercial hatcheries obtain their starter culture stocks from the MAS and use them to develop their intermediate and mass cultures.

Boiled, filtered seawater is used in the laboratory cultures but chlorinated seawater is used for all other steps. Optimal salinity is 22

Table 1. Summary of the processes of *N. oculata* culture.

	Culture	Source of stock	Vol. of inoculation	Initial density (cells/ml)	Peak density (cells/ml)	Length of cycle (days)	Culture volume
1.	Laboratory culture						
	Maintenance	125-ml flask	0.1 ml	$3 - 4 \times 10^3$	$3 - 4 \times 10^6$	14 - 30	100 ml
	Stock	125-ml flask	2 ml	$6 - 8 \times 10^4$	$3 - 4 \times 10^6$	5 - 6	100 ml
2.	Starter culture						
	3-L bag	125-ml flask	300 ml	$3 - 4 \times 10^5$	$20 - 25 \times 10^6$	7	3 L
	20-L bag	3-L bag	3 L	$3 - 4 \times 10^6$	$18 - 20 \times 10^6$	5	20 L
3.	Intermediate culture						
	1.3-m^3 tank	20-L bag	60 L	$2.5 - 3 \times 10^6$	$10 - 12 \times 10^6$	5 - 6	Begins with 0.5 m^3
4.	Mass culture						
	8-m^3 tank	1.3-m^3 and 8-m^3 tanks	1 m^3	$5 - 6 \times 10^6$	$10 - 12 \times 10^6$	4 - 5	Begins with 2 m^3, upscaled to 6 m^3

- 25 ppt for *N. oculata* and 26 - 31 ppt for *T. tetrathele*. Hence, the seawater used for *N. oculata* culture is diluted with fresh water before boiling or chlorination, and no dilution is needed for the *T. tetrathele* seawater.

The procedures used for *N. oculata* and *T. tetrathele* culture are summarized in Tables 1 and 2 respectively, and are described in more detail below.

Laboratory Culture

Two types of laboratory culture are conducted. They are **maintenance culture**, used to maintain axenic stocks year-round in the laboratory, and **stock culture**, used to produce enough stock for inoculation of the starter cultures.

Culture procedure

Stock culture and maintenance culture are both conducted in 125-ml flasks in an air-conditioned laboratory. Boiled, filtered seawater enriched with Walne's medium is used. The composition of Walne's medium is given in Table 3. Each flask is filled with 100 ml of seawater and approximately 0.2 ml of Walne's medium.

The stock for laboratory culture is obtained from a healthy stock culture or maintenance culture. Two ml of *N. oculata* stock or 10 ml of *T. tetrathele* stock is added to each 100-ml stock culture to give an initial density of $6 - 8 \times 10^4$ cells/ml for *N. oculata* and $3 - 4 \times 10^4$ cells/ml for *T. tetrathele*. For maintenance culture, only 0.1 ml of *N. oculata* or 2 ml of *T. tetrathele* is added to each 100-ml culture. The flasks are stoppered with non-absorbent cotton after inoculation.

Table 2. Summary of the processes of *T. tetrathele* culture.

	Culture	Source of stock	Volume of stock	Initial density (cells/ml)	Peak density (cells/ml)	Length of cycle (days)	Culture volume
1.	Laboratory culture						
	Maintenance	125-ml flask	2 ml	$6 - 8 \times 10^3$	$3 - 4 \times 10^5$	14 - 30	100 ml
	Stock	125-ml flask	10 ml	$3 - 4 \times 10^4$	$3 - 4 \times 10^5$	6 - 8	100 ml
2.	Starter culture						
	3-L bag	125-ml flask	300 ml	$3 - 4 \times 10^4$	$4 - 5 \times 10^5$	7	3 L
	20-L bag	3-L bag	3 L	$6 - 8 \times 10^4$	$4 - 5 \times 10^5$	6	20 L
3.	Intermediate culture 1.3-m^3 tank	20-L bag	60 L	$6 - 8 \times 10^4$	$1 - 2 \times 10^5$	6 - 7	Begins with 0.5 m^3, upscaled to 1 m^3
4.	Mass culture 8-m^3 tank	1.3-m^3 and 8-m^3 tanks	1 m^3	$5 - 10 \times 10^4$	$1 - 2 \times 10^5$	5 - 6	Begins with 2 m^3, upscaled to 6 m^3

Culture conditions and growth

All the flasks are kept under constant illumination using two 34-watt fluorescent lamps ("True lite") suspended 15 - 20 cm above the flasks. To minimize the risk of contamination, no aeration is provided. Instead, all the flasks are shaken daily to prevent the cells from clumping at the flask bottom. The peak density is $3 - 4 \times 10^6$ cells/ml for *N. oculata* and $3 - 4 \times 10^5$ cells/ml for *T. tetrathele*. In stock cultures, the peak density for *N. oculata* is attained five to six days after inoculation, while that of *T. tetrathele* occurs in six to eight days. Then the cultures are ready for use as stock for starter culture.

In maintenance culture, both species can be kept for up to 30 days before their growth declines. Hence, subculture is done for the purpose of stock maintenance once every 20 - 30 days. In addition, two to three maintenance culture flasks of each species are placed in a refrigerator as backups about two weeks after inoculation. This stock is replaced once every two months.

Starter Culture

Starter culture begins in 3-liter bags and is then upscaled to 20-liter bags. The latter is used as stock for intermediate culture in outdoor tanks.

Culture procedure

The green microalgae starter cultures are conducted in 3- and 20-liter polyethylene bags. The use of polyethylene bags is convenient because it eliminates both cleaning and sterilization of culture containers and the risk of contamination from previous cultures. When hung in the air, the bag allows for better growth of the microalgae because light can penetrate from all directions.

To each 3- and 20-liter starter culture, 6 and 40 ml of Walne's medium is added, respectively. Laboratory stock culture is used to inoculate the 3-liter starter culture. Three flasks are needed for each 3-liter starter culture. The initial density is 3 - 4 x 10^5 cells/ml for *N. oculata* and 3 - 4 x 10^4 cells/ml for *T. tetrathele*. For the 20-liter starter culture, the 3-liter starter culture is used as stock. One 3-liter bag inoculates one 20-liter bag. The initial density is 3 - 4 x 10^6 cells/ml for *N. oculata* and 6 - 8 x 10^4 cells/ml for *T. tetrathele*.

Culture condition and growth

After inoculation, the polyethylene bag is tied and hung outdoors under a shed with transparent roofing. Additional illumination is provided in the evening with several 34-watt fluorescent lamps. All the cultures are provided with strong aeration.

Culture of the 3-liter bags takes seven days, yielding a final density of 20 - 25 x 10^6 cells/ml for *N. oculata* and 4 - 5 x 10^5 cells/ml for *T. tetrathele*. The 20-liter cultures take five days to reach their peak density of 18 - 20 x 10^6 cells/ml for *N. oculata* while the *T. tetrathele* 20-liter cultures take six days to reach 4 - 5 x 10^5 cells/ml.

Table 3. Composition of Walne's medium.

$NaNO_3$	100.0	g
Na_2 EDTA	45.0	g
H_3BO_3	33.6	g
$NaH_2PO_4 \cdot 2H_2O$	22.6	g
$FeCl_3 \cdot 6H_2O$	1.30	g
$MnCl_2 \cdot 4H_2O$	0.36	g
Vitamins Primary Stock*	100.00	ml
Trace Metals Primary Stock**	1.00	ml
Distilled water	to 2	L
Utilization	2 ml/L	
*Vitamins Primary Stock		
B_1	1.00	g
B_{12}	0.05	g
Distilled water	1	L
**Trace Metals Primary Stock		
$ZnCl_2$	2.1	g
$CoCl_2 \cdot 6H_2O$	2.0	g
$(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$	0.9	g
$CuSO_4 \cdot 5H_2O$	2.0	g
Distilled water	100.0	ml

Intermediate Culture

Culture procedure

Intermediate culture is conducted outdoors in 0.5 to 2-m^3 fiberglass tanks. All tanks are sterilized by thoroughly mopping them with a towel soaked with a 10% formalin solution and allowed to dry before use. A nutrient mix consisting of inorganic fertilizers is added at the rate of one part/m^3 of culture. "One part" consists of the following: 50 g ammonium sulfate, 5 g disodium hydrogen phosphate and 3 g urea.

The dosage of nutrient mix used in Singapore is considered very low, only 50% or

less than that used in Taiwan, Japan and the Philippines (Liao et al. 1983). However, there was no difference in culture performance when higher doses were used (Lim, unpublished data).

A 1.3-m^3 tank is filled with 0.5 m^3 of seawater and 0.5 part of nutrient mix. Three, 20-liter starter culture bags are then added. The initial cell density is 2.5 - 3 x 10^6 cells/ml for *N. oculata* and 6 - 8 x 10^4 cells/ml for *T. tetrathele*. After two days, more seawater and another 0.5 part of nutrient mix is added, resulting in a final volume of 1 m^3. The microalgae is then allowed to multiply for another two to four days.

Culture conditions and growth

Tanks are located in an open area and provided with vigorous aeration. In the MAS, they are placed atop a 2-m high platform next to the mass culture tanks. This enables us to transfer the intermediate culture to the mass culture tanks by gravity. The intermediate culture is ready for transfer when densities of 10 - 12 x 10^6 cells/ml for *N. oculata* and 1 - 2 x 10^5 cells/ml for *T. tetrathele* are attained. Each cycle of *N. oculata* culture takes five to six days. This is slightly shorter than the six to seven days needed for *T. tetrathele* culture.

Mass Culture

Culture tank

As the algae production cycles are relatively short, four to six days, the approach to **mass culture** in Singapore is to use many medium-sized tanks. A convenient size for mass culture is 5 - 20 m^3. Concrete or fiberglass tanks, either circular or rectangular, are used.

To develop a new mass culture, an 8-m^3 tank is filled with 1 m^3 of chlorinated water, inoculated with an equal volume of stock from another mass culture, and then supplied with two parts of nutrient mix. The mass culture stocks must be replenished with a new intermediate culture once every four to five cycles. The initial density is 5 - 6 x 10^6 cells/ml for *N. oculata* and 5 - 10 x 10^4 cells/ml for *T. tetrathele*.

The day after inoculation, more chlorinated water and another two parts of nutrient mix are added, resulting in a 4-m^3 culture. After two more days, a final 2 m^3 of seawater is added. To keep ammonia (NH_3-N) levels down, no more nutrient mix is added at this stage. The microalgae is then allowed to grow for one to two more days to reach its maximum density.

Culture conditions and growth

Conditions for mass culture are the same as those for intermediate culture. Tanks are sited in an open area and provided with strong aeration. The cell density and the occurrence of ciliates are monitored. The culture is ready when it contains 10 - 12 x 10^6 *N. oculata* cells/ml or 1 - 2 x 10^5 *T. tetrathele* cells/ml. The whole cycle takes four to five days for *N. oculata* and five to six days for *T. tetrathele*.

Cultures contaminated with ciliates are discarded to avoid contaminating other cultures. There is no effective way to eliminate ciliates, however the risk of contamination can be minimized in the following ways:

The filamentous blue-green algae, *Oscillatoria* sp., is another important contaminant in the mass cultures. Excessive growth will inhibit the growth of *N. oculata* and *T. tetrathele*, and the source of contamination is unknown. *Oscillatoria* sp. can be separated from the cultures after aeration has been stopped for about 1 hour. The upper layer of the culture containing mainly the green microalgae is then pumped into another tank for subculture. The remaining water containing mainly the heavier *Oscillatoria* sp. cells is then discarded.

ROTIFER CULTURE

Rotifers are one of the most important feeds for marine fish larvae because of their unique swimming motion, small size and digestibility. Furthermore, rotifers are suitable for mass culture because of their short culture period, tolerance of intensive culture conditions and rapid reproductive rate. The rotifers cultured in Singapore are S-type, which have pointed anterior spines, a mean lorica length of 160 - 190 μm and a width of 110 - 125 μm.

In Singapore, rotifers are cultured primarily for the production of Asian seabass, *Lates calcarifer*, fry. Two methods are used: **semi-continuous culture** using the green microalgae, *N. oculata* or *T. tetrathele*, as feed, and **batch culture** using green microalgae and baker's yeast. Semi-continuous culture is more common in the commercial fish hatcheries.

Culture Tanks

Rotifers are cultured in fiberglass or concrete rectangular tanks. As the culture cycle for rotifers in tropical areas is rather short, usually less than ten days, small- to medium-sized tanks, 5 - 10 m^3, are used for ease of cleaning. The tanks are equipped with a drain pipe (10 cm dia.) at the short side of the tank for harvesting. Perforated pipes are mounted on the tank bottom to provide strong aeration. Before use, the tanks are washed with 10% formalin and rinsed thoroughly with fresh water.

Culture Procedure

Procedures for the two rotifer culture methods are shown in Figure 2 and described below.

Semi-continuous culture using green microalgae as feed

Nannochloropsis oculata or *T. tetrathele* is used to feed the rotifers. The cell density is 10 - 12 x 10^6/ml for *N. oculata* and 1 - 2 x 10^5/ml for *T. tetrathele*. Since rotifers grow best at 25 ppt, the salinity is reduced with fresh water when *T. tetrathele* is used. No salinity adjustment is required when *N. oculata* is used.

To start a new culture in a 6-m^3 tank, the tank is filled with 2 m^3 of microalgae (Day 0) and inoculated with 80 - 100 million rotifers from another tank, resulting in an initial density of 40 - 50/ml. The tank is topped off with 3 m^3 of green microalgae the next day (Day 1). On Day 2, the rotifer density increases to 80 - 120/ml, at which time they are ready for harvesting. About half of the rotifers, 200 - 300 million, are harvested daily. After every harvest the amount of water removed is replaced by an equal volume of microalgae; harvesting continues for about five days. Thereafter, the total crop is harvested and the tank is washed and prepared for a new culture. The whole cycle takes about eight days, and produces 1,400 - 2,000 million rotifers per

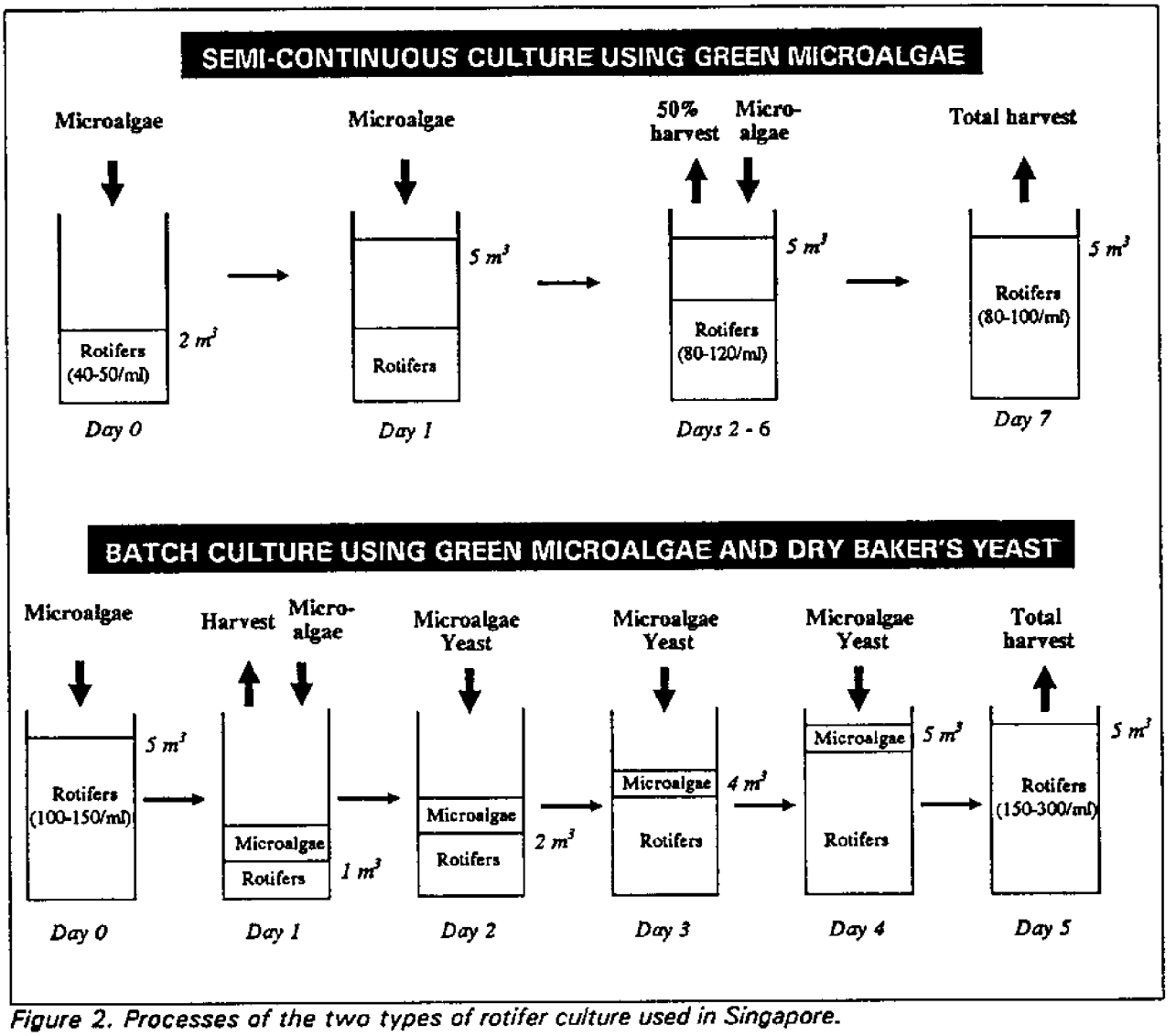

Figure 2. Processes of the two types of rotifer culture used in Singapore.

cycle, or 175 - 250 million per day. The total volume of microalgae used is 17.5 m^3 per cycle, or 2.2 m^3 per day. Therefore, 80 - 114 million rotifers are produced per m^3 of microalgae.

Batch culture using green microalgae and dry baker's yeast as feed

Rotifers produced with this method are fed only green microalgae the day before being fed to fish larvae. This ensures that the rotifers are high in highly unsaturated fatty acids (ω3 HUFAs), which are essential to marine fish larvae (Watanabe et al. 1983). This enrichment procedure is incorporated into the mass culture system.

To develop a new culture in a 6-m^3 tank, rotifers harvested from another culture are added to a tank containing green microalgae. The inoculum varies but it is usually 100 - 150 million rotifers more than what will be re-

quired to feed the fish larvae. The initial density is 100 - 150 rotifers/ml of microalgae.

The day after inoculation (Day 1), some rotifers are harvested to feed larvae, leaving only 1 m^3 of water in the tank. The tank is supplied with 1 m^3 of microalgae per day until the total volume reaches 5 m^3 on Day 4. During the period from Day 2 to Day 4, the rotifers are also fed dry baker's yeast; 0.3 g/million rotifers/day. Yeast is given twice daily, in the morning and in the late afternoon. The entire crop of rotifers is harvested on Day 5; density at harvest is 150 - 300/ml. Production is therefore 750 - 1,500 million per six-day cycle, or 125 - 250 million per day. The volume of microalgae used for rotifer culture is 9 m^3 per six-day cycle, or 1.5 m^3 per day. Therefore, 83 - 167 million rotifers are produced per m^3 of microalgal culture.

Monitoring Culture Conditions

Cultures are monitored daily. This involves estimating rotifer density, the percentage of individuals bearing eggs and the occurrence of ciliates, especially *Euplotes* sp., a hypotrichid ciliate. Cultures contaminated with *Euplotes* sp. or which show a drastic decline in density are terminated and restarted after the tank is thoroughly cleaned. Inoculation stocks are selected on the basis of reproduction and ciliate contamination. Only those with a high percentage of egg-bearing rotifers and which are free from ciliate contamination are used.

Euplotes sp. contamination is a major problem. Excessive growth of this ciliate depletes the microalgae and inhibits the growth and reproduction of the rotifers (Reguera 1984). Furthermore, introduction of ciliates to fish larviculture tanks via contaminated rotifers may also affect the performance of the fish larvae. *Euplotes* sp. usually occur in the latter stages of the culture cycle and are more common if the batch culture method, which involves feeding baker's yeast, is used. Rothbard (1975) reported that eradication of *Euplotes* sp. could be achieved by addition of 20 - 30 ppm formalin into the culture water one day before inoculation of rotifers. At the MAS, *Euplotes* sp. contamination is minimized if the microalgae used for rotifer culture are grown in chlorinated water.

Harvesting

During harvesting, the rotifer culture water is siphoned through a coarse plankton net (200-μm mesh) to remove copepods, dead algae, detritus and dirt particles. Then it is passed through a fine 40-μm mesh plankton net which sits inside a plastic container. The rotifers are then washed gently before use.

DESIGN OF A LIVE FEEDS PRODUCTION SYSTEM FOR SHRIMP LARVICULTURE

Fry Production Capacity

The average production capacity of a commercial shrimp hatchery in Singapore is five million banana shrimp fry or two million tiger shrimp fry (both at PL7, that is, seven day-old postlarvae) per run. At one run per month and ten runs per year, the annual production is 50 million banana shrimp fry or 20 million tiger shrimp fry. The stocking density of shrimp nauplii is 150,000/m^3 for banana shrimp and 100,000/m^3 for tiger shrimp. Based on survival rates of 50% for banana shrimp and 30% for tiger shrimp from nauplius to PL7, the production rate is 75,000

fry/m^3 for banana shrimp and 30,000 fry/m^3 for tiger shrimp. For a hatchery to have a fry production capacity of five million banana shrimp or two million tiger shrimp, the volume that must be devoted to shrimp larviculture is 70 m^3.

Design Criteria

The density of *S. costatum* is 30,000 - 40,000 cells/ml at harvest while the feeding density is 10,000 cells/ml. Hence, one volume of diatom culture is required daily for every 3.5 volumes of larviculture. The daily *S. costatum* requirement for 70 m^3 of larviculture is therefore 20 m^3. Mass culture is also required to inoculate new mass cultures. Five to 10 percent of the total volume of the new mass culture must be added. Hence, another 2 m^3 of mass culture algae is required for this purpose. The total daily requirement of mass culture to supply 70 m^3 of shrimp fry culture is therefore 22 m^3. Since *S. costatum*'s culture cycle is about four days (including one day for tank cleaning and drying), 88 m^3 of water at the shrimp hatchery must be devoted to diatom production.

The starter stock culture is used as stock for mass culture once every two to three cycles. At an inoculation rate of 5 - 10% of the volume of mass culture, the daily amount of starter stock culture needed to support 22 m^3 of mass culture is therefore about 0.6 - 1.2 m^3.

Required Facilities

Based on the above design criteria, the facilities required for the production of 22 m^3 of diatom culture are as follows:

- Eight 14-m^3 tanks. Two tanks are harvested per day, producing 22 m^3 of *S. costatum*. About 20 m^3 is used for larviculture and 2 m^3 for starting new mass cultures.
- Six 1-m^3 tanks for starter stock culture. One or two tanks are harvested per day, producing 0.8 - 1.6 m^3 of diatoms to support one to two mass culture tanks (up to 22 m^3).

No additional tanks are required for starter stock culture because shrimp spawning tanks can also be used for this purpose.

DESIGN OF A LIVE FEEDS PRODUCTION SYSTEM FOR SEABASS LARVICULTURE

Fry Production Capacity

As Asian seabass is the most common marine fish species produced in Singapore's marine fish hatcheries, it is used as a model fish for the design of the live feeds production system. The average production capacity of a seabass hatchery is one million fry (Day 20) per run, or 10 million fry per year (one run per month for 10 months). The stocking density is 30,000/m^3 and the mean survival rate from hatchling to fry (Day 20) is 41.7% (Lim et al. 1986). The production rate is therefore 12,500 fry/m^3, and 80 m^3 is needed to produce one million seabass fry per run.

Rotifer Production System

Design criteria

Of the two rotifer culture methods developed by the MAS, commercial hatcheries prefer semi-continuous culture using green microalgae alone over the batch culture

method using green microalgae and baker's yeast, for the following reasons:

- Production using semi-continuous culture is more uniform, 200 - 300 million per tank per partial harvest and 400 - 500 million per tank at the end of the culture. Production using batch culture fluctuates greatly, from 750 million to 1,500 million per tank.
- There is a high risk of ciliate contamination when baker's yeast is used. This leads to low production or culture collapse. The semi-continuous method using only green microalgae as feed is therefore more reliable than the batch technique.

Batch culture, which is more efficient in the use of microalgae than semi-continuous culture (83 - 167 million rotifers/m^3 of microalgae versus 83 - 114 million rotifers/m^3), is used only when the production of green microalgae cannot keep up with the demand from rotifer culture. Hence, the design of the rotifer production system is based on the semi-continuous culture method.

Rotifers are used to feed seabass larvae from Day 2 to Day 15. Density is maintained at 10 - 15/ml from Day 11 to Day 15, with a feeding rate of 15 - 20/ml/day during this peak feeding period. Hence 1,200 - 1,600 million rotifers/day are needed to grow one million seabass fry in 80 m^3 of water. An additional 100 million rotifers are required daily for inoculation purposes. The total rotifer requirement is thus 1,300 - 1,700 million/day.

Required facilities

Rotifer production using the semi-continuous culture method at the MAS is 1,700 million/5 m^3 of culture in a 6-m^3 rectangular tank over a period of eight days. Hence the average daily production is approximately 42.5 million/m^3 of culture water. To meet the daily requirement of 1,300 - 1,700 million rotifers, the required volume of rotifer culture is 30 - 40 m^3.

As each culture cycle takes about eight days, eight culture tanks are needed, so a new rotifer tank is begun daily. The optimal tank size is therefore 6 m^3, each with a 5-m^3 capacity. Hence eight 6-m^3 tanks are needed to produce 1,300 - 1,700 million rotifers. These can produce 1,700 million rotifers/day from the partial harvest of five tanks (each producing 250 million rotifers) and the total harvest of one tank (450 million rotifers).

Green Microalgal Production System

Design criteria

Both *N. oculata* and *T. tetrathele* are cultured in Singapore's fish hatcheries. The selection of culture species depends on the weather conditions and the needs of the hatchery. Due to its faster reproduction, *N. oculata* is usually cultured under normal conditions. However, because *T. tetrathele* is 20 - 30 times larger than *N. oculata*, it is hardier and its cultures are not as easily taken over by ciliates. *Tetraselmis tetrathele* is therefore more suited to production in the rainy season from September to December. For these reasons, the design of the green microalgal production system is based on the requirements and growth patterns of both species.

Mass culture

The requirements of green microalgae for seabass fry production are as follows:

i) For larviculture: The maximum density during mass culture is 10 - 12 million cells/ml for *N. oculata* and 0.1 - 0.2 million cells/ml for *T. tetrathele*. The required den-

sity for seabass larviculture is 300,000 cells/ml for *N. oculata* or 4,000 cells/ml for *T. tetrathele*. This is about 2.7% of the maximum density during mass culture. To maintain this density in 80 m^3 of larviculture water, the daily requirement of microalgae is about 2.2 m^3.

ii) For rotifer production: The daily microalgae requirement for 8 x 5 m^3 of rotifer culture is 2 m^3 for Day 0, 3 m^3 for Day 1 and 2.5 m^3 each for Days 2 - 6. The total daily requirement of algae for rotifer production is therefore 17.5 m^3 (Fig. 2).

iii) Stock for mass culture: The mass culture can be subcultured four to five times before new intermediate culture stock is needed. The ratio of stock to mass culture is 1:6, hence, about 4 m^3 of mass culture is required daily to inoculate new mass cultures.

So, to produce one million seabass fry per run, about 24 m^3 of green microalgae is needed every day.

Intermediate culture

Intermediate culture is used as stock for mass culture once every four to five mass culture cycles. The ratio of intermediate culture to mass culture is 1:6, thus, 0.042 m^3 of intermediate culture is required for every m^3 of mass culture, and 1 m^3 is needed for the daily production of 24 m^3 of mass culture.

Starter culture

The inoculum for intermediate culture is obtained from 20-liter starter cultures at the ratio of 60 liters/m^3 of intermediate culture. Hence the daily requirement for production of 1 m^3 of intermediate culture is three 20-liter bags of starter culture. Since a 3-liter starter culture bag is used to inoculate one 20-liter bag, the daily requirement of 3-liter starter cultures is also three bags.

Laboratory culture

At the inoculation ratio of three 125-ml flasks of stock culture to one 3-liter starter culture, the requirement of stock culture is nine flasks per day.

Required facilities

Based on the design criteria, the required facilities for the daily production of 24 m^3 of microalgae to grow one million seabass fry are as follows:

i) Laboratory culture: Each laboratory stock culture cycle lasts five to six days for *N. oculata* and six to eight days for *T. tetrathele*. To produce nine flasks of stock culture per day, it is necessary to maintain at least 54 flasks of *N. oculata* or 72 flasks of *T. tetrathele* in the laboratory. The amount of maintenance culture needed for stock maintenance is only two to three flasks for each species, since subculturing is done only once every 20 - 30 days.

ii) Starter culture: With starter culture, it takes five to seven days before peak densities are reached. At least 21 bags each of the 3- and 20-liter starter cultures must be maintained to produce three of each per day (to support the intermediate culture).

iii) Intermediate culture: The intermediate culture step lasts five to six days for *N. oculata* and six to seven days for *T. tetrathele* cultures. Eight 1.3-m^3 tanks are required for intermediate culture and 2 m^3 from two tanks of intermediate culture are used every two days to start a new mass culture.

iv) Mass culture: Mass cultures require four to five days for *N. oculata* and five to six days for *T. tetrathele*. To produce 24 m^3 of mass culture, a total of twelve 15-m^3 mass culture tanks are required. Two mass culture tanks, each producing 12 m^3 of microalgae,

are used daily to support the production of one million seabass fry per run.

RESEARCH EFFORTS

Development of an Intensive Microalgal Culture System

Most microalgal production in Singapore's hatcheries takes place in large outdoor tanks. This method utilizes natural light to power photosynthesis. This method has several problems, however, including contamination with competing algal species, infestation with protozoa and infection by pathogenic organisms. Due to the limited light availability, the maximum density attained is low, 10 - 12 million *N. oculata* cells/ml and 0.1 - 0.2 million *T. tetrathele* cells/ml, and large volumes of microalgae must be cultured to obtain enough cells for hatchery use. This type of culture requires a great deal of space and is unsuitable for Singapore which has a limited amount of land for farming.

Recent research efforts were therefore directed toward developing a reliable intensive microalgal culture system for fish and shrimp hatcheries. The central concept of the intensive microalgal culture system is to culture algae outdoors in 100-liter polyethylene bags which are provided with sufficient illumination, carbon dioxide and an enriched culture medium (instead of inorganic fertilizer mix).

Preliminary experiments were conducted on *N. oculata* using D1 medium (Table 4) in 3-liter polyethylene bags provided with irradiation from a solar lamp (1,500 lux) and supplemented with 1% carbon dioxide. Results indicated that from an initial density of five million cells/ml, the culture grew to 67 million cells/ml in six days, and 213 million cells/ml in 20 days (unpublished data). This is much higher than the 10 - 12 million cells/ml obtained with the conventional outdoor culture system. More research is required to assess the economic feasibility of the system.

Table 4. Composition of D1 medium.

Urea	102.0	mg
KH_2PO_4	33.0	mg
$FeSO_4 \cdot 7H_2O$	20.0	mg
$ZnSO_4$	4.0	mg
$CoCl_2 \cdot 6H_2O$	0.12	mg
$MnSO_4 \cdot 4H_2O$	4.8	mg
$CuSO_4 \cdot 5H_2O$	4.8	mg
$Na_2B_4O_7 \cdot H_2O$	10.0	mg
$Na_2MoO_4 \cdot 2H_2O$	3.0	mg
V_2O_5	0.1	mg
$NiSO_4 \cdot 7H_2O$	0.2	mg
Na-EDTA	29.0	mg
Tris-HCl	50	mM
Sterilized seawater	to 1	L

Use of Dry Microalgal Feed for Rotifer Culture

A major constraint of the present rotifer production system is the need to maintain large volumes of live microalgae. This is tedious and consumes a great deal of the hatchery's facilities and space. An experiment was conducted to determine the feasibility of using dry microalgal feeds instead of live microalgae. A commercial feed, ALGAL 361, was tested. Total replacement of the live microalgae with ALGAL 361 produced more rotifers having a comparable fecundity, in terms of egg density (Fig. 3). Further work is being done to assess the nutritional value of the rotifers produced with dry microalgae.

HUFA Enrichment Strategy for Rotifers

The performance of marine fish larvae is closely associated with the composition of the HUFAs in their feeds (Watanabe et al. 1983). Research is being conducted to develop an enrichment strategy to improve the HUFA content in rotifers. Rotifers are fed different HUFA-rich diets before they are fed to fish larvae. The food values of these enriched rotifers are analyzed in terms of their lipid contents and the performance of the fish larvae.

Development of a Small Strain of Rotifers for Marine Fish Larviculture

Some species of marine fish such as greasy grouper and golden snapper have small mouths and cannot eat rotifers during the early period of larviculture. At present, green mussel eggs and larvae are produced as feed for these species. This is a tedious process involving much labor and time to treat the adult mussels and to process the eggs and larvae before feeding. Furthermore, the mussel eggs and larvae, if not properly washed, can pollute the fish culture water. Consequently, research is being conducted to isolate a small strain of S-type rotifer, one with a mean lorica length of less than 155 μm, for first feeding marine fish larvae.

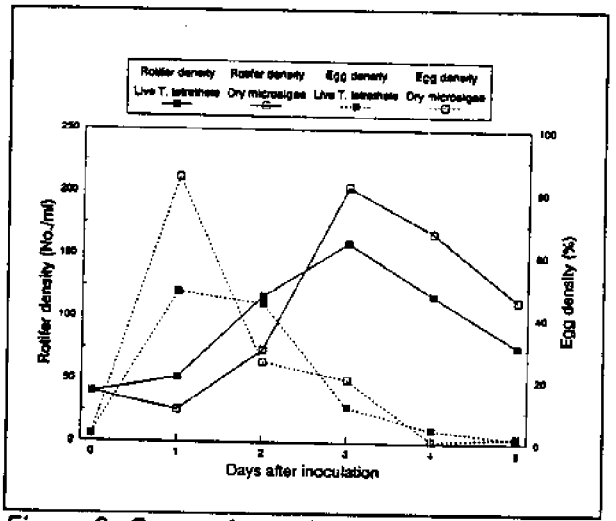

Figure 3. Comparison of the performance of rotifer cultures given live microalgae (T. tetrathele) and dry microalgal feed (ALGAL 361). Egg density is expressed as a percentage of the respective rotifer density.

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Expert Systems and Their Use in Aquaculture

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INTRODUCTION

Expert systems are rapidly becoming an integral part of applications in a number of domains ranging from traditional manufacturing processes to applications in outer space. Expert systems have been shown to improve traditional approaches by as much as an order of magnitude. There are a number of areas, including aquaculture, in which the return on investment in an expert system can be tremendous. This paper will discuss how expert systems can benefit aquaculture, including live feeds production.

Aquaculture, the growing of fish and their feeds in controlled environments, has been practiced for decades in various parts of the world. Culturing techniques have been developed for a wide range of terrains, climatic zones and aquatic species. However, a large part of this expertise continues to reside in a few individuals, each of whom has expertise about culture techniques specific to his or her climatic zone, systems and species. Capturing, codifying and effectively applying this knowledge is clearly a desirable task; one that is especially suited for expert systems.

INTENSIVE CULTURE SYSTEMS AND THEIR PROBLEMS

Our present focus is on intensive culture systems because their problems are generally more acute than regular culture systems. An expert system can play an effective role in extensive systems as well. An intensive aquaculture system is one in which fish are grown at densities far exceeding those found in nature or traditional pond aquaculture. Some of these systems achieve population densities as high as one pound of fish per gallon of water. These densities can only be achieved through strict water management techniques, pure oxygen injection, tight temperature control and timed feedings. The resulting protein production per unit of land is greater than from any other form of agriculture.

Increasing stocking density, however, can increase the potential for disease and partial or total crop loss. Should any portion of the life support equipment (pumps, sensors, oxygen, feeds, valves, etc.) in an intensive system fail, the operator could have less than an hour to implement an appropriate remedy.

To successfully operate an intensive culture system, one must have knowledge about the species grown, the control system used and

the various probes and sensors. Knowledge of optimal growing patterns is typically available from a limited number of expert researchers and operators. In a number of aquaculture facilities, production is a direct function of the time these people spend monitoring the various aspects of the growing process. In an emergency, the absence of an expert can result in a complete loss of the product. In addition to biological information, the expert usually needs to understand the mechanical processes controlling his or her system. Finally, he or she is forced to learn about how and when the various probes and sensors fail. This last piece of knowledge is often obtained at considerable cost!

Once the aquaculturist obtains the requisite knowledge, he or she deals with biological and mechanical processes that are inherently complex. Large amounts of data need to be processed to determine an appropriate control strategy. This data could include the dissolved oxygen level, pH, temperature, and other factors specific to the organism being cultured. One must also keep in mind that the sensor technology is not 100% fail safe. The combination of all these factors may account for the fact that experts are constantly in demand, whether they are growing rotifers or salmon. Expert systems can relieve the pressure on human experts and increase productivity at the same time.

HOW AN EXPERT SYSTEM APPROACH CAN ADDRESS THESE PROBLEMS

An expert system is an approach to computer programming in which a problem in a particular domain is solved using the same rules of thumb that a human would use. The framework of a rule-based expert system is shown in Figure 1. A collection of rules about a related subject is called a **knowledge base.** Separate knowledge bases can exist to focus on separate problems. For example, a knowledge base about dissolved oxygen probe failure can embody all the expertise of an operator who knows exactly when the membrane on the probe needs to be cleaned, etc.

The knowledge base for growing Tilapia may contain the following simple rules:

- If dissolved oxygen is below 6.0 ppm, then dissolved oxygen is low.
- If dissolved oxygen is low, then open oxygen injector valve.

The knowledge base rules, or heuristics, are identified after intensive sessions with the domain expert. In this case, the expert is a Tilapia producer.

The knowledge base is maintained separately from the **inference engine** which makes use of these rules and interfaces with the **real world.** The real world in this case consists of sensors, probes and switches to various pumps controlling flows (e.g. water flow, air flow). Conventional programming techniques do not separate the knowledge about the domain and the control structures that interface with the real world. This distinction in expert systems is one of the great strengths and a main advantage of this techni-

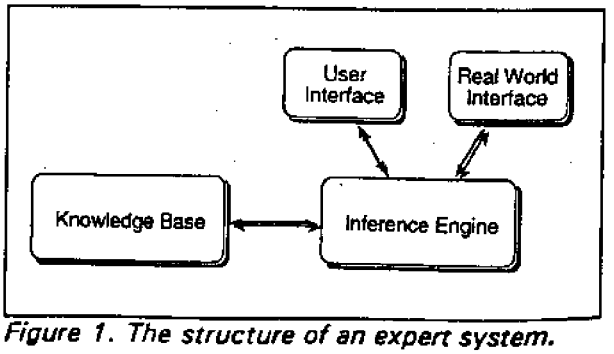

Figure 1. The structure of an expert system.

que. As a result, the reasoning processes governing the system's action are very transparent. This transparency, combined with the clear separation of the knowledge base from the inference engine, enables the development of expert systems in small, increasingly intelligent building blocks. This iterative development introduces a powerful paradigm for dealing with complex biological systems where assumptions are based on experience at least as often as on experimentally proven facts. Consider, for example, the above rule in which a dissolved oxygen level less than 6.0 ppm is considered low. The value 6.0 ppm may be an initial assumption which, through iterative development, might be changed to 5.7 ppm.

The greatest power that an expert system gives the aquaculturist is the ability to magnify and distribute his or her knowledge throughout an organization. This involves capturing many or all of the parameters or rules about growing a particular species in a rule-based manner as shown above. Rules about temperature, dissolved oxygen and pH are quite simple for the expert system to handle since automated probes exist to measure all of these parameters. In cases where one is attempting to measure parameters for which probes do not currently exist, the expert system approach focuses on identifying the relationships between measurable parameters and immeasurable ones.

Aquaculturists monitor toxic metabolic wastes which accumulate and can kill fish or inhibit their growth. Producers may remove them using sophisticated biological and mechanical filters. Chemical tests are conducted daily and the results are evaluated by experts. In automated facilities, these experts currently formulate control and management strategies based on a constant flow of information fed to them by automatic sensors. In those facilities that are less automated, humans play a large role as sensory devices. Sensors provide data on water levels, flow, temperature and pH for every tank in the system. If correct management procedures are not followed or an instrument fails, systems will rapidly deteriorate. Expert systems have the capacity to transform large amounts of data into information and use this information to decide on a particular strategy, by applying the same reasoning as a human expert under similar circumstances.

It may be more practical, efficient and accurate to monitor rotifer production system parameters using an expert system. A real time processor which constantly gathers and analyzes data from a variety of sensors is invaluable. Incorporating into the analysis an expert's reasoning as to what alarm conditions warrant waking up the operator versus those that the system itself could rectify would be even more powerful. An expert system performs in just this manner.

Expert system based "smart" alarms are far more useful and effective than traditional "dumb" alarms; smart alarms can offer explanations as to why they occurred. This inherent capacity to easily explain and justify their conclusions and actions is a unique and powerful feature of expert systems. This is possible because the expert system keeps track of all the rules that have led to the alarm's occurrence. Backtracking through these rules gives a picture of why the system reasoned as it did. For example, the following rules are part of UMECORP's RIAX (Recirculating Intensive Aquaculture eXpert) knowledge base.

- If filter pump flow switch failure, then activate alarm 7. **(R1)**
- If no filter pump flow is present, **(R2)**

and filter pump is activated,
and filter pump pressure is present,
then filter pump flow switch failure.

If alarm 7 occurred, the operator could query the system as to why it thought there was a problem with the filter switch. The system would then check which rules were investigated and find that rules **R1** and **R2** both fired. The explanation given by the system would be that because (1) no filter pump flow was present, and (2) the filter pump is activated, and (3) filter pump pressure is present, it concluded that there was a filter pump flow switch failure (**R2**). This prompted it to activate alarm 7 (**R1**). Such an approach helps the operator understand the system's rationale, which is especially important if that rationale is based upon another expert's knowledge. It also helps the original expert, whose expertise is embodied in the system, verify that his or her reasoning processes still hold true and appropriately alter the system if they do not (iterative development paradigm).

CASE STUDY: RIAX — RECIRCULATING INTENSIVE AQUACULTURE EXPERT

RIAX (Recirculating Intensive Aquaculture eXpert) is an intelligent monitor and control system developed by UMECORP to grow *Tilapia mozambique* in an intensive recirculating system. RIAX was a project started with 40 Tilapia in a 100-gallon system about nine months ago. The 24-hour monitoring and control has allowed us to support 80 larger and healthier fish in the same amount of water. Our original experiment was designed only as a growout experiment but we unexpectedly discovered that one of the specimens was a female, thereby adding some aspects of a hatchery system to our experiment.

RIAX works with the UMECORP Expert Controller (EC), a stand alone microcomputer that constantly retrieves input through its data line from operators and automatic sensors. Figure 2 shows a schematic of the RIAX system. RIAX analyzes these inputs like a human expert would using modifiable rules stored in the EC's battery backed-up memory. As new, and sometimes unexpected, conditions develop in the aquaculture environment, RIAX will make intelligent conclusions and report suggested actions to operators on a video display terminal. Where appropriate, RIAX will also intelligently control system activators such as switches, pumps, alarms and valves, and activate a remote electronic pager or dial a telephone to deliver an alarm.

RIAX can be a vital component in a recirculating intensive system. It can drive all system elements to their maximum efficiency and optimize production in the face of changing inputs such as feed, temperature, water chemistry and other parameters.

RIAX is an appropriate solution for aquaculture when:

- A large crop investment could be jeopardized in a very short period of time as result of adverse environmental changes.
- Increasing the number of growout tanks or raceways increases management complexity. As the size of the aquatic or biological system increases, there is an explosive increase in the number of tasks to be performed on a multistage process.
- The burden on a limited number of experts and/or their time becomes too great. Operators can easily be stretched between urgent tasks and different event types occurring at the same time.

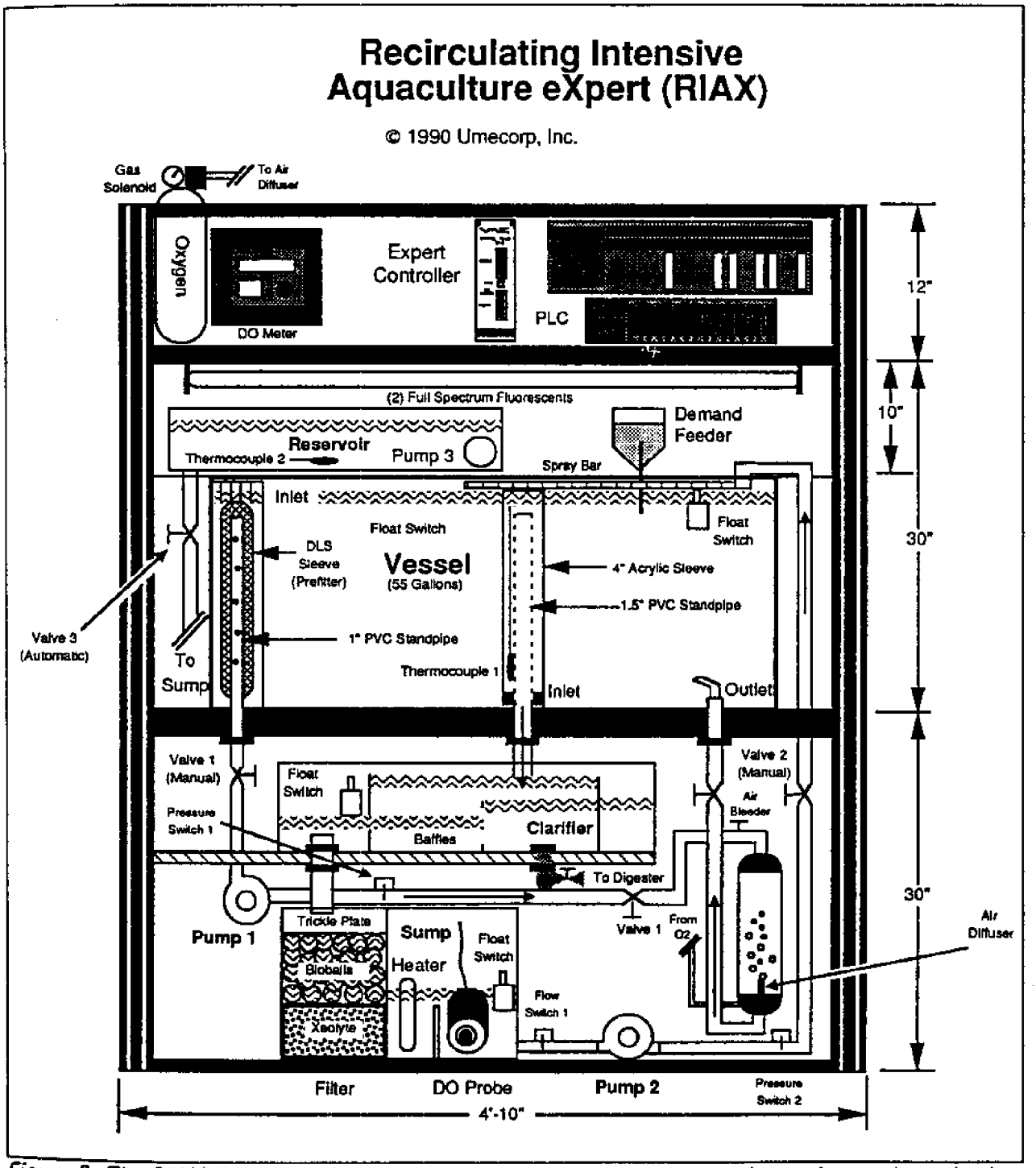

Figure 2. The RIAX demonstration system uses an expert system to monitor and control a recirculating intensive aquaculture system. The Expert Controller (EC) uses rules based on an expert's knowledge and experience to deliver control strategies to system activators through programmable logic controllers (PLCs). Inputs are sensed both automatically and by operator observation. The EC can also be programmed to send data to a computer simulation, run tests and examine the results to decide on appropriate responses. Trial culture species is Tilapia mozambique.

RIAX is a multi-function system. It monitors mundane system tasks 24 hours a day. It guides inexperienced operators through important maintenance and emergency procedures such as when to perform a water change. RIAX can also deliver predictive advice about complex problems. Forecasting biological, chemical and mechanical events before they occur gives operators and systems managers ample warning to deal with spurious or large-scale events. It can also be set up to support a wide variety of manufacturers' equipment.

The RIAX knowledge-based control and advisory system can do things that traditional set-point control systems cannot due to their lack of flexibility. It can also do things that operators cannot do because of humans' limited ability to deal with rapidly occurring events requiring immediate response. RIAX can make conclusions by combining the flexibility of human observations and reasoning with the speed and precision of mechanical instrumentation readings to produce action recommendations not possible with either one alone.

RIAX has four primary modular knowledge bases:

1) the startup knowledge base,
2) the run knowledge base,
3) the operator-assisted maintenance knowledge base, and
4) the equipment diagnostic knowledge base.

The startup knowledge base focuses on all the aspects of starting the system and is typically used after a shut down has occurred due to some flaw. The run knowledge base consists a variety of heuristics on the optimal environment for growing Tilapia. The operator-assisted maintenance knowledge base has rules to help an operator interact with the system and diagnose a problem when it occurs. The equipment diagnostic knowledge base currently has rules about pumps, float switches, dissolved oxygen probes and network interface equipment.

CONCLUSIONS

An expert system can play an important role in several areas of aquaculture including:

- rotifer and microalgal production;
- fingerling production;
- finfish production;
- and marine shrimp hatchery processes.

In each of these cases, an expert system incorporated into the control system of the growing process will result in a more robust system, eliminating loss and increasing both size and quality of yields. The ability of an expert system to produce smart alarms which can explain their origin helps ensure that alarms are valid and can be trusted by human operators — an alarm that cannot be trusted is worse than no alarm at all.

Capturing, codifying and effectively applying the knowledge of human experts results in substantially better production management on a 24-hour basis. Because this expertise resides in English-like rules, it is easy to review, understand and modify. A substantial side benefit of using an expert system is the rapid improvement of the aquaculturist's expertise. This results from direct observation of the results of reasoning processes, and iterative improvements to the system and one's own knowledge. An expert system approach to aquaculture also distributes expertise to various parts of a large installation as well as to new installations very economically.

Based on direct experience developing the RIAX system and the qualities of expert

systems in general, it is quite clear that knowledge-based control allows for easier management and maintenance of control strategies in aquaculture. Expert system technology represents an exciting and important tool in the development and commercialization of aquaculture.

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Commercial Production of Microalgae at Coast Oyster Company

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ABSTRACT

Techniques are described for the culture of microalgae on a commercial scale for feeding larval and early post-metamorphic molluscan shellfish at Coast Oyster Company. Efficient production enables this system to meet the needs of a hatchery which is currently producing more than 20 billion eyed larvae per year. Each stage of algae production is described, the sum of which produces 100,000 liters of harvestable algae per day during the hatchery season. Excess and off-season production is centrifuged, preserved and marketed. The expanded tank culture system is discussed with particular reference to the High Intensity Discharge (HID) Lighting system, which has been used successfully since 1982. The relative costs of the major algal production system components are as follows: labor 37%, chemicals and lab supplies 19% and energy for lighting and heating water 14%.

INTRODUCTION

Pacific oysters, *Crassostrea gigas*, have been cultured on the Pacific Coast of North America since their introduction from Japan in 1902 (Beattie 1982, Steele 1964). From the 1930s through the 1970s, oyster seed stock was purchased directly from Japan and shipped on freighters every spring to supply the majority of the industry's needs. The seed were always wild-caught natural stocks and, as a result, the quantity and quality varied annually. Some years resulted in a complete failure of the natural recruitment while other years provided all of the needs of the industry. Naturally reproducing stocks were found in a few areas of the west coast of North America by the 1960s, but as with the Japanese natural stocks, the quantity and quality of the seed oysters was inconsistent. By the 1970s France began to compete with others for the available supplies of Pacific oyster seed from Japan. This quickly drove up the price and limited the supply available to west coast growers. The natural alternative was the development of hatcheries. Figure 1 is a graphic representation of the recent history of Pacific oyster seed supplies on the west coast of North America.

Several researchers have made significant contributions to the development of hatcheries (Breese and Malouf 1975, Helm and Millican 1977, Dupuy et al. 1977, Galtsoff

1964, Loosanoff and Davis 1963, Walne 1974) but the majority of progress and development has occurred as a result of the many people who have committed themselves to commercial hatcheries as employees.

Coast Oyster Company, which today is the world's largest oyster company with 15 million dollars worth of sales per year and over 20,000 acres of available land, began operations in 1946. In 1974, it became one of the first companies to establish a commercial-scale *C. gigas* hatchery in the United States. Production of over one billion eyed oyster larvae was achieved in 1979. In the late '70s and '80s, the "remote setting" technique for eyed larvae was developed by Coast and others in the hatchery business. This revolutionary technique allowed larvae to be grown in hatcheries, then shipped as larvae to sites remote from the hatchery for setting or seed production. Several manuals have been written describing "remote setting" techniques (Jones and Jones 1983, 1988; Roland and Broadley 1990).

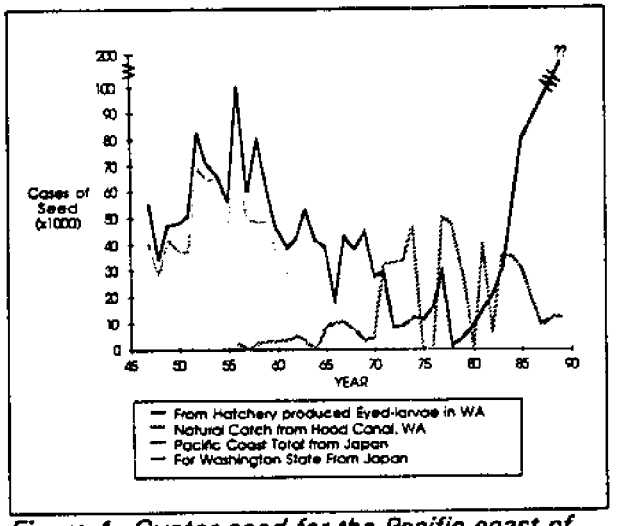

Figure 1. Oyster seed for the Pacific coast of North America (from Chew 1990). Reprinted with permission of the author.

Several researchers at Oregon State University (Lund 1972, Carlson 1982, Henderson 1983) have investigated some of the important details involved with hatchery-reared larvae. In 1984, the 10 billion eyed larvae level was reached, and by 1989 more than 28 billion eyed larvae had been produced by Coast alone. Not only was Coast supplying all of its own needs, it also was offering oyster seed and eyed larvae to many other oyster farms on the west coast of the United States and several foreign countries.

Any large shellfish hatchery has tremendous needs for algae. Algal supplies must not only be of high quality, but they must be reliable and produced in large volumes. The algae production system described in this paper is for feeding broodstock, larvae, and seed of all of the bivalve species currently grown by Coast Oyster Company, which include the Pacific oyster, *C. gigas*, the Manila clam, *Tapes philippinarum*, and the Kumamoto oyster, *C. gigas* var. *kumamoto*.

ALGAL SYSTEM PHILOSOPHY

The algae production system now in place at Coast Oyster Company is the result of many years of experimentation at Coast and adaptations from systems described in the literature (Guillard 1975, Ukeles 1965, Pruder and Bolton 1979, Liang 1979). Several types of lighting sources and arrangements, inoculation volumes, transfer schedules, monitoring schemes, and CO_2 levels have been tested, for example.

As with any hatchery, the algal species cultured must be appropriate for the target species. It must be nutritious for the broodstock, which must be conditioned for as long as two months before spawning in the

hatchery. It must also be nutritious for the larvae, affording them the maximum growth rate, the highest possible survival to metamorphosis, and the highest level of reserves to enable them to successfully reach the next stage of their life cycle in the greatest possible numbers. The chosen species of algae must then be appropriate for the juvenile stage in the hatchery. In the case of Coast Oyster Company, and most others who do "remote setting," the hatchery portion of the juvenile stage is a very short period of time, lasting only three to five days in most cases. At this point, the seed is put out into embayments for further growout.

The quality and quantity of eggs taken from broodstock shellfish depends considerably on the glycogen reserves present in the animal when conditioning for spawning commences. The most significant role for the algae being fed to broodstock during conditioning seems to be for maintenance rather than for reproduction. Furthermore, because juveniles spend very little time in hatcheries using the remote setting technique, the type of algae fed probably plays an insignificant role. If a species of algae is adequate for larvae, it is probably adequate for juveniles, at least for this short term growout.

Another common type of shellfish hatchery grows relatively few larvae but produces millions of single seed, sometimes as large as 1 cm. They select algal species tailored for the growout of juveniles, many of which are inappropriate for raising larvae.

By far the most important role of algal species selection at Coast is for the feeding of larvae. Currently, there are three species in culture which provide for all of the hatchery's needs. These were selected by a combination of literature review (Enright et al. 1986, Whyte 1987, Davis and Guillard 1958) and feeding trials at Coast. *Skeletonema* sp. (clonal designation ARC-3) and *Chaetoceros calcitrans* (CCAL) are small cells which are ideal for feeding very young larvae from first feeding until they reach about 150 μm in size. These species, of course, can also be fed to broodstock, older larvae, and juveniles. *Thalassiosira pseudonana* (UW3H) is best for larvae larger than 150 μm and is also given to broodstock and juveniles. This 150 μm limit for *T. pseudonana* 3H has applied to all of the species we have cultured over the years at Coast. Whyte (1987) describes the importance of understanding the nutritional content of algae as well as the energy content when selecting algal strains.

The system that will be described for culturing all of these algal species is designed to grow them at their maximum rate and offer the highest algal yields or number of cells produced. All species are grown in a batch system with the same basic design. The major key to achieving high yields is to transfer all cultures when they are still in the exponential phase of growth. This is after only 48 hours for the species cultured at the Quilcene Hatchery site. Transfer schedules are strictly adhered to for consistency. High quality is maintained by (1) monitoring pH, cell density at transfer, culture color; (2) microscopic examination for protozoan contaminants; and (3) bacterial screening of all primary cultures. Without a doubt, the most important part of any algal system is not only the system itself, but the people who operate it. Consistency in quality and quantity of algae can only be achieved if there is a consistency of quality personnel who truly care about what they are producing.

ALGAL SYSTEM WATER SUPPLY

The seawater that is now employed at Coast to grow algae is taken from a saline well that was drilled adjacent to the hatchery below the intertidal beach level. The water is pumped out of the well from 30 feet below the beach surface in a substrate of sand and gravel. This well has supplied the hatchery with a consistent supply of water year-round. The salinity ranges from 24 - 28 ppt, the pH is about 6.8 - 7.4, and the temperature ranges from 12 to 18°C. Contaminants in the water are almost nonexistent prior to culture. Regardless, all expanded algal cultures are treated with chlorine to ensure a contaminant-free medium.

PRIMARY CULTURES

The primary cultures for all species are held in 1,000-ml Erhlenmeyer flasks. The culture level in each flask is 650 - 700 ml. The flasks are all grown on shelves with overhead "vita lights," (Duro-test) which are full spectrum fluorescent tubes. All culture media is made up with "F/2" nutrient solution. Media for flasks is autoclaved to ensure sterility. Normally, 50 ml of algae culture is inoculated into 650 ml of culture medium. The volume of the inoculum varies somewhat with the density of the culture and the expected growth rate after inoculation. A typical starting density in these flask cultures is 2×10^5. A typical final density after two days of culture is 3×10^6. All cultures are monitored for density by a colorimeter (Chemtrix Type 24) correlated with cell counts by a hemacytometer.

Because of the sensitivity of molluscan larvae to bacteria, these cultures are monitored for varieties of bacteria. TCBS bacterial media is used to monitor *Vibrio*. Vibrios are notorious as the primary disease-causing organisms for many marine animals. Elston (1990), describing Vibriosis in larval and juvenile molluscs, states that "probably all species are subject to the disease, although some may be more susceptible than others." He further states that algal stocks are one of the significant sources for the introduction of Vibrios to a hatchery system. Marine Agar media is used to detect other general bacteria, some of which can be harmful at low levels. If significant levels of bacteria are present on any of these media, the primary cultures are removed from the system. All flask transfers are done under a sterile hood to help prevent outside contamination.

20-LITER CARBOYS

Flask cultures are inoculated directly into 20-liter carboys for the next stage. At this and all subsequent stages, the culture medium is treated with a minimum of 10 ppm chlorine for at least one hour before it is neutralized with sodium thiosulfate in preparation for inoculation as recommended by Elston (personal communication). Approximately 1 liter of inoculum is added to 14 liters of culture medium, resulting in a starting density of about 2×10^5. Within 48 hours, these cultures have achieved a density of 6×10^6 and are ready for the next transfer. Again, the "vita-lite" fluorescent tubes are used for illumination. As with the flask cultures, lighting is continuous.

From the carboy stage through expanded tank cultures, an automatic CO_2 injection system is employed. This system injects CO_2 into the air system once an hour to deliver about 10% CO_2, measured at the time of injection. As long as the cultures are normal and healthy,

this rate of CO_2 addition will maintain the pH in the cultures at 7.5 - 8.5.

EXPANDED TANK CULTURES

The 3,000-liter and 20,000-liter expanded tank cultures employ the same basic strategy as the other stages. Maximum growth rate, log phase harvesting or transfer, and short turnover time. The water treatment, nutrient, and CO_2 addition are the same as in the carboy system. The primary difference is the lighting source, due to the size of the cultures. The tanks are housed in a covered greenhouse to help minimize environmental variations that occur in this area in an outdoor facility. The roof is transparent, allowing us to utilize ambient light, however, the primary source of light is the array of High Intensity Discharge (HID) light fixtures (Lumark model MHSS-SA23-M-1000MT) suspended over each tank. The fixtures have Metal Halide lamps, which have been used by Coast since 1982. The 3,000-liter tanks each have one light fixture with a 1,000-watt lamp (Sylvania model M-1000) suspended 0.5 meters above the water surface. Each 20,000-liter tank has four 1,000-watt lamps suspended 1.5 meters above the culture surface. Both tanks give the highest yield of algae when filled to a depth of about 1.5 m.

This lighting system is primarily responsible for the fact that our small tanks grow from an initial density of 1×10^5 to 3×10^6 in only 48 hours, at which time they are transferred to the 20,000-liter tanks. All transfers are done with centrifugal pumps; each large tank receives about 1,300 liters of inoculum. The lighting system is also the primary reason that the 20,000-liter tank cultures grow from an initial density of 2×10^5 to 3×10^6 in 48 hours before being fed. There are two reasons for the success of the HID lighting system. First, the intensity of the lights is extremely high. The surface of the culture water in the 3,000-liter tanks directly under the lamps was measured with a photometer (Licor model LI 185A) and found to be twice that of natural sunlight. Secondly, the color spectrum is beneficial for the growth of plants. The important spectral region for photosynthesis is in the red and blue spectrum (Fig. 2). Thousand-watt Metal Halide lights have a good balance of red to blue wavelength (Fig. 3) compared to other lighting sources.

As with any commercial-scale production facility, the fewer units of culture dealt with at each step and the fewer steps to production scale, the lower the costs of production. Table 1 outlines the number of units of culture at each culture stage for all of the species currently grown.

The algae system at Coast is geared to produce about 100,000 harvestable liters of algae per day during the production season, which lasts from March 1 through October 31.

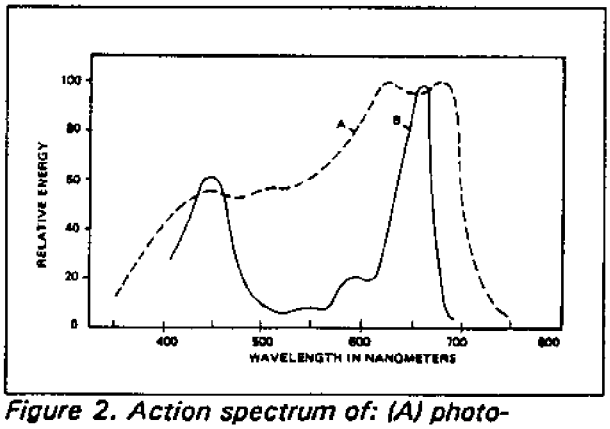

Figure 2. Action spectrum of: (A) photosynthetic response, and (B) chlorophyll synthesis (from Sylvania Engineering Bulletin O-352).
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Table 1. Units devoted to microalgal culture at Coast Oyster Company.

Vessel	Units inoculated per day	Units in system
Flasks	12	24
Carboys	9	18
3,000-liter tanks	4 - 5	18 available for culture
20,000-liter tanks	6 - 7	20 available for culture

Most of this algal yield is fed as liquid algae to the following three areas:

- Broodstock 5% of total
- Larvae 42% of total
- Seed 53% of total

CENTRIFUGATION AND PRESERVATION OF ALGAE

For the past several years, Coast has been developing preserved diets with the intent of feeding remote set larvae from a stored source that can be fed on demand. Originally, algae cells were simply centrifuged (AML Industries model B-30H) into a paste form, refrigerated, and fed after resuspension in seawater. This provided adequate nutrition, but the shelf life was only about 10 days. More recently, Coast has developed the algal paste into a form that is preserved and can be held in refrigeration for up to one year. It is then resuspended in seawater and mixed in a blender for 30 - 60 seconds before use. The blended mixture can be added directly to a tank at a predetermined density or added to a holding tank and pumped over to the animals for feeding.

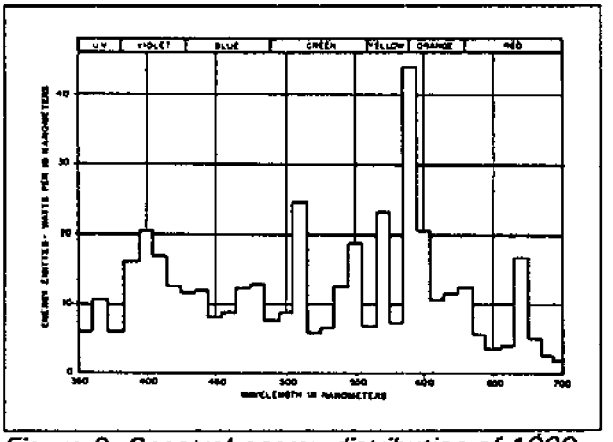

Figure 3. Spectral energy distribution of 1000-watt metalarc clear lamp (from Sylvania Engineering Bulletin O-344).
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As with any algal substitute, in our experience at Coast, live algae has always been a better food source. However, live feed systems are costly and not 100% reliable. During our down-time at the hatchery, in the winter months, and when excess algae is available, centrifugation and preservation is an excellent alternative to live algae production. These preserved diets are now used routinely to feed seed at Coast and are sold to many others who report good success in a variety of different systems feeding a variety of species. Recommendations for handling and feeding these preserved diets are available from the author.

CONCLUSIONS

Algae can be grown efficiently in a commercial hatchery system. During the 1990 hatchery season, the entire algal production system accounted for 18% of the total hatchery expenditures. The major categories in algae cost were labor (37%), lab supplies and chemicals (19%), and energy for heating water and lighting (14%). The supply of algae at Coast meets the hatchery demand in terms of both quantity and quality and also provides a marketable product.

Acknowledgments

I wish to thank Pam Johnsen and especially Christine Edwards for their hard work and dedication to the production of algae at Coast Oyster. I am also grateful to the late Verne Hayes, and more recently to John Petrie for their contributions to my growth as a person and a manager. I want to express my greatest thanks to my wife, Meg, for her patience and support during the years I have spent at the hatchery.

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Development of a Micro-computer Automated Algal Chemostat: Overview from Bench to Production Scale

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ABSTRACT

A 225-liter computer automated, bench-scale algal chemostat was developed and its production and culture maintenance capabilities were investigated. During a five-month test period, the system operated in a semi-continuous fashion resulting in an average daily productivity of 72 g/m^3/day, an average specific growth rate of 0.66/days and a maximum standing crop of 190 g/m^3.

The Turbo Pascal program "Supervisor" precisely controlled and monitored all daily activities through an interface with an ADC-1 data and acquisition unit. The organization of "Supervisor" created a flexible programming environment which allowed for quick modifications and will enable system expansion with relative ease. Based on the stability and reliability of the computer control strategies, the outlook for commercial application of automated technologies appears very promising.

INTRODUCTION

As natural sites for the cultivation of bivalves, fish, and crustaceans become rarer due to decreasing water quality, the number and size of hatcheries, nurseries, and grow-out facilities will have to increase to sustain production levels. However, the limiting factor in determining the carrying capacity of such facilities is and has been the mass production of algae (DePauw et al. 1983, 1984; Walsh 1987). The batch culture technique commonly used in most facilities is a rather simple and reliable method, but makes inefficient use of the production capabilities and manpower of the facility.

The use of semi-continuous and continuous algal production systems allows dominant cultures to be maintained easily by manipulation of environmental parameters including temperature, light, pH, CO_2 enrichment, and dilution rate. Additionally, algae can be maintained in the exponential growth phase, allowing for indefinite culture periods and increasing the amount of algae produced per unit volume (Ukeles 1971, Palmer et al. 1975, Trotta 1981). Historically, however, chemostats have been widely employed as

research tools in studying the growth kinetics of algal cultures (Caperon 1967; Droop 1968, 1974; Caperon and Meyer 1972; Eppley 1981; McCarthy 1981). Under laboratory conditions, with careful control of the pertinent parameters, cultures can be maintained for extended periods of time without premature collapse or contamination by undesirable algal species, bacteria, or zooplankton (Fogg 1975). Subsequently, small-scale chemostatic cultures have proven to be reliable.

For commercial applications, this technique must be upscaled. Large-scale continuous cultures are harder to implement due to their higher costs, the manpower needed for maintenance, and the inability to maintain control of steady state conditions (DePauw et al. 1983). Under any conditions, the control of an algal chemostat requires technological training, which is not commonly found in the aquacultural industries. Due to the lack of proper control at the large-scale level, serious contamination with undesirable algal species and/or bacteria commonly leads to the demise of a productive chemostatic system. Unless this type of system is well optimized, delays in algae production can not be avoided and ultimately, interruptions in production will cost money.

Implementation of computer controlled processes in large-scale chemostat operations is necessary to optimize production capabilities and flexibility while minimizing costs. Procedural decisions are based on the information received from monitoring devices. Additionally, operational modifica-

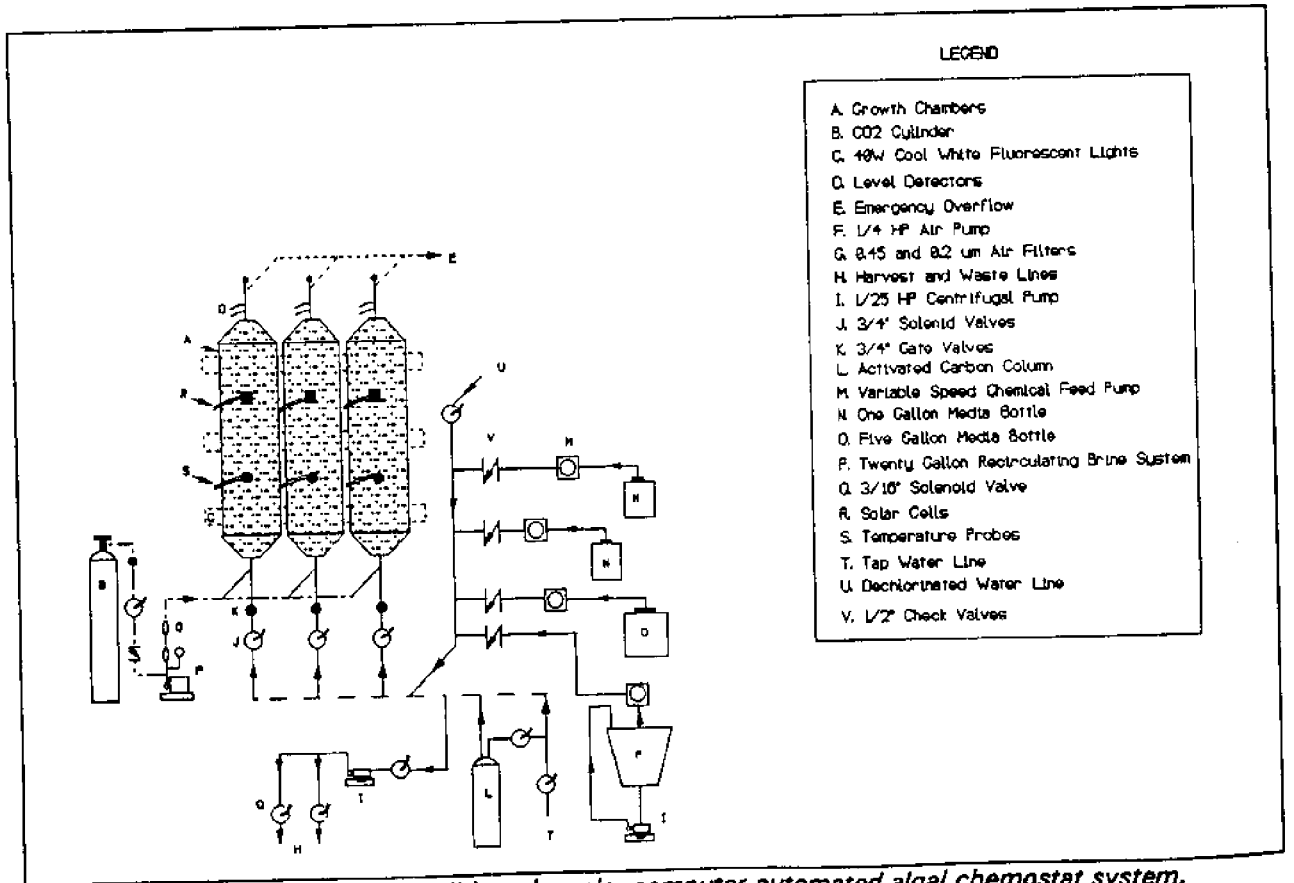

Figure 1. Schematic of the overall bench-scale, computer automated algal chemostat system.

tions can be accomplished through simple program changes, minimizing system downtime. A system of this type is robust and "forgiving" in that computer or system interruptions are not catastrophic to the algal culture. The algal culture will continue to grow under batch conditions until the normal operational mode is restored. The feasibility of a bench-scale micro-computer automated algal chemostat has been demonstrated by Rusch (1989) and commercial-scale evaluation is warranted. This paper discusses the bench-scale chemostat and describes the movement from bench- to production-scale.

BENCH-SCALE ALGAL CHEMOSTAT

Experimental Apparatus

The bench-scale chemostat, detailed in Rusch (1989), is schematically illustrated in Figures 1 and 2. The system consisted of three 20-gallon fiberglass-reinforced polymer growth chambers (Solar Components Corporation, Manchester, New Hampshire, USA) connected to a central harvesting and dosing apparatus. Each chamber contained only one solenoid valve for solution additions and algal suspension withdrawals, allowing for expansion with only slight increases in system complexity and cost.

All inputs (nutrients, brine, water, and air/CO_2) and withdrawals were accomplished through a single port located at the bottom of the chambers. Constant volumes were maintained using stainless steel level detectors. The 3 - 5% CO_2/air volume ratio was maintained by discrete CO_2 injections into the filtered (0.2 μm) air line. Six 40-watt cool white, fluorescent lights, placed horizontally behind the chambers, supplied a continual

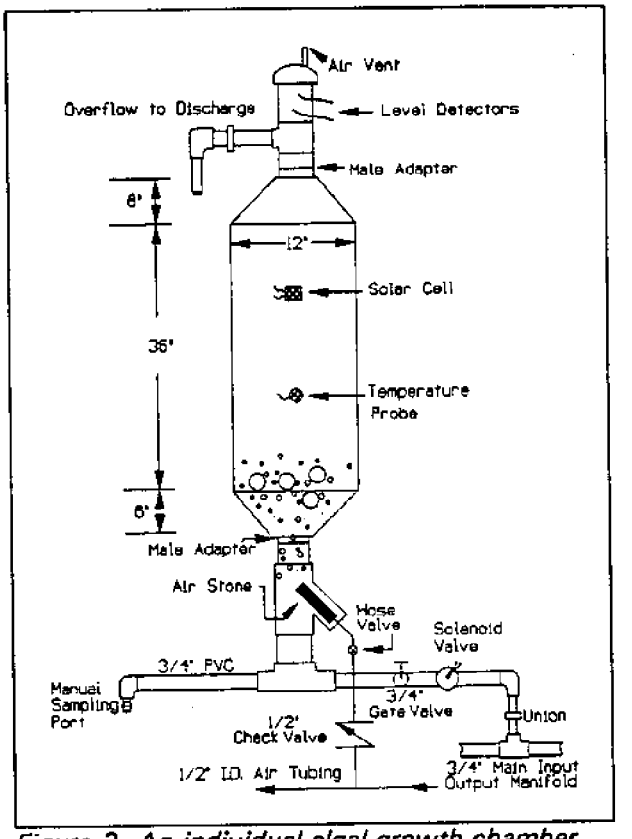

Figure 2. An individual algal growth chamber.

surface irradiance of approximately 6,000 lumens/m^2. Solar cells and temperature probes were attached to the outside of the chambers for independent monitoring of each culture.

System Monitoring and Control

The individual components of the bench-scale system were interrelated, controlled, and monitored by a Zenith Z-184 Supersport laptop micro-computer interfaced to monitoring devices via a Remote Measurement Systems ADC-1 data/acquisition unit (Remote Measurement Systems, Inc., 1983). The ADC-1 unit accommodated 16 analog inputs used to receive signals from precision measurement devices, four digital inputs, six controlled

outputs for intermittent operation of the level detectors, 32 BSR units to control devices requiring simple 'on/off' signals, and RS-232 communications (Fig. 3).

Operational success was determined to a large extent by the computer's ability to make procedural decisions concerning harvesting and dosing of the chambers. Harvest volume was determined using inexpensive solar cells which generated their own voltage depending on the light transmittance through the chambers. The cells exhibited a linear relationship between light intensity (lumens/m^2) and algal suspension concentration (R^2 = 0.9845, 0.8528, and 0.9687 for chambers 1, 2, and 3, respectively), making them a reliable and inexpensive method for cell density estimations.

System control and monitoring was accomplished through the user friendly, menu-driven, computer program "Supervisor" (Rusch 1989). After program initiation, the operator had the freedom to either activate the supervisor or manually select the desired processes via the specified toggle keys (Fig. 4).

The supervisor control sequence contained a stack of commands enabling the chemostat to operate in a self-sustaining mode, with the exclusion of solution replenishment. The supervisor procedure served two functions. First, a bubble sort stack procedure, which prioritized command execution chronologically, and loaded all of the necessary commands into the stack. With this type of chronological command execution, each process was programmed independently, utilizing time of day execution and condition verification loops to avoid conflicts. Second, a stack supervisor monitored the internal clock and the time associated with the top command in the stack. When the two times matched, the function or procedure associated with that command was called, allowing all processes to be performed at precisely the same time every day. The bubble sort stack procedure added delayed operations to the stack and relinquished control back to the stack supervisor immediately. Each operational process was written independently as either a procedure or function, creating a flexible programming environment ideal for system expansion and/or modification.

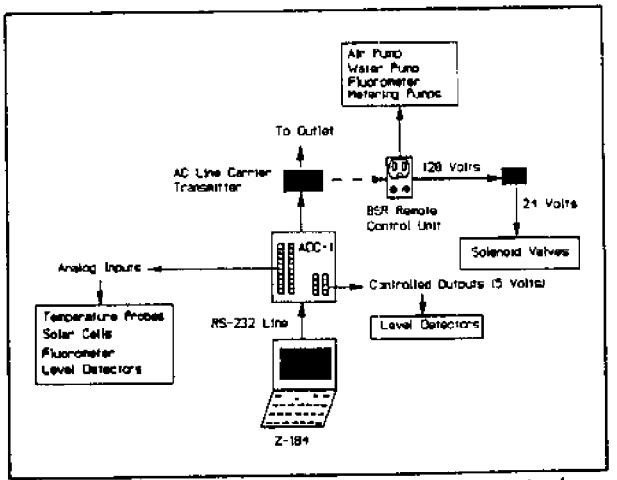

Figure 3. Schematic of the computer control system for the algal chemostat.

The control strategy established an environment conducive to long term algal production with minimal human intervention. The intricate feedback system allowed the computer to detect problems early, making the system more reliable.

Production Performance

During the experimental period between March and July, 1989, *Chlorella minutissima* Fott et Novakova was produced semi-continuously. The computer harvested each chamber four times a day for an approximate 38% volumetric turnover. The system was put on a bi-monthly disinfection cycle to control sidewall growth. Reinoculation of a disin-

fected chamber occurred via automatic culture movement from another growth chamber, eliminating the need for maintaining additional cultures.

Daily monitoring data was collected and stored in a text file by the computer. The data was analyzed to evaluate the operational conditions and productivity of the system and to check for irregular documentation patterns indicating a software or information retrieval problem. Once the system exhibited stable production patterns, a kinetics study was performed to determine daily specific growth rates and harvestable biomass (dry).

The results of the kinetics study are illustrated in Figure 5. Biomass steady state conditions were not reached; subsequently, the maximum biomass of 190 g/m^3, observed for chamber 2, was considered a conservative estimate of the maximum sustainable standing crop. The overall specific growth rate (μ)* averaged 0.66/day. Based on the maximum biomass concentration and average growth rate, 72 g/m^3/day dry weight could be reliably

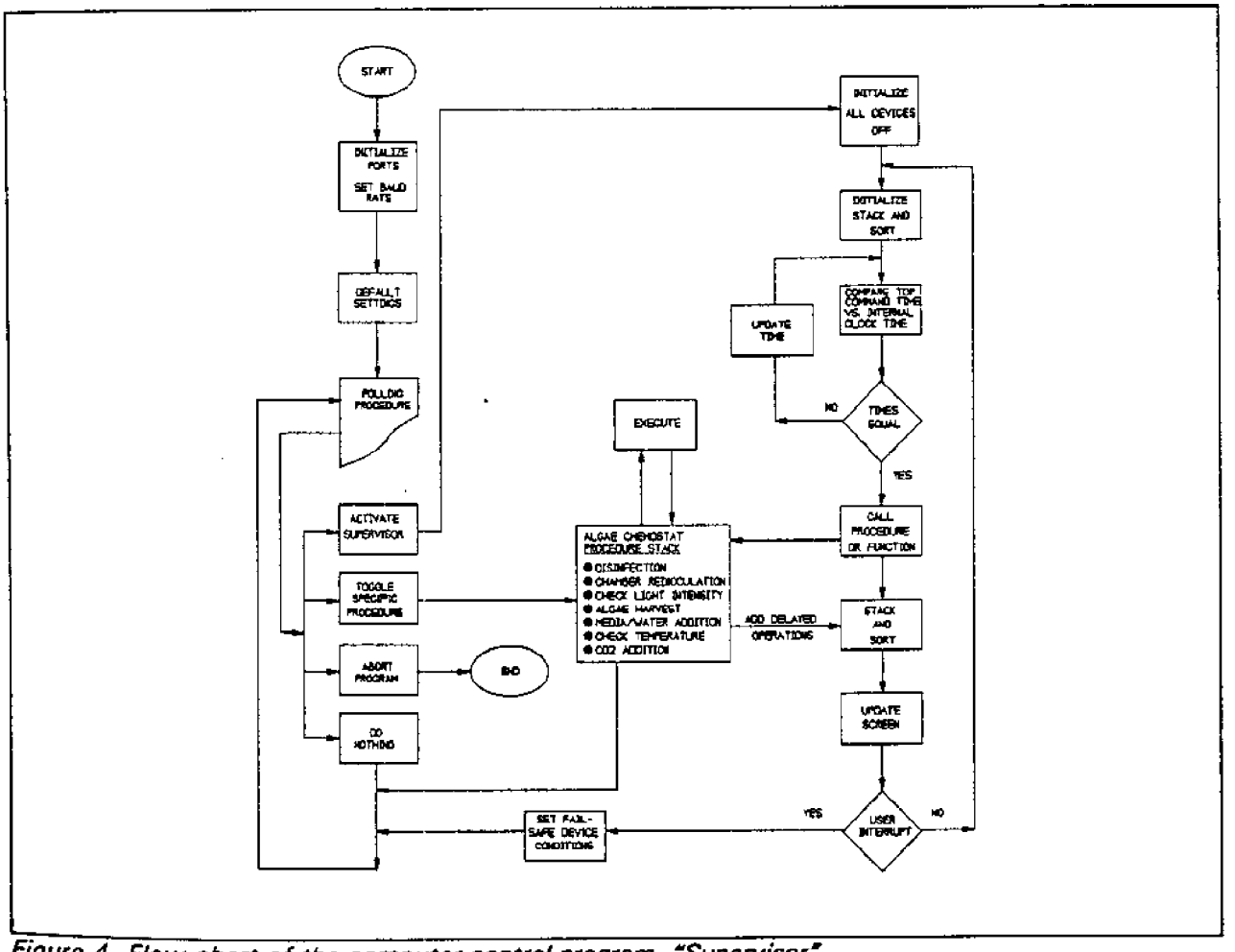

Figure 4. Flow chart of the computer control program, "Supervisor".

* $\mu = \ln \frac{\frac{x_2}{x_1}}{t_2 - t_1}$, where x_2 = biomass concentration at time t_2; x_1 = biomass concentration at time t_1 or beginning time; $t_2 - t_1$ = elapsed time between selected interval (days)

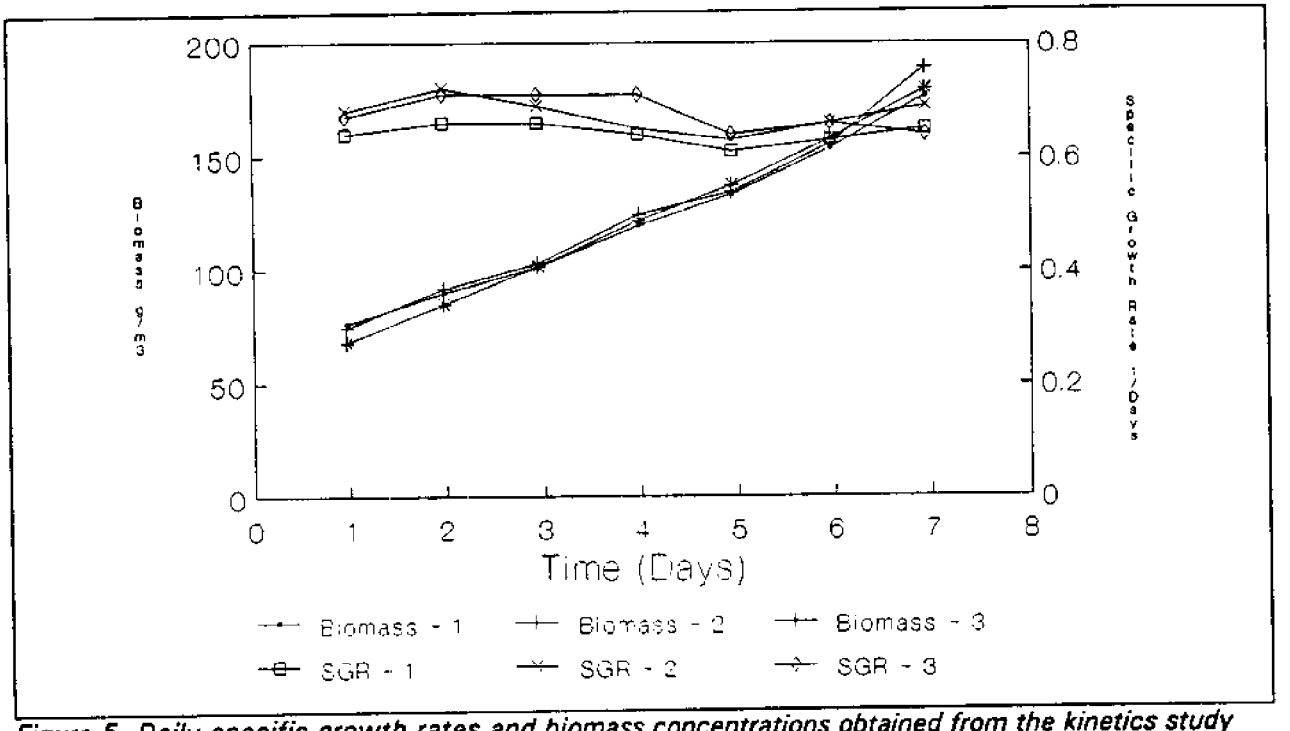

Figure 5. Daily specific growth rates and biomass concentrations obtained from the kinetics study (SGR = specific growth rate, the numbers refer to the three chambers).

harvested from this three-chamber system. While the system was not optimized for maximum productivity, the study does show the capabilities of the three-chamber chemostat.

A first order computer model was developed and calibrated using data from the kinetics study to allow biomass production estimates at any time during the operation period. Figure 6 illustrates a sample simula-

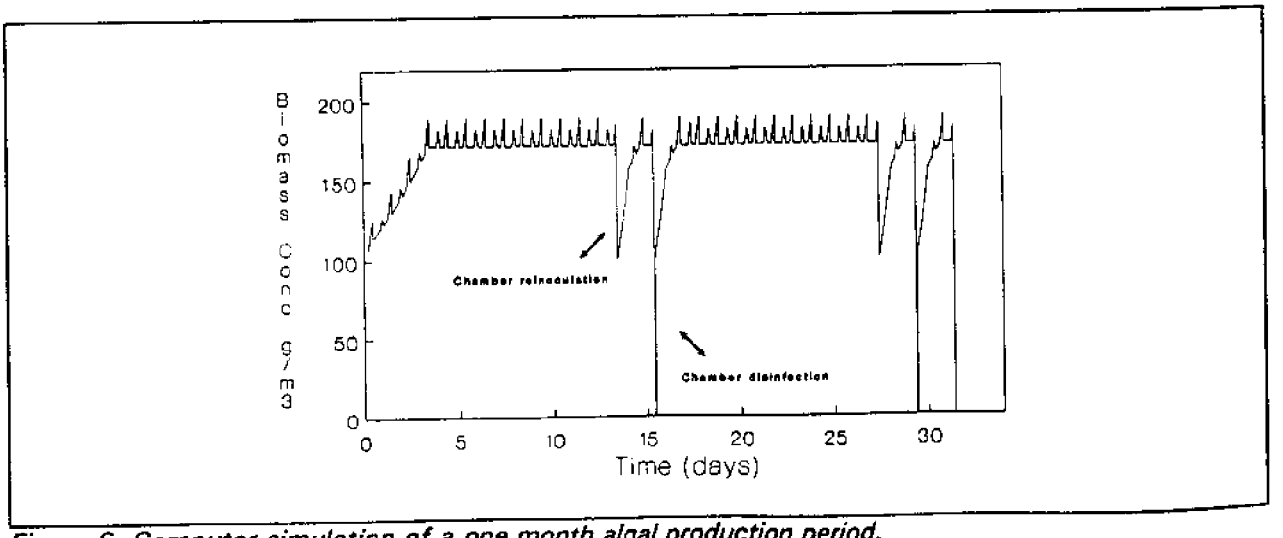

Figure 6. Computer simulation of a one month algal production period.

tion period of 35 days during which time the chambers were harvested four times daily and disinfected twice monthly, representing the same operational conditions of the actual bench-scale system. Daily biomass concentrations fluctuated between 172 and 190 g/m^3, resulting in the typical saw-toothed curve common to semi-continuous cultures. Predicted harvestable biomass (dry) averaged 82 g/m^3 /day with lower averages observed during the first four days due to the initial start-up conditions. The simulation came close to the actual observed harvestable biomass of 72 g/m^3/day. The higher simulation value is the result of completely harvesting the chambers during the disinfection cycle. By changing the input values, various growth, harvesting and disinfection scenarios can be simulated.

Overall Evaluation of the Bench-Scale Algal Chemostat

System evaluation was concerned with the reliability of the control program and overall algal production capabilities. Computer automation increased the operational flexibility of the system while maintaining precise control over the daily processes, dramatically reducing labor requirements. Additionally, the use of the micro-computer resulted in a rather passive chemostat system, requiring only weekly replenishing of stock solutions and intermittent system checks. The organization of the stack and supervisor sequence allowed modifications to be implemented quickly and will facilitate easy expansion to production scale applications.

The three-chamber system reliably produced *Chlorella minutissima* for the five-month test period. James et al. (1988) reported optimized yields of 117 g/m^3/day for *Chlorella* MFD-1, only 38% greater than the yields obtained for this chemostat under less than optimal conditions.

PROJECTION TO PRODUCTION-SCALE ALGAL CHEMOSTAT

The short term goal of this research is to extend the computer control technologies to a production-scale system capable of replacing indoor batch cultures. The ultimate goal, however, is to replace all batch cultures (indoor and outdoor ponds) with computer automated chemostats.

Presently, the researchers at Louisiana State University's Civil Engineering Aquatic Systems Laboratory are working to develop a production-scale computer automated chemostat which will take full advantage of the computer control technologies developed for the bench-scale system. In order for a large-scale system to be efficient and cost effective, the following design criteria must be met:

- system simplicity
- contamination minimization,
- self-cleaning abilities,
- and system stability and reliability.

All four of these criteria can be met through a properly designed system. The rationale for this design is system modularity. In other words, make each component (dosing block, growth chambers, monitoring block, etc.) a unique entity which can be economically mass produced and easily replaced in case of failure.

Of primary importance at the production level is the physical and operational simplicity of the system. As illustrated in Figure 1, the bench-scale system required four chemical feed pumps, two centrifugal pumps, and ten solenoid valves for solution and culture move-

ment. Not only does each electronic component increase the initial cost, but the probability of failure makes such a system uneconomical on a large scale.

The complexity of the bench-scale system was mainly associated with the harvesting and dosing apparatus. Subsequently, this component has been re-designed enabling all withdrawals and additions to be accomplished using the same air/vacuum pump which runs the entire system. As with the bench-scale system, the production system will consist of a series of growth chambers, each containing only one solenoid valve, allowing expansion with additional chambers without a significant increase in electrical components or cost.

Some researchers have expressed doubt as to whether chemostats/continuous cultures can be utilized for mass algal production. The basis of the skepticism, however, stems from the perceived inability to maintain the level of control required for such a system to be successful. Commercial success will be influenced by factors such as the biological aspects of the algal species itself, bacterial contamination, technical expertise, etc. As was demonstrated with the bench-scale system, the hardware components and computer control unit will not be the limiting factors. Based on the success of the bench-scale system, the authors feel a large-scale continuous system is quite feasible given the proper algal species and control strategy.

Acknowledgments

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The Status of Phytoplankton Production in Japan

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ABSTRACT

Some species of phytoplankton are indispensable as feed for the larvae and juveniles of marine fish and invertebrates in Japan. Furthermore, the amount of phytoplankton needed will increase as larval rearing technology improves. However, serious problems, such as the propagation of unfavorable organisms and sudden culture collapses during mass production often disrupt microalgae production. Although various measures are taken to deal with such situations at fish farming centers, we cannot find definitive solutions to these problems.

On the other hand, condensed suspensions of useful phytoplankton have been commercially developed and used as supplementary feeds in Japan. They save labor and their nutritive value is high. Special culture tanks are used in some private companies to produce these commodities.

This paper describes the production status of the major species of phytoplankton used as feed in Japan, and briefly discusses their problems and future prospects.

INTRODUCTION

Thirty years have passed since the first successful larval rearing of economically valuable marine fishes and invertebrates in Japan. During these years, the food value of many species of phytoplankton have been investigated in physiological experiments, feeding experiments and nutritional analyses. As a result, some species were selected as useful food organisms. Nowadays, these phytoplankton are used to feed zooplankton as well as the larvae of molluscs, crustaceans, sea cucumbers and sea urchins. They are also fed to experimentally cultured marine animals such as sea hares and sea squirts. Since the amount of phytoplankton required increases with each new advance in larval rearing technology, producing sufficient quantities is difficult. Therefore, efficient production methods need to be established immediately. Breeding efforts are also expected to give rise to more suitable strains in the future.

PHYTOPLANKTON USED AS FEED IN JAPAN

The main species of phytoplankton cultured in fish farming centers and the names of

Table 1. Species of useful phytoplankton used to rear the larvae and juveniles of aquatic animals in Japan.

Species	Aquatic animals reared
Chaetoceros calcitrans	Bivalves, sea urchins, sea squirts
C. gracilis	Crustaceans, bivalves, sea cucumbers
C. simplex	Crustaceans, bivalves, sea cucumbers, sea urchins, sea squirts
C. ceratosporum	Crustaceans, bivalves, sea cucumbers
Isochrysis galbana *Pavlova lutheri*	Bivalves, sea cucumbers, sea hares
Nannochloropsis oculata	Rotifers
Tetraselmis tetrathele *Tetraselmis* sp.	Rotifers, crustaceans
Nitzschia sp.	Abalones
Navicula sp.	Abalones, turbans
Amphora sp.	Abalones, sea cucumbers
Cocconeis sp.	Abalones
Licmophora sp.	Abalones
Phaeodactylum tricornutum	Spiny lobsters

the animals to which these phytoplankton have been fed are listed in Table 1. Phytoplankton are selected as feed according to the following criteria: they should be cultured easily in large vessels, have a high nutritional value for the target species, be smaller than the mouth of the target species and have a thin cell wall so as to be digested easily, remain suspended in seawater, and finally, they should not cause water pollution or red tides.

A brief description of the useful species of phytoplankton cultivated in Japan and their specific qualities follows.

Chaetoceros

Chaetoceros calcitrans, *Chaetoceros gracilis*, *Chaetoceros simplex* and *Chaetoceros ceratosporum* are all cultivated in Japan. They are the most popular and useful feed species for the larvae of bivalves and crustaceans. *Chaetoceros calcitrans* is a small species which reproduces at relatively low temperatures (10 - 20°C). *Chaetoceros ceratosporum*, by contrast, is a high temperature-tolerant species (Tanaka 1982), and may be cultured in outdoor tanks in the summer.

Chaetoceros is high in ω3 HUFAs (Ackman et al. 1968) and its overall nutritional value is also high. This species can be mass

produced in seawater which has been enriched with inorganic fertilizers and/or fowl droppings, so its production cost is comparatively low. Furthermore, since individual cells seldom form chains during cultivation, they are easily suspended in seawater. However, because their population growth is not always constant (e.g. their lag phases are sometimes too long and their stationary phases are sometimes too short), their consistent mass production in outdoor tanks is difficult.

Nannochloropsis

Nannochloropsis oculata was confused with a marine species of *Chlorella* in almost all previous Japanese scientific reports, but was taxonomically transferred to *Nannochloropsis* in 1986 (Maruyama et al. 1986). Therefore, *N. oculata* is customarily called "marine *Chlorella*" in Japan.

Nannochloropsis oculata is the most important cultured feed for *Brachionus plicatilis* because of its high levels of vitamin B_{12} and eicosapentaenoic acid (EPA) (Table 2). Vitamin B_{12} is essential for rapid population increase in rotifers (Scott 1981, Hirayama and Funamoto 1983), and EPA improves the nutritional value of rotifers for the larvae and juveniles of marine fish (Watanabe et al. 1983). The food value of *N. oculata* for other aquatic animals, however is low. This is because it has a tough cell wall which may prevent animals from digesting it thoroughly.

Nannochloropsis oculata can be extensively cultured year-round in Japan in large outdoor tanks filled with enriched seawater. Furthermore, chemical fertilizers can be used in its mass production. However, the most serious problem in the mass production of *N. oculata* is that cell density suddenly decreases during rainy seasons, preventing us from supplying sufficient *N. oculata* to the rotifers. This phenomenon is poorly understood and severely disrupts the rotifer production process. Predation by the protozoa *Paraphysomonas* sp. (Kanematsu et al. 1989) and cell dissolution by the bacteria *Cytophaga* sp. have been reported as the causes of this phenomenon. Chlorination and sterilization with the antibiotic Terramycin are effective, but cannot definitively prevent the problem. Other possible causes include insufficient light, especially for deep cultures, and contamination with blue-green algae, diatoms and benthic microalgae.

Table 2. Fatty acid composition of some species of phytoplankton used as feed in Japan (% total fatty acids).

	EPA	Total ω3 HUFAs
Tetraselmis tetrathele	6.4	8.1
Nannochloropsis oculata	30.5	42.7
Pavlova lutheri	13.8	23.5
Isochrysis aff. *galbana*	0.5	3.3
Isochrysis galbana	3.5	22.5
Phaeodactylum tricornutum	8.6	9.6
Skeletonema costatum	13.8	15.5

Isochrysis and *Pavlova*

Isochrysis galbana and *Pavlova lutheri* are used as feed for the larvae of bivalves and sea cucumbers. Like *Chaetoceros*, their nutritional value is rated as high. However, they cannot be mass produced in outdoor vessels for the following reasons: they require various vitamins and a stable temperature, and they cannot multiply in a chlorinated medium (Baynes et al. 1979). Therefore, we usually

culture these species in relatively small bottles (10 - 30 liters) in temperature-controlled rooms. Recently, some fish farming centers have begun using *Isochrysis* aff. *galbana* as feed for bivalves instead of *I. galbana*. Although *I.* aff *galbana* can increase rapidly in outdoor tanks in hot weather, its nutritional value is inferior to that of *I. galbana* because of its low EPA content (Table 2).

Tetraselmis

Although *Tetraselmis suecica* is the most popular food organism for cultured bivalves in England (Laing and Helm 1981), *Tetraselmis tetrathele* is generally used to feed the larvae of penaeid shrimp and the rotifer, *B. plicatilis*, in Japan. *Tetraselmis tetrathele* is both eurythermal (5 - 33°C) and euryhaline (6 - 53 ppt), so it can be mass produced in place of *N. oculata* in rainy and hot seasons. *Tetraselmis tetrathele* reproduces rapidly in seawater enriched with fertilizers like those used to culture *N. oculata*. *Tetraselmis tetrathele*, however, has a high linolenic acid content, but much fewer amounts of EPA and ω3 HUFAs than *N. oculata* (Table 2). Thus, the food value of rotifers cultured with *T. tetrathele* is low, and mixed or secondary culture with *N. oculata* or ω yeast is necessary to improve the nutritional value of these rotifers.

Benthic Microalgae

Some species of benthic microalgae are important feed for juvenile gastropods and bivalve spat. The value of *Nitzschia* sp. and *Navicula* sp. for abalone juveniles, *Haliotis discus hannai*, is high (Uki and Kikuchi 1979). Moreover, *Cocconeis* sp., *Amphora* sp. and *Licmophora* sp. are also used as feed in abalone hatcheries. These algae are neither isolated nor cultured separately. They are grown on plastic plates which are put in outdoor tanks with flowing seawater systems. If the productivity of the culture grounds is estimated to be high, no fertilizer is added. The nutritional composition of these algae is poorly known.

COMMERCIAL PHYTOPLANKTON PRODUCTION

Condensed suspensions of freshwater *Chlorella*, *N. oculata*, and *Monodopsis subterraneus* were commercially developed as feed for *B. plicatilis*. These commodities have been used as supplemental feed in many fish farming centers and the amount used is gradually increasing due to their convenience and improvements in their quality. A brief description of their features follows.

Freshwater *Chlorella*

Some strains of freshwater *Chlorella* (*C. vulgaris*, *C. ellipsoidea*, *C. reguralis*, etc.) are heterotrophic or mixotrophic. For this reason, they can be mass produced easily in aseptic, dark conditions, and the production costs are comparatively low. Furthermore, it was reported that a freshwater species of *Chlorella*, *Chlorella vulgaris* K-22, could store various levels of vitamin B_{12} added to the culture medium (Maruyama et al. 1989) in its cells. As stated above, vitamin B_{12} is an essential nutrient for rotifer growth, so this enriched *Chlorella* would appear to be a good feed for rotifers. On the other hand, freshwater *Chlorella* doesn't contain ω3 HUFAs, so the nutritional quality of rotifers fed this algae is

low. Hence, secondary culture of the rotifers with *N. oculata* or fatty acids is necessary.

Nannochloropsis oculata

This commodity consists of a condensed suspension of *N. oculata* whose cell walls have been digested by enzymes. Thus, it can be used to feed brine shrimp, *Artemia salina*, other species of zooplankton which are used as feed (*Daphnia pulex*, *Moina macrocopa*, etc.) and penaeid shrimp larvae, in addition to rotifers. The EPA content of the cells is the same as that of the *N. oculata* mass produced at fish farming centers.

Monodopsis subterraneus

This species of phytoplankton belongs to the freshwater family Eustigmatophyceae and contains a high level of EPA. Condensed suspensions are marketed as feed for *B. plicatilis*.

MASS PRODUCTION OF PHYTOPLANKTON USED AS FEED

A brief description of the mass production methods for phytoplankton used at fish farming centers and in some private companies in Japan follows.

Mass Production Tanks

Transparent circular polycarbonate vessels (30 - 100 liters) and outdoor concrete tanks (10 - 200 m^3) with heaters and aeration systems are usually used for mass producing phytoplankton at fish farming centers. Since the specialized tanks cannot be used as multipurpose tanks, their maintenance is generally difficult. Simple tanks are usually used in the larval rearing process.

On the other hand, special culture tanks are used at some private companies which make health foods. Figure 1 shows the outdoor freshwater *Chlorella* culture tanks at Chlorella Ind. Co. Ltd. The depth of the tanks is shallow and cultures are agitated by stirrers. For these reasons, the growth rate of *Chlorella* is high and stable under suitable conditions. Additionally, freshwater *Chlorella* is cultured heterotrophically or mixotrophically in tanks under dark and sterile conditions with continuous culture methods.

Figure 2 shows the outdoor *Spirulina* culture tanks at Dainippon Ink and Chemicals, Inc. in Thailand. Generally, because the cell wall and cell membrane of *Spirulina* are more fragile than those of single-celled phytoplankton such as *Chlorella*, it is necessary to stir the culture water slowly and uniformly so as not to damage them. If pooling or stagnation occurs, self-shading and a lack of nutrients causes autolysis and promotes the growth of wild algae, protozoa and other unfavorable organisms. This tank has flow rectifying devices which prevent the formation of puddles or stagnant water and provides for uniform flow of the water into the corners of the tank. Furthermore, the tank does not require a great deal of floor space and costs less than semicircular tanks (Shimamatsu and Tominaga 1980, Shimamatsu 1987).

The systems described below have been developed and tested recently.

A photoautotrophic bioreactor is now commercially available. This apparatus is used to promote photosynthesis in a variety of plant cells, algae and microbes. A sunbeam is effectively transmitted from the condenser through optical fiber cables and is then scattered into the reactor tank (Mori 1985).

Various other bioreactors useful for small-scale culture are on sale in Japan.

To mass produce benthic microalgae, a culture vessel composed of a transparent pipe and a brush was developed (Tanaka 1988) (Fig. 3). Its construction is very simple, hence its maintenance is easy. The vessel has a flowing seawater design, so algal growth depends on the productivity of the culture grounds. Cells adhere to the brush where they grow rapidly, and are harvested when the brush is drawn through wires.

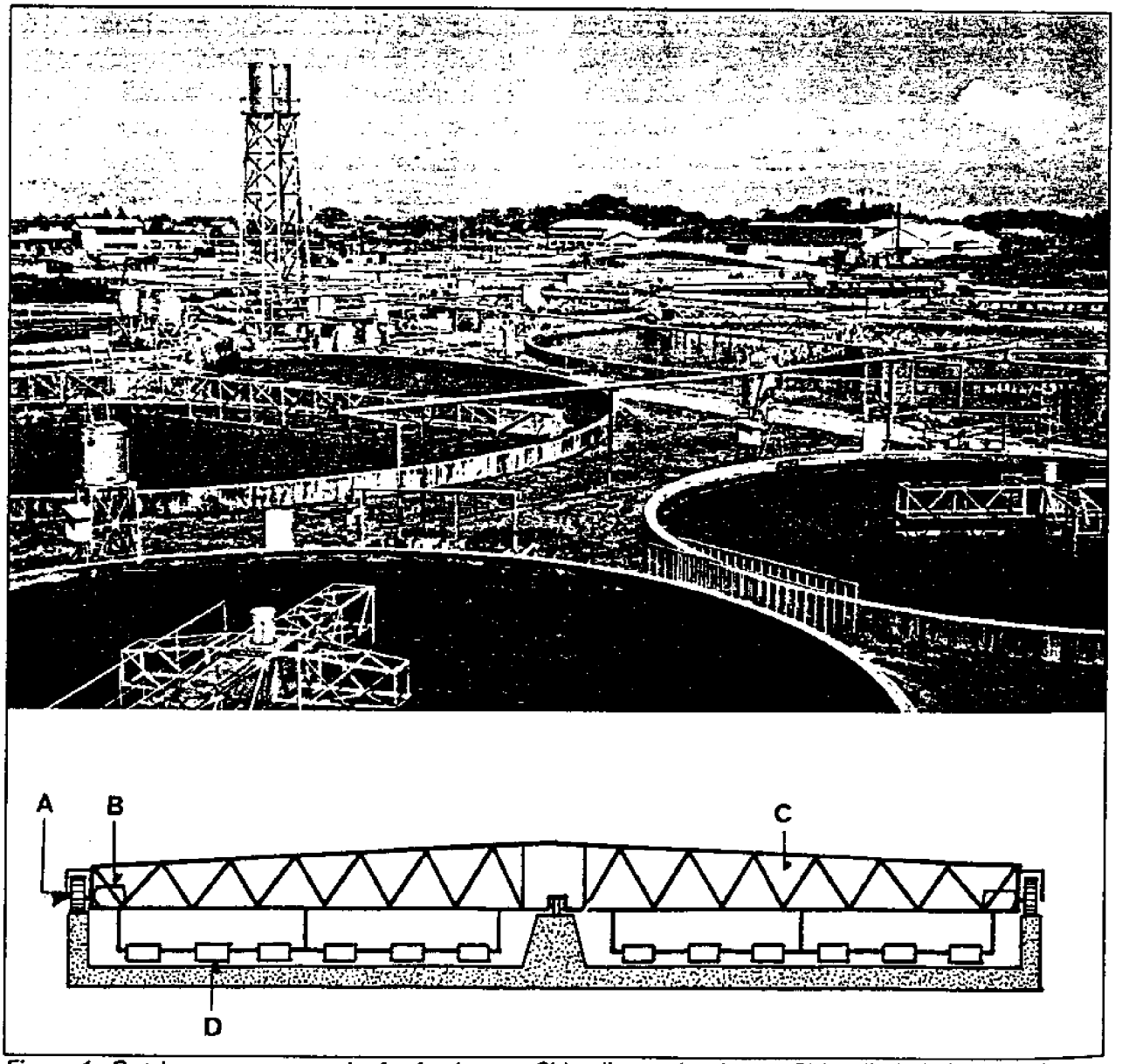

Figure 1. Outdoor concrete tanks for freshwater Chlorella *production at Chlorella Ind. Co. Ltd. A: Driving wheel, B: Motor, C: Agitator, D: Wings for agitation. (Photo provided by Chlorella Ind. Co. Ltd.)*

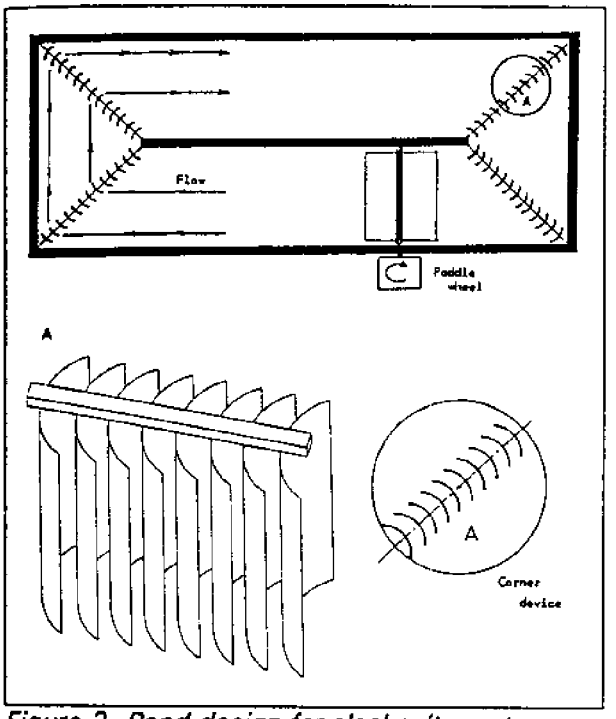

Figure 2. Pond design for algal culture at Dainippon Ink and Chemicals, Inc. (Shimamatsu and Tominaga 1980, Shimamatsu 1987).

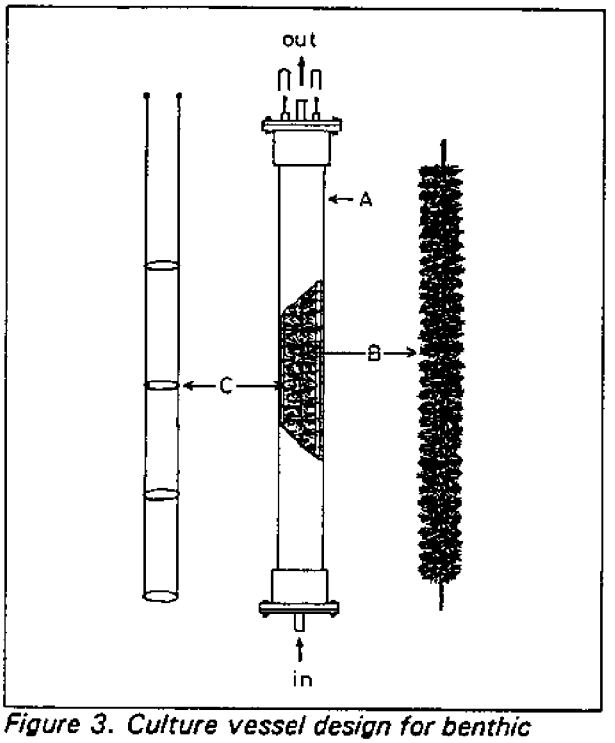

Figure 3. Culture vessel design for benthic microalgae (Tanaka 1988). A: Transparent pipe, B: Brush, C: Wire

Medium for Mass Production

Filtered seawater enriched with chemical fertilizers is the most common medium for the mass production of phytoplankton in large outdoor tanks. Ammonium sulfate, urea, superphosphate and Clewat 32 (a commercial mix of chelated metals) are the major fertilizers. Furthermore, sodium silicate is used in the culture of diatoms. Table 3 shows the composition of the media used in the mass production of *N. oculata* at some fish farming centers. Alternatively, seawater enriched with various reagents such as PES medium (Provasoli et al. 1957) and Guillard F medium (Guillard and Ryther 1962), is generally used for small-scale culture (less than 30 liters). Artificial seawater is seldom used because of its complex composition.

Methods of Sterilizing Seawater

The removal of unfavorable organisms by filtration and/or sterilization is indispensable for unialgal culture. We usually filter seawater with sand and/or glass fiber filters before sterilization, and chlorination is the most popular means of sterilization in large-scale cultures (less than 1,000 liters). A 3 - 10-ppm hypochlorite solution (about 12% chlorine w/v) is added to seawater; a large volume can be treated with this method. However, seawater thus sterilized will not support good growth of *I. galbana*, *P. lutheri* and some species of diatoms.

Sterilization by ultraviolet irradiation and ozone is useful and effective in small-scale cultures (less than 1,000 liters). This method has been gradually introduced to many fish

Table 3. Composition of the media used for the mass production of *N. oculata* in some prefectural fish farming centers.

Fertilizer added per 1,000 L seawater	Fish farming centers					
	Nagasaki	Hiroshima	Kyoto	Ishikawa	Yamagata[1]	Yamagata[2]
Ammonium sulfate (g)	50	100	100	100	50	100
Superphosphate (g)	30	20	15	15	15	15
Urea (g)	25	10	10	10	50	10
Clewat 32[3] (g)	5	4		1		

[1] From April to October.
[2] From November to March.
[3] Clewat 32 is a commercial product composed of micro-nutrients.

farming centers because the capacity of sterilizing machines has been improved. Finally, autoclaving is useful for reliable sterilization of small volumes (less than 20 liters) such as those used for stock cultures.

PROBLEMS AND FUTURE PROSPECTS

Problems

These are the serious problems encountered during the production of phytoplankton feed in Japan:

(1) Some species, especially those belonging to the genera *Nannochloropsis* and *Chaetoceros*, are collected from the water adjacent to fish farming centers, and their taxonomy has not been investigated. For this reason, many have been classified incorrectly and confusion has resulted.

(2) Although many bioreactors and environmental control systems have been developed, the majority of phytoplankton culture is labor-intensive. Mechanization should be important in the future. Continuous or semi-continuous culture methods seem to be useful in small-scale cultures (less than 200 liters).

(3) Recently, the larval rearing of cold- and warmwater species of marine fish and invertebrates has become possible; Japanese hatchery technology has advanced to a very high level. Therefore, high (25 - 35°C) or low (5 - 10°C) temperature-tolerant algae with high nutritional values are needed badly.

(4) Appropriate methods for removing contaminating organisms have not been developed yet.

(5) The reason *N. oculata* populations suddenly decrease during mass production in the rainy and warm seasons remains unclear. Therefore, we cannot take suitable measures to solve this problem.

(6) To preserve phytoplankton without changing their subtle biological characteristics, preservation by freezing or freeze-drying is preferred. However, these methods have never been applied to the preservation of food organisms.

Future Prospects

Many types of artificial diets (including baker's yeast and ω yeast have been developed and their nutritive value has been improved. Recently, it has become possible to obtain these diets easily and cheaply. In the future, the gradual replacement of live feeds with artificial diets will be promoted. However, if the specific nutritional requirements of the reared animals are unknown, we will be unable to develop perfect diets. Furthermore, an excess of artificial diets induces water pollution. Thus, it will be difficult for inert diets to take the place of live feeds entirely. It is more likely that they will be used together with phytoplankton or zooplankton even in the future. Therefore, efficient techniques for the mass production of useful phytoplankton should be established immediately. Furthermore, additional strains which are useful feeds must be bred in the future.

Recently, cellular and genetic engineering techniques have become popular in the breeding of many plants. Moreover, the protoplasts of some algae (including seaweed) have been isolated and breeding experiments have been carried out in some institutes. If the protoplasts of phytoplankton can be isolated and made to reproduce, then cell fusion of useful strains and the introduction of useful genes seems to be possible.

On the other hand, the use of condensed phytoplankton is very convenient and can drastically reduce labor costs. It will, therefore, be used in more fish farming centers in the future. Furthermore, we hope that the quality of the commodities will be improved and that condensed suspensions of other useful phytoplankton, such as diatoms, will be developed.

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The Development of a Phytoplankton Production System as a Support Base for Finfish Larval Rearing Research

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ABSTRACT

The initial focus of research in the Finfish Program at The Oceanic Institute was the control of maturation and spawning in two species; the milkfish, *Chanos chanos*, and the striped mullet, *Mugil cephalus*. Success in this area required a reliable live feeds production system to support larval rearing research. Live larval feeds are cultured in two stages. Phytoplankton are cultured in the first stage. Two species have been produced and tested, *Nannochloropsis oculata* (Eustigmatophyceae) and *Tetraselmis tetrathele* (Prasinophyceae). The algae are fed to the rotifer, *Brachionus plicatilis*, in the second stage. While *B. plicatilis* is the organism ingested by fish larvae, the nutritional value of this package is strongly influenced by the algae that it is fed. This paper deals with the development of a system for algae culture and the problems encountered. Progress made in rotifer culture techniques and larval nutrition at The Oceanic Institute is described in Tamaru et al. (this volume).

INTRODUCTION

The control of reproduction is a significant barrier to progress in fish culture. First, maturation and spawning in captivity must be accomplished. The next major hurdle is the development of successful larval rearing techniques. The complexity of these techniques can vary greatly, depending on the behavior and food requirements of each stage.

In the simplest form of larval rearing, larvae are weaned to a dried or formulated feed immediately following yolk absorption. A slightly more complicated system has developed in bivalve larviculture in which different species of algae may be raised as the primary food for the larvae. In addition, some organisms, such as penaeid shrimp, may be reared on algae during the early larval stages but must be weaned onto zooplankton in later stages.

The culture of some species of finfish requires maintenance of a more complex series of culture systems. In the simplest terms, a food pyramid must be established and controlled to provide the right food organism for each stage of larval development. This may include

culture systems for different species of phytoplankton as well as zooplankton.

Finfish larval rearing often depends on the production of phytoplankton as well as zooplankton. Phytoplankton serve as feed for the zooplankton which, in turn, nourish the fish larvae. Different sizes and types of zooplankton may be raised for different larval stages. Feeds production at all stages must be consistent in quality and quantity for the duration of the hatchery cycle if larval rearing is to be successful.

Phytoplankton and zooplankton production can occupy the majority of the space and labor allocated to larval rearing. Phytoplankton production generally requires the most space. Therefore, any improvements in the production of algae have the potential to improve overall hatchery production.

Improvements in production at The Oceanic Institute's finfish hatchery have taken place gradually over the past six years. The overall goal of the program has been to develop techniques for the maturation, spawning and larval rearing of the milkfish, *Chanos chanos*, and the striped mullet, *Mugil cephalus*. Previous larval rearing efforts indicated that phytoplankton and zooplankton culture systems would be necessary in the development of hatchery techniques. Because of this, research in algae culture has developed as a support activity within the Finfish Program. This places limits on the types of research questions that can be addressed. These limitations, however, have helped to focus efforts toward developing technologies that are practical, have immediate applications and can be readily transferred to other sites.

The finfish hatchery system at The Oceanic Institute is based, in part, on the Japanese style of larval rearing as practiced by Mr. Hiroki Eda. His efforts have provided a foundation for understanding larval culture systems. Mr. Eda's contributions and insights are gratefully acknowledged.

OVERVIEW OF THE FINFISH PROGRAM

The early work done in the Finfish Program at The Oceanic Institute dealt with identifying the conditions under which milkfish, *Chanos chanos*, could be brought through the later stages of maturation and induced to spawn. Maturation and spawning soon became a reality and questions relating to egg viability and larval rearing had to be answered.

Previous work with striped mullet and some preliminary work with milkfish suggested that live rotifers and brine shrimp, *Artemia salina*, would be suitable larval feeds. This was supported by a growing literature based on the use of rotifers, brine shrimp and copepods as feed for the larval stages of many species of marine fish. Brine shrimp offered the advantage of being commercially available as cysts which could easily be hatched to nauplii in 24 to 48 hours. Species or stages requiring a smaller food particle size would have to be fed smaller organisms, such as rotifers. Rotifer production required the production of algae.

Consistency in the quality and quantity of algae produced has resulted in the establishment of a reliable system for rotifer production. This is one of the key factors contributing to the success of the Finfish Program over the past six years. Reliable production levels for the larvae of milkfish and striped mullet were eventually established. Once consistency in larval rearing was established, changes in system design, hatchery management techniques and nutritional quality could

be tested and these factors have been customized for each species. Advances in larval fish biology have allowed the program to explore the potential of culturing other species, such as the threadfin or "moi," *Polydactylus sexfilis*, and the mahimahi, *Coryphaena hippurus*. Other new species are also being considered for future research.

Consistent algal production was not achieved overnight. Continuous improvements in the facilities and culture techniques have been made since our facility was dedicated in 1979. These have led to improved production. We seldom have a production surplus, however, as production increases are usually absorbed by the increasing demands of larval rearing.

Because algae culture is a part of the Finfish Program, the bulk of our efforts have centered on fish reproduction and larval rearing. The current system does not necessarily reflect the state-of-the-art in terms of system design or the latest in scientific technology. It has kept ahead of the demands of larval rearing by anticipating future needs and incorporating new concepts to help meet those needs. Furthermore, the basic algae culture routines have been kept simple so that the technology can be easily transferred to other situations.

THE ALGAE CULTURE SYSTEM AND ASSOCIATED PROBLEMS

A number of questions and problems had to be dealt with as the algae culture system evolved. These revolved around the problem of producing high quality algae on a consistent basis. An algae culture room equipped with air conditioning, shelves, fluorescent lights a variety of glassware, fiberglass cylinders and aeration lines was available at the start. Fresh water is provided by municipal sources and seawater is available from two wells, the Sea Life Park (SLP) well located along the Makapuu shoreline, and The Oceanic Institute (OI) well located further inland. The SLP well is shallow, approximately 10 m deep and provides seawater at 32 ppt. The OI well is approximately 80 m deep and draws water with a salinity of 35 ppt. This water has a higher manganese and iron content than the SLP well water.

Culture Vessels

Indoor cultures are brought up through a series of vessels. Cultures are started in 2-liter flasks (Fig. 1). The second stage consists of 20-liter carboys. Glass carboys have tradi-

Figure 1. Two-liter flask culture illuminated by a single fluorescent light.

tionally been used for this stage; for the past five years, however, we have used polyethylene bags (Fig. 2). This has eliminated cleaning and breakage problems. The final indoor stage is the 160-liter fiberglass cylinders (Fig. 3).

Outdoor cultures are taken through a series of fiberglass tanks containing 500 liters, 5,000 liters (Fig. 4) and 25,000 liters (Fig. 5). Transfers between stages and for harvesting are made with submersible pumps.

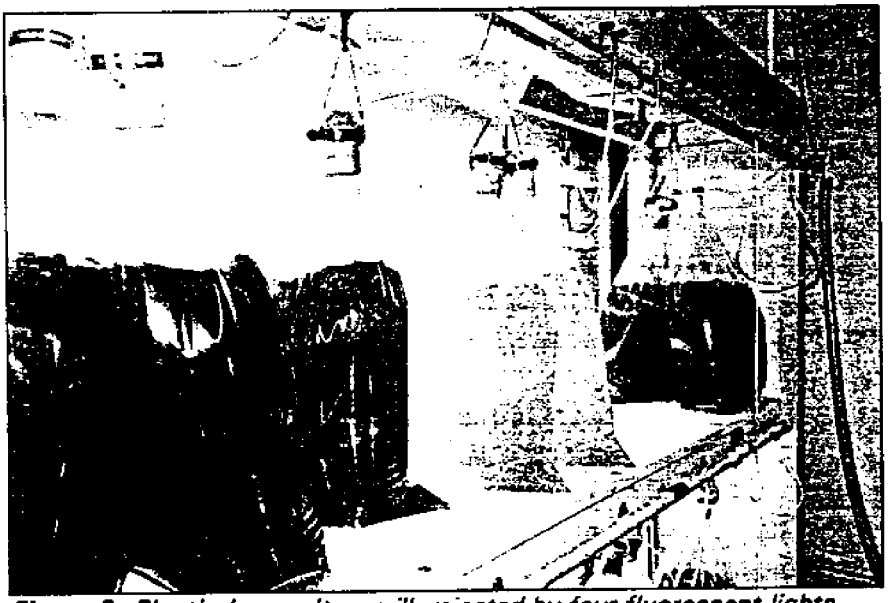

Figure 2. Plastic bag cultures illuminated by four fluorescent lights.

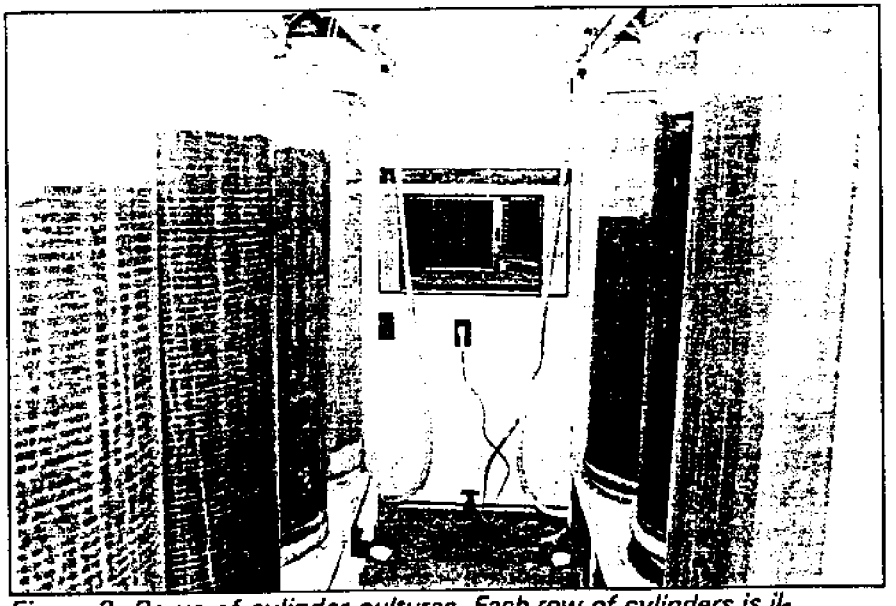

Figure 3. Rows of cylinder cultures. Each row of cylinders is illuminated by eight fluorescent lights (not shown).

Temperature Control

Air conditioning or some other form of temperature control is important in the management of algae cultures. Many species have a distinct range for optimal growth. Some species grow faster at higher temperatures, but these cultures are much more difficult to manage. They may peak too quickly and crash

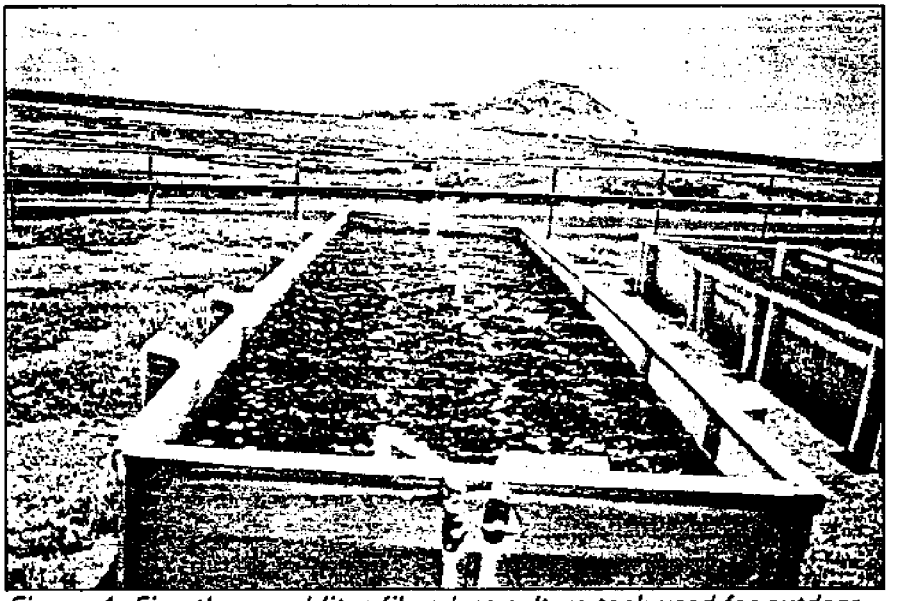

Figure 4. Five thousand-liter fiberglass culture tank used for outdoor phytoplankton production.

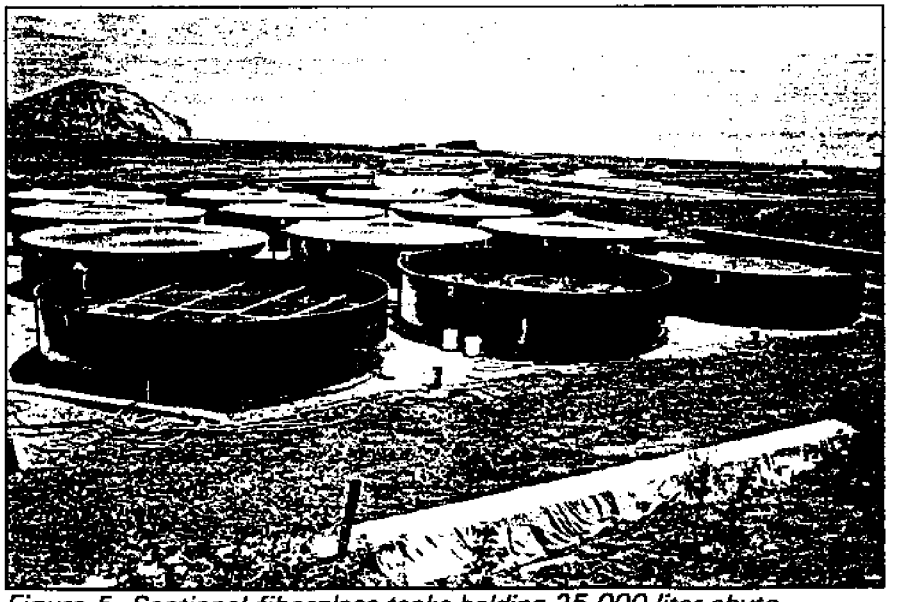

Figure 5. Sectional fiberglass tanks holding 25,000-liter phytoplankton cultures; the final stage of outdoor phytoplankton production. The airline distribution system is visible in the first tank.

before they can be used. Higher temperatures may also have adverse effects on the nutritional quality of the algae.

It is important to manage cultures so that they are in the active growth phase when they are transferred or used as food. At 22 - 25°C, cultures will peak in four to five days consistently. Because of this, inoculation, transfers and harvesting can be planned, allowing for continuous production.

Lower temperatures may also impact the growth of contaminating organisms; the growth of some protozoans and bacteria is slowed at low temperatures. This allows the algae, which are maintained in exponential growth, to reach a harvestable density before contaminants become a problem.

The temperature of outdoor cultures cannot be controlled. These cultures experience higher temperatures during summer periods, due to the higher atmospheric temperatures and longer day length. In Hawaii, summer temperatures in outdoor cultures may regularly climb to 30°C whereas winter temperatures may routinely reach 26°C. Experience has shown that summer cultures of *N. oculata* tend to be much more unstable than winter cultures. The winter season, however, presents other problems for culture growth.

Light Energy

Illumination for indoor algae cultures is provided by fluorescent lights. Single tubes provide approximately 11,000 lux for flask cultures. Carboy and cylinder cultures are equipped with multiple fluorescent tubes which provide approximately 14,000 lux of radiant energy. Excess heat from the lights is minimized by air conditioning.

The indoor cultures are probably light-limited when maximum cell densities are reached. When aeration and mixing are increased, increasing the exposure of cells to light, more dense cultures are achieved. Simply increasing the illumination for carboy and cylinder cultures has also increased maximum density, however, after a certain point, the amount of heat generated by additional lighting makes such modifications impractical.

Outdoor cultures depend upon ambient solar irradiation which varies during the year. In Hawaii, the light:dark photoperiod varies from 11 hours light:13 hours dark during the winter to 13.5 hours light:10.5 hours dark during the summer. Summer cultures experience more sunlight and warmer temperatures. While the increase in available light helps cultures to grow, the warmer temperatures can have adverse effects on some species of algae. Species with a lower temperature optimum may be more prone to crashes during summer months. This is particularly true if cultures are not used immediately and have to be held for a few days after reaching harvest density. Winter cultures may be slower in reaching a usable harvest density due to excessive cloud cover. During periods of consecutive cloudy days, a culture may not grow at all. Furthermore, heavy rain can dilute cultures and cause them to overflow the tanks. Cultures which do grow to harvest density appear to be much more stable than summer cultures; they can routinely be held for several days after reaching harvest density.

Nutrients

The standard nutrient medium used for algae culture is the "Medium-F" presented by Guillard and Ryther (1962). There are a number of variations for this medium including the "F/2 medium" described by McLachlan

(1973). Widespread use has led to the commercial availability of the F/2 medium.

Experience with freshwater and marine algae has shown that higher nutrient levels, particularly nitrogen and phosphorus, may extend the longevity of a culture. This was suggested by Bischoff and Bold (1963) in work with edaphic algae cultures. Using a triple nitrogen (3N) variation of Bold's Basal Medium (BBM 3N) allows maintainance of some cultures for several months without subculture.

Increasing the levels of nitrogen and phosphorus in the standard F/2 medium improved growth in some species of algae. Cultures were stable and usable for longer periods of time, with growth continuing at a decreased rate after the initial period of exponential growth.

It has recently been observed that the trace metal solution of the Medium-F may be inadequate under certain conditions. The specific trace metal component that may be limiting has not yet been identified. Higher culture densities can be attained by increasing the concentration of trace metals in the medium. The problem appears to be related to the source of culture water and may be a site specific problem. It may be that modifications should be made to the trace metal formulation depending on the characteristics of the available seawater. For this reason, we have developed and are currently testing a more complete trace metal mix. This formulation is based on published recipes (McLachlan 1973; Pringsheim 1946; Provasoli 1963, 1968) and analysis of commercially available mixtures. The final formulation should provide a complement of trace metals that are suitable for the species of algae cultured at The Oceanic Institute. Unfortunately, this mixture may not be ideal for the seawater available at other hatcheries.

The current OI Algae Culture Medium used for indoor and outdoor culture is based on Miquel's enrichment solution as modified by Allen and Nelson (1910) for the culture of diatoms. A combination of the high levels of nitrogen, phosphorus and iron provided by the medium, and the trace metal mixture we have developed has enabled us to consistently achieve higher rates of growth in *N. oculata* and *T. tetrathele* with the sources of water that have been tested.

Outdoor cultures utilize the same high levels of nitrogen and phosphorus as indoors, but cheaper, commercially available agricultural-grade components are also utilized. These are generally readily available in other countries, making technology transfer simpler.

Filtration and Sterilization

Filtration and sterilization of incoming seawater is probably the most important means of preventing contamination in indoor algae cultures. There are also various methods of removing contaminants from culture media, including filtration, autoclaving and hypochlorite disinfection.

Stock cultures are maintained in media that has been sterilized via an autoclave. Stocks are transferred regularly using standard microbiological techniques adapted for algae isolation and culture. Guillard (1973), Hoshaw and Rosowski (1973) and Pringsheim (1946) present a number of methods that have been used to isolate and maintain pure cultures of algae. In order to minimize exposure to contaminating organisms, stock cultures are maintained separately from the indoor algae culture room.

All water used in the algae culture room is filtered through 5- and 1-μm cartridge filters. An ultraviolet sterilization system was used in the past. These systems may lose their effectiveness if not properly monitored and maintained. Previous occupants of the algae culture room were not consistent in their use of the ultraviolet sterilizer or the cartridge filters and there were problems with contaminants which became established. Currently, filters are used to remove large particulates but ultraviolet sterilization is not being used.

Carboys and cylinders are filled with seawater and disinfected with sodium hypochlorite. After aerating for 24 hours, the residual hypochlorite is neutralized with sodium thiosulfate according to the recommendations of Hemerick (1973). After another 24 hours the seawater is ready for enrichment and an inoculum.

Outdoor cultures are prepared in a similar manner. A 35-μm mesh bag filter or a 5-μm cartridge filter is used to remove larger particles from the incoming seawater. The 500- and 5,000-liter tanks are disinfected with sodium hypochlorite and neutralized. This minimizes contamination. The 25,000-liter cultures are not chlorinated. This is the final culture stage, and errors in chlorination or dechlorination can have devastating effects on rotifer production and/or the fish larvae, so chlorination has been eliminated from this stage. If contaminants have not been a problem up to this point, they generally do not become one.

All culture vessels are cleaned and disinfected after use. A mild solution of hydrochloric acid is used to remove any residues on the sides of the cylinders and tanks. All vessels are then rinsed well before being refilled with seawater.

Species Selection

A number of factors should be considered when selecting a species of algae to be cultured. In finfish larval rearing, the primary concern is that the alga be easily cultured and satisfy the nutritional requirements of the larvae. Because rotifers must be cultured as an intermediate stage, it is also important that the rotifers grow well on that alga.

Many species of algae have been grown and tested as aquacultural feeds. Their varied sizes and shapes affect their suitability for different organisms. High lipid algae are able to provide for the caloric needs of larvae. Furthermore, highly unsaturated fatty acids, or HUFAs, are considered to be important in developing larvae and proteins are essential to growth and development. These and many other factors can be considered when selecting an algal species.

A unicellular eustigmatophyte called "Japanese chlorella," or *Nannochloropsis oculata*, was tested for milkfish and mullet larviculture. It was relatively easy to culture and appeared to outcompete contaminating organisms. Rotifers grew well on *N. oculata* and larvae exhibited survivals of 20 - 30%. As a result, the OI larval rearing system was centered around *N. oculata*.

Experiments were later conducted to determine if rotifer production could be improved by culturing different species of algae. *Tetraselmis tetrathele*, *Tetraselmis chuii*, *Chaetoceros gracilis* and *Isochrysis galbana* were considered. Because preliminary experiments indicated that *T. tetrathele* may have some advantages over *N. oculata*, further experiments are being conducted with this species.

Contaminants

Contamination is a major problem in a number of algae culture systems. Facilities that have adequately addressed this issue have been able to maintain consistent production for years. For example, the Biological Laboratory, National Marine Fisheries Service in Milford, Connecticut has adapted sterile microbiological techniques for all phases of their indoor culture system (Ukeles 1973, Wickfors 1990). Their methods are quite involved, requiring the sterilization of all media and materials used in the inoculation, transfer and harvesting of cultures in test tubes, flasks and carboys. Some cultures have been growing continuously for over four years and yields are comparable to those achieved in bioreactors. The Milford system would not be cost effective on a commercial scale, but it does demonstrate the potential of a well managed system.

Contaminants have not been eliminated from the system at The Oceanic Institute. Many problems are inherent in the original design and operation of the system. Problems with contaminants have been minimized, however, by preventing them from becoming established in production cultures. This is accomplished with sterilization and disinfection and by maintaining cool temperatures. Cultures are managed as batch cultures and are moved through the system as quickly as possible. In this way, contaminants rarely become numerous enough to cause problems.

Holding cultures beyond the normal growth cycle allows contaminants to become established, so we have developed a protocol for dealing with such cultures. Tanks that are ready for harvesting but will not be used immediately are concentrated by means of a continuous centrifuge. The cells are harvested as a paste and stored in a refrigerator for later use. The algae may then be used as feed for rotifers or as inocula.

One of the most important characteristics of species like *N. oculata* is their ability to simply outcompete contaminating algae and protozoans. *Nannochloropsis oculata* may not be palatable to grazing protozoans. It may also be more effective in competing for nutrients. However, the medium being used is so nutrient-rich it seems unlikely that the algae could effectively tie up all of the excess for its own use.

One alternative explanation is chemical interactions, or allelopathy. Some species can inhibit the growth of other organisms by releasing "toxic" compounds. This type of interaction may explain why cultures of *N. oculata* remain relatively free of contaminants when cultures of other species are easily taken over.

Backup Cultures

One of the most important components of a good larval rearing run is good communication. Everything depends on food being available on a regular basis. Because rotifer production is dependent on phytoplankton production, shortcomings in phytoplankton production can have a significant impact on larval rearing success. Increased demands for rotifers must be communicated to those responsible for rotifer production and the needs of rotifer production must be communicated to those who produce the phytoplankton. Because indoor phytoplankton production must begin seven weeks before spawning, any changes by the rotifer production team must be anticipated and communicated as early as possible.

In the absence of adequate communication and/or foresight, it is important to have backup cultures at all stages. In this way, increasing demands can be addressed almost immediately and adjustments can be made in the production schedule to supplement those needs. Backup cultures are routinely maintained at 30 to 50% in excess of what is required. By staying ahead of the anticipated needs of the rotifer culture and larval rearing groups, adequate amounts of algae are almost always available.

STAGES OF ALGAE CULTURE

Algae culture at The Oceanic Institute takes place in three stages: stock culture maintenance, indoor culture and outdoor culture. Each stage is dependent on the earlier stages to provide clean cultures of good quality.

Algae Culture Schedule

All algae culture activities must be planned two months before the first anticipated spawn. Spawning schedules and experimental designs for larval rearing trials which relate to algae production must be carefully outlined so that all stages of production can be properly coordinated.

Algae production begins approximately seven weeks before the first anticipated spawn. Between seasons, the algae culture room is cleaned and repairs and modifications completed. Flask cultures started from test tubes take about ten days to reach a harvestable density. Each successive stage of culture may take five or six days before it can progress to the next. Bringing up cultures from test tubes to carboys/bags takes about 21 days, and cylinders take another seven days to reach harvest density.

The outdoor routine is based on a four- or five-day cycle at each of the three stages. This adds another 14 days to the production routine. Rotifers must be brought into the production routine one or two weeks before the first spawn.

The growth of indoor and outdoor cultures follows the growth pattern exhibited in Figure 6. First there is a lag or acclimation phase, which is followed by the logarithmic or exponential growth phase. The density or biomass of cells increases at the fastest rate during this phase. The duration of the lag phase can usually be shortened by transferring cultures while they are still in the exponential growth phase.

The transition phase is characterized by a declining growth rate. This usually leads to a stationary phase in which no net growth or increase in biomass occurs. Photosynthesis and cell division may still occur during the stationary phase, but the number of new cells produced will be approximately equal to the number of cells settling out of the water column and/or dying. Therefore, no notice-

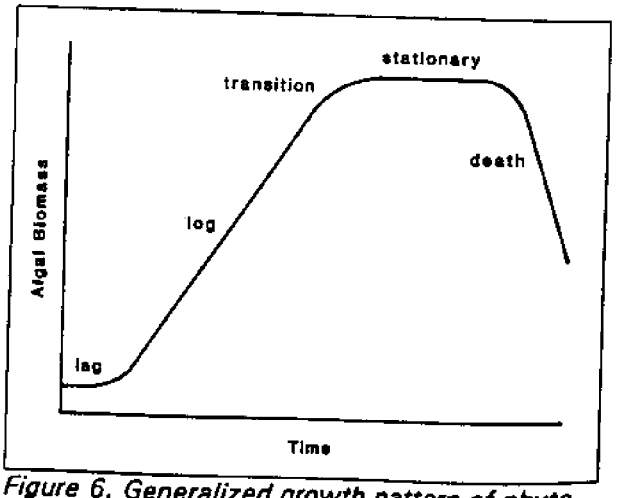

Figure 6. Generalized growth pattern of phytoplankton cultures.

able growth occurs. If cultures are held long enough, they may enter the death phase or "crash," a rapid decrease in biomass.

Cultures at all stages follow the same growth pattern. All other conditions being equal, the rate of growth and the final culture density at the stationary phase may vary depending on the size and shape of the culture vessel, as well as the amount of aeration or mixing.

Algae Stock Culture Room

Stock cultures are maintained in a Stock Culture Room. This room is located and maintained separately from the normal culture facility. It is equipped with an air conditioner to maintain relatively constant temperatures throughout the year. Six shelves are available for cultures and some small-scale experiments. Each shelf is illuminated by a 4-ft. long fluorescent tube. A refrigerator is used for storing certain chemicals and autoclaved media. Finally, an incubator is also available for maintaining cultures on a long term basis.

Seawater for use in stock culture maintenance comes from the SLP well which provides seawater at 32 ppt. This water is considered to be abiotic. The standard OI Indoor Algae Culture Medium is used to maintain stock cultures. All of the marine species cultured do well on this medium. When freshwater species are cultured, Bold's Basal Medium 3N is used (Bischoff and Bold 1963), and *Spirulina* cultures are grown with Zarouk's Medium (Zarouk 1966).

Temperature in the Stock Culture Room varies with the season. In general, it fluctuates between 20 and 25°C over the course of a year. Lighting is constant. The incubator is maintained at 15°C with a short day cycle.

Stock Culture Maintenance Routine

Each month, two or three tubes of each species are subcultured; more tubes are prepared for species in greater demand. Media is autoclaved in the test tubes beforehand, and aliquots from each selected tube are transferred to four new tubes using standard sterile microbiological techniques. Descriptions of some of these techniques modified for algal isolation and culture may be found in Guillard (1973), Hoshaw and Rosowski (1973) and Pringsheim (1946). After all of the transfers have been completed, the tubes are set in racks beneath fluorescent lights. For the next month, each new culture is inverted once each day. Growth in the new culture tubes can usually be observed within a week and is allowed to continue for a month before the next set of transfers takes place. At this time, two or three tubes of each species are selected for transferring, while test tubes which show no growth are discarded. Cultures for starting production flasks are selected from the remaining culture tubes.

In addition to stock culture maintenance, the stock culture room is also used for isolation work. Interesting algal species observed in ponds or from natural blooms in the ocean are sometimes brought in for isolation and subsequent culture. New cultures acquired from outside sources are usually re-isolated before they are used. In this way, pure cultures are readily available and unwanted organisms are excluded from the Stock Culture Room. On rare occasions, stock cultures may need to be re-isolated because of contamination.

Although our focus is primarily on marine species, some freshwater algae have also been isolated and maintained. Liquid and solid media can be prepared according to the needs of the project.

By maintaining clean stocks of algae, contamination problems which have previously impeded consistent, long-term algal production have been virtually eliminated. Contaminants are recognized early in the production cycle and those cultures are replaced by new stocks, which are prepared on a monthly basis. In addition, stock cultures have been given to a number of other research, commercial and educational facilities.

Indoor Culture System

Indoor algae culture takes place in three stages. Culture volumes range from 2-liter flasks, to 20-liter carboys/bags to 160-liter cylinders. Seawater for algae culture is available from the SLP well which has a salinity of 32 ppt.

The temperature of the algae culture room is 20 - 25°C. The temperature varies with the season, being higher during the summer months. Illumination is constant; provided by 6-ft. long, high output fluorescent tubes.

The OI Indoor Algae Culture Medium is used for all stages of indoor cultures. This formulation contains high nitrogen and phosphorus levels and a complete range of trace metals.

Indoor Production Routine

The first stage is the preparation of 2-liter flasks. All flask cultures are filled with media and autoclaved prior to inoculation. The initial inoculation takes place in the Stock Culture Room. The contents of a single test tube are added to a flask of autoclaved medium. This "primary flask" is taken to the algae culture room and aerated. Flask cultures started from test tubes generally take 10 to 14 days to reach harvest density. The primary flask inoculates three to six additional 2-liter flasks, the "secondary flask cultures." These transfers may be staggered over several days so that harvestable flask cultures are available every day.

When sufficient secondary flask cultures have been prepared, carboy cultures can be started. New flask cultures are inoculated with approximately 400 ml from another flask culture. The remaining 1400 ml is used to start a carboy or bag culture. The preparation of enough flask cultures for the production routine takes about 21 to 28 days.

Flask cultures reach the highest densities in the indoor culture system. Inoculation is approximately 10 to 20 million cells/ml and flasks reach a harvest density of 100 to 150 million cells/ml in five to seven days. The general growth pattern observed for flask cultures is presented in Figure 7A. Maintaining cultures at these higher densities allows us to inoculate at higher densities and results in shorter times to reach harvest density. After 21 to 28 days, when enough secondary flask cultures have been prepared, carboy/bag cultures can be started. Four hundred milliliters from one flask is used to inoculate another flask while the remaining 1,400 ml is used to start a carboy or bag culture.

Carboy/Bag Cultures

These cultures were originally housed in glass carboys which hold 16 to 20 liters. Over the past few years, we have begun using plastic bags, which are somewhat easier to handle and do not require cleaning. The hazard of broken or chipped glass edges is also eliminated. The 5-ml thick polyethylene bags measure 16 x 24 inches. They must be stored and handled to avoid puncturing during the assembly process.

The preparation and management of cultures is the same whether glass or plastic is used.

The bags are filled with 16 to 18 liters of seawater that has been passed through 5- and 1-μm cartridge filters. The water and bag are disinfected with commercial bleach, a 5.25% solution of sodium hypochlorite. It is heavily aerated overnight and neutralized with sodium thiosulfate the following day; then the solution is again aerated overnight. Media and algae may be added after the second day. Approximately 1,400 ml of culture is added. These cultures will reach harvest density in five to seven days.

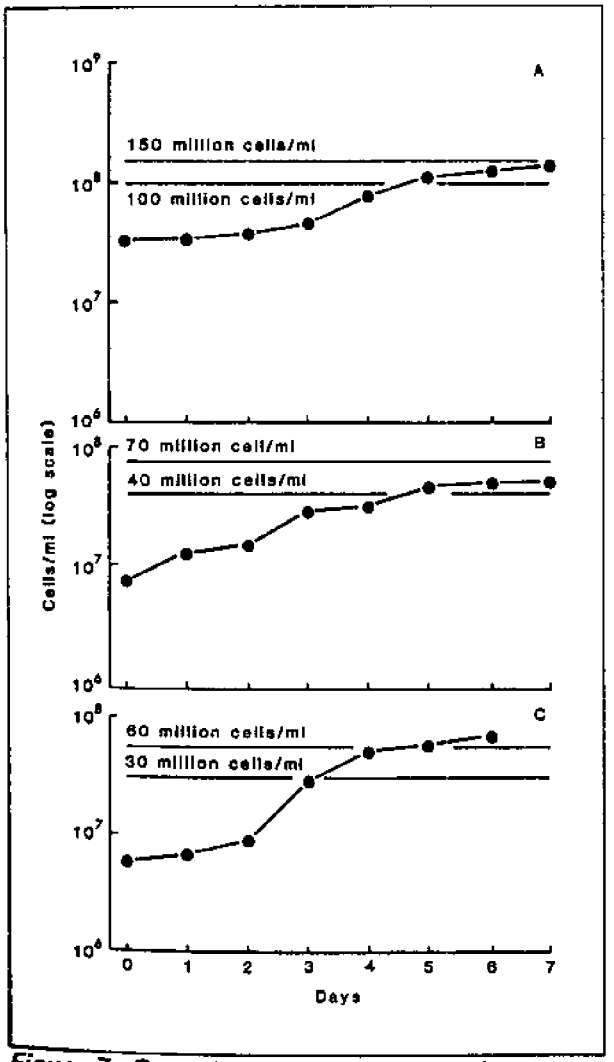

Figure 7. Growth pattern of indoor cultures during 1990/1991. (A) Two-liter flask culture, (B) Twenty-liter carboy culture, (C) 160-liter cylinder culture. The harvest density ranges are indicated on each graph.

Carboy cultures are inoculated at a density of 7 to 10 million cells/ml. Harvest density is between 40 million and 70 million cells/ml. The typical growth pattern observed in carboys can be seen in Figure 7B. If all of the disinfection steps are properly taken, contamination is rarely, if ever observed at this stage. If contamination is observed, new cultures must be brought up immediately to replace contaminated ones.

The bag cultures are used as inocula for two different phases of production. In the indoor system they are used to inoculate 160-liter cylinders that are prepared in the same way as the carboy/bag cultures. The cylinders are cleaned and filled with seawater. Sodium hypochlorite is added to disinfect the system; after 24 hours the residual hypochlorite is neutralized with sodium thiosulfate. After an additional 24 hours, nutrients may be added. A single bag inoculates each cylinder.

Cylinders are inoculated at a density of 5 to 8 million cells/ml. They are usually harvested at 30 to 60 million cells/ml in seven days. The growth pattern is shown in Figure 7C. This graph is from 1990/1991 data and is somewhat atypical of normal cylinder growth. The growth rate was higher than normally observed — harvest density was reached in three to five days rather than six to seven days, as previously observed. In addition, many of the final densities exceeded the 60 million cells/ml that is usually expected.

Cylinder cultures are used for small-scale larval rearing and rotifer culture experiments as well as in the larval rearing system. They also serve as an emergency source of algae in the rotifer production system.

Bag cultures are also used to inoculate the outdoor 500-liter tanks. The total indoor production period from flask to the harvest of carboys/bags and/or cylinders takes between 28 and 35 days.

Outdoor Production System

The outdoor system consists of three stages. Fiberglass tanks are used exclusively. These range in volume from 500 liters to 25,000 liters. The seawater for outdoor cultures may come from the SLP well, salinity 32 ppt, or from the OI well, which has a salinity of 35 ppt.

The nutrient medium for outdoor cultures is based on that used indoors. However, agricultural-grade fertilizers, available through commercial sources, are used instead of laboratory-grade reagents. The formulation is high in nitrogen and phosphorus and this seems to promote rapid growth and sustain cultures for several days when they are not used immediately. A complete trace metal mixture is also added.

Outdoor Production Routine

Cultures are started from carboys/bags from the algae culture room. The algae in each tank is brought up to a maximum density of 20 to 30 million cells/ml before proceeding to the next step. Each stage takes four to five days. Thus, approximately 14 days are necessary before the microalgae is ready to be fed to rotifers.

The first stage is the inoculation of up to five 500-liter tanks with culture from the indoor production system. These tanks are cleaned and dried beforehand. Seawater is added and disinfected with sodium hypochlorite. This mixture is aerated overnight and neutralized with sodium thiosulfate. The seawater is nutrified and the inoculum added.

The growth performance of the 500-liter tank cultures is presented in Figure 8A. Inoculation density varies between 2 million and 5 million cells/ml; the target harvest density is 20 million cells/ml. This generally takes three to four days during the summer months. Cultures usually take longer, four to five days, to reach the same density during winter months. Cloudy weather and rain sometimes prevent cultures from reaching 20 million cells/ml.

The 500-liter cultures are used as the inoculum for the 5,000-liter tanks. Approximately 4,000 liters of seawater is added to each tank and disinfected with sodium hypochlorite. This mixture is neutralized with

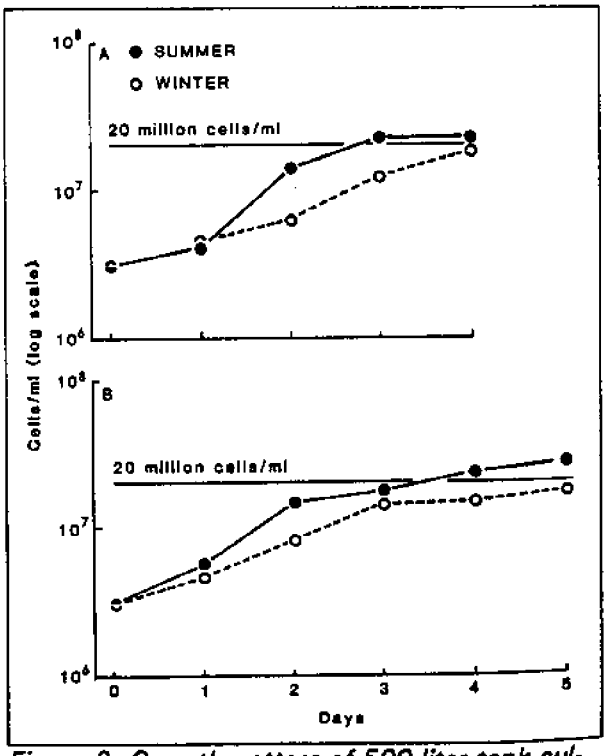

Figure 8. Growth pattern of 500-liter tank cultures (A) and 5000-liter tank cultures (B) in the outdoor phytoplankton production system. Target harvest density is 20 million cells/ml. Growth patterns are presented for summer and winter seasons.

sodium thiosulfate after 24 hours. Following neutralization, the nutrients may be added, followed by an inoculum from a 500-liter tank.

The target density at harvest for the 5,000-liter tanks is 20 million cells/ml. This is usually attained in four to five days during the summer months. During the winter period, the cultures may take five to six days to reach the target density. Also, in the winter 15 million cells/ml may be the inoculation density.

Most of the four 5,000-liter tanks are used during normal production. However, additional 5,000-liter tanks may be maintained as backups. These cultures may be used as a source for new cultures or they may be fed to rotifers.

The 25,000-liter tanks are used for the third stage of outdoor production. Up to five 25,000-liter tanks may be required during the peak of the larval rearing season. The tank walls are scrubbed and disinfected between cultures. Approximately 20,000 liters of seawater is added to the tank. Nutrients are added, followed by 5,000 liters of inoculum.

Cultures in the 25,000-liter tanks are usually inoculated with 1 million to 5 million cells/ml. They reach the target density of 20 million cells/ml in four to five days during the summer months. During the winter, the cultures may not reach the target density until day six. Representative growth curves for 25,000-liter cultures are in Figure 9.

Approximately 6,000 liters of algae is required each day to maintain rotifer cultures, so each tank can be used for three days of feeding, after which they are drained and cleaned in preparation for a new culture. Algae production is staggered to provide a continuous supply of algae.

Yearly Growth Patterns in Outdoor Production Systems

The summer and winter growth patterns for the outdoor production system are distinctly different. The slower growth rate and lower harvest density in the winter are generally attributed to shorter days, lower light intensities and lower temperatures.

Light levels are not monitored, however, salinity and culture temperature are monitored. A decrease in salinity accompanied by lower temperatures would be indicative of a cloudy day with reasonably heavy rainfall. An increase in salinity and higher temperatures would suggest a day with high solar irradiation.

The number of days per month of increasing or decreasing salinity is presented in Figure 10. This data is from 1989. Corresponding temperature data is presented in Figure 11A. Heavy rains, increased cloud cover, lower temperatures and lower levels of solar irradiation occurred during the months of January and February and early in March of 1989. The production data for 1989 is presented in Figure 11B. The values represent the percentage of cultures each month that reached the target density of 20 million cells/ml. Less than 30% of the cultures

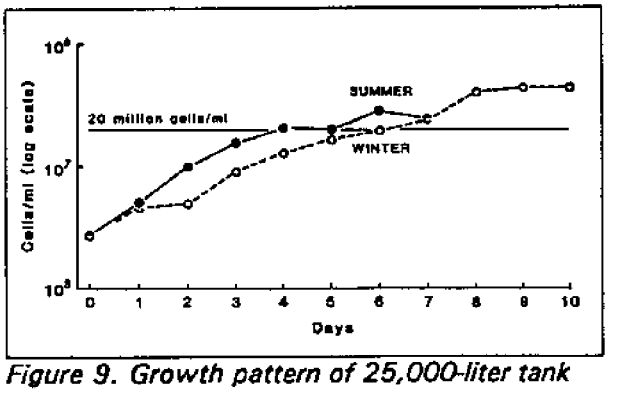

Figure 9. Growth pattern of 25,000-liter tank cultures. Target harvest density is 20 million cells/ml. Growth patterns are presented for summer and winter seasons.

reached the target density from January through March.

Late March and April appear to be transition periods to summer conditions. May, June, July and August are typical of the summer periods with high incident light levels and higher temperatures. The larval rearing season ends in September or October. More than 80% of the cultures reached the target density of 20 million cells/ml during this period.

The number of outdoor production cultures is reduced after the end of the larval rearing season, between October and November. Cultures are not begun and harvested on a regular schedule. Temperatures do begin to decrease in November. While the weather may become more rainy and cloudy, these conditions do not present a problem until December and January when cultures for the next larval rearing season are again brought outdoors.

SUMMARY

The phytoplankton production system of The Oceanic Institute has supported the increasing demands of rotifer production for larval rearing research for the past six years. Production goals have been established at a minimum density of 20 million cells/ml for the eustigmatophyte *Nannochloropsis oculata*. Except for periods of poor weather, these goals have been consistently achieved throughout the year. The data presented in this paper is primarily from the 1989 larval rearing seasons for mullet and milkfish. During that year, approximately 2 million liters of algae were produced and the demand for algae continued to increase. When space is not available for increasing the production area, attempts have been made to increase production by refining techniques for managing cultures; thereby improving growth rates and harvest densities.

There are seasonal differences in the growth of outdoor cultures. Winter cultures appear to be limited by cooler temperatures and less available light. It appears that growth

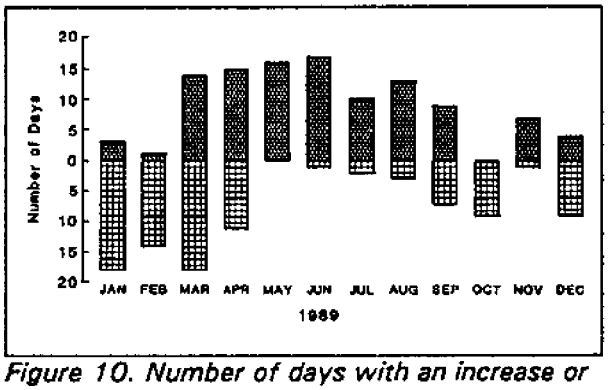

Figure 10. Number of days with an increase or decrease in salinity of algae cultures. The data is summarized per month for 1989. An increase in salinity suggests warm days with high light intensities. A decrease in salinity is caused by rain and suggests cooler weather with heavy cloud cover.

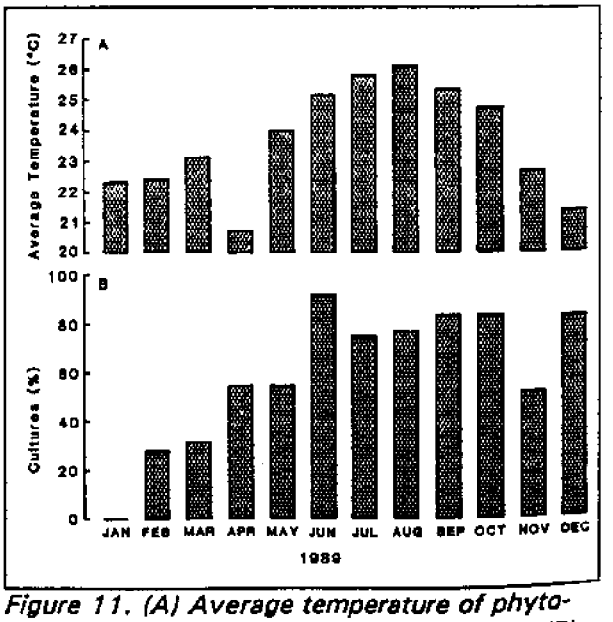

Figure 11. (A) Average temperature of phytoplankton cultures for each month in 1989. (B) Percent of cultures which reach the target density of 20 million cells/ml. The data is summarized for each month in 1989.

during the winter months can be improved by increasing light. This can be achieved by increasing light intensity or lengthening the photoperiod. Providing additional light in an efficient manner appears to be the next major hurdle for both the indoor and outdoor production systems.

This paper has discussed the problems encountered by the phytoplankton production team at The Oceanic Institute along with resolutions which have been implemented. It is hoped that they will provide some insight for those who are developing new production systems. The most serious problems must be addressed first. For example, the problem of light limitation was secondary until problems with water quality, nutrients, aeration and contamination were resolved. To optimize production, each problem area must be clearly identified, defined and resolved according to the local situation.

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Heterotrophic Microalgae Production: Potential for Application to Aquaculture Feeds

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ABSTRACT

Phytoplankton comprise the base of the food chain in the marine environment and, as such, are essential to all life in the oceans. Therefore, it is not surprising that a number of marine organisms seem to have an absolute requirement for microalgae in their diet at some stage in their development. Recently, research has indicated that this requirement is due at least partially to the need for long chain ω3 highly unsaturated fatty acids (ω3 HUFAs) synthesized by the algae. Due to the importance of phytoplankton feeds to aquaculture, many producers of bivalve mollusks and crustaceans must devote significant portions of their time and resources to growing algae. In many cases, the algal production systems used are inefficient and expensive. Martek Corporation, a U.S. company devoted to microalgal biotechnology, is engaged in a program to develop inexpensive, nutritious microalgal feeds for aquaculture through the use of heterotrophic (without light) growth conditions.

BACKGROUND: HETEROTROPHY VS. PHOTOAUTOTROPHY

Algae are, for the most part, photoautotrophs, meaning that they derive their energy from light and the carbon necessary for building biomass from carbon dioxide by the process of photosynthesis. Heterotrophs, on the other hand, acquire both their energy and carbon requirements from organic compounds in the form of sugars, fats, organic acids or any number of other substrates. Since the metabolic pathways by which heterotrophs derive energy from organic compounds are virtually universal, many photoautotrophic algae have been found to be capable of heterotrophic growth (Droop 1974, Neilson and Lewin 1974). Even among those algae that display no heterotrophic capability, many can grow at an increased rate by simultaneously utilizing organic carbon and light energy (mixotrophic growth; Droop 1974).

Photoautotrophic cultures of algae useful as feeds for aquaculture are usually produced through one of two methods:

Outdoor ponds. Algal production in outdoor ponds is relatively inexpensive since it relies on natural sunlight for energy. However, this method is only suitable for a few, fast-growing species due to problems with contamination by predators, parasites and "weed" species of algae. Furthermore, production outdoors requires a large area of suitable land and is subject to weather unpredictability.

Indoor culture. This method allows the grower to produce unialgal cultures while maintaining some control over contamination. However, due to space, energy and skilled labor requirements, it is more expensive than outdoor culture.

Heterotrophy has certain advantages over photoautotrophy when it comes to the production of biomass.

Production Costs

For photosynthetic algae, light can be thought of as an essential nutrient, without which the cells cannot grow. When light (or any other essential nutrient) becomes limited in supply, it will slow growth and possibly affect the biochemical content of the cells. In algal production systems, light is almost invariably the growth-limiting nutrient. This is because as the algae grow they effectively shade each other from the light source. In most algal feed production systems, either indoors or in open ponds, growth becomes light-limited when the culture cell density reaches ca. 5×10^6 cells/ml. This density corresponds to ca. 100 mg of dry biomass per liter of culture. Higher densities of 5 g/liter or more can be attained by increasing the light intensity, but the energy cost of this approach is too expensive for indoor culture and is only appropriate outdoors for a few algal species at a limited number of locations (Kyle and Gladue 1990, Soong 1980).

By contrast, industrial fermentations of heterotrophic yeast and bacteria often produce cultures with densities of 40 - 140 g/liter. Heterotrophic algal densities in this range have been attained at Martek and at other facilities (Soong 1980). At these densities the same amount of algae normally produced in a 10,000 liter tank at a hatchery could be grown in a bench-top 10-liter fermentor. Besides the obvious savings in the amount of space and water used for algal cultures, there would be a concomitant savings in the labor and pumping capacity involved in handling large volume cultures.

Production costs for outdoor ponds of photosynthetically grown algae are in the range of ca. US$4 - 20/kg dry biomass (DePauw and Persoone 1988). However, these outdoor culture systems must cope with contamination by predators and weed species of algae, and rely on good weather and sunlight. The hidden costs of outdoor culture include poor batch-to-batch consistency and unpredictable culture "crashes" caused by changes in weather, sunlight and water quality. In addition, many algal species that are desirable as aquaculture feeds are not suitable for outdoor culture (Ryther and Goldman 1975).

Production costs for indoor photosynthetic algae culture range from $160 to more than $200/kg of dry biomass (DePauw and Persoone 1980). These high costs are partially offset by the advantage of being able to control the algal species being grown and the conditions of culture. However, indoor culture requires a tremendous amount of space and time and is often subject to the culture crashes that plague outdoor culture.

Heterotrophic production of microalgae can be performed for less than $20/kg of dry

biomass using large fermentors as culture vessels (Soong 1980). To date, this approach has been economically feasible with very few strains of algae. However, calculations based on standard models for large scale fermentations (Kalk and Langlykke 1986) for some of the heterotrophic algae being tested at Martek project costs of $2 - 25/kg. Improvements in growth rates and process conditions could lower these production costs to less than $1/kg, as is the case with some bacteria (Crueger and Crueger 1989).

Contamination Control

Contamination of algal cultures, either by predators such as rotifers, copepods and protozoa, or by unwanted "weed" species of algae, is a constant threat with both open pond and indoor photosynthetic algal production systems. This often means loss of an entire crop. Contamination of a heterotrophic culture by fungi or bacteria can be equally devastating, but the methods used to prevent such an occurrence are more sophisticated. Heterotrophic cultures are grown in fermentors that are specially designed to allow steam sterilization of the medium and vessel prior to inoculation. Furthermore, air entering and leaving the vessel is filter sterilized to prevent transport of any microbes. These methods have proven effective in industrial microbiology over the past 50 years (Crueger and Crueger 1989).

Quality Control

Quality control of photosynthetic algal feeds depends on water quality, control of nutrients required by the algae, and physical parameters that affect growth such as light, temperature and pH. In both indoor tanks and outdoor ponds these factors can be extremely difficult to control (DePauw and Persoone 1988). With the two to three orders of magnitude decrease in volume afforded by heterotrophic production, as well as the sophisticated control equipment that is standard with most fermentors, quality control can virtually eliminate batch to batch variations.

Nutritional Value

It has been repeatedly demonstrated that the nutritional value of algal feeds varies significantly between species and even between different strains of the same species (Enright et al. 1986, Ryther and Goldman 1975). This variation can affect growth rates, mortality, time to maturation and disease resistance of the fish or shellfish consumers (see Volkman et al. 1989, for references). Mixtures of algal strains usually support better growth than any one strain alone (Enright et al. 1986). Yet, due to space limitations and the growth characteristics of different algae, most aquaculture operations can only afford to supply a few different strains for feed. These strains are not always the most nutritious so much as the easiest or least expensive to grow. The reduction in cost allowed by heterotrophic production should allow more experimentation with mixtures of different strains as feeds. Eventually, specific diets promoting optimal growth of bivalves and crustaceans at each growth stage may be formulated.

HETEROTROPHIC PRODUCTION SYSTEMS: FERMENTORS

Fermentation technology developed over the past five decades for the large-scale industrial production of bacteria and yeasts can be readily adapted to heterotrophic algal

production. The same fermentor vessels used for other microorganisms are suitable for growing heterotrophic algae (Fig. 1). Off-the-shelf instrumentation is available for monitoring and controlling a number of parameters that affect cell growth and biochemical content. In addition, a wealth of information is available in the industrial microbiological literature on methods for optimizing productivity and minimizing costs. All that prevents the routine fermentative production of microalgae is the lack of knowledge concerning their heterotrophic potential.

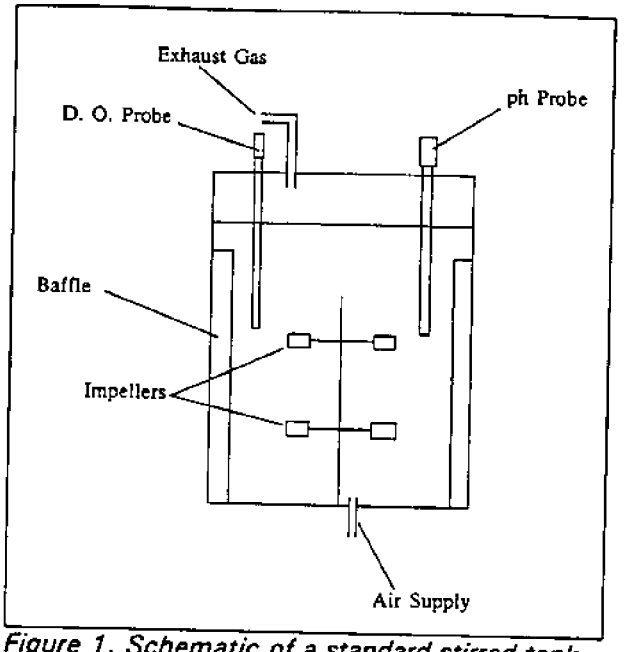

Figure 1. Schematic of a standard stirred-tank fermentor. Instrumentation for measurement and control is available for the following parameters; temperature, pressure, air flow rate, turbidity, pH, dissolved oxygen and agitation speed.

HETEROTROPHIC POTENTIAL OF MICROALGAE

Microalgae are generally considered to be strictly photosynthetic organisms. However, a number of studies have demonstrated that there are a significant number of species with heterotrophic or mixotrophic growth capability (Droop 1974, Hellebust and Lewin 1977, Neilson and Lewin 1974). Furthermore, these heterotrophic species exist within nearly every taxonomic class of algae (Table 1).

Most of the studies that have investigated heterotrophy in algae have approached the question from an ecological point of view (Droop 1974, Hellebust and Lewin 1977). The primary concerns have been the amount of organic carbon removed from the aquatic environment by algae (compared to bacteria or protozoa) and whether algae can supplement photosynthesis by uptake of organic compounds. In most cases, these studies have tested algal growth in media containing low concentrations of organic matter, as would be found in the natural environment. Many algae have been labelled "obligate" photoautotrophs as a result of their inability to grow heterotrophically under these conditions. However, "obligate" photoautotrophy is only useful to describe algal behavior for the exact conditions under which the tests were performed. In many cases, algae labelled as "obligate" photoautotrophs have subsequently been shown to have heterotrophic growth ability. Two examples will illustrate this point.

1) *Brachiomonas submarina* and some strains of *Haematococcus pluvialis* can reduce nitrate when growing photosynthetically but are unable to do so in the dark. When supplied with a reduced nitrogen source such as ammonia, however, they are capable of heterotrophic growth (Neilson and Lewin 1974).

2) *Prymnesium parvum* and *Chroomonas* (*Pyrenomonas*) *salina* are unable to grow heterotrophically on the low concentrations of glycerol that might be found in the natural

Table 1. Algal genera used as aquaculture feeds and/or capable of heterotrophic growth.

Algal class	Genus	Used as feed	Dark growth
Bacillariophyceae	*Actinocyclus*	+	
	Amphora		+
	Bellerochea	+	
	Coscinodiscus		+
	Chaetoceros	+	
	Cylindrotheca	+	+
	Cyclotella	+	+
	Ditylum	+	
	Melosira		+
	Nitzschia	+	+
	Navicula		+
	Phaeodactylum	+	
	Skeletonema	+	
	Thalassiosira	+	
Chlorophyceae	*Ankistrodesmus*		+
	Astrephomene		+
	Brachiomonas	+	+
	Bracteococcus		+
	Carteria	+	
	Chlamydobotrys		+
	Chlamydomonas	+	+
	Chlorella	+	+
	Chlorococcum	+	
	Chlorogonium		+
	Dunaliella	+	
	Gonium		+
	Haematococcus		+
	Neochloris		+
	Nannochloris	+	
	Oocystis		+
	Polytoma		+
	Polytomella		+
	Prototheca		+
	Scenedesmus	+	+
	Spongiochloris		+
	Stephanosphaera		+
	Stichococcus		+
	Volvulina		+
Chrysophyceae	*Chromulina*	+	
	Monas		+
	Ochromonas		+
	Poterioochromonas		+

Table 1. Continued.

Algal class	Genus	Used as feed	Dark growth
Cryptophyceae	*Chilomonas*		+
	Chroomonas	+	+
	Cryptomonas (*Pyrenomonas*)	+	+
	Hemiselmis	+	
	Rhodomonas	+	
Cyanophyceae	*Anabaena*		+
	Anacystis		+
	Aphanocapsa		+
	Calothrix		+
	Chlorogloea		+
	Fremyella		+
	Lyngbya		+
	Nostoc		+
	Oscillatoria		+
	Phormidium		+
	Plectonema		+
	Spirulina	+	
	Tolypothrix		+
	Westiellopsis		+
Dinophyceae (Pyrrophyta)	*Crypthecodinium*		+
	Gymnodinium	+	
	Gyrodinium		+
	Gonyaulax	+	
	Heterocapsa	+	
	Oxyrrhis		+
	Prorocentrum	+	
	Scrippsiella (*Peridinium*)		+
Euglenophyceae	*Astasia*		+
	Euglena		+
Eustigmatophyceae	*Nannochloropsis* (Marine *Chlorella*)	+	
Prasinophyceae	*Micromonas*	+	
	Pyramimonas	+	
	Tetraselmis	+	+

Table 1. Continued.

Algal class	Genus	Used as feed	Dark growth
Prymnesiophyceae (Haptophyceae)	*Coccolithus* (*Emiliania*)	+	
	Cricosphaera	+	
	Dicrateria	+	
	Isochrysis	+	
	Monochrysis (*Pavlova*)	+	
	Prymnesium		+
	Pseudoisochrysis	+	
Xanthophyceae	*Botrydiopsis*		+
	Bumilleriopsis		+
	Chlorellidium		+
	Olisthodiscus	+	
	Tribonema		+

Information in this table was adapted from the following references; DePauw and Persoone 1988, Droop 1974, Guillard 1975, Hellebust and Lewin 1977, Neilson and Lewin 1974, Ukeles 1980.

environment, although they can do so when supplied with very high concentrations (0.25 M) (Droop 1974).

There are three main hypotheses explaining the biochemical basis of obligate photoautotrophy in algae in experimental settings (Droop 1974, Neilson and Lewin 1974). These hypotheses can also be used to devise methods to circumvent the biochemical deficiencies responsible for lack of growth in the dark. The postulated causes and the strategies used to attack them are listed below.

Enzymatic deficiencies: Some algae lack the enzymes necessary for the catabolism of certain organic substrates (Droop 1974, Neilson and Lewin 1974). This is generally true of the blue-green algae (cyanobacteria), which lack one or more of the enzymes of the tricarboxylic acid cycle and thus cannot grow on acetate (although at least one blue-green, *Chlorogloea fritschii*, is an exception). However, the lack of an enzyme involved in catabolism of one substrate will not necessarily inhibit growth on other substrates. Therefore, one strategy to induce enzyme-deficient algae to grow heterotrophically is to supply a medium containing a variety of carbon sources, capable of being catabolized by different pathways.

Permeability barriers: Before it can be catabolized, a substrate must first be transported across the cell membrane into the cell. Many algae apparently lack efficient uptake mechanisms for organic carbon sources (Droop 1974, Hellebust and Lewin 1977). To overcome this nutrient transport deficiency algae can be tested for heterotrophic growth on substrates that fit one of the following criteria:

- Substrates that are widely utilized by algae, as reported in the literature (Hellebust and Lewin 1977, Neilson and Lewin 1974).

- Substrates that are capable of some degree of passive diffusion across cell membranes, such as glycerol, acetic acid and lactic acid (Neilson and Lewin 1974).
- Substrates found naturally in the alga's environment. Algae are more likely to have transport mechanisms for compounds that they frequently encounter in nature. For example, epiphytic diatoms, growing upon seaweed, have been shown to be capable of heterotrophic growth on amino acids and small organic acids excreted by the seaweeds (Lewin and Lewin 1960, Hellebust and Lewin 1977).

Limited respiratory capacity: Algae survive daily dark periods by respiring their reserve materials to provide the energy necessary for cell maintenance. However, the energy produced during respiration may not be sufficient for growth or active transport of organic substrates. In a natural setting, this characteristic would be of value to the cell. Limited respiratory capacity would prolong the supply of the reserve material, while still providing enough energy for cell maintenance until the cells were again exposed to light (Neilson and Lewin 1974).

Algae with limited respiratory capacity can sometimes be induced to grow heterotrophically if they are provided with a high energy carbon source as well as sufficient growth factors, such as vitamins, amino acids (or other reduced nitrogen sources) and purines and pyrimidines as building blocks for nucleic acids. The inclusion of growth factors in the medium may stretch the limited respiratory capacity of the algal cells by eliminating the need to synthesize many of these building blocks.

RESEARCH PROGRAM AT MARTEK

The algal feeds research program at Martek seeks to develop inexpensive, highly nutritious algal feeds for aquaculture. This goal is being approached from two directions:

- Algae known to grow heterotrophically are being tested as feeds.
- Algae currently used as feeds are being tested for heterotrophic growth potential.

Our approach for each algal strain follows a stepwise pattern.

Axenic cultures are established. This is done by plating, antibiotic treatment, micropipetting or a combination of all three methods (Guillard and Keller 1984).

Heterotrophic growth capability is assessed. Screening is performed in test tube cultures using media designed to support dark growth of fastidious strains. These media are designed on the basis of the hypothetical causes of "obligate" photoautotrophy as outlined above.

Heterotrophic growth parameters are determined. Those strains that exhibit heterotrophic growth in the screen (as well as known heterotrophs from the literature) are grown in shake flask cultures to determine growth rates, nutrient yields (grams of biomass per gram of substrate for carbon, nitrogen, phosphorus, silicon, vitamins, etc.) and biochemical contents (protein, lipid, carbohydrate and $\omega 3$ fatty acid content). All of this information is valuable in making decisions to scale-up production of an alga.

Fermentation scale-up potential is assessed. Those algae that exhibit desirable qualities (e.g., high growth rate, high $\omega 3$ fatty acid content) are cultured in bench scale fermentors (2 liters) to determine their ability to attain high biomass densities (10 g dry weight

/liter) and to estimate production costs for large-scale culture (200 - 200,000 liters).

Feeding tests are performed. Test quantities of the algae are produced in fermentors (2 - 200 liters). Biomass is sent to various research and commercial facilities for testing as feed for a number of cultured fish and shellfish.

Martek has identified over 80 strains of heterotrophic algae from within its culture collection of 800+ strains. Testing of these heterotrophs as feeds is just getting underway. Preliminary results indicate that some of these strains may be suitable as feeds for oysters and rotifers.

CASE STUDY 1: *POTERIOOCHROMONAS MALHAMENSIS*

Poterioochromonas (formerly *Ochromonas*) *malhamensis* is a flagellated chrysophycean alga that is unusual in its ability to grow more rapidly heterotrophically than photosynthetically (Droop 1974). By using basic process optimization techniques the biomass productivity of this alga was increased by more than ten-fold. The process changes leading to this improvement consisted of changing the carbon source from sucrose to glucose, instituting pH control, adding a specific type of yeast extract and switching from a magnetically driven to a top-driven fermentor design. At this point the alga was deemed suitable for scale-up to production levels (Table 2).

CASE STUDY 2: UNIDENTIFIED DIATOM

As part of a continuing screening program for microalgal producers of ω3 HUFAs, diatoms were isolated from seaweed. These diatoms were screened for rapid heterotrophic growth and for the presence of significant quantities of the ω3 HUFA, eicosapentaenoic acid (EPA). One of these strains was chosen for growth optimization in our bench-scale fermentors. Automatic control of dissolved oxygen allowed biomass densities to be increased five-fold and increased the content of EPA (Fig. 2). Adjustments in nutrient feed rates and inoculum preparation effected additional improvements in productivity (Table 3).

Table 2. Process development for *P. malhamensis* production.

Process improvement	Species growth rate (per hour)	Maximum density (g/liter/hour)	Productivity* (g/liter/hour)
Initial conditions	0.05	2	0.10
Sucrose → glucose	0.06	2	0.12
pH control	0.06	4	0.24
Add yeast extract	0.12	4	0.48
Change fermentor design	0.12	12	1.44

*Productivity is idealized as the specific growth rate x the maximum density to indicate the maximum potential for continuous culture. This concept is used to estimate production costs for either continuous or batch culture.

DISADVANTAGES OF HETEROTROPHIC ALGAL PRODUCTION

Whereas photosynthetic production of algae is labor and space intensive, heterotrophic production is capital and technology intensive. The cost of a fully instrumented bench-top fermentor with a two liter capacity is in the range of US$5 - 10,000 while a 10,000 liter production fermentor would cost more than $1 million (Kalk and Langlykke 1986). In addition to the fermentor itself, other facilities necessary for production include a steam generator, an air compressor and a clean room for maintenance and transfer of axenic cultures of the algae.

Because of these requirements, it is unlikely that heterotrophic algal production would be feasible for most individual aquaculture facilities. Instead, due to the importance of economies of scale, existing fermentation facilities may be leased for large production runs.

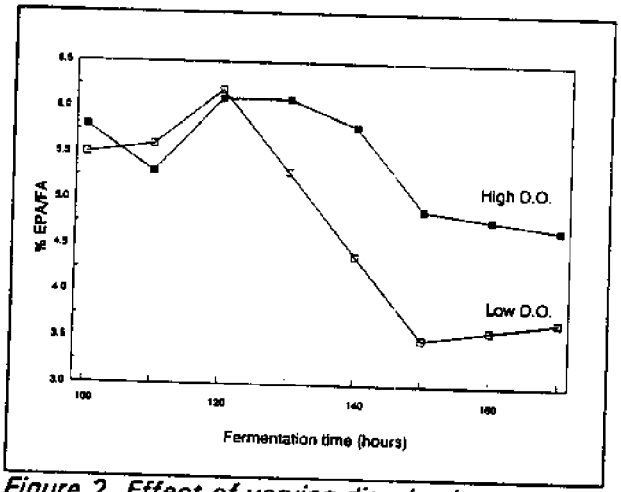

Figure 2. Effect of varying dissolved oxygen concentrations on EPA content in an unidentified heterotrophic diatom. Two fermentors were run parallel with D.O. controlled at less than 10% (Low D.O.) or greater than 50% (High D.O.) of saturation.

FUTURE POSSIBILITIES

Many reports have demonstrated the importance of the long chain ω3 HUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), to the growth and maturation of marine fish and shellfish (Ben-Amotz et al. 1987, Langdon and Waldock 1981, Olsen 1989, other references in Volkman et al. 1989 and Devresse et al. 1990). Most species of commercially important fish and shellfish seem to have a limited ability to synthesize these fatty acids and must obtain them through their diet (Ben-Amotz et al. 1987, Langdon and Waldock 1981, Sowizral et al. 1990). Algae, at the base of the food chain, are the primary source of EPA and DHA in the marine environment and are essential components in the diets of many marine organisms. Unfortunately, those algae that are the best sources of EPA and DHA are not always the easiest strains to grow and producers often must settle for algal species that do not supply optimum amounts of these essential fatty acids (Ben-Amotz et al. 1987, Ryther and Goldman 1975).

By selecting heterotrophic algal strains high in EPA or DHA it should be possible to provide inexpensive and nutritious algal feeds for fish and shellfish. Some of the heterotrophic algae emerging from the screening program mentioned above have significant levels of ω3 HUFAs, ranging from 2 - 25% of the total fatty acid content. If the production costs for these strains can be sufficiently reduced, it may even become economically feasible to feed these algae to other live feed organisms, such as rotifers and *Artemia*, to increase their ω3 HUFA content.

The strict control that can be exercised over heterotrophic cultures using standard fermentation technology may lead to improved

Table 3. Process development for unidentified diatom.

Process Improvement	Species growth rate (per hour)	Density (g/liter)	Productivity* (g/liter/hour)	% EPA/FA
Initial conditions	0.12	2	0.24	1 - 2
Automatic D.O. control	0.12	10	1.20	4 - 5
Glucose gradient feed	0.12	16	1.92	4 - 5
Silicate gradient feed	0.12	30	3.60	4 - 5
Adjusted inoculum size	0.12	45	5.40	4 - 5

*See Table 2.

feeds for aquaculture. By controlling certain chemical and physical parameters, growers should be able to influence the biochemical contents of the algal strains. Examples include the effect of dissolved oxygen on EPA content described above and the effects of nutrient depletion on fat, protein and carbohydrate contents (Wikfors 1986).

Additionally, one of the factors affecting suitability of an alga as food for shellfish is the thickness of its cell wall. Certain strains of diatoms (e.g., *Phaeodactylum tricornutum*) have been shown to be poor feeds due to their indigestibility, despite having high levels of ω3 HUFAs (Ben-Amotz et al. 1987, Ryther and Goldman 1975). Studies at Martek have shown that the silicon content and, presumably, the thickness of the silicic cell wall of diatoms can be modified by limiting the concentration of dissolved silicate in the growth medium. The ability to control the silicon content may improve the suitability of many diatoms as feeds.

SUMMARY

- A wide variety of algal species can grow heterotrophically or mixotrophically.
- The potential exists for inducing heterotrophic growth in many more species.
- Heterotrophic production of algal feeds would be less costly than current methods of photosynthetic production.
- Well established fermentation technology may allow more stringent control of and improvement in the nutritional quality of heterotrophically produced algal feeds.

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The Status of Mass Production of Live Feeds In Korean Hatcheries

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ABSTRACT

There are now 10 hatcheries under the control of the NFRDA and 78 privately owned hatcheries in Korea. Larviculture is being conducted for several species of finfish and for abalone, pearl oyster, Japanese tiger shrimp, fleshy shrimp, etc.

Rotifers are used to feed finfish larvae and *Nannochloropsis oculata* and *Chlorella* sp. are provided to the rotifers. Feeds for bivalve larvae include *Pavlova lutheri*, *Isochrysis galbana*, *Chaetoceros calcitrans* and *Chaetoceros simplex*, while *Tetraselmis tetrathele* and *Skeletonema costatum* are fed to shrimp larvae.

In the case of marine finfish, tanks for fish larvae, rotifers and *Chlorella* culture are present in the approximate ratio of 1 : 2 : 6, respectively. The type of tank used to mass produce rotifers is commonly a 25-ton concrete tank and both zooplankton and phytoplankton are usually cultured according to the batch culture method.

INTRODUCTION

Studies on the production of marine larvae in Korea began at the National Fisheries Research & Development Agency (NFRDA). The establishment of the aquatic animal hatchery at the Yosu branch of the NFRDA paved the way for the larval production of animals such as abalone, *Haliotis* spp., top-shell, *Turbo cornutus*, and tiger puffer, *Takifugu rubripes*. There are now 10 hatcheries under the control of the NFRDA (Fig. 1, Table 1) and 78 privately owned hatcheries in Korea.

Before the 1980s, species for which larval production was a high priority were shellfish such as abalone, *Haliotis discus* and

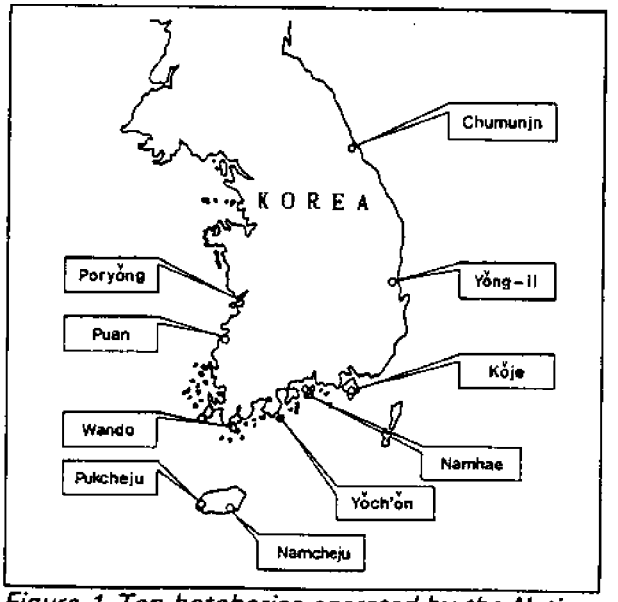

Figure 1. Ten hatcheries operated by the National Fisheries Research & Development Agency.

Table 1. The status of NFRDA hatcheries and larval production statistics for 1990.

Hatcheries	Year established	Scale (m^2)		Production	
		Site	Floor space	Species	Number of larvae
Pukcheju	1973	9,217	1,713	*Haliotis discus*	500,000
				Pagrus major	200,000 (larvae) 3 x 10^6 (fert. eggs)
				Oplegnathus fasciatus	
				Turbo cornutus	10,000
Chumunjin	1979	6,442	1,914	*Haliotis discus hannai*	400,000
				Pleuronichthys cornutus	
				Arctoscopus japonica	
				Sebastes schlegeli	10,000
				Halocynthia roretzi	
				Strongylocentrotus intermedius	10,000
Yoch'on	1980	14,378	2,003	*Paralichthys olivaceus*	10^7 (fert. eggs)
				Haliotis discus hannai	300,000
				Takifugu rubripes	
				Pagrus major	100,000
				Acanthopagrus schlegeli	100,000
				Portunus trituberculatus	600,000
Yong-il	1981	8,880	1,083	*Haliotis discus hannai*	300,000
				Halocynthia roretzi	
				Paralichthys olivaceus	20,000
				Anthocidaris crassispina	150,000
Koje	1982	14,447	2,954	*Haliotis discus hannai*	100,000
				Penaeus japonicus	5,200,000
				Takifugu rubripes	
				Paralichthys olivaceus	65,000 (larvae) 32 x 10^6 (fert. eggs)
				Halocynthia roretzi	
Wando	1984	12,973	2,878	*Haliotis discus hannai*	200,000
				Paralichthys olivaceus	20,000
				Pagrus major	50,000
				Acanthopagrus schlegeli	
				Sebastes schlegeli	50,000
				Mugil cephalus	30,000

Table 1. Continued.

Hatcheries	Year established	Scale (m^2)		Production	
		Site	Floor space	Species	Number of larvae
Poryong	1985	38,922	2,709	*Haliotis discus hannai*	100,000
				Paralichthys olivaceus	
				Pagrus major	
				Sebastes schlegeli	50,000
				Mugil cephalus	100,000
				Penaeus japonicus	
				Penaeus chinensis	6,350,000
Puan	1986	14,877	3,145	*Haliotis discus hannai*	100,000
				Sebastes schlegeli	50,000
				Pagrus major	
				Penaeus chinensis	500,000
				Portunus trituberculatus	1,800
Namcheju	1988	17,450	2,528	*Paralichthys olivaceus*	70 x 10^6 (fert. eggs) 50,000 (larvae)
				Pagrus major	200,000

Haliotis discus hannai; arkshell, *Anadara broughtonii*; pearl oyster, *Pinctada fucata*; and the tunicate, *Halocynthia roretzi*. Techniques for the production of larval marine finfishes such as bastard halibut, *Paralichthys olivaceus*; red sea bream, *Pagrus major*; rock bream, *Oplegnathus fasciatus*; black porgy, *Acanthopagrus schlegeli* and tiger puffer, *Takifugu rubripes*; and crustaceans such as the Japanese tiger shrimp, *Penaeus japonicus*; fleshy shrimp, *P. chinensis*; and blue crab, *Portunus trituberculatus* have only been developed since 1983.

As the culture of these species has advanced, demand for better mass culture techniques for microalgae and zooplankton to feed the early larval stages has increased.

STATUS OF MASS CULTURE OF LIVE FEEDS

Hatcheries have special facilities for the culture of live feeds (Table 2). In the case of marine finfish, tanks for fish larvae, rotifer and *Chlorella* culture are present in the approximate ratio of 1 : 2 : 6, respectively. The amount, timing and types of feeds given *Acanthopagrus schlegeli* and *Pagrus major* larvae are shown in Tables 3 and 4, respectively.

Phytoplankton Culture

Pearl oysters and arkshells are the species of bivalves now produced at hatcheries in Korea. Increasing amounts of *Pavlova lutheri* and *Chaetoceros calcitrans* are used as feed (four parts *P. lutheri* to every part *C. calcitrans*) (Table 5). F/2 medium and Provasoli

Table 2. Live feeds production at NFRDA hatcheries.

Hatcheries	Production area (m^2)	Live feeds production area (m^2)	Capacity of live feeds culture tanks (m^3)	Number of culture tanks for feeds	Remarks
Pukcheju	1,313.2	270	170	1	
Chumunjin	1,101.5	34.7	—	—	
Yoch'on	1,907.6	308	100 50 30	3 1 1	Phytoplankton Zooplankton Zooplankton
Yong-il	680.2	15	—	—	
Koje	2,720	560	1 4	50 10	Phytoplankton Zooplankton
Wando	2,400	250	7 2	7 3	Phytoplankton Zooplankton
Poryong	2,490	179 (indoors 272 (outdoors)	6 2 150 70	12 20 2 3	Phytoplankton Zooplankton
Puan	2,517	800	12 4 2 5	2 3 50 12	
Namcheju	1,465	191	26 26 20	3 3 3	Phytoplankton Zooplankton Zooplankton
Namhae	2,174	331 (indoors) 160 (outdoors)	17 33 43 70 150	13 1 1 2 1	

ES medium (Table 6) are generally used. The culture process is outlined in Table 7.

Because the production of live feeds should keep pace with the growth of the target species, we use serial culture, periodically upscaling by 15 - 20% as the target species grows. Seawater is sterilized by boiling, but if a great deal is needed, it is sterilized with UV irradiation.

Chlorella sp. is used primarily to feed the rotifer, *Brachionus plicatilis*. This is because *Chlorella* sp. grows rapidly, is easy to culture, and is euryhaline. Either Complesal® or Biwang® medium is used for the intermediate culture of *Chlorella* sp. in one-ton fiberglass

Table 3. Amount, timing and species of feeds given cultured *Acanthopagrus schlegeli* .

Days after incubation	1	10	20	30	40	50	60	70
Total length (mm)	1.85	3.17	5.95	8.18	14.37	25.00	34.30	51.20
Feed R	2 → 5 → 10 → 15 ind./ml							
A			4 → 6 ind./ml					
T				50 → 100 g/1,000				
M					100 → 300 → 400 g/1,000			

R: rotifers, A: brine shrimp, T: short-necked clam, M: minced meat

Table 4. Amount, timing and species of feeds given cultured *Pagrus major* .

Days after incubation	1	10	20	30	40	50	60	70	80
Total length (mm)	2.6	3.8	9.2	11.2	19.4	21.5	23.5	34.2	53.4
Feed R	2 → 5 → 10 → 15 ind./ml								
A			4 → 6 ind./ml						
T				40 → 150 g/1,000					
M					100 → 300 → 400 → 500 g/1,000				

R: rotifers, A: brine shrimp, T: short-necked clam, M: minced meat

reinforced plastic tanks (Table 8). Tanks are filled with filtered seawater, then *Chlorella* sp. and nutrient medium are added and aeration is begun. Mass culture tanks differ between hatcheries, but 100-ton concrete tanks are generally used. The nutrient medium contains ammonium sulfate (100 g/ton), superphosphate (15 g/ton) and urea (5 g/ton).

Table 5. Production of *Pinctada fucata* and *Anadara broughtonii* larvae at NFRDA using *Pavlova lutheri* and *Chaetoceros calcitrans* as feed.

Days after hatching	Size	Survivors (ind./ton)	Survival rate (%)	Density of microalgae after addition (cells/ml)	No. feedings/day
0 - 1	65 - 70 μm	400 x 10^4	100	1 x 10^3	1
1 - 3	70 - 80 μm			2.5 x 10^3	2
4 - 6	80 - 90 μm			5 x 10^3	2
7 - 9	90 - 110 μm			7.5 x 10^3	2
10 - 12 (10)	110 - 130 μm	300 x 10^4	75	10 x 10^3	2
13 - 15	130 - 160 μm			12 x 10^3	3
16 - 18	160 - 180 μm			13 x 10^3	3
19 - 21 (20)	180 - 210 μm	200 x 10^4	50	17 x 10^3	3
30	0.6 - 0.7 mm			25 x 10^3	3
40	1 mm	100 x 10^4	25	25 x 10^3	4
50	2 mm			25 x 10^3	5
60	3 mm	10 x 10^4	25	25 x 10^3	6

The harvest density is 8 - 10 x 10^6 cells/ml. In the winter, when it is cooler than 8°C, heaters are used occasionally to speed growth.

The main problem encountered in the mass culture of *Chlorella* sp. is occasional rapid declines in cell number. These "crashes" are accompanied by a change in the color of the culture medium to yellowish-brown. The cause of this phenomenon has not been elucidated, however their occurrence is correlated with sudden increases in temperature or exceptionally long culture periods under low light conditions (caused by continuous rain or contamination with diatoms). Water is filtered prior to use with a 3 - 5-μm filter.

ZOOPLANKTON CULTURE

Rotifer Culture

The type of tank used to mass produce rotifers in Korea differs in accordance with the production method, culture volume, duration of culture, etc., but 25-ton concrete tanks are commonly used.

There are two culture methods (Fig. 2): the total harvesting method (batch), which uses small tanks, and the partial harvesting method (semi-continuous), which employs large tanks. Recently, the former has begun to be used in conjunction with a variety of tank sizes. Due to the consistent purity of the inocula, it is more efficient than the partial harvesting method and cultures can remain stable for long periods of time. Also, planned

Table 6. Composition of media used for phytoplankton culture.

F/2 medium (Guillard and Ryther 1962)		
$NaNO_3$	150	mg
Na_2HPO_4	8.69	mg
Ferric EDTA	10	mg
$MnCl_2$	0.22	mg
$CoCl_2$	0.11	mg
$CuSO_4 \cdot 5H_2O$	0.0196	mg
$ZnSO_4 \cdot 7H_2O$	0.044	mg
$Na_2SiO_3 \cdot 9H_2O$	60	mg
$Na_2MoO_4 \cdot 2H_20$	0.012	mg
B_{12}	1.0	μg
Biotin	1.0	μg
Thiamine · HCl	0.2	mg
Seawater	1,000	ml
Provasoli ES medium (Provasoli 1968)		
$NaNO_3$	105	mg
Na_2 glycerophosphate	15	mg
Na_2 EDTA	24.9	mg
$Fe(NH_4)_2 \cdot (SO_4)_2 \cdot 6H_20$	10.5	mg
H_3BO_3	3	mg
$FeCl_3 \cdot 6H_2O$	0.15	mg
$MnCl_2 \cdot 4H_20$	0.6	mg
$ZnCl_2$	0.075	mg
$CoCl_2 \cdot 6H_20$	0.015	mg
B_{12}	3	μg
Biotin	1.5	μg
Seawater	1,000	ml

production is possible when total harvesting method is used. If there is a requirement of 10 tons of rotifers/day, five 10-ton tanks are prepared and one tank is harvested each day. The advantage of the semi-continuous method is that rotifers can be harvested whenever they are needed.

The first step in the mass production of *B. plicatilis* is to add seawater to an undiluted *Chlorella* sp. culture. The algal density is then adjusted to 8 - 10 x 10^6 cells/ml. Tanks larger than 50 tons are not heated, but cultures in 20-ton tanks are maintained at 25°C with a heater. Strong aeration is provided.

The inoculation density is 30 - 50 rotifers/ml, and after four to five days increases to 100 - 200/ml. At this point, the tank is harvested. A portion is reserved to begin a new culture and the remainder is fed to fish.

In the absence of *Chlorella* sp., baker's yeast is fed at the rate of 1 - 2 g/100 rotifers /day. If rotifer density is low or if the culture is heavily contaminated with protozoa, more yeast is added.

Yeast is provided twice daily, in the morning and in the evening. Occasionally there will be three feedings/day, but this is rare. If baker's yeast which has been enriched with fat or oil is to be used, 0.25 - 1 g/million rotifers is fed. Harvesting is accomplished with a 58 - 63-μm plankton net and an underwater pump (0.25 kw, 0.4 kw) or by gravity.

Artemia

To hatch *Artemia* eggs, pour seawater into a 500-liter incubator and add 500 g of dry cysts. If the water can be maintained at 28°C, the eggs will hatch into nauplii in 18 - 24 hours.

Ordinarily, the nauplii are collected and given to larvae and fingerlings along with

Table 7. Culture process for bivalve larval feeds.

	Stock culture	Intermediate culture	Rapid culture	Mass culture
Culture container	100 - 500-ml Erlenmeyer flask	2 - 5-liter round bottom flask	20-liter bottle	100-liter FRP
			Polyethylene (vinyl)	
Seawater filtration	Membrane filter (0.45 μm)		Cartridge filter through three steps 25 → 5 → 1 μm	
Seawater sterilization	Autoclave (121°C, 15 min.)		100°C, 10 min.	
Aeration	None	Compressed, filtered air	Add CO_2 gas (2 - 5%) to compressed, filtered air	
Illumination (lux)	500 - 1,000	3,000	5,000 - 6,000	6,000 - 7,000
Inoculation density (cells/liter)	2×10^6	2×10^6	2 x 106	2×10^6
Culture period (days)	7	7	7	7
Maximum density (cells/liter)	$40 - 50 \times 10^6$	$80 - 90 \times 10^6$	$70 - 100 \times 10^6$	$30 - 70 \times 10^6$
Inside/outside	Inside	Inside	Inside	Inside

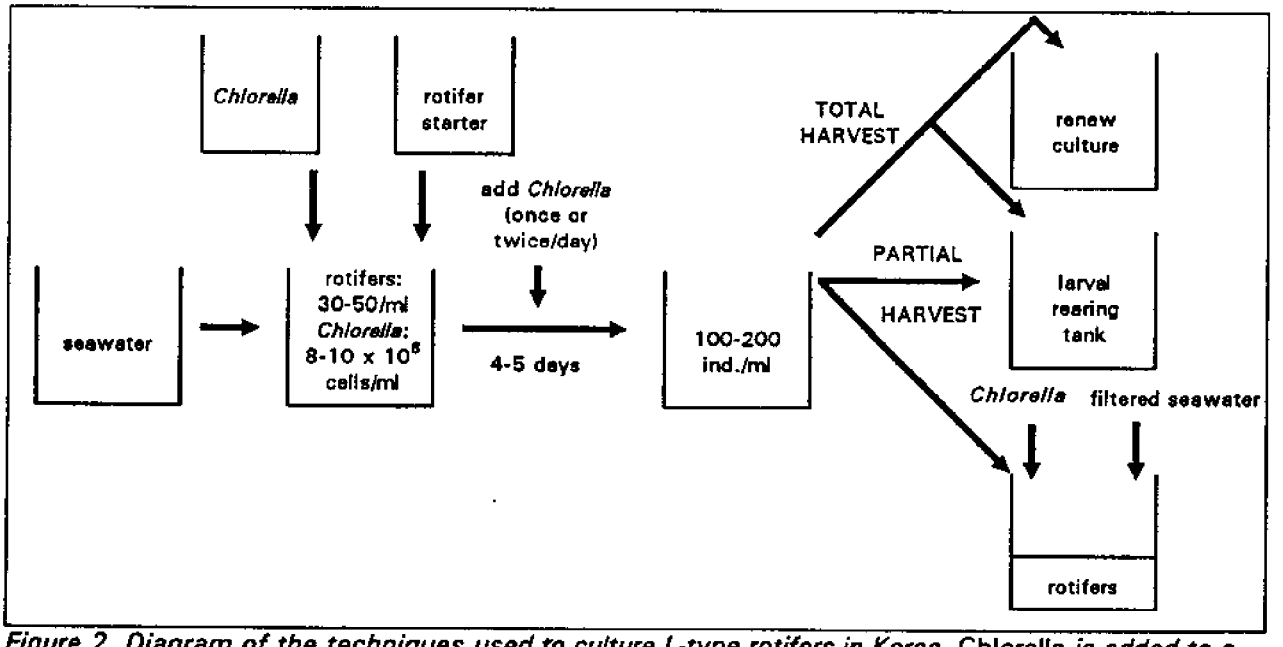

Figure 2. Diagram of the techniques used to culture L-type rotifers in Korea. Chlorella *is added to a density of 1 - 2 x 10^6 cells/ml.*

Table 8. Composition of media used for *Chlorella* culture.

Complesal		
Total nitrogen	10	%
Soluble phosphate	4	%
Soluble potassium	6	%
Soluble boron	0.05	%
Soluble manganese	0.1	%
Soluble magnesium	0.3	%
Soluble copper	0.02	%
Soluble iron	0.05	%
Soluble molybdenum	0.01	%
Biwang		
Total nitrogen	11	%
Soluble phosphate	3	%
Soluble potassium	5	%
Soluble magnesium	0.03	%
Soluble boron	1	%
Soluble manganese	0.02	%
Soluble iron	0.03	%
Soluble zinc	0.02	%
Soluble copper	0.01	%

other types of feeds. Additionally, some hatcheries enrich their *Artemia* by secondary culture with oil and ω yeast, emulsified oil plus *Chlorella* sp., etc.

Part III

MICROALGAE DISCUSSION GROUP A
Culture Situations, General System Description I

This was the first of five discussion groups to focus on the culture of microalgae to feed rotifers and the larvae of bivalves and crustaceans. To begin, everyone was asked to characterize the facility about which they would provide information for the remainder of the discussions.

Twelve culture situations were represented from the United States, Thailand, northern China, Taiwan, South Korea, Singapore and Japan (Table 1). Most participants represented research institutes, hence their scale of operations was generally small, ranging from "laboratory" to "experimental" to "semi-commercial." One U.S. participant, Jim Donaldson of Coast Oyster Company, was producing algae on a commercial scale. Other commercial-scale operations were described by Jiao Fen Chen from the northern region of the People's Republic of China, Huei-Meei Su from Taiwan and Hassanai Kongkeo of Thailand.

The vast majority of systems were batch type, photosynthetic tank culture. In addition, those attending learned about two rather unconventional systems — Ray Gladue of MARTEK was producing algae heterotrophically in fermentors, and Kelly Rusch of Louisiana State University provided data on a 600-liter semi-continuous, computer-controlled system.

Those participants culturing algae for bivalves produced oysters, clams, cockles and abalone with *Thalassiosira pseudonana*, *Skeletonema* sp., a variety of species in the genus *Chaetoceros* (*C. calcitrans*, *C. muelleri*, *C. simplex*, *C. ceratosporum*, and *C.* sp.), *Chlorella minutissima*, *Isochrysis galbana*, *Isochrysis* aff. *galbana*, *Pavlova viridis*, *Phaeodactylum tricornutum*, *Nitzschia* sp., *Gomphonema* sp. and *Tetraselmis subcordiformis*.

Finfish producers were producing the rotifer, *Brachionus plicatilis*, with a variety of algae, including: both *Nannochloris oculata* and *Nannochloropsis oculata*, *Chlorella* sp., *Chlamydomonas* sp., *Tetraselmis tetrathele* and *T. chui*.

Finally, penaeid shrimp (*P. chinensis*, *P. monodon*, *P. merguiensis*, *P. japonicus*), *Metapenaeus ensis*, and *Portunus trituberculatus* were the crustaceans cultured by the participants in this group. Again a range of microalgae was cultured to feed the larvae of these species, including a number of diatoms, a species of *Tetraselmis* and some blue-green algae (Table 1).

The largest operation was Coast Oyster Company, which cultivated 20 20,000-liter tanks of *Thalassiosira pseudonana* (3H clone) year-round in a greenhouse (Table 2). With the exception of some 2-liter fermentors at MARTEK, the smallest harvestable culture vessels were the 100-liter cylinders used at the National Fisheries Research & Development Agency (NFRDA) in South Korea and the 500-

Table 1. Scale and type of culture, species cultured, and target species.

Country/location	UNITED STATES				
	Washington (Coast Oyster Company)	Hawaii (The Oceanic Institute)	Louisiana (Louisiana State University)		Maryland (MARTEK)
Production scale	Commercial	Experimental	Laboratory	Laboratory and "semi-commercial"	Experimental
Microalgal species cultured	*Thalassiosira pseudonana* 3H clone, *Skeletonema* sp., *Chaetoceros calcitrans*	*Nannochloropsis oculata*	*Chlorella minutissima*	*Chaetoceros muelleri*	Unidentified dinoflagellate, Unidentified spp., others (30 spp. total)
Target species	*Crassostrea gigas, C. gigas kumamoto, Tapes philippinarum* (broodstock, larvae, postlarvae)	*Brachionus plicatilis*	Bivalves	Bivalves	Postlarval molluscs and shrimp
Type of culture	Batch	Batch	Semi-continuous	Semi-continuous	Heterotrophic. Batch and semi-continuous.

Table 1. Continued.

Country/location	THAILAND (Typical backyard hatchery)	NORTHERN CHINA				
Production scale	Commercial	Commercial				
Microalgal species cultured	*Chaetoceros calcitrans, Skeletonema costatum, Tetraselmis* sp.	*Isochrysis galbana*	*Pavlova viridis*	*Chaetoceros muelleri*	*Phaeodactylum tricornutum*	*Tetraselmis subcordiformis*
Target species	*Penaeus monodon, P. merguiensis*	*Argopecten* adults and larvae, *P. chinensis* larvae	*Argopecten* larvae, *P. chinensis* larvae	*P. chinensis* larvae	*Argopecten* broodstock	*Argopecten* postlarvae
Type of culture	Batch and semi-continuous	Batch				

Table 1. Continued.

Country/location	TAIWAN						SINGAPORE	
Production scale	Commercial						Laboratory and commercial	
Microalgal species cultured	*Nannochloropsis oculata*	*Tetraselmis chui*	*Chlorella* sp.	*Spirulina platensis* (dried)	*Skeletonema costatum*	*Isochrysis* aff. *galbana*	*Nannochloropsis oculata, Tetraselmis tetrathele*	*Skeletonema costatum*
Target species	*B. plicatilis*	*B. plicatilis*, penacid larvae	*B. plicatilis*	Penaeid larvae		Hard clam	*B. plicatilis*	*Penaeus merguiensis, P. monodon*
Type of culture	Batch						Batch	

Table 1. Continued.

Country/location	SOUTH KOREA							
	National Fisheries Research & Development Agency				National Fisheries University of Pusan			
Production scale	Experimental	Commercial			Laboratory			
Microalgal species cultured	*Chaetoceros calcitrans, C. simplex, Isochrysis galbana, Pavlova lutheri,* and *Phaeodactylum tricornutum*	*Tetraselmis tetrathele, Skeletonema costatum*	*Nannochloropsis oculata, Chlorella* sp.	*Nitzschia* sp. and *Navicula* sp.	*Nannochloris oculata, Chlorella* sp.	*Skeletonema costatum*	*Pavlova lutheri*	*Isochrysis galbana, Isochrysis* aff. *galbana*
Target species	*Anadara broughtoni, Pinctada fucata* and *Crassostrea gigas*	*Penaeus chinensis* larvae	*Brachionus plicatilis*	Abalone	*Brachionus plicatilis*	*Penaeus chinensis*	*Anadara broughtoni, Pinctada fucata*	*Pinctada fucata*
Type of culture	Batch				Batch			

Table 1. Continued.

Country/location	JAPAN						
	Seikai National Fisheries Research Institute					National Research Institute of Aquaculture	
Production scale	Laboratory		Laboratory and commercial			Lab.	Semi-commercial
Microalgal species cultured	*Chaetoceros calcitrans, C. simplex, C. ceratosporum, C.* sp.	*Pavlova lutheri, Isochrysis galbana, Isochrysis* aff. *galbana* (Tahiti strain)	*Tetraselmis tetrathele*	*Nannochloropsis oculata* and *Chlamydomonas* sp.	*Nitzschia* sp. and *Gomphonema* sp.	*Navicula husteadtiana*	*Synecococcus* sp.
Target species	Many bivalves, *Penaeus japonicus, Metapenaeus ensis*	Bivalves	*Brachionus plicatilis,* penaeids	*Brachionus plicatilis*	Abalone larvae	*Portunus trituberculatus, Penaeus monodon, P. japonicus*	
Type of culture	Batch	Semi-continuous for some spp., batch for mass culture	Batch			Batch	

and 600-liter vessels used in experimental settings by Masachika Maeda at the National Research Institute of Aquaculture (NRIA) in Japan and Louisiana State University, respectively. Backyard shrimp hatcheries in Thailand were characterized as having either approximately 10 1,000-liter fiberglass

Table 2. General system descriptions: culture enclosures.

Culture enclosures	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone)	Hawaii (*Nanno-chloropsis oculata*)	Louisiana (*Chaetoceros muelleri*)	Maryland (Unidentified sp.)		
Size (largest)	20,000 liters	25,000 liters	600 liters	Fermentors: 2 - 200 liters	1,000 or 4,000 liters	7,200 liters (4 x 3 x 0.8 m; 0.6 m water depth)
Shape	Round	Round	Round with conical bottom. Covered (air tight, with vent)	Sealed cylinders, internal agitation	1,000 liters: round 4,000 liters: square or rectangular	Most rectangular
Construction materials	Fiberglass	Fiberglass	Fiberglass	Stainless steel	1,000 liters: fiberglass 4,000 liters: concrete	Cement
Number harvested	20	3 - 5	N/A	N/A (Exp. scale)	1,000 liters: approx. 10 4,000 liters: approx. 4	36 (max.)

Table 2. Continued.

Culture enclosures	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nannochloropsis oculata, Tetraselmis tetrathele, Skeletonema costatum*)	JAPAN
		NFRDA (*Pavlova lutheri*)	NFUP (*Nannochloris oculata*)		SNFRI (*Nitzschia* sp. and *Gomphonema* sp.)
Size (largest)	10,000 - 40,000 liters (2 m deep)	100 liters	6,000 liters	10,000 liters	10,000 liters
Shape	Rectangular	Round cylinders	Round	Round	Round
Construction materials	Concrete ponds	FRP	Concrete	FRP	Transparent polycarbonate, FRP sometimes
Number harvested	Four is best	> 20	8	> 8	> 20

Chaetoceros calcitrans tanks or four 4,000-liter concrete tanks.

With the exception of Thailand's inland backyard hatcheries, water was obtained from saline wells or coastal areas. In some small-scale operations, artificial seawater was used (small scale only) (Table 3). In Thailand, 250 ppt water from salt farm evaporation ponds is trucked to hatcheries and diluted prior to use. The hypersaline water is free of bacteria.

Filtration and/or chlorination were the most prevalent types of water treatment (Table 3). A range of climates was also represented, but most operations were able to grow algae

Table 3. General system descriptions: water, climate, stock culture room.

	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone)	Hawaii (*Nanno-chloropsis oculata*)	Louisiana (*Chaetoceros muelleri*)	Maryland (Unidentified sp.)		
Water source	Well	Two wells	Laboratory: artificial	Artificial	Hypersaline water from salt farm evaporation ponds: 250 ppt, no bacteria	Coastal area
Water treatment	Chlorine only	35-μm mesh bags	UV sterilization	Steam sterilization	Dilute with freshwater, treated with chlorine after transport to hatchery	1. If fairly clean: sand filtration and settling, then chlorine 2. If turbid: treat with alum, then filter, settle, chlorinate
Water transport	Pump	Pump transports to holding tank, then gravity	N/A	N/A	Trucked to hatchery	Pumped to a holding tank before filtration
Climate/site characteristics	Temperate. Greenhouse, year-round production (water is heated)	Subtropical. Outside, 20 - 28°C, year-round production	Environmental control room	Inside lab	Tropical. Outside below sheds with transparent roofs, or partially covered with roofing tiles in the afternoon, year-round production	Temperate (production March-June). Greenhouse, ambient temp.
Stock culture room	Sterile chamber used during transfer of stock. Kept in flasks at 20°C	Stock kept in test tubes (25 spp.). Water filtered to 1 μm, then autoclaved	Kept in flasks at 20°C. Water deionized prior to mixing with sea salts	Axenic, laminar flow hood is used	Stock provided by government free, also can be obtained cheaply from large hatcheries. 1 - 2 liters purchased at a time	Size varies

year-round. The majority of the facilities maintained stock culture rooms; however, Lian Chuan Lim noted that *Skeletonema costatum* stocks can be obtained year-round from Singapore's coastal waters.

Table 3. Continued

	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nannochloropsis oculata, Tetraselmis tetrathele, Skeletonema costatum*)	JAPAN	
		NFRDA (*Pavlova lutheri*)	NFUP (*Nannochloris oculata*)		SNFRI (*Nitzschia* sp. and *Gomphonema* sp.)	NRIA (*Synecococcus* sp.)
Water source	Coastal water	Coastal water	Coastal water	Coastal water	Coastal water	Coastal water
Water treatment	Sand filtration and cloth bag	3-step cartridge filtration: 25-μm, 5-μm and 1-μm	20- and 1-μm cartridge filtration	Filtration, chlorination	Sometimes stored for > 2 years. Chlorine, ozonization, sand and glass fiber filtration	Sand filter, then chlorinate
Water transport	Pump	Gravity and manpower	Pump	Pump	Pump and buckets	Pump
Climate/site characteristics	Subtropical. Outside, year-round production	Inside, 25 ± 1°C	Temperate. Outdoor culture from spring to fall	Tropical. Year-round production	Temperate. Year-round production	Temperate. Year-round production
Stock culture room	Flasks in a growth chamber at 20°C, 500 - 1,000 lux. Stock obtained from the harbor year-round	Room: 20°C, 100 - 500 Erlenmeyer flasks. Water filtered to .45 μm then autoclaved. Illumination 500 - 1,000 lux	15 - 20 °C	*Nannochloropsis* and *Tetraselmis*: 100-, 125-ml flasks in air conditioned room. Illumination 1,000 lux. *Skeletonema*: stock obtained from coastal waters year-round	Kept in small test tubes, use Provasoli's enriched seawater and artificial seawater. Has used cryopreservation (-196°C) for *Tetraselmis*, *N. oculata*, and diatom stocks	20°C, not sterile, photoperiod

MICROALGAE DISCUSSION GROUP B
General System Description II

In this session, participants were asked to describe the culture procedures used at their respective facilities and to discuss the pros and cons of different culture strategies. Information was sought about upscaling procedures, vessel preparation, monitoring, harvesting procedures and personnel training (Table 1).

Although many culture operations don't have well defined seasons, the scaling-up time represents the amount of advance notice a microalgal production unit must have prior to full-scale larviculture. The longest scale-up time reported was 60 days at The Oceanic Institute's finfish larval rearing facility where they use relatively large, 20,000-liter tanks (Table 1). Other times ranged from six to 30 days. In most cases, four or five different sized vessels were used during upscaling, from 25 ml to 25,000 liters. Smaller vessels were often sterilized prior to use by means of an autoclave, whereas chlorine was the most popular means of cleaning the larger vessels. In some cases only a freshwater rinse was necessary.

All participants reported conducting regular microscopic examinations of their cultures (Table 1). Cell density, pH, salinity and the presence of contaminating organisms (protozoa and sometimes bacteria) were monitored.

Contamination was a key concern of practically all members of the group. This is probably the primary reason operators are running batch systems instead of semi-continuous ones. Concerns were voiced about algal predators such as protozoa as well as pathogenic bacteria such as *Vibrio*. The latter pose a threat primarily to the algae consumer, not the algae culture itself.

Maintenance of sanitary procedures, of course, is the most common and probably the most important means of minimizing contaminants. Jim Donaldson stressed the importance of keeping ahead of contamination, which is inevitable in open systems, by growing algae at accelerated rates, harvesting during the late exponential phase and starting anew. Lian Chuan Lim considered water treatment (to kill protozoan cysts) secondary to tank disinfection. He also stressed the importance of tank siting—tanks should be distanced from major sources of contamination such as trees—and using filters in the aeration system. Finally, Sung Bum Hur noted that the water circulation system can be an important tool in controlling contamination. Strong aeration at the bottom discourages ciliates.

The maximum cell densities attained, of course, vary greatly with the cell size of the species cultured. For example, *Tetraselmis tetrathele*, which is considerably larger than *Nannochloropsis oculata*, does not reach the same cell densities in culture as *N. oculata*. It is not surprising, however, that the highest harvest density reported was that achieved with an unidentified diatom grown heterotrophically: 2.5×10^8 cells/ml (Table 2). The density of photosynthetic cultures is usually limited by light availability, whereas this is not a problem for algae produced in fermentors

Table 1. Scaling-up time, culture vessel preparation, monitoring/quality control procedures.

	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone)	Hawaii (*Nanno-chloropsis oculata*)	Louisiana (*Chaetoceros muelleri*)	Maryland (Unidentified sp.)		
Scale-up time	14 days	60 days	7 - 9 days	6 - 10 days	10 - 12 days	20 - 30 days
Vessel sizes	1-liter axenic, 20-, 3,000-, 20,000-liter	25-ml, 2-, 20-, 500-, 5,000-, 25,000-liter	25-ml, 500-ml, 4-, 600-liter	50-ml, 2-, 20-, 200-liter	1-, 20-, 500-, 1,000-, 4,000-liter	20-, 100-, 2,000-, 7,000-liter
Culture vessel preparation	Freshwater rinse daily and HCl once/year	Freshwater rinse.Glassware autoclaved.Carboys and tanks less than 25,000 liters chlorinated	Chlorine rinse	Freshwater rinse, detergent every 3 months	Chlorine rinse and sun dry	20- and 100-liter vessels cleaned with HCl. Chlorine is used for 2,000- and 7,200-liter tanks
Monitoring/ quality control procedures	Macroscopic and microscopic. pH, bacteria (TCBS and marine agar), cell density (with color meter), temperature	Microscopic. pH, temperature, salinity, optical density	Microscopic and macroscopic. pH, temperature, density (with solar cell), salinity	Microscopic. pH, DO, temperature, bacteria (with agar plates)	Microscopic. Temperature, turbidity and color	Microscopic. Contamination, cell density checked at harvest

Table 1. Continued.

	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nanno-chloropsis oculata*)	JAPAN	
		NFRDA (*Pavlova lutheri*)	NFUP (*Nannochloris oculata*)		SNFRI (*T. tetrathele* and *I. galbana*)	NRIA (*Synecococcus* sp.)
Scale-up time	12 days	6 - 8 days	21 days	20 - 30 days	7 - 10 days	14 days
Vessel sizes	100-ml, 1-, 15, 500-, 10,000-liter	5-, 20-, 100-liter	100-ml, 1-, 15, 100-, 1,000-liter	125-ml, 3-liter, 20-liter bag, 1,300-, 8,000-liter	10-ml, 500-ml, 1-, 10-, 500-, 1,000-liter	10-ml, 500-ml, 10-, 500-liter
Culture vessel preparation	Freshwater rinse, flasks autoclaved	5- and 20-liter vessels autoclaved. 100-liter vessel rinsed with chlorine	100-ml, 1- and 15-liter autoclaved. 100-liter boiled. 1,000-liter rinsed and sun dried	125-ml rinsed with freshwater and autoclaved. Bags are disposable. 1,300- and 8,000-liter treated with 10% formalin, rinsed and sun dried	Smaller vessels autoclaved. Others rinsed with freshwater.	Detergent and rinse
Monitoring/quality control procedures	Macroscopic and microscopic	Microscopic. pH, contamination	Microscopic. pH, density, contamination	Microscopic. Cell density, contamination (ciliates)	Microscopic. pH, bacteria (marine agar)	Microscopic and macroscopic (color)

Table 2. Harvesting, training.

	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone)	Hawaii (*Nanno-chloropsis oculata*)	Louisiana (*Chaetoceros muelleri*)	Maryland (Unidentified sp.)		
Density at harvest (cells/ml)	3 x 10^6 (all species)	20 x 10^6 (target)	40 - 50 x 10^6 (target)	2.5 x 10^8 (20 - 45 g dry weight/liter)	5 - 10 x 10^4	1.8 - 2 x 10^6
Duration of harvest (batch); rate of harvest (semi-cont.)	2 days	3 days	35 - 50%/day	4 hours (200 liter)	1 day	3 - 5 days
Harvesting procedure and equipment	Direct pump. Centrifuge used if batch is to be preserved.	Direct pump	Computer controlled: algae pulled from tanks by vacuum	Sharple's centrifuge, then refrigerate the thick paste (50% solid)	Direct pump	Direct pump
Training	On the job (1 month)	On the job	N/A	On the job	On the job	Apprenticeship, regular workshops

Table 2. Continued.

	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nanno-chloropsis oculata*)	JAPAN	
		NFRDA (*Pavlova lutheri*)	NFUP (*Nan-. nochloris oculata*)		SNFRI (*T. tetrathele* and *I. galbana*)	NRIA (*Synecococcus* sp.)
Density at harvest (cells/ml) range	8 - 15 x 10^4	50 x 10^6	10 - 25 x 10^6	10 - 20 x 10^6	1 - 2 x 10^6 (*Tetraselmis*) 7 - 8 x 10^6 (*Isochrysis*)	5 x 10^6
Duration of harvest (batch); rate of harvest (semi-cont.)	2 days	2 days	2 - 4 days	5 - 7 days	7 - 10 days	≥ 10 days
Harvesting procedure and equipment	Gravity, concentrated with cloth bag	Gravity, manpower	Pump and gravity	Pump	Pump or continuous centrifuge, manual	Direct pump
Training	On the job	On the job	Periodic courses	N/A	N/A	2-3 months training in the lab

(see Gladue, this volume). Ray Gladue noted that most indoor, photosynthetic cultures are usually .1 g/liter dense, while algae grown in fermentors can exceed densities of 40 g/liter. *Nannochloropsis oculata* producers in Hawaii and Singapore harvested at 10 - 20 x 10^6 cells/ml, and a similar density was reported

for *Nannochloris oculata* in South Korea and *Skeletonema costatum* in Taiwan.

Harvests from batch cultures (except for the fermentor) took anywhere from one to 10 or more days (Table 2). Harvest was accomplished most often by direct pumping, and sometimes the algae was concentrated by centrifuge or by means of a cloth bag, in the case of *S. costatum* in Taiwan. Coast Oyster Company centrifuges excess algae and chemically preserves it (it keeps for up to a year) to sell or to use as feed for oyster broodstock and juveniles.

Most participants reported that their personnel are trained primarily "on the job," although a few conduct regular training workshops (Table 2).

The session was concluded with the question, "How would you improve your microalgae production system?" Responses ranged from specific requests (Maeda Masachika wants a practical means of culturing diatoms with blue-green algae inside shrimp larvae tanks; Lian Chuan Lim seeks an indoor, intensive microalgae production system) to general concerns about costs and reliability. Jim Donaldson noted that as a commercial operator, his primary concern is cost. One way he found to improve output cheaply was to increase algal density by adding an additional light above each tank. Ray Gladue is similarly interested in improving culture density, whereas Jiao Fen Chen's greatest obstacle is minimizing contamination with airborne protozoa. Labor is cheap in China, but high levels of contaminants force her to harvest cultures early. Mi Seon Park from the Korean Fisheries Research & Development Agency and Vernon Sato of The Oceanic Institute are primarily concerned about contamination in outdoor cultures. Finally, Kelly Rusch's goal is the development of a production system which is less labor-intensive than traditional systems.

MICROALGAE DISCUSSION GROUP C
Culture Conditions and Reliability

Reliability of production was a key issue raised in Discussion Group B. To follow up on this issue, participants in session C were asked to:

- specify the conditions under which they grew their algae;
- characterize any problems they had concerning reliability (both consistency of production and quality);
- and discuss ways of dealing with unreliable systems.

Culture Conditions

The operational parameters discussed included: light intensity, photoperiod, pH, temperature, salinity, aeration/mixing, CO_2 addition and the types and amounts of nutrients added (Table 1). Except for the one case of heterotrophic production, algae were cultured either with natural sunlight and photoperiod or with continuous artificial lighting ranging in intensity from 6,000 to 12,000 lux. In addition, Jim Donaldson noted that he had achieved very good growth with metal halide lamps.

Photosynthetic algae cultures tend to become more basic as they progress, but culturists can counteract this tendency by providing aeration. Carbon dioxide, naturally present in the air, enhances the buffering capacity of the culture. In heterotrophic systems, the pH is regulated with a pHstat, which automatically adds an acid or a base to keep the pH at a constant level. On the whole, producers maintained pH's between 7.4 and 10, but a pH close to 8 was common (Table 1).

Few of the systems described had rigidly controlled water temperatures. Masonori Okauchi reported the widest range in temperature: 10 - 33°C; however, 20 - 30°C was more common (Table 1). Salinity likewise wasn't strictly regulated and varied between 10 and 35 ppt.

All systems used "moderate" to "violent" aeration for mixing and as a source of carbon (Table 1). In addition, supplemental CO_2 was added periodically in three cases. Guillard's F/2 medium (Guillard and Ryther 1962) or variations thereof were commonly used. In Singapore, urea (3 g/m^3) is often added to *Nannochloropsis oculata* cultures, and Clewat 32, a chelated metal mix, is a common additive to microalgae media in Japan.

System Reliability

The frequency of "crashes" that is, the complete failure of a culture, ranged from "rare" to two to three/month (Table 2). It is important to note, however, that crashes may be rare in a system only because declining batches are terminated before they have the opportunity to collapse. This is the case in northern China.

Table 1. Culture conditions.

Culture parameters	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone)	Hawaii (*Nanno-chloropsis oculata*)	Louisiana (*Chlorella minutissima*)	Maryland (Unidentified sp.)		
Light intensity (lux)	Metal halide and natural light. 8,000 - 12,000	Natural (system is light-limited, especially in the winter)	6,000 - 8,000	N/A	>10,000	10,000
Photoperiod	Continuous	Varies with season (natural)	Continuous	N/A	Natural (in summer is controlled by covering with roofing tiles)	Natural
pH	7.5 - 8.5	7.4 - 9.5	7.5 - 8.5	7.5 - 8.5	7.5 - 8.5	Natural (seawater)
Temperature (°C)	18 - 22 (no temperature control in room, water temp. adjusted at inoculation)	19 - 32	23 - 26	29	28 - 30	25 - 30
Salinity (ppt)	24 - 28 (well)	32	20	15 - 30	25 - 30	10 - 30
Aeration /mixing	Moderate aeration.Single airline in the center	Maximized	"Violent" air injection into bottom of each chamber	1. 1 vol. sterile air/vol. liquid/minute 2. Impeller	Strong aeration	Airstones
CO_2 addition	Injected for 1 min./45 min., 5 - 10% (mixed with air)	Not used	3 - 5%. 10 sec./min. (computer controlled)	Not used	Not used	Not used
Nutrient medium (types, amounts)	F/2 variation	F/2 or F/2 with increased amounts of N and P (this increases longevity)	F/2 (not sterile)	ASW with heterotrophic nutrients added (primarily glucose). 2 g glucose/g algae harvested	Per 1,000 liters of seawater: KNO_3 300 g Na_2HPO_4 30 g $FeCl_3$ 5 g Na_2SiO_3 1.5 g EDTA 20 - 40 g (if available)	$NaNO_3$:60 g/ton KH_2PO_4:4 g/ton Vit. B_1:100 mg/ton Vit. B_{12}: 0.5 mg/ton

Subjectively ranking the severity of crashes is difficult, but when asked to rate the impact of such occurrences, group members characterized them as anywhere from "not severe" to "moderate" to "devastating." Causes included human error, temperatures that were either too high or too low, and rain, but an informal survey of participants yielded contamination as the primary cause of culture crashes (Table 3).

To prevent crashes, participants often keep backup cultures of the same or a different species and follow sanitary procedures to the greatest degree possible—filtering air and/or water and chlorinating and dechlorinating tanks and water. The importance of site selec-

Table 1. Continued.

Culture parameters	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nannochloropsis oculata*)	JAPAN	
		NFRDA (*Pavlova lutheri*)	NFUP (*Nanno-chloris oculata*)		SNFRI (*Tetraselmis tetrathele*)	NRIA (*Synecococ-cus* sp.)
Light intensity (lux)	Natural	6,000 - 7,000	Natural	Natural	Natural	Natural
Photoperiod	Natural	Continuous	Natural	Natural	Natural	Natural
pH	Natural	Approximately 8.2	Approximately 8	> 7.8	8 - 10	7.6 initially
Temperature (°C)	20 - 30	25	20 - 30	25 - 30	25 optimum 10 - 33 range	25
Salinity (ppt)	20 - 30	31 - 32	30 - 35	25	30 - 35	30 - 35
Aeration/ mixing	Moderate aeration	Aeration	Aeration	Strong aeration	Aeration, approximately 5,000 - 8,000 liters/min.	Aeration used but not needed
CO_2 addition	Not used	2 - 5% compressed, filtered gas added	Not used	Not used	Not used	Not used
Nutrient medium (types, amounts)	Urea: 4-30 g/ton Ammonium sulfate: 4-20 g/ton Calcium superphosphate: 4-15 g/ton	F/2	F/2 or Erdshreiber medium	Ammonium sulfate: 50 g/m^3 Superphosphate: 5 g/m^3 Urea:3 g/m^3	Ammonium sulfate: 100 g/ton Superphosphate: 20 g/ton Clewat 32: 4 g/ton Fe-EDTA:20 g/ton	Enriched seawater

tion was again raised, as was the fact that good species/strain selection can prevent many culture problems. If one can find a hearty, fast-growing species suited to local conditions, reliable microalgae culture is easier to achieve. This, along with covering or enclosing tanks as much as possible, was considered by the group to be the most promising approach to reducing contamination (Table 3).

To reduce human error, participants suggested adopting computer-operated systems, improving training programs, requiring a high degree of accountability from the staff and monitoring cultures closely. Finally, culturists reported that they were generally pleased with the quality of their algae; several rated their quality as "consistent" or "good."

Table 2. Reliability — frequency, severity, causes, and prevention of culture crashes.

	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone)	Hawaii (*Nanno-chloropsis oculata*)	Louisiana (*Chlorella minutissima*)	Maryland (Unidentified sp.)		
Frequency of crashes (estimate)	Rare. < 5 tanks/2,000	1 - 3/ year	1/5 months — infrequent	Perhaps 1% of the runs	2 - 3/month during rainy season	Infrequent
Severity of crashes	Can be severe	Depends on the timing. Can be devastating	Back up within a few days	Discard	80% mortality	Not severe
Common causes	Insufficient chlorine neutralization, protozoans	1. Human error 2. Contamination	Temporary lack of temperature control	Bacteria. Fungus is rare (result of human error). Equipment malfunction.	1. Rain (low light intensity) 2. Contamination 3. Poor quality of starter	Protozoa
Safeguards (original and added)	Sanitary procedures for protozoans	Backup cultures	1. Better temp. control 2. Disinfection cycle added to computer control program 3. Air is well filtered	Sterilization	Provide shelter, backup cultures	Rotate species
Quality consistency	Good	Varies with season	Good	Very high. High EPA content	Maintained over 10 generations	Fairly consistent

Table 2. Continued.

	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nanno-chloropsis oculata*)	JAPAN	
		NFRDA (*Pavlova lutheri*)	NFUP (*Nannochloris oculata*)		SNFRI (*Tetraselmis tetrathele*)	NRIA (*Synecococcus* sp.)
Frequency of crashes (estimate)	High if there is a delay in subculturing	Occur in summer only (*Chlorella*)	5/season (occur only in spring and fall)	Rare	5/season	5/season
Severity of crashes	Severe	Discard	Moderate	Moderate	Mild in comparison with *N. oculata*	Moderate
Common causes	High temperature, light intensity, bad inoculum	Temperature (*Chlorella*)	Low temperature	Ciliate contamination as a result of human error	Protozoan and rotifer contamination	Protozoan contamination due to human error (not chlorinating the water)
Safeguards (original and added)	Stock culture kept indoors or collected from nature	1. Sterilization 2. Backups (*Chlorella*)	Stock culture is kept indoors	1. Sited away from trees 2. Water chlorinated 3. Air filtered	1. Sterilization 2. Non-motile cells used to start new batches	Backup cultures
Quality consistency	Consistent at the exponential stage	Good	Fairly consistent	Consistent	Consistent	Consistent, however the nutritional quality of this species is not high

Table 3. The causes of microalgal culture crashes and potential solutions.

	Causes[†]	Potential Solutions	Comments
1.	**Contamination** with: a. protozoa b. inhibitory or pathogenic bacteria c. blue-green algae d. other algal species e. *Artemia*, rotifers	-*Cover or enclose tanks as much as possible -*Better species/strain selection (the higher the growth rate the smaller the chance of contamination) -Place algae tanks upwind of rotifer tanks -Site selection -Close monitoring -Increase salinity -Staircase circulating system kills some protozoa -Biofilter (will reduce psammophilic protozoa) -Add malachite green (to inhibit blue-green algae) -Add germanium dioxide (to inhibit diatoms) -Better water treatment (e.g. use a biofilter and UV or UV and ozone) -Biological control of predators/competitors	-Cost-effectiveness of most of these solutions will have to be assessed -Some treatments (adding a predator, increasing salinity, adding chemicals, etc.) may reduce growth of the desired species of algae
2.	**Environmental conditions** (temperature, cloud cover, rain, wind)	-Cover or enclose -Site selection -Better species/strain selection -Storage of algae during seasons which are best for growth	Cost-effectiveness to be evaluated
3.	**Human error**	-Computer control/expert systems -Better training programs -Accountability and close monitoring at every step	Cost-effectiveness to be evaluated
4.	**Metabolite build-up**	-Waste can be siphoned from tank bottom	

[†]Ranked in order of importance.

*Considered the most promising approaches, in general.

MICROALGAE DISCUSSION GROUP D
Production Levels and Requirements

This discussion group focused on the following points: "How much algae are you producing?"; "How does this compare with what you'd like to be producing?"; and "What resources are needed to maintain current production levels?"

Production

It was difficult for most group members to estimate how much algae their systems were capable of producing; in many cases, production was gauged to meet the demands of larval rearing. To meet its production goals, Coast Oyster Company typically grows 720 kg dry weight of *Thalassiosira pseudonana* (3H clone) every eight months (Table 1). The **potential** yearly production of a 200-liter fermentor was estimated to be 1,200 kg dry weight of an unidentified diatom, and the average algal output of one of Thailand's backyard shrimp hatcheries is approximately 5×10^{12} cells of *Chaetoceros calcitrans* per month.

The intensity of production is reflected by the harvest densities. The diatoms *Chaetoceros calcitrans* and *Skeletonema costatum* are cultured at fairly low densities. In Thailand, the density of the former is $5 - 10 \times 10^4$ cells/ml, while *S. costatum* in Taiwan is harvested at approximately 12×10^4 cells/ml (Table 1).

It's noteworthy that almost all of the culturists present indicated that they were able to meet the demands of larval rearing. Algal shortages were uncommon, largely due to the fact that some redundancy was built into the systems, allowing producers to absorb any unexpected losses. Coast Oyster Company, the world's largest oyster hatchery with a yearly production capacity exceeding 20 billion larvae, routinely produces an excess of microalgae that it concentrates and preserves to use later as feed for juvenile and adult oysters.

Requirements

Participants were asked to detail the resources required to maintain their present production levels. The categories were: space, equipment, energy, labor and chemicals and other supplies. Additionally, producers were asked to estimate the cost of constructing the feeds production portion of their hatcheries.

Louisiana State University's system had minimal space requirements, 1.5 - 1.8 m^2, while others usually fell somewhere between 50 m^2 and 500 m^2 (Table 2). The two largest operations, in terms of space, were Coast Oyster Company (790 m^2) and The Oceanic Institute (< 10,000 m^2).

After start-up, the equipment needs of a microalgal production facility are fairly small and may include a host of materials for the stock culture room (glassware, pipets, cleaning materials, microscope slides, fluorescent lights, etc.), in addition to flexible tubing, polyethylene bags, airstones, etc. Costs for such items ranged from $70/year at a

Table 1. Production estimates.

	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone)	Hawaii (*Nanno-chloropsis oculata*)	Louisiana (*Chlorella minutissima*)	Maryland (Unidentified sp.)		
Maximum production (per season or year)	740 kg dry weight/8 months	40×10^{12} cells/year	N/A	1,200 kg dry weight/year (50 weeks/year, 3 runs/week in a 200-liter fermentor) [Potential]	1×10^{13} cells/month	4.8×10^{15} cells/year
Minimum production	700 kg dry weight/8 months	30×10^{12} cells/year	N/A	—	3×10^{12} cells/month	1.4×10^{15} cells/year
Average production	720 kg dry weight/24,000 tons/8 months	2.2×10^{6} liters/year, 8,000 - 10,000 liters/day	N/A	—	5×10^{12} cells/month	3.1×10^{15} cells/year
Maximum density (cells/ml)	3.5×10^{6}	38×10^{6}	N/A	$4 - 5 \times 10^{4}$ g dry weight/ml	10×10^{4}	2×10^{6}
Minimum density	2.0×10^{6}	17×10^{6}	N/A	$1.5 - 2.0 \times 10^{4}$ g dry weight/ml	3×10^{4}	1.2×10^{6}
Average density	3.0×10^{6}	20×10^{6} (target)	Average productivity: 7.2×10^{7} g dry weight /ml/day	$3.5 - 4.0 \times 10^{4}$ g dry weight/ml	5×10^{4}	1.6×10^{6}

Table 1. Continued.

	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nannochloropsis oculata*)	JAPAN	
		NFRDA (*Pavlova lutheri*)	NFUP (*Nannochloris oculata*)		SNFRI (*Tetraselmis tetrathele*)	NRIA (*Synecococcus* sp.)
Maximum production (per season or year)	9,000 tons/year	—	—	3.5×10^{16} cells/year	3×10^{14} cells/year	2×10^{14} cells/year
Minimum production	3,000 tons/year	—	—	2.0×10^{16} cells/year	1.5×10^{14} cells/year	—
Average production	6,000 tons/year	On demand production	On demand production	2.8×10^{16} cells/year	2.3×10^{14} cells/year	—
Maximum density (cells/ml)	15×10^{4}	70×10^{6}	30×10^{6}	25×10^{6}	2×10^{6}	5×10^{6}
Minimum density	8×10^{4}	30×10^{6}	10×10^{6}	10×10^{6}	1×10^{6}	5×10^{5}
Average density	12×10^{4}	50×10^{6}	20×10^{6}	12×10^{6}	$1.5 - 1.8 \times 10^{6}$	1×10^{6}

Table 2. Estimated production requirements.

	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone)	Hawaii (*Nanno-chloropsis oculata*)	Louisiana (*Chlorella minutissima*)	Maryland (Unidentified sp.)		
Land/space (m^2)	790	< 10,000	1.5 - 1.8	2.8 for fermentor, 150 for ancillary equipment	500	500
Construction ($US)	Unknown, approx. 20% of hatchery cost	Unknown	3,000 (main cost was computer)	100,000	2,500	10,000 - 20,000
Equipment ($US)	Unknown, approx. 20% of hatchery costs	1,500+/year	$500.00	10 - 20% of costs	500/month	400/year
Energy ($US)	20,000/year	Unknown	N/A	—	25/month	200/year
Labor (hours/week)	112	≥ 160	2 - 3	20 (hypothetical)	56	70
Chemicals, other supplies ($US)	25,000/year	2,000/year	$4,000/year (mainly artificial salt)	40% of cost (media only, based on estimates from the literature of larger scale production)	150/month	1,400/year

Table 2. Continued.

	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nannochloropsis oculata*)	JAPAN	
		NFRDA (Commercial bivalve hatchery)	NFUP (*Nannochloris oculata*)		SNFRI (*Tetraselmis tetrathele*)	NRIA (*Synecococcus* sp.)
Land/space (m^2)	50 - 100	500	50	500	10 - 25	500
Construction ($US)	Cement ponds: 5,000 - 10,000	200,000	7,000	20,000	8,000 - 10,000	Small (semi-commercial)
Equipment ($US)	70/year	300/month	1,000/year	1,000/year	1,000 - 1,200/year	Small
Energy ($US)	100/year	270/month	150/year	1,000/year	4,000 - 4,500/year	Small
Labor (hours/week)	7	63	20	28	14	7
Chemicals and other supplies ($US)	60/year	250/month	100/year	250/year	400 - 500/year	Small

Taiwanese shrimp hatchery to $500/month for a backyard hatchery in Thailand (Table 2).

Energy requirements for pumping, illumination and temperature control were estimated to run from as high as $20,000/year at Coast Oyster Company to as low as $100/year at the Taiwanese shrimp hatchery (Table 2).

In terms of labor, The Oceanic Institute and the Louisiana State University *Chlorella minutissima* chemostat system had the highest and lowest requirements, respectively (Table 2). More than 160 hours/week go into producing algae at The Oceanic Institute, whereas the chemostat system required only two to three hours/week for mixing stock solutions and performing other tasks the computer didn't control. This discrepancy is partially due to automation in the chemostat system, but also reflects the fact that The Oceanic Institute harvests 8,000 - 10,000 liters of algae/day. The Louisiana State University system, by contrast, was composed of three 20-gallon growth chambers, hence only a total of 227 liters was under cultivation. Most of the other participants reported that less than 100 hours/week was devoted to growing algae at their facilities.

Finally, the most significant requirement in the "chemicals and other supplies" category is for the culture media. Expenditures for fertilizers and other supplies were lowest in Taiwan at $60/year and highest for Coast Oyster Company, $25,000/year. Additionally, the cost of culture media for heterotrophic systems is estimated to be 40% of total operating costs.

MICROALGAE DISCUSSION GROUP E
Costs

Estimates of the true cost of culturing feed for a hatchery are difficult to come by, largely because the feed and target species production units almost always share space, equipment, supplies, energy and labor. Teasing apart the separate components is a difficult task, and it seems producers and researchers have had little incentive to do so. Participants did agree, however, that the cost of growing algae is significant, and reducing that cost would go a long way toward helping commercial larviculturists.

Algal production costs may be determined indirectly by estimating the percentage of total hatchery costs used to grow algae. The highest numbers were for the research-scale hatchery at the National Fisheries University of Pusan (NFUP) in South Korea (30 - 40%) and for a commercial shrimp hatchery in Singapore (25 - 30%) (Table 1). Coast Oyster Company spends about 18% of its annual operating budget to grow algae, the cost of which is approximately US$ 50/kg dry weight. By contrast, the cost of producing algae heterotrophically on a large scale has been projected to be anywhere from US$ 2 to 25/kg dry weight.

Another estimate of the resources devoted to algal production may be derived from the ratio of algae culture volume to target species culture volume. The highest algae:target species ratios were for penaeid shrimp larvae (10 - 15:1 in Taiwan, 10:1 in Thailand) and rotifers (8 - 10:1 at SNFRI in Japan) (Table 1). This indicates that these facilities probably devote a great deal more resources to producing algae than to growing shrimp larvae or rotifers.

Group members were also asked to rank their main expenditures. The majority of culturists listed chemicals and supplies as their most significant cost, while the cost of labor was second (Table 1). Other important costs were for energy and facilities.

However, near the end of the session, when the entire group was asked to rank the major costs of producing microalgae, the majority of the votes were cast for the "labor" category (Table 2). "Supplies and chemicals" did come in second in the group ranking, followed by "facilities" and "energy."

When asked about the most promising means of reducing costs, culturists mentioned increased automation, streamlining the upscaling process, cryopreservation of stock cultures, and adopting continuous culturing techniques as some ways to reduce labor requirements (Table 2). Expenditures for supplies and chemicals, they suggested, could also be minimized by shopping around for the cheapest dealer and by using agricultural-grade fertilizers, rather than reagent-grade chemicals, where possible.

Participants also discussed the possibility of creating centralized facilities devoted solely to producing algae for sale to hatchery operators. A large, automated, centrally located enterprise could potentially sell microalgae much more cheaply than a hatchery could produce it. Thailand is approaching this;

Table 1. Percent total hatchery costs used for algae production, ratio of algae culture volume to target species culture volume.

	UNITED STATES				THAILAND (*Chaetoceros calcitrans*)	N. CHINA (*Isochrysis galbana*)
	Washington (*Thalassiosira pseudonana* 3H clone; 1990 data)	Hawaii (*Nannochloropsis oculata*)	Louisiana (*Chlorella minutissima*)	Maryland (Unidentified sp.; projections based on 5,000 - 500,000 liter fermentors)		
% Total hatchery operating costs used for algae production	18 (algae production cost is approx. $50/kg dry weight)	1/6 labor force devoted to algal production	N/A	N/A (production cost: $2 - 25/kg dry weight)	Approx. 15%	1/3 labor force devoted to algal production
Ratio of algae culture volume : target species culture volume	1 : 3 (oyster larvae)	4 - 5 : 1 (rotifers) : 1 (fish larvae)	N/A	N/A	10 : 1 (*Penaeus* larvae) [calculated from the total volume of *Chaetoceros* water used in each batch]	4 : 1 (*Argopecten* broodstock) 1 : 2 (*Argopecten* larvae)
Ranking of major costs (% total algae production cost)	1. Labor (37%) 2. Overhead (30%) 3. Lab supplies and chemicals (19%) 4. Energy (14%)	1. Labor 2. Energy 3. Supplies and equipment	1. Supplies and chemicals (energy and capital not included)	1. Carbon source (15 - 45%) 2. Capital (18 - 30%) 3. Other chemicals and equipment (5 - 25%) 4. Energy (10 - 20%) 5. Labor (2 - 5%)	1. Chemicals and supplies (35%) 2. Labor (24%) 3. Maintenance (18%) 4. Energy (6%) 5. Starter cultures (4%)	1. Supplies and chemicals (70%) 2. Labor (20%) 3. Energy and misc. (10%)

government centers provide free starter cultures to small shrimp hatcheries, freeing them of the task of stock culture maintenance. Further, in Japan and the United States at least, a number of companies are devoted to microalgae production (primarily for non-aquacultural purposes), and one can purchase a product which has been dried or otherwise processed and use it as feed (see Snell this volume and Okauchi this volume). Improvements need to be made in the quality and price of such algae, but this is certainly a promising approach for the future.

Table 1. Continued.

	TAIWAN (*Skeletonema costatum*)	SOUTH KOREA		SINGAPORE (*Nannochloropsis oculata*)	JAPAN	
		NFRDA (*Pavlova lutheri*)	NFUP (*Nannochloris oculata*)		SNFRI (*Tetraselmis tetrathele*)	NRIA (*Synecococcus* sp.)
% Total hatchery costs used for algae production	2%	N/A	30 - 40%	25 - 30%	N/A	Small
Ratio of algae culture volume : target species culture volume	10 - 15 : 1 (penacid larvae)	5 : 1 (1 mm spat)	3 - 4 : 1	1.5 - 2.0 : 1	4 : 1 (*Penaeus japonicus* larvae) 8 - 10 : 1 (Rotifers)	5 : 1
Ranking of major costs (% total algae production cost)	1. Facility (44%) 2. Labor (37%) 3. Stock (9%) 4. Supplies (6%) 5. Energy (4%)	1. Facility 2. Supplies 3. Energy	1. Facilities and supplies (50%) 2. Labor (30%) 3. Energy (10%) 4. Other (10%)	1. Labor (50%) 2. Facilities and equipment (30%) 3. Energy (10%) 4. Supplies (10%)	1. Supplies and chemicals (45 - 55%) 2. Energy (30 - 40%) 3. Equipment (10 - 20%)	1. Supplies and chemicals (60%) 2. Labor (20%) 3. Energy (20%)

Table 2. Primary costs associated with microalgal production and some potential solutions.

	Main Costs†	Potential Solutions
1.	Labor	-Computer control/Automation -Streamline scale-up -Reduce the number of culture units -Cryopreservation of stock cultures -Continuous culture -Buy pre-mixed nutrients
2.	Supplies and chemicals	-Shop around -Use agricultural-grade fertilizers
3.	Facilities	-Minimize space requirements -Continuous centrifugation -Increase efficiency -Continuous culture
4.	Energy	-Take advantage of natural light -Utilize different species in different seasons

†Ranked in order of importance (see text).

ROTIFER DISCUSSION GROUP A
Culture Situations, General System Description I

Participants spent this first session characterizing their culture situations and providing basic system descriptions. All group members described their scale of operation, identified the strain of rotifer produced, listed the species for which the rotifer, *Brachionus plicatilis*, was being cultured (their "target species," a variety of marine finfish larvae), and described the type of culture and culture enclosures used.

Ten culture situations were described from the United States (Hawaii, Texas and California), Japan (Ehime Prefectural Fish Farming Center, EPFFC), South Korea (Sung Ji Industry), the southern region of the People's Republic of China (Zhanjiang Experimental Station), Singapore (the Marine Aquaculture Section of the Primary Production Department, Ministry of National Development), Thailand, Taiwan, and Kuwait. The latter was a continuous chemostat operated by Charles James (James and Abu-Rezeq 1989a, b; 1990) and described by Terry Snell from the University of Tampa in Florida. Although not directly relevant to the topic of live feeds culture in the United States and Asia, this system was included because of its uniqueness and the potential it holds for rotifer culture worldwide.

Production scales were either "experimental," "government," or "commercial" (Table 1). Furthermore, everyone was culturing S-type *B. plicatilis*, but some facilities were also producing L-types, either separately or together with the S-type rotifers.

A variety of target species were listed (Table 1). These included mullet, milkfish, Pacific threadfin, mahimahi, red drum, white seabass and California halibut in the United States and red sea bream, Japanese flounder, Japanese sweet fish, rock fish, sea bass, grouper and golden snapper in Asia.

The majority of culture was either semi-continuous (5 cases) or batch (2 cases) (Table 1). Thai and Taiwanese hatcheries, meanwhile, can utilize either batch or semi-continuous methods, and Charles James' setup was operated continuously. The chemostat system was composed of six 1-m^3 units that were operated indoors under constant conditions.

By far the largest systems represented were the EPFFC in Japan described by Tsuneo Morizane and Sung Ji Industry in South Korea described by Geon Gil Pi. These facilities have eight 50,000-liter tanks and 16 32,000-liter tanks under cultivation, respectively (Table 1). The systems in the United States were considerably smaller, experimental-scale operations. The Oceanic Institute, for example, manages 14 1,200-liter tanks of *B. plicatilis*; Hubbs-Sea World Research Institute has three 8,500-liter pools; and the University of Texas at Austin Marine Sciences Lab has an undisclosed number of 150-liter tanks. The Zhanjiang Experimental Station and commer-

Table 1. Production scale, rotifer strains cultured, target species, type of culture.

	UNITED STATES			JAPAN	KOREA
	HAWAII (The Oceanic Institute)	TEXAS (UTA Marine Sciences Institute)	CALIFORNIA (Hubbs-Sea World Marine Lab.)	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Production scale	Experimental	Experimental	Experimental	Commercial	Commercial
Rotifer strain	S	S and L	S	S and L	S and L S + L
Target species	Mullet, milkfish, Pacific thread-fin and mahimahi	Red drum	White seabass, California halibut	Red sea bream, Japanese flounder, Japanese sweet fish	Sea bream, Japanese flounder, rock fish (*Sebastes*)
Type of culture	Batch (2 days)	Batch (3 days)	Semi-continuous (2 - 8 weeks)	Semi-continuous (2 - 4 weeks)	Semi-continuous (1 - 2 weeks)
ENCLOSURES					
Shape	Round	Cylindrical	Round	Rectangular	Rectangular
Dimensions	1 m diameter	46 x 92 cm	3.7 m diameter	5 x 6 x 2 m	6 x 3 x 2 m
Capacity (liters)	1,200	150	8,500	50,000	32,000
Construction Material	Fiberglass	Fiberglass	Plastic lined tanks	Concrete, epoxy resin	Concrete, FRP coated
Number	14	—	3	8	16
Arrangement/ placement	Rows	Stacked rows	Rows	Rows	Rows

Table 1. Continued.

	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
Production scale	Government	Government	Commercial	Commercial	Experimental
Rotifer strain	S + L	S	S	S	S and L
Target species	Sea bass	Grouper, sea bass, golden snapper	Sea bass, grouper, freshwater prawns	11 spp., see Liao et al., this volume	Red sea bream, sea bass
Type of culture	Semi-continuous (1 week)	Semi-continuous (1 week)	Batch and semi-continuous	Semi-continuous and batch	Continuous (many months)
ENCLOSURES					
Shape	Rectangular	Rectangular	Square and rectangular	Rectangular	Conical
Dimensions	3 x 10 x 1.2 m, 16 x 7 x 1.2 m, 6 x 5 x 1.2 m	5 x 1.5 x .8 m	2 x 5 x 1.0, 4 x 4 x 1.25	4 x 10 x 1 m, 10 x 30 x 1 m	Unknown
Capacity (liters)	30,000, 60,000, 145,000	6,000	10,000 - 20,000	40,000 - 300,000	1,000
Construction Material	Concrete	Concrete, epoxy resin	Concrete	Concrete	Fiberglass
Number	4 @ 30,000 L, 6 @ 60,000 L, 2 @ 145,000 L	8	4	Varies	6
Arrangement/ placement	Rows	Rows	Rows	2 rows	Rows

Table 2. Water source, treatment, and transport; climate and stock culture.

	UNITED STATES			JAPAN	KOREA
	HAWAII (The Oceanic Institute)	TEXAS (UTA Marine Sciences Institute)	CALIFORNIA (Hubbs-Sea World Marine Lab.)	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Water source	Seawater - well, Freshwater - municipal	Ocean	Ocean	Ocean	Ocean
Water treatment	None	100- and 1-μm	Sand filter, 5- and 1-μm	Sand filter	Sand and oyster shell filter
Water transport	To tank-pump, To harvest-gravity	To tank-pump, To harvest-gravity	To tank and harvest-pump	To tank -pump, To harvest-gravity	To tank-pump, To harvest-gravity
Indoor/outdoor	Indoor	Indoor	Indoor/greenhouse	Indoor	Indoor in winter; Outdoor: May - Nov.
Climate	Sub-tropical	Temperate	Temperate	Temperate	Temperate
Stock culture	No	Yes	No	No	Yes
Filter suspended solids	None	None	Yes. Settled first, then siphoned	Yes. Through a hanging, spongy plastic pore mesh	Yes. Airlifted and filtered though oyster shell basket

Table 2. Continued.

	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
Water source	Ocean	Ocean	Ocean	Ocean	Ocean
Water treatment	Sand filter	Sand filter	Sand filter and chlorinate	Sand filter	Sand filter
Water transport	To tank-pump, To harvest-gravity	To tank-pump, To harvest-gravity	To tank-pump, To harvest gravity	To tank-pump, To harvest-gravity	To tank-pump, To harvest-gravity
Indoor/outdoor	Outdoor	Outdoor	Outdoor	Outdoor	Indoor
Climate	Sub-tropical	Tropical	Tropical	Sub-tropical	Arid
Stock culture	No	No	Yes	No	Yes
Filter suspended solids	Yes. Siphoned into bamboo basket, then through a plastic basket with nylon mesh	None	Yes	None	Yes. Airlifted through a 20-liter plastic basket

cial Taiwanese finfish hatcheries culture rotifers in larger tanks: 30,000-, 60,000- and 145,000-liter tanks and 40,000-liter to 300,000-liter tanks, respectively. Finally, the tank sizes reported for Singapore and Thailand ranged from 6,000 to 20,000 liters.

With the exception of The Oceanic Institute, which has a saline well, culturists pump their water directly from the ocean

(Table 2). Most also filter the water through sand before use, and utilize gravity for harvesting.

Almost all of the systems located in tropical or sub-tropical regions of the world cultivate their rotifers outside, while most of the temperate facilities conduct operations inside or in greenhouses. Finally, six of the 10 systems represented do not maintain stock cultures of their rotifer strains, and four of the 10 do not filter suspended solids from the culture medium.

References

James, C.M. and T. Abu-Rezeq. 1989a. Intensive rotifer cultures using chemostats. Hydrobiologia. 186/187: 423-430.

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ROTIFER DISCUSSION GROUP B
General System Description II

Participants provided detailed descriptions of culture techniques, including upscaling, culture vessel preparation, monitoring, harvesting and enrichment.

Upscaling procedures and duration vary depending on the culturist, the type and scale of culture, and a number of other factors. Producers with shorter scale-up times generally begin with large tanks that are inoculated with rotifers from previously harvested tanks. Those with relatively long scale-up times begin with stock cultures in small vessels.

Participants reported upscaling times ranging from a low of two days for the government hatchery in Singapore and James' chemostat system in Kuwait, to a high of 17 days for a commercial hatchery in Thailand and the Zhanjiang Experimental Station (Table 1). In the case of Thailand, cultures are begun from stock in 1-liter vessels and transferred to indoor 20-liter and 1,000-liter containers over a period of 12 days before they are moved outside to 10,000-liter tanks. These require approximately five days before they reach harvest density and can then be operated as batch or semi-continuous systems.

Between runs, culture vessels are usually scrubbed or rinsed with chlorine. Workers at The Oceanic Institute, however, scrub their tanks with freshwater only. The Zhanjiang Experimental Station utilizes both potassium permanganate and chlorine, and finfish hatcheries in Singapore mop their tanks with 10% formalin and rinse with freshwater (Table 1).

To promote good reproduction and prevent crashes, rotifer producers often regulate a variety of parameters, including temperature, salinity, pH and density (Table 2). In addition, indicators of rotifer activity such as swimming speed and percentage of females carrying eggs are monitored regularly.

Water is heated except in Southern China, Singapore and Thailand (Table 2); The Oceanic Institute maintains cultures at 28°C. Participants noted that they ordinarily add freshwater to lower salinity; salinities ranging from 10 ppt to full strength seawater were reported. Different strains grow better at different salinities, although a salt content somewhat lower than that of natural seawater usually results in the best reproduction. One important consideration, however, is the salinity of the water in which the target species is being grown; dramatic changes in salinity can cause the rotifers to cease swimming and sink (Lubzens 1987). At the Zhanjiang Experimental Station, however, they grow rotifers at 10 - 15 ppt that suffer no apparent ill effects when transferred to the full strength seawater in sea bass larval rearing tanks.

All participants also said that they monitor pH, but it is not always controlled (Table 2). The other parameters monitored were as follows: density (10 reports); percentage of females bearing eggs (9); swimming speed (7); levels of contaminants, especially ciliates (9); ammonia (1); turbidity (1); coloration (1); and degree of foam formation (1).

Table 1. Scale-up procedures and culture vessel preparation.

	UNITED STATES			JAPAN	KOREA
	HAWAII (The Oceanic Institute)	TEXAS (Commercial batch culture in plastic bags)	CALIFORNIA (Hubbs-Sea World Marine Lab.)	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Scaling up time (days)	14	3	2.5 (average)	7 - 14	7 - 14
Scaling up procedure	Start at 500 liters; grow 2 - 3 days and scale up until reach final size (1,200 liters)	Initial inoculation with 10 liters algae and 50 liters water; then add rotifers after 3 days	40/ml up to 640/ml, 360/ml average	Always large scale	Start at 1,000 liters at the beginning of the season only; from then on semi-continuous system
Culture vessel preparation	Freshwater wash and scrub	Rarely used twice, but if used twice chlorine is used to sterilize	Chlorine and/or steam gun	Chlorine wash	Chlorine wash

Table 1. Continued.

	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
Scaling up time (days)	15 - 17	2	17	5 - 7	2
Scaling up procedure	Transfer to larger tank on day 4 - 5 and again on day 8 - 10; harvest on day 15 - 17	For 6-m^3 tank start in 2 m^3 (day 0); then 5 m^3 the next day. 50% harvested on day 2 and for the next 5 days	Indoors start at 1 liter; then 20 liters; then 1,000 liters (12 days). Then outdoors start at 10,000 liters (5 days)	2-ton tank changed every 5 - 7 days	None; stocking density 50/ml and only a few days to first harvest
Culture vessel preparation	Potassium permanganate and chlorine 60 m^3; 145 m^3	Tanks mopped with a cloth soaked with 10% formalin and rinsed thoroughly with freshwater	Chlorine and sun dry	Chlorine and if there is bacterial contamination use dilute HCl	Washed with sodium hypochloride and dried

At this point, discussion focused on the fact that producers have few techniques with which to easily and reliably assess the health of their rotifer cultures (see Snell et al. 1987). Tracking density is fairly simple but may not allow one to detect problems in time to prevent a crash. Two other popular indices, percentage of egg-bearing females and swimming speed, are more sensitive but also more labor-intensive.

Terry Snell has been researching quantifiable assays of rotifer health that also lend

Table 2. Monitoring/quality control procedures.

Parameters monitored	UNITED STATES			JAPAN	KOREA
	HAWAII (The Oceanic Institute)	TEXAS (Commercial culture in plastic bags)	CALIFORNIA (Hubbs-Sea World Marine Lab.)	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Temperature	Submersible heater to 28°C	Gas heat	Submersible heater to 25°C	Heat exchanger/ boiler	Heat with boiler
Salinity (ppt)	Add tap water to lower to 25 - 28	Add tap water to lower to 25 - 28	Full-strength seawater	Not controlled in most cases	Add tap water to lower to 25 - 28
pH	pH monitor	pH monitor	pH monitor	pH monitor	pH monitor
Other	Density, percentage carrying eggs; swimming speed; ciliates or other protozoans	Density, percentage carrying eggs; swimming speed; ciliates or other protozoans	Density is monitored with a particle counter, percentage carrying eggs, swimming speed, protozoan contamination; ammonia checked daily with probe	Density, percentage carrying eggs; swimming speed; ciliates or other protozoans	Density, percentage carrying eggs; swimming speed; ciliates or other protozoans; presence of surface oil membrane

Table 2. Continued.

Parameters monitored	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
Temperature	Not controlled	Not controlled	Monitored, but not controlled	Monitored. Electric heat in winter	Submersible heater to 28°C
Salinity (ppt)	Lower to 10 - 15	22 - 25	Controlled at 22	Add tap water to lower to 20 - 25	25 - 28 ppt culture medium pumped in continuously
pH	pH not controlled	Not controlled	pH monitored, but not controlled	Not controlled	pH monitor
Other	Density, percentage carrying eggs, swimming speed; coloration, turbidity and degree of foam formation	Density, percentage carrying eggs and ciliate contamination (especially *Euplotes*) are monitored. Regarding quality control: tank with poor growth will be harvested and restarted; tank with ciliates discarded.	Density is controlled and contamination and swimming speed are monitored. The presence of luminescent bacteria during certain seasons can indicate poor conditions.	Density, contamination, percentage carrying eggs	Density, percentage carrying eggs; swimming speed; ciliates or other protozoans

themselves to automation. He remarked that while un-ionized ammonia is a good, sensitive indicator of stress, probes for this parameter require frequent calibration; hence it is a somewhat labor-intensive process. He is presently investigating the use of fluorescent probes to quantify the activity of the enzyme

Table 3. Harvesting, enrichment and indicators of activity.

	UNITED STATES			JAPAN	KOREA
	HAWAII (The Oceanic Institute)	TEXAS (Commercial culture in plastic bags)	CALIFORNIA (Hubbs-Sea World Marine Lab.)	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Harvest procedure	Rotifers are gravity fed into a 70-μm nitex screen bag that is 30 cm in diameter and 70 cm deep	Rotifers are gravity fed into a 48-μm bag that is 30 cm in diameter and 70 cm deep	Rotifers are pumped into a 70-μm bag that is 55 cm square	Rotifers are gravity fed into a 63-μm bag that is 40 cm in diameter and 1.5 m deep	Rotifers are gravity fed into a 50-μm bag that is 1 m in diameter and 50 - 70 cm deep
Method of transfer	Rotifers are put into a container with *Nannochloropsis* and carried by hand to the larval rearing tank	The rotifer bags are carried in water and put in a known volume of water and counted just prior to feeding	Rotifers are washed in the harvest tank and transported to an automatic feeder where *Nannochloropsis* is added	Rotifers are washed and carried by basket to a secondary culture tank.	Rotifers are washed and carried by basket to a secondary culture tank.
Enrichment	No	No	*Nannochloropsis* for 12 hours	1. *Nannochloropsis* (12 - 24 hours) + squid liver oil 2. Direct method	*Nannochloropsis* for 1 day

esterase in cultured rotifers (see Snell, this volume; Snell and Moffat, in preparation).

Along these same lines, Masachika Maeda and Masaaki Fukuda were optimistic about the use of RNA/DNA ratios to measure protein synthesis in cultured rotifers; the higher the levels of synthesis, the better the health of the rotifers. Finally, Kazutsugu Hirayama suggested that swimming speed may be the best overall indicator of rotifer health.

Discussion then turned briefly to harvesting and enrichment procedures. Most culturists gravity feed their culture water through plankton netting with a mesh size ranging from 50 to 70 μm (Table 3). An enrichment stage, the purpose of which is to pack the rotifers with a nutritious substance which will benefit the target species, often follows; *Nannochloropsis oculata* is usually used. Following rinsing and/or enrichment, the rotifers are transferred to the larval tanks or used to inoculate new cultures. Transfer, in most cases, is accomplished by hand.

References

Snell, T.W., M.J. Childress, E.M. Boyer and F.H. Hoff. 1987. Assessing the status of rotifer mass cultures. J. World Aquacult. Soc. 18: 270-277.

Table 3. Continued.

	S. CHINA Zhanjiang Experimental Station	SINGAPORE Marine Aquaculture Section	THAILAND	TAIWAN	KUWAIT C. James System
Harvest procedure	Rotifers are gravity fed into a 50-μm mesh nylon bag that is 15 cm in dia. and 1 m deep	Rotifers are gravity fed through a 250-μm plankton net and collected in a 40 μm plankton net to remove undesirable particles	Rotifers are gravity fed into a 50-μm bag that is 40 cm in dia. and 1 m deep	Rotifers are gravity fed into a 63-μm bag that is 40 cm in dia. and 70 cm deep	Rotifers are gravity fed into a 63-μm bag that is 40 cm in dia. and 1 m deep
Method of transfer	Rotifers are washed in a bag, transferred to a bucket and carried to larval rearing tank	Concentrated rotifers are transferred to plastic buckets and carried to larval rearing tanks	Washed first, transferred to a bucket and carried to larval rearing tank	Rotifers are put into a container with *Nannochloropsis* and carried by hand to the larval rearing tank	Rotifers are washed in a bag, transferred to a bucket and introduced to a 500-liter tank containing 40 x 10^6 cells *Nannochloropsis* /ml before they are fed to larvae
Enrichment	No	Yes - with HUFA-rich booster diet before feeding	Yes - with *Nannochloropsis* for 12 hours	Sometimes. When enriched they use *Nannochloropsis* for 6 - 12 hours	*Nannochloropsis* only

ROTIFER DISCUSSION GROUP C
Culture Parameters and Reliability

The key issues raised in Discussion Group C were:

- the acceptable ranges for culture parameters such as temperature, salinity and pH;
- and the frequency and severity of crashes, causes, and means of avoiding them.

Feed Types and Culture Parameters

Group members began by describing the type of food they provided their rotifers (Table 1). With the exception of Singapore, all systems were using a combination of algae and yeast. One or a combination of the following algae were utilized: *Nannochloropsis oculata*, *Nannochloris oculata*, *Chlorella ellipsoidea*, *Tetraselmis subcordiformis*, *T.* sp. and *T. chui*. Baker's, marine and ω yeasts also were used. In addition, the Hubbs-Sea World Marine Lab adds Menhaden oil and vitamins C and E to its rotifer cultures.

Participants were very interested in the topic of feed. They conferred about which species of algae or type of yeast worked best and the benefits of oil and vitamins. For example, several participants believe that oil is beneficial for both rotifer and fish larval growth, but specific experimental evidence is lacking. Some expressed the view that microalgae not only feed rotifers but can improve water quality and increase the stability of cultures, possibly through one or a combination of the following mechanisms: moderating pH, taking up nutrients from the water and limiting the propagation of bacteria and protozoa.

Culturists were asked to list the acceptable ranges for the following parameters: DO, illumination, pH, temperature, salinity and un-ionized ammonia. Acceptable dissolved oxygen levels ranged from 3 to 8 ppm; however most culturists preferred to keep DO above 4 ppm (Table 1). All rotifers were grown in lighted conditions, either natural or artificial. The Oceanic Institute and Thailand, which have indoor systems, reported light levels of 8,000 and 2,000 to 3,000 lux, respectively. Photoperiods were either continuous or 12 L:12 D for indoor systems or natural in the outdoor systems. Kunihiko Fukusho related that he had compared cultures grown outside in full sunlight to those grown indoors in intermediate and dark conditions. The outside cultures performed the best.

Acceptable pH levels fell between 6.9 and 9 (Table 1). Most of the temperature ranges, meanwhile were in the 20s. Salinities were between 20 and 35 ppt. The wide range reported by Dr. Su of Taiwan, 20 - 35 ppt, can be accounted for by differences in the salinities at which target species are grown. Furthermore, all participants provided some type of aeration, usually with airstones. No other means of mixing was described.

Only the staff at Hubbs monitors ammonia regularly. Don Kent remarked that total ammonia levels in his system reach 80

Table 1. Culture parameters.

	UNITED STATES			JAPAN	KOREA
	HAWAII The Oceanic Institute	TEXAS UTA Marine Sciences Lab.	CALIFORNIA Hubbs-Sea World Marine Lab.	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Feed	*Nannochloropsis oculata* + yeast	Algae + oil emulsion + yeast	*Nannochloropsis oculata* + yeast, lecithin and oil, vitamins C and E	*Nannochloropsis oculata* + omega yeast + baker's yeast	*Chlorella ellipsoidea* + *Nannochloris oculata* + omega yeast
DO (ppm)	7 - 8	Not available	3 - 5 (average 4)	4.0 - 5.0	4.0 - 5.0
Light Intensity	8,000 lux	Not available	Natural	Natural	Natural
Photoperiod	24 hours	Continuous	Natural	Natural	Natural
pH	7.5 - 8	7.6 - 8.0	6.9 - 7.5	7.0 - 8.0	7.0 - 8.0
Temperature (°C)	28 (controlled)	25 - 30	22 - 26 (controlled)	22 (in winter) - controlled) 23 - 28 (in summer) - (natural)	L-type: 23 S-type: 28 (controlled in winter only) or 22 - 28 (controlled in winter only)
Salinity (ppt)	25 - 28	20 - 30	34	32	30 - 34
Aeration/ mixing	Vigorous aeration with 3 (2-inch) airstones per tank	Yes. 1 (6-inch) airstone per bag	Vigorous. 8-inch diameter diffuser; 4 per pool	8 (15-cm) airstones per tank	6 (30-cm) airstones per tank
Un-ionized ammonia	Not monitored	Not monitored	Up to 80 ppm total ammonia	Not monitored	Not monitored

ppm; this translates to roughly 0.5 to 0.8 ppm un-ionized ammonia (NH_3). Un-ionized ammonia levels as low as 0.3 ppm, however, can cause decreases in swimming activity (Snell et al. 1987).

Culture Crashes

Rotifer cultures are susceptible to sudden declines in population numbers and/or reproductive rate. Certain phenomena are correlated with these so-called "crashes," but the problem is complex and still not well understood. Participants reported fairly low frequencies of crashes, one to three per season or one every three to four weeks (Table 2). Keep in mind, however, that it is common practice to harvest those cultures which begin to decline **before** they can fail completely. Hence the crash estimates are, in a sense, underreported.

The severity of culture crashes varied from "minor" to "total loss" (Table 2). The impact of a crash, in turn, is a function of the severity of the culture failure, timing and a system's ability to absorb such losses.

Participants generated a long list of possible crash causes (Table 2). Operators postulated that the primary causes of crashes in their systems were: a shortage of microalgae (3 responses), poor water quality (2), ciliates or other protozoan predators (2), suboptimal temperatures (1) or unknown (1). The presence of males in the system is also correlated with crashes; however, this is probably just an early manifestation of poor culture conditions. Genetic problems, human error,

Table 1. Continued.

	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
Feed	*Tetraselmis subcordiformis* or *T.* sp. + baker's yeast	*Nannochloropsis oculata*	*Nannochloropsis oculata* or *Tetraselmis* + baker's or marine yeast	*Tetraselmis chui*, *Nannochloropsis oculata* and yeast	*Nannochloropsis oculata* + yeast
DO (ppm)	Continuous aeration; oxygen saturation	> 4	4 - 8	>4	5 - 6
Light intensity	Natural	Natural	2,000 - 3,000 lux	Natural	Fluorescent, 6,000 lux
Photoperiod	Natural	Natural	Natural	Natural	12 : 12 (LD)
pH	8 - 8.3	7.5 - 8.5	7 - 9	7 - 8	Algae 6.5; rotifers ≈8.0
Temperature	20 - 30 (natural)	25 - 30 (natural)	20 - 25 (shaded) or 28 - 32 (without shade)	25 - 32 (natural, controlled in winter)	25 (controlled)
Salinity (ppt)	20 - 27	22 - 25	22	20 - 35	30
Aeration/ mixing	1 (3-cm) airstone/m^2	Strong aeration; perforated PVC; 4-mm hole; 80 ml/liter/min	Strong; 4 (4-inch) airstones per m^2	Airstone	Moderate aeration; perforated PVC
Un-Ionized ammonia	Not monitored	Not monitored	Not monitored	Not monitored	Occasionally monitored. Below 0.2 ppm

general problems with the weather, poor nutrition or low-quality feed, disease as a result of adding fish oil and an accumulation of metabolic wastes were also mentioned.

Certain pathogenic or toxic strains of bacteria have been identified as detrimental to rotifer cultures (Yu et al. 1990; Maeda and Hino, this volume). Regarding the effect of ciliates, Kunihiko Fukusho noted that they can be harmful to rotifer eggs and that in Japan, ciliates appear at 10 - 15°C but disappear when the temperature is raised to about 20°C. However, the group reached a consensus that ciliates were usually problematic only in semi-continuous cultures.

In general, producers deal with crashes by maintaining extra cultures that they can harvest if other tanks fail (Table 2). Other safeguards include the use of batch culture, sanitary procedures to exclude as many contaminants as possible, temperature regulation and water exchange. Finally, the quality consistency of rotifers produced ranged from "none" and "fair" to "consistent" and "good."

References

Yu, J.P., A. Hino, R. Hirano and K. Hirayama. 1990. The role of bacteria in mass culture of the rotifer *Brachionus plicatilis*. *In*: R. Hirano and I. Hanyu (Eds.). The Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines. pp. 29-32.

Table 2. Reliability—quantity and quality.

	UNITED STATES			JAPAN	KOREA
	HAWAII The Oceanic Institute	TEXAS UTA Marine Sciences Lab.	CALIFORNIA Hubbs-Sea World Marine Lab.	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Frequency of crashes	2 - 3 per season	Every 3 - 4 weeks	1 per month	0 or 1/season (total)	Rare
Severity of crashes	1/4 - 1/2 minor = total loss	Minor - total loss	Up to total loss	Decrease in density	Decrease in density
Common causes	1. Ciliates 2. Food quality and quantity 3. Males in system 4. Genetic problems 5. Human error	1. Unknown 2. Ciliates 3. Poor water quality	1. Water quality 2. Algae shortages 3. Temperature too high or low	1. Water quality 2. Food quality and quantity	1. Temperature 2. Nutrition 3. Weather 4. Fish oil leads to disease 5. Contamination (ciliates)
Safeguards (original and added)	1. Batch cultures 2. Computer database with parameters for later predictions	1. Remove ciliates 2. Redundancy 3. Batch culture	1. Heaters 2. Improve harvesting techniques 3. Water exchange 4. Waste removal 5. Abundant high quality algae 6. Diminish sexual reproduction	1. Redundancy 2. Change water frequently 3. High quality algae	1. Redundancy 2. Culture outdoors 3. Improve water quality
Quality consistency	Average - good	Fair	None	Unknown	Fair

Table 2. Continued.

	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
Frequency of crashes	Rare	Rare	2 - 3/season	Rare	Very rare or none
Severity of crashes	Total loss	Moderate	60% loss	Growth rate decrease	Decrease in density to 100/ml
Common causes	1. Shortage of microalgae due to cold weather 2. Food quality	1. Lack of microalgae 2. Protozoan contamination	1. Predators (protozoa) 2. Quantity of algae 3. Metabolic waste	1. Lack of microalgae 2. Protozoan contamination 3. Waste of rotifer	Electrical/ mechanical failure
Safeguards (original and added)	1. Try to maintain proper temp. by using plastic protective cover 2. Work hard	Algae cultured with chlorinated water	1. Well-treated seawater 2. Add *Spirulina* powder 3. Clean tank bottom	1. Redundancy 2. High quality algae 3. Change water frequently	Ensure electrical supply
Quality consistency	In general, very reliable	Fairly consistent	Over 10 generations	Consistent	Consistent for many months

ROTIFER DISCUSSION GROUP D
Production Levels and Requirements

Participants estimated the number of rotifers they produce and compared that to the number they need. The session concluded after each culturist detailed the resources necessary for maintaining current production levels.

Production Levels

It should be emphasized that the facilities represented are primarily concerned with raising fish, not rotifers. Only Charles James' experimental system was operated for the sole purpose of maximizing rotifer production. Hence, most operators could only report what their systems had produced, not what they might potentially produce. Participants provided estimates for the following: total production/day (maximum, minimum and average), production/ml/day (maximum, minimum and average), inoculation density, harvest density and number of rotifers required /day.

Representatives of the Ehime Prefectural Fish Farming Center and Sung Ji Industry reported producing, on average, the most rotifers/day; 6,000 and 4,800 million, respectively (Table 1). The average production levels for a government-operated finfish hatchery in Singapore and the chemostat in Kuwait were about the same, 1,900 million and 1,122 million rotifers/day, respectively. Finally, The Oceanic Institute, Hubbs-Sea World Marine Lab. and Thailand had average daily outputs of 400, 305 and 166 million rotifers/day, respectively.

In order to gauge the intensity of production, group members were asked how many rotifers they produced/ml of culture water on a daily basis* (Table 1). The highest yield, 187 rotifers/ml/day, was achieved in Charles James' system (Table 1). This yield is remarkable, and indicates the great potential of the continuous chemostat system approach to raising rotifers. Sung Ji Industry was second, with an average productivity of 150 rotifers /ml/day (except in the winter), while other estimates fell between 10 and 75/ml.

Southern China reported the lowest inoculation density, 5 - 50/liter. Densitites also tended to be fairly low, ranging from 10 to 50/ml, in Singapore, Thailand, Taiwan and Kuwait, but were higher in the United States, Japan and South Korea (Table 1). The highest average stocking density was at Hubbs: 366/ml. Harvest densities, with the exception of Southern China and Hubbs, were between 100 and 200/ml. Harvest density at the Zhanjiang Experimental Station ranges from 200/ml to 500/ml, while the researchers at Hubbs harvest an average of 590 rotifers from every ml of culture water.

When asked how many rotifers they needed per day, two operators indicated that they were not, on average, able to meet the demands of finfish larval rearing (Table 1). Of those providing estimates, only Taiwan, Kuwait and Singapore reported culturing an excess of rotifers, and the EPFFC appears to

*Production/ml/day for semi-continuous systems = (rotifers collected by partial harvesting) - (rotifers inoculated) + (total harvest)

Table 1. Production levels and densities.

	UNITED STATES		JAPAN	KOREA
	Hawaii (The Oceanic Institute)	California (Hubbs-Sea World Marine Lab.)	Ehime Prefectural Fish Farming Center	Sung Ji Industry
MAXIMUM production (million rotifers/day)	500	536	8,000	6,400
MINIMUM production (million rotifers/day)	100	100	4,000	3,200
AVERAGE production (million rotifers/day)	400	305	6,000	4,800
MAXIMUM productivity (rotifers/ml/day)*	100	47	50	200 (100 in winter)
MINIMUM productivity (rotifers/ml/day)	50	12	20	100 (50 in winter)
AVERAGE productivity (rotifers/ml/day)	75	25.7	30	150 (70 in winter)
Stocking density	100/ml	Average: 366/ml	100/ml	70 - 100/ml
Harvest density	150 - 200/ml	Average: 590/ml	100 - 200/ml	100 - 200/ml
Production requirements (rotifers/day)	One billion/day, all year	400 million/day	6 - 8 billion/day	Unknown

Table 1. Continued

	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
MAXIMUM production (million rotifers/day)	Unknown	2,100	333	1,200	1,428
MINIMUM production (million rotifers/day)	Unknown	1,700	66	800	378
AVERAGE production (million rotifers/day)	Unknown	1,900	166	1,000	1,122
MAXIMUM productivity* (rotifers/ml/day)	Varies with demand	40	40	15	238 (up to 998 in experiments)
MINIMUM productivity (rotifers/ml/day)	Varies with demand	30	20	10	63
AVERAGE productivity (rotifers/ml/day)	10	35	30	12	187 (up to 856 in experiments)
Stocking density	5 - 10/liter	40 - 50/ml	10 - 50/ml	10 - 30/ml	50/ml
Harvest density	200 - 500/ml	80 - 120/ml	100 - 200 cells/ml	100 - 200/ml	187/ml/day
Production requirements (rotifers/day)	Depends on scale of production	1,700 million	Varies according to target species and production scale	10^9/day	One billion/day

* Production/ml/day for semi-continuous systems = (rotifers collected by partial harvesting) - (rotifers inoculated) + (total harvest)

Table 2. Estimated production requirements.

	UNITED STATES		JAPAN	KOREA
	Hawaii (The Oceanic Institute)	California (Hubbs-Sea World Marine Lab.)	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Land (ha)	0.5	< 0.2	0.2	0.9
Construction and equipment (start-up costs)	Unknown	Unknown	US$ 281,000	US$ 10 million
Equipment and supplies	10x ocular microscope, pipets (1 ml), plastic buckets,nitex screens	Pumps, pools, harvesters	Aeration, pump, heater, freezer	Tanks, blowers
Energy	Electricity and physical labor	Solar and electrical	Electrical, oil	Boiler (300,000 kcal)
Type of feed	*Nannochloropsis oculata* + baker's yeast	Yeast + algae + fish oil	*Nannochloropsis oculata* + omega yeast	*Nannochloropsis oculata* + baker's yeast or omega yeast
Algal density maintained in rotifer tank	5 x 10^6/ml	0.5 x 10^6/ml	1 - 4 x 10^6/ml	20 x 10^6/ml (initial denisty)
Amount of yeast (g/million rotifers/day)	0.5	0.3 - 1.0	0.5 - 0.7	0.5 - 1.2
Labor (hours/week)	196	40	84	36 - 40
Chemicals, other supplies	Fertilizers (NO_3, urea, phosphates)	1. Menhaden oil 2. Vitamins C and E 3. Filter	1. Fertilizers 2. Chlorine	1. Fish oil 2. Vitamins A,D, and E

be just keeping up with its rotifer demand. Some reasons culturists have difficulty producing enough rotifers were explored in Discussion Group C.

Requirements

We then asked about the resources each system required to maintain current production levels. The categories were land, start-up costs for construction and equipment, necessary equipment, energy type, feed types and amounts, labor, and chemicals and other supplies. Responses were, of course, quite different for the different production scales and economic settings.

Land estimates ranged from a minimum of .004 ha in Taiwan to a maximum of 0.9 ha at Sung Ji Industry (Table 2). Start-up costs also varied greatly among systems. Of those able to give estimates, costs were between US$ 500, in Thailand and US$ 10 million at Sung Ji in South Korea. The major equipment needs are tanks, piping, pumps, blowers and heaters. Smaller equipment such as microscopes, pipets, buckets and plankton netting (nitex screen) are also necessary. Finally, the majority of the facilities had an electric energy source.

In terms of feed requirements, most producers maintain the density of *Nannochloropsis oculata* at 0.5 to 5 million cells/ml in the rotifer tanks (Table 2). If yeast is used, it is added at a rate of 0.3 to 1.0 g/million rotifers/day.

Labor requirements for rotifer culture, exclusive of microalgae production, ranged

Table 2. Continued.

	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
Land (ha)	Depending on the demand	0.01	0.1 (US$ 2,000)	.004	0.005
Construction and equipment (start-up costs)	Unknown	US$ 13,000	US$ 2,000	US$ 40,000	US$ 10,000
Equipment and supplies	17 6 x 2 m^2 tanks = 204 m^2	Tanks (8 x 6 m^3 tanks), air blower	Four 10-ton tanks, aeration and water supply systems	Heater in winter, aeration	10X microscope, pipettes, tanks, buckets
Energy	Electricity and diesel	Electricity	Electricity with standby diesel generator	Electricity	Electricity
Type of feed	*Chlorella* sp., *Tetraselmis subcordiformis*, + baker's yeast	*Nannochloropsis oculata*, *Tetraselmis tetrathele*	*Nannochloropsis oculata*, yeast	*Tetraselmis chui* or *Nannochloropsis oculata* + yeast	*Nannochloropsis oculata* + yeast
Algal density (maintained in rotifer tank)	10^4 - 10^6 cells/ml	*N. oculata*: 5 x 10^6/ml *T. tetrathele*: 5 x 10^4/ml	1 - 2 x 10^6 cells/ml/day	*N. oculata*: 5 x 10^6 cells/ml *T. chui*: 10^5 cells/ml	20 million cells/ml
Amount of yeast (g/million rotifers/day)	1	None	1	0.5 - 1	0.3 - 0.5
Labor (hours/week)	210 (includes microalgae production)	20	56	35	7
Chemicals and other supplies	$KMnO_4$ + chlorine	Formalin	Fertilizers, chlorine	Fertilizer, chlorine	Fertilizer

from a low of seven hours/week for the chemostat system in Kuwait which produced an average of 1.122 billion rotifers/day, to a high of 196 hours/week at The Oceanic Institute, which produces approximately 400 million rotifers /day (Table 2). The low labor requirement for the Kuwaiti system was due largely to automated feeding (James, pers. comm.).

A listing of chemicals and other supplies is also in Table 2. Commonly used chemicals include chlorine for disinfection, vitamins and oil as feed supplements and fertilizers for growing algae.

ROTIFER DISCUSSION GROUP E
Requirements II, Cost Reduction

Participants were asked to detail the resources they would need for the sustained, reliable production of one billion rotifers/day. The group then concluded with a discussion of some possible ways to lower the cost of rotifer culture.

Requirements to Produce One Billion Rotifers Per Day

To produce one billion rotifers per day, Chen Xing Qian of the Zhanjiang Experimental Station said that he would require only one person working ten hours/week (Table 1). This is slightly higher than Charles James' estimate of seven hours/week. Five participants estimated they would need one person working 20 hours/week, and three participants each would require one, two and three full-time workers, respectively.

Because all the different components of a hatchery share an energy supply, most of the culturists were unable to estimate the amount of electricity or diesel fuel needed to grow one billion rotifers (Table 1).

The lowest algae estimate for the sustained production of one billion rotifers per day was 5 m^3 for the chemostat system in Kuwait (Table 1). This attests to the system's remarkable efficiency. Ten m^3 is the next lowest figure; this is for the government hatchery in Singapore. Zhanjiang Experimental Station and Sung Ji Industry would require 40 m^3 and 45 to 60 m^3 of microalgae, respectively. The Ehime Prefectural Fish Farming Center, Hubbs, Thailand and The Oceanic Institute, furthermore, estimated that they would need 50, 85, 100 and 105 m^3, respectively.

A very detailed list of equipment and supply requirements was provided by Clyde Tamaru of The Oceanic Institute (Table 1). The list includes a dissecting microscope, graduated cylinders, tubs, buckets, tanks, an air blower, submersible heaters, nitex screening, PVC and airstones. The blocks and planks listed are used in the construction of platforms and allow the tanks to be harvested by gravity. In addition, the Hubbs estimate includes one harvest concentrator/rinsing tank (see Orhun et al. this volume) and the list for a Taiwanese hatchery includes a blender for preparing yeast.

It's not surprising that the smallest volume of rotifer culture water necessary to produce one billion rotifers per day, 6 m^3, is for the continuous chemostat system (Table 1). The estimates for The Oceanic Institute and Sung Ji Industry systems were more than twice that size; 14 and 15 m^3, respectively, while the others were even bigger; from 30 m^3 to 400 m^3.

Decreasing the Cost of Production

Finally, each group member suggested two mechanisms for lowering rotifer production costs (Table 2). Feed quality and quantity

Table 1. Estimated resources required to produce one billion rotifers/day[1].

	UNITED STATES		JAPAN	KOREA
	Hawaii (The Oceanic Institute)	California (Hubbs-Sea World Marine Lab.)	Ehime Prefectural Fish Farming Center	Sung Ji Industry
Labor[2]	Three full-time people	One full-time person	One half-time person	One half-time person
Energy (type and amount)	Electricity (amount unknown)	2.5-hp continuous	Unknown	Unknown
Algae (m^3)	105	85	50	45 - 60
Equipment and supplies (type and amount)	One dissecting microscope, 1 glass pipette (1-ml), 1 roll nitex screening (60-μm), 4 plastic buckets (5-gallon), 3 plastic tubs (15-gallon), 2 graduated cylinders (1-liter, 500-ml), 12 fiberglass tanks (1,200-liter), 12 ball valves (1 1/2-inch), 100 ft. roll air line tubing, ball airstones (2-inch), 30 - 50 gang valves, 144 concrete hollow tile blocks, 200 ft. of wood planks (2 x 4 inches), 40 ft. PVC piping (1/2-inch), 12 submersible heaters (1-kw), 1 blower (5-hp)	Five pools (8,500-liter), 1 blower (2-hp), 5 heaters (2,000-watt), 1 harvest pump (1/2-hp, rubber impeller), 1 siphon pump (3/4-hp), 1 harvest concentrator/ rinsing tank (300-liters)	Eight tanks (50,000 liters); 3 blowers (25-kw); heating system (boiler, circulating water pump, pipelines, heat exchanger, control system); 1 microalgae transferring pump (2.2 kw) with pipelines, seawater, tap water and air supply system; 1 mixer (20-liter); 1 freezer, air tubes, airstones	Unknown
Rearing facilities — rotifer tanks only — (m^3)	14.4	42.5	400	15

[1]Estimated resources must include any redundancy needed to ensure that you always end up with one billion rotifers per day.
[2]Labor = number of persons required from the time you harvest the algae until the time you harvest the rotifers.

Table 1. Continued.

	S. CHINA	SINGAPORE	THAILAND	TAIWAN	KUWAIT
	Zhanjiang Experimental Station	Marine Aquaculture Section			C. James System
Labor	One 1/4-time person	One half-time person	One half-time person	Two full-time people	One 1/4-time person
Energy (type and amount)	Electricity, diesel fuel	Electricity, US$2.00/day	N/A	Heaters in winter (24 2,000-watt), aeration. US$ 400/month	Electricity
Algae (m^3)	40	10	100	140 m^3	5
Equipment and supplies (type and amount)	Two 145 m^3 tanks, 6 plastic buckets, nylon mesh, 4 blowers	Eight 3.75-m^3 tanks, 1 water pump, 1 air blower, chemicals, hosing, harvesting nets, etc.	N/A	Two pumps (1-hp), blender for yeast	pH meter, metering pump, timer, heater, etc.
Rearing facilities — rotifer tanks only — (m^3)	290	30	50	80	6

Table 2. Potential solutions for lowering production costs.

1.	Need an inexpensive source of concentrated algae or another food source, e.g. yeast enriched with squid oil (12)
2.	Automate system to reduce labor requirements — move toward a chemostat system. Labor is the biggest cost factor in rotifer production (10)
3.	Improve harvesting techniques (2) Prevent rotifer disease (2) Genetic improvement of rotifers (2)
4.	Collection and preservation of rotifer eggs (1) Centralized rotifer production facility to supply a large number of fish producers (1)

[1]Each participant was asked to suggest two mechanisms to lower rotifer production costs.
[2]Mechanisms are listed in order of importance, number of votes indicated in parentheses.
[3]Representatives of Thailand and Taiwan were not present to make their suggestions.

seemed to be foremost is most people's minds. The solution receiving the most votes was finding an inexpensive source of high-quality feed, such as concentrated algae or enriched yeast. The majority of finfish larviculturists probably prefer to feed their rotifers algae but they cannot always afford to do so.

Second on the list was the need for increased automation to reduce labor, the largest cost factor in rotifer production. Furthermore, improving harvesting techniques, preventing rotifer disease and genetically improving rotifer strains each received two votes. Better harvesting techniques are needed primarily to reduce labor. Harvesting nets often become clogged, so someone needs to oversee the operation constantly. Several private companies in Japan have invented automatic harvester/concentrators in reply to this need.

Finally, two other cost-reduction measures, collection and preservation of resting eggs and utilizing a centralized production center to supply a number of fish producers, were also mentioned. The former would eliminate the need for stock culture maintenance. The latter, by contrast, would take advantage of economies of scale and relieve fish growers of the burden of rotifer and microalgae production altogether.

FINAL DISCUSSION GROUP

On the last day of the workshop, participants gathered in the conference room for the Final Discussion Group session. First, representatives of the microalgae and rotifer groups summarized the key issues raised in sessions A - E. Members of the "rotifer group" took this opportunity to question the "microalgae group" about its findings, and vice versa. The session ended with a general discussion of the future of live feeds production and research.

Microalgae

Kelly Rusch summed up the findings of the microalgae discussions. She noted that batch culture was, by far, the most common culture technique, largely due to its reliability. Most participants utilized the same general routine, scaling up gradually from stock cultures in test tubes to larger and larger volumes of microalgae, then harvesting. Culturists did differ with respect to the number of steps needed to reach a given volume. Overall, each microalgae production system was tailored to meet the demands of a particular culture facility while operating within site-specific constraints.

The group agreed that the most important problems encountered in the large-scale production of microalgae are contamination and cost. The worst contaminants are protozoa, while bacteria, other algal species and zooplankton can also be troublesome. Probably the best solution to contamination is to enclose tanks wherever possible; however, selection of a species or strain with a high growth rate was also considered a promising approach. In fact, a common theme throughout the microalgae discussion groups was that careful or fortuitous species/strain selection can prevent or minimize a number of problems, including contamination, and also reduce costs. A suitable species or strain must be hearty and have a high growth rate in the climate in which it is cultured and be nutritious for the target species.

One reason algae costs are high is simply that the requirements for algal biomass are very high. The ratio of microalgae culture volume to target species culture volume can be 5 - 10 : 1, hence a large portion of a hatchery's resources must often be devoted to microalgae culture.

The primary factors contributing to the high cost of algae production are: 1) labor and 2) supplies and chemicals. Labor can be reduced by greater automation/computer control and by moving toward more continuous systems. The latter allows one to grow a given amount of microalgae in a smaller volume of water. The use of heterotrophic production technology was also proposed as a possible future trend that could reduce the cost of microalgal feeds. It has been projected that certain species of algae could be cultured on a large scale for $20/kg dry weight in fermentors.

Participants were hopeful that centralized microalgae production centers would be developed in the future, freeing larviculturists of the need to grow microalgae. The government of Thailand has taken a step in this

direction, providing free starter cultures to the owners of small shrimp hatcheries.

To conclude, Kelly Rusch proposed that in order to meet the increasing worldwide demand for aquacultured products dependent upon cultured microalgae, we must change the way we are growing microalgae. She proposed moving away from traditional batch systems toward continuous systems in order to improve efficiency and boost production levels.

Rotifers

The summary presented by Terry Snell reviewed the following topics, which were covered in the rotifer sessions:

1) feed,
2) production methods,
3) culture crashes,
4) strain selection, and
5) future research needs.

In the area of feed, the nutritional requirements of rotifers remain poorly characterized. Finfish producers are primarily concerned with improving the quality and quantity of their rotifers, and decreasing the cost of production. With regard to the former, participants talked about supplementation and enrichment with omega yeast, vitamins E, D, C and B_{12}, and marine oils. Also mentioned was the possibility of adding vitamin B_{12}-producing bacteria to cultures and finding new species of algae for places or seasons in which *Nannochloropsis oculata* doesn't grow well. Finally, in the future it may be feasible to reduce costs by using dried or condensed algae as feed.

Interestingly, there was singular lack of uniformity among production methods. There is no standard technique for the mass culture of rotifers, although most producers did use semi-continuous methods. The variety of approaches reflect site-specific needs, taking into account different physical and chemical environments and socio-economic factors.

Although culture crashes are an important issue for finfish hatcheries, this problem remains poorly understood. Certain key factors associated with crashes have been identified. They include:

- un-ionized ammonia and other water quality parameters,
- feed quality,
- pathogenic bacteria,
- and ciliates.

An "early warning system" to detect stress and prevent culture collapses is needed. Participants indicated that the following variables should be monitored: rotifer swimming speed, density, percentage of rotifers carrying eggs, un-ionized ammonia concentration, and bacterial, ciliate and protozoan concentrations.

Potential solutions to crashes include: redundancy within the production system, rinsing cultures well, changing water frequently, improving feed quality, biological control of ciliates and possibly bacteria by addition of predators, the importance of knowing when a problematic culture should be terminated and the use of resting eggs or cryopreserved rotifers for restarting cultures.

There is also a clear need for both larger and smaller *B. plicatilis* strains. Some species have mouths that, at the first feeding stage, are smaller than the smallest S-type rotifers. On the other hand, there are economically valuable species with relatively large mouths that could feed more efficiently on larger rotifers. While the size of a particular strain can be altered by its feed, there is a limit to this approach. Through more intense investiga-

tions of natural stocks and artificial selection, culturists hope to find extra small and "jumbo" strains of rotifers. Terry Snell also cited a need for a Rotifer Reference Center to facilitate the exchange of useful *B. plicatilis* strains.

According to the discussion group participants, future rotifer research should focus on the following:

- the role of bacteria in rotifer cultures;
- improving the design of batch cultures;
- increasing automation to minimize labor;
- moving toward more continuous systems;
- obtaining new strains for culture, possibly through genetic engineering;
- reliable mechanisms to detect stress in mass cultures;
- eliminating protozoa;
- feed — including improved feed quality, development of more digestible yeast, vitamin B_{12}-producing yeast, and concentrated and spray-dried algae;
- disease;
- improved techniques to increase resting egg production to eliminate the need for stock cultures;
- and development of standard reporting procedures for production data.

Terry Snell concluded by stressing the need for increased funding of rotifer-related research.

Conclusion

The final session ended with brief discussions of the potential for producing *Nannochloropsis oculata* heterotrophically, the concentration of fertilizers added to algae cultures, and the potential for formulated feeds to replace live larval feeds. Research is currently underway to produce *Nannochloropsis* using heterotrophic methods. In order for this technique to be practical for a given species, it must have a high growth rate and reach high densities under heterotrophic conditions.

With regard to formulated feeds, several participants expressed the opinion that great advances were being made in the area of formulated feeds, especially in Japan. One problem, however, is that the larvae lack digestive enzymes until they reach approximately 6 mm; hence they are unable to digest the protein in formulated feeds. In the near future, the group concluded, live feeds will continue to be important in finfish rearing, but someday advances in formulated feed technology may make rotifer culture obsolete.

APPENDIX I: Workshop Participants

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APPENDIX II: Agenda

Monday, January 28

TIME	SPEAKER	TOPIC
8:30 - 9:00	**Welcome and Introductions**	
9:00 - 9:30	Dr. Fukusho Kunihiko	A review of the research status of zooplankton production in Japan
9:30 - 10:00	Dr. Terry Snell	Improving the design of mass culture systems for the rotifer, *Brachionus plicatilis*
10:00 - 10:30	**Break**	
10:30 - 11:00	Dr. Donald Kent	Practical approach to high density production of the rotifer, *Brachionus plicatilis*
11:00 - 11:30	Mr. Tsuneo Morizane	A review of automation and mechanization used in the production of rotifers in Japan
11:30 - Noon	Dr. Clyde Tamaru	Improving the larval rearing of Striped mullet (*Mugil cephalus*) by manipulating quantity and quality of the rotifer, *Brachionus plicatilis*
Noon - 1:30	**Lunch**	
1:30 - 3:00	Microalgae Discussion Group A: General System Description I Rotifer Discussion Group A: General System Description I	
3:00 - 3:30	**Break**	
3:30 - 5:00	Microalgae Discussion Group B: General System Description II Rotifer Discussion Group B: General System Description II	

Tuesday, January 29

TIME	SPEAKER	TOPIC
8:30 - 9:00	Prof. Jiao-Fen Chen	Commercial production of microalgae and rotifers in China
9:00 - 9:30	Mr. Geon Gil Pi	Design and operation of a large-scale rotifer culture system at a Sung-Ji Industry farm, South Korea
9:30 - 10:00	Dr. Connie Arnold	Various methods for the culture of the rotifer, *Brachionus plicatilis*, in Texas
10:00 - 10:30		**Break**
10:30 - 11:00	Dr. Masachika Maeda	Environmental management for mass culture of the rotifer, *Brachionus plicatilis*
11:00 - 11:30	Dr. Huei-Meei Su	Mass culture and utilization of live feeds in Taiwan
11:30 - 1:00		**Lunch**
1:00 - 2:30	Microalgae Discussion Group C: Culture Conditions/Reliability Rotifer Discussion Group C: Culture Conditions/Reliability	
2:30 - 3:00		**Break**
3:00 - 4:30	Microalgae Discussion Group D: Production Levels/Requirements Rotifer Discussion Group D: Production Levels/Requirements	

Wednesday, January 30

TIME	SPEAKER	TOPIC
8:30 - 9:00	Dr. Kazutsugu Hirayama	The nutritional improvement of baker's yeast for the growth of the rotifer, *Brachionus plicatilis*
9:00 - 9:30	Dr. Sung Bum Hur	The selection of optimum phytoplankton species for rotifer culture during cold and warm seasons and their food values for marine finfish larvae
9:30 - 10:00	Dr. Hassanai Kongkeo	An overview of live feeds production system design in Thailand
10:00 - 10:30		**Break**
10:30 - 11:00	Prof. Xing-Qian Chen	Research and production of live feeds in China
11:00 - 11:30	Dr. Lian Chuan Lim	An overview of live feeds production design in Singapore
11:30 - Noon	Mr. Adithya Padala	The application of expert systems to aquaculture
Noon - 1:30		**Lunch**
After lunch	**FREE TIME**	

Thursday, January 31

TIME	SPEAKER	TOPIC
8:30 - 9:00	Mr. Jim Donaldson	Commercial production of microalgae at Coast Oyster Company
9:00 - 9:30	Ms. Kelly Rusch	Development of a micro-computer automated algal chemostat: Overview from bench to production scale
9:30 - 10:00	Dr. Masonori Okauchi	The status of phytoplankton feed production in Japan
10:00 - 10:30		**Break**
10:30 - 11:00	Mr. Vernon Sato	Development of a phytoplankton production system as a support base for finfish larval rearing research
11:00 - 11:30	Mr. Ray Gladue	Heterotrophic microalgae production: potential for application to aquaculture feeds
11:30 - Noon	Dr. Park Mi Seon	Status of mass production of live feed in Korean hatcheries
Noon - 1:30		**Lunch**
1:30 - 3:00	Microalgae Discussion Group E: Costs Rotifer Discussion Group E: Costs	
3:00 - 3:30		**Break**
3:30 - 5:00	Final Session	

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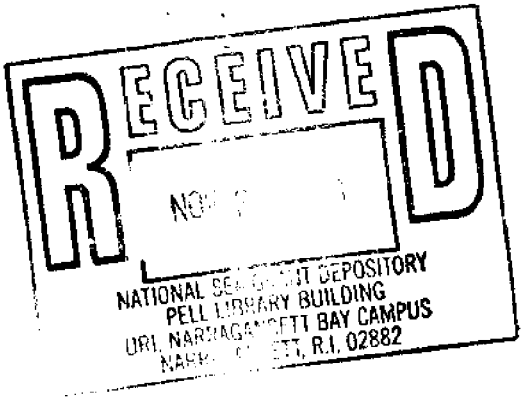

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