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 reptiles 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-
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 UMERCORP’s RIAAX (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 a 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.
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).

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 CO2 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 Erhlemeyer 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 $2 \times 10^4$. A typical final density after two days of culture is $3 \times 10^6$. 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. TCB 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 $2 \times 10^5$. Within 48 hours, these cultures have achieved a density of $6 \times 10^6$ 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 CO₂ injection system is employed. This system injects CO₂ into the air system once an hour to deliver about 10% CO₂, measured at the time of injection. As long as the cultures are normal and healthy,
this rate of CO₂ 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 CO₂ 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 x 10⁵ to 3 x 10⁶ 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 x 10⁵ to 3 x 10⁶ 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 (Licon 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.

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*Figure 2. Action spectrum of: (A) photosynthetic response, and (B) chlorophyll synthesis (from Sylvania Engineering Bulletin O-352). Reprinted with permission from Sylvania Lighting Centers.*
Table 1. Units devoted to microalgal culture at Coast Oyster Company.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Units inoculated per day</th>
<th>Units in system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flasks</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Carboys</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>3,000-liter tanks</td>
<td>4 - 5</td>
<td>18 available for culture</td>
</tr>
<tr>
<td>20,000-liter tanks</td>
<td>6 - 7</td>
<td>20 available for culture</td>
</tr>
</tbody>
</table>

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.

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.

*Figure 3. Spectral energy distribution of 1000-watt metalarc clear lamp (from Sylvania Engineering Bulletin O-344). Reprinted with permission from Sylvania Lighting Centers.*
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.

LITERATURE CITED


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/m³/day, an average specific growth rate of 0.66/days and a maximum standing crop of 190 g/m³.

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, CO₂ 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 down-
time. A system of this type is robust and "forgiving" in that computer or system inter-
ruptions are not catastrophic to the algal cul-
ture. The algal culture will continue to grow
under batch conditions until the normal oper-
ational 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 Cor-
poration, 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 expa-
sion with only slight increases in system com-
plexity and cost.

All inputs (nutrients, brine, water, and
air/CO₂) and withdrawals were accomplished
through a single port located at the bottom of
the chambers. Constant volumes were main-
tained using stainless steel level detectors.
The 3 - 5% CO₂/air volume ratio was main-
tained by discrete CO₂ injections into the
filtered (0.2 µm) air line. Six 40-watt cool
white, fluorescent lights, placed horizontally
behind the chambers, supplied a continual
surface irradiance of approximately 6,000
lumens/m². 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 lap-
top micro-computer interfaced to monitoring
devices via a Remote Measurement Systems
ADC-1 data/acquisition unit (Remote Mea-
urement 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/m²) 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.

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. Re inoculation of a disi-
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/m³, 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/m³/day dry weight could be reliably

\[ \mu = \ln \left( \frac{x_2}{x_1} \right) \frac{1}{t_2 - t_1} \]

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 4. Flow chart of the computer control program, “Supervisor”.*
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 5. Daily specific growth rates and biomass concentrations obtained from the kinetics study (SGR = specific growth rate, the numbers refer to the three chambers).

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/m³, resulting in the typical saw-toothed curve common to semi-continuous cultures. Predicted harvestable biomass (dry) averaged 82 g/m³/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/m³/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 minuissima for the five-month test period. James et al. (1988) reported optimized yields of 117 g/m³/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.

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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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Aquatic animals reared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetoceros calcitrans</td>
<td>Bivalves, sea urchins, sea squirts</td>
</tr>
<tr>
<td>C. gracilis</td>
<td>Crustaceans, bivalves, sea cucumbers</td>
</tr>
<tr>
<td>C. simplex</td>
<td>Crustaceans, bivalves, sea cucumbers, sea urchins, sea squirts</td>
</tr>
<tr>
<td>C. ceratosporum</td>
<td>Crustaceans, bivalves, sea cucumbers</td>
</tr>
<tr>
<td>Isochrysis galbana</td>
<td>Bivalves, sea cucumbers, sea hares</td>
</tr>
<tr>
<td>Pavlova lutheri</td>
<td></td>
</tr>
<tr>
<td>Nannochloropsis oculata</td>
<td>Rotifers</td>
</tr>
<tr>
<td>Tetraselmis tetrathele</td>
<td>Rotifers, crustaceans</td>
</tr>
<tr>
<td>Tetraselmis sp.</td>
<td></td>
</tr>
<tr>
<td>Nitzschia sp.</td>
<td>Abalones</td>
</tr>
<tr>
<td>Navicula sp.</td>
<td>Abalones, turbans</td>
</tr>
<tr>
<td>Amphora sp.</td>
<td>Abalones, sea cucumbers</td>
</tr>
<tr>
<td>Cocconeis sp.</td>
<td>Abalones</td>
</tr>
<tr>
<td>Licmophora sp.</td>
<td>Abalones</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>Spiny lobsters</td>
</tr>
</tbody>
</table>

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 B₁₂ and eicosapentaenoic acid (EPA) (Table 2). Vitamin B₁₂ 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.

**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

<table>
<thead>
<tr>
<th>Species</th>
<th>EPA</th>
<th>Total ω3 HUFAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraselmis tetrahele</td>
<td>6.4</td>
<td>8.1</td>
</tr>
<tr>
<td>Nannochloropsis oculata</td>
<td>30.5</td>
<td>42.7</td>
</tr>
<tr>
<td>Pavlova lutheri</td>
<td>13.8</td>
<td>23.5</td>
</tr>
<tr>
<td>Isochrysis aff. galbana</td>
<td>0.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Isochrysis galbana</td>
<td>3.5</td>
<td>22.5</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>8.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td>13.8</td>
<td>15.5</td>
</tr>
</tbody>
</table>
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 tetrahele_ is generally used to feed the larvae of penaeid shrimp and the rotifer, _B. plicatilis_, in Japan. _Tetraselmis tetrahele_ 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 tetrahele_ reproduces rapidly in seawater enriched with fertilizers like those used to culture _N. oculata_. _Tetraselmis tetrahele_, 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. tetrahele_ 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 B₁₂ added to the culture medium (Maruyama et al. 1989) in its cells. As stated above, vitamin B₁₂ 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 \textit{N. oculata} or fatty acids is necessary.

\textbf{Nannochloropsis oculata}

This commodity consists of a condensed suspension of \textit{N. oculata} whose cell walls have been digested by enzymes. Thus, it can be used to feed brine shrimp, \textit{Artemia salina}, other species of zooplankton which are used as feed (\textit{Daphnia pulex}, \textit{Moina macrocopa}, etc.) and penaeid shrimp larvae, in addition to rotifers. The EPA content of the cells is the same as that of the \textit{N. oculata} mass produced at fish farming centers.

\textbf{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 \textit{B. plicatilis}.

\section*{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.

\section*{Mass Production Tanks}

Transparent circular polycarbonate vessels (30 - 100 liters) and outdoor concrete tanks (10 - 200 m\textsuperscript{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 \textit{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 \textit{Chlorella} is high and stable under suitable conditions. Additionally, freshwater \textit{Chlorella} is cultured heterotrophically or mixotrophically in tanks under dark and sterile conditions with continuous culture methods.

Figure 2 shows the outdoor \textit{Spirulina} culture tanks at Dainippon Ink and Chemicals, Inc. in Thailand. Generally, because the cell wall and cell membrane of \textit{Spirulina} are more fragile than those of single-celled phytoplankton such as \textit{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.

\[\text{Figure 1. Outdoor concrete tanks for freshwater Chlorella production at Chlorella Ind. Co. Ltd. A: Driving wheel, B: Motor, C: Agitator, D: Wings for agitation. (Photo provided by Chlorella Ind. Co. Ltd.)}\]
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 chlorine 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.

<table>
<thead>
<tr>
<th>Fertilizer added per 1,000 L seawater</th>
<th>Fish farming centers</th>
<th>Nagasaki</th>
<th>Hiroshima</th>
<th>Kyoto</th>
<th>Ishikawa</th>
<th>Yamagata(^1)</th>
<th>Yamagata(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulfate (g)</td>
<td></td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Superphosphate (g)</td>
<td></td>
<td>30</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Urea (g)</td>
<td></td>
<td>25</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Clewat 32(^3) (g)</td>
<td></td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^1\) 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.

REFERENCES

