PROGRESS REPORT: 1970-1982
RESEARCH IN RECIRCULATING AQUACULTURE SYSTEMS FOR GROWING COMMERCIALLY VALUABLE BIVALVES

Conducted through the
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College of Marine Studies
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Foreword

In 1970 the University of Delaware, with National Sea Grant Program support, embarked on a forward-looking research program to develop controlled-environment systems (called mariculture or aquaculture) for growing oysters and clams to market size at commercially competitive prices. Our progress to date in the husbandry of molluscs is significant and compares favorably to the history associated with the successful husbanding of poultry, beef cattle, or swine. For example, the commercial-broiler industry had its beginnings in Delmarva to produce "hothouse" chickens in the early 1920s. However, it took approximately 40 more years of research carried out in colleges of agriculture throughout the country to develop a comprehensive process resulting in the multi-million dollar industry of today.

This progress report on controlled-environment mariculture at the University of Delaware was written to provide a readable and reasonably brief summary of the progress and problems encountered during the past 12 years. The paper that forms the body of this report was invited by the organizers of the Massachusetts Institute of Technology (MIT) Seminars in Biotechnology and Genetic Engineering and was presented in March 1982 at Cambridge, Massachusetts. The review and suggestions for improvement made by researchers on the project have added important insights and accuracy to the paper.

Before highlighting research accomplishments, other aspects of the program deserve comment. It is the only aquaculture research program in the nation supported by Sea Grant for 12 continuous years. Annual investment, while not large by industrial standards, averaged $365,000. Financial support has come not only from the Office of Sea Grant, but also from the State of Delaware, the University of Delaware, industry sponsors, private foundations, and individuals. Based on its record of accomplishments, the mariculture program received sufficient budget support from the university to continue research.

A further indication of prolonged institutional commitment is the dedication of an entire laboratory devoted to controlled-environment systems research. This laboratory, known as the Otis H. Smith Laboratory, houses not only mariculture, but also salt-tolerant plant research.

As a result of more than a decade of research in mariculture, the University of Delaware has earned a national and an international reputation as a leader in recirculating-system aquaculture. The university has organized and hosted the first (1975) and second (1981) international conferences on aquaculture nutrition. These conferences were formally sponsored by the United States and Japan with worldwide scientific participation. In addition, there have been several visiting scientists who have come to study and work in our laboratory.

Our expertise also has resulted in numerous requests for information and technology transfer. For example during 1981 alone, 95 tours of the research and prototype facilities were conducted, 29 requests for algae were accommodated, and 41 technical consultations were provided.

Since 1974, 24 students have earned masters or doctoral degrees for research on problems connected with recirculation-system aquaculture. At this moment, five additional graduate students and one postdoctoral researcher are at work in the Otis H. Smith Laboratory on other problems concerned with the culture of bivalves or algae. As an indication of the regional value of our laboratory, both the University of Maryland and Rutgers University are providing funds to support activities in the Otis H. Smith Laboratory that benefit their own educational and research programs.

Beginning in 1970, an interdisciplinary research team systematically attacked the objective:

> *To develop a commercially viable, controlled-environment, recirculating mariculture system to produce bivalve molluscs, particularly oysters and clams.*

Inherent in this definition are these requirements:

1. A seawater supply that is non-toxic and supportive of the total life-cycle;
2. A dependable supply of molluscs, including desirable broodstock and their offspring; and
3. A nutritionally adequate and ample feed supply.

None of these requirements was controlled in 1970. We had not yet "brought the chicken into the barnyard." Since that time, there have been notable research and development accomplishments. Selected of these follow and are presented chronologically so that the reader may appreciate the evolution and maturation of the program.

1970-71

- Conditioned broodstock, induced spawning of parents at will, reared larvae to setting, and grew oysters on natural plankton in a flow-through system for six months during the natural growing season.
- Fed starch and protein to oysters, but with little success.

1971-72

- Developed improved methods for setting larvae.
- Developed methods for prevention of gas bubble disease.
- Completed design and construction of the Mariculture Laboratory on the Fish Products site and published a specification manual (1973).
- Built recirculating system, loaded with finfish, and monitored seawater quality.

1972-73

- Designed and built the first algal production unit.
- Maintained in recirculating seawater postlarval clams and oysters reared in the laboratory and fed them different diets of cultured algae; growth of the clams during the first six months exceeded that in nature by 50% (Hartman, 1974).
- Established a water-quality laboratory and demonstrated the use of an ammonia electrode in seawater analyses.
- Identified alkalinity problems in the recirculating system and implemented a solution.

1973-74

- Continued growth studies of bivalves raised in recirculated seawater and fed bivalves selected species of laboratory-produced algae; the shellfish (clams and oysters) approached market size. This was the first time anywhere that oysters and clams had been grown successfully in a recirculating seawater system (Epifanio and Mootz, 1975).
- Developed preliminary biological specifications for construction of a commercial system.
- Completed studies of tolerance of clams and oysters to their own waste products, particularly ammonia, and determined the feeding rates of oysters.
- Characterized the chemical kinetics of small-scale marine biological filters.
- Demonstrated the use of chloride, calcium, and divalent electrodes to maintain major ion balance in recirculating seawater.
- Collected preliminary data on production of ammonia by shellfish.

1974-75

- Raised to edible market size small numbers of clams *(Mercenaria mercenaria)* and oysters *(Crassostrea virginica)* that exhibited yearly growth rates greater than the reported growth of the species in Delaware Bay.
- Designed, constructed, and began operation of a controlled, four-bushel-design-capacity shellfish prototype.
- Developed algal culture and hatchery techniques sufficient to provide algae and bivalves on a regular and reliable basis; algae culture densities were ten times higher and growth rates four times faster than previously achieved.
- Identified single alga that supported oyster growth as well as a diet of four algal species, thus simplifying culture of food for bivalves.
- Completed studies of nitrification kinetics; a new modular nitrification filter design permitted preferential growth of desired algal species.
- Isolated and characterized bacteria from algal cultures and recirculating seawater systems; no definite correlations could be established with algal and bivalve growth.
- Completed analysis of fishery laws of Delaware, Maryland, and Virginia that might have impacted the feasibility of controlled-environment mariculture as a commerical enterprise.

1975-76

- Developed hatchery procedures to the point of sustaining an oyster/clam hatchery capability of 24×10^6 spat/month.
- Formulated diets developed and tested; no success in supporting rapid oyster growth.
- Achieved significant reductions in make-up water, waste treatment, and make-up nutrients in the small prototype.
- Demonstrated that the oyster, *Crassostrea virginica*, could be grown on a two-part algal diet at very high rates under high temperatures using large amounts of algae at very high concentrations; oysters reached 70 mm in height in 6-½ months.
- Detected no human or pathogenic viruses or bacteria in operation of mariculture laboratory.

1976-77

- Assembled a large (30 ft. X 120 ft.) greenhouse to house a 50-bushel-capacity controlled environment.
- Grew algae in 9000 L pools with full solar radiation and on normal day/night cycles; determined the importance of controlling pH and dissolved oxygen to prevent "algal crashes" (death).
- Quantified optimal ration size for bivalves, yielding an equation predicting ration size from animal weight.
- Analyzed chemistry of several algal species but revealed no differences in chemical composition of those supporting growth of oysters versus those not supporting growth.
- Analyzed trace metal concentrations in the recirculating culture system and adjusted trace metal additions to prevent build-up of toxic materials in oyster tissue.

1977-78

- Received U.S. Patent #4,065,875 "Selective Destruction of Certain Algae."
- Received U.S. Patent #4,080,930 "Oyster Rearing Process."
- Obtained $1.2 million from the Economic Development Administration to construct a new mariculture laboratory.
- Determined the specifications for oxygen and carbon dioxide concentrations, pH, and light intensity required to support mass algal growth.
- Tested the food value of non-algal diets and showed that certain yeast could be substituted for part of an algal diet with no loss of growth for clams.
- Defined minimum calcium requirements for normal oyster growth.
- Studied shell structure of wild versus controlled-environment oysters and discovered no structural difference.

1978-79

- Received U.S. Patent #4,133,294 "Process for Marking Molluscs."
- Completed the design of the new mariculture laboratory, including a controlled-environment production prototype; began construction.
- Measured the growth efficiency of bivalves fed algal diets under different experimental conditions and showed that growth efficiency is a function of temperature, ration size, and dietary composition.
- Improved algae culture vessel configuration to enhance gas exchange, thereby improving culture stability.
- Initiated investigation of Broadkill River water to ascertain oyster growth factors found in natural system.
- Identified unbalanced exponential growth in unicellular algae.

1979-80

- Conducted preliminary economic analysis prior to prototype start-up and established 80 percent of production cost of oysters associated with the production of algae, at the time, the only known suitable oyster feed.
- Developed and tested tubular algal reactors for inclusion in the oyster-production prototype.
- Found, through taste panel evaluations, no differences in flavor and texture, but discovered differences in color and appearance between maricultured and wild hard clams.
- Investigated the role of silt in improving the growth of *Crassostrea virginica.*
- Initiated a study of alternative ways to mitigate gas-exchange problems in the algae cultures through addition of organics.
- Defined environmentally induced, unbalanced growth in four algal species (*Thalassiosira pseudonana, Phaeodactylum tricornutum, Isochrysis galbana,* and *Isochrysis aff. galbana* (T-ISO)).

1980-81

- Moved into the new Otis H. Smith Mariculture Laboratory and set up integrated chemical, microbiological, algae, and hatchery services in support of research and prototype operations.
- Developed a set of specifications; constructed and operated a prototype to meet those specifications, based on research to date.
- Carried out comprehensive monitoring of materials and energy inputs into the prototype system as well as the performance of various age classes of oysters.
- Evaluated both the taste and chemistry of oysters grown in a controlled environment and found them to be equal or superior to oysters grown in the natural environment.

- Considered the role of algal and bacterial coupling in intensive aquatic production systems.
- Continued work on investigation of Broadkill River water to ascertain oyster growth factors found in natural system, reaffirming the role of silt in the growth of *Crassostrea virginica.*

1981-Present (Work in Progress)

- Detailed analysis of progress to date is being documented in a report of this first phase of research. This report will be available in June 1982. Conclusion of prototype operation: met food supply and water quality specifications; specified oyster growth was not achieved.
- Continuing research to develop artificial diets and methods of feeding them.

The operation of the mariculture prototype has demonstrated the technical feasibility of the overall concept. There are numerous fundamental problems that have been identified as a result. Notable among these are the following:

1. Development of alternative or supplementary feeds that can be supplied at far lower cost than that of algae cultivated *in situ.*
2. Identification of the growth factor(s) that appear to be needed to stimulate oyster growth from the 2-inch to 3-inch (commercial) size.
3. Genetic improvement of the wild-type molluscs, specifically for growth-intensive, controlled environments.
4. Development of specifications for rearing practices that promote fastest growth.
5. Application of technology to commercially high-value molluscs such as scallops and soft clams.

As is evident from these research problems, a commitment to a commercially viable process continues to influence our research planning. These problems now await our investigation and solution before a second-generation prototype merits development.

At the time we initiated the mariculture-research program, there was little known about the fundamental requirements for life support in an "unnatural" or hothouse environment. We have been at the cutting edge of research leading to the domestication of the oyster. Given the time and resources equivalent to that devoted to poultry or other agricultural commodities, the stated objective can be met. Once accomplished, it is our aspiration to continue problem-solving in support of those applying the new technology. The university is at its best when contributing to and expanding the knowledge base and educating for the future.

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PROGRESS AND PROBLEMS WITH RECIRCULATING SYSTEM AQUACULTURE

An Invited Paper Presented at

Seminars in Biotechnology and Genetic Engineering
MIT Sea Grant Marine Science Seminar Series
March 19, 1982

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I have taken this occasion, as a research administrator and to some extent a research policy maker, to describe the progress we have made at the University of Delaware in recirculating-system aquaculture for shellfish, and also to look into the future and suggest potential applications of the acquired knowledge as well as areas requiring further research.

Historical Perspective

This paper will be a brief history of aquaculture research at the University of Delaware. In 1966 the National Sea Grant College and Program Act became a federal statute and in 1968 Delaware began its Sea Grant funded research to improve the oyster industry in the Delaware Bay, which had been ravaged by the MSX disease (Ref. 1) and adversely affected by at least a century of water quality degradation in the estuary.

Our approach to this problem was influenced by the report published in June 1968 by the American Cyanamid Company titled *New Engineering Approaches for the Production of Connecticut Oysters* (Ref. 2). We planned our initial Sea Grant research in the 1968-1970 period to learn as much as we could about recirculating systems for growing shellfish. This approach required that biologists join forces with engineers in order to build and operate a recirculating system for the controlled, intensive culture of oysters. Engineers were also involved in systems studies to help guide the research as quickly as possible from the laboratory to commercial production of various bivalve species. I was interested to re-read a statement made by Dr. Donald Maurer, a biologist and a founding member of the research team. In our April 1970 Sea Grant proposal he wrote:

> One important point, however, which is the singular thrust of the University's Sea Grant Program, is the concentrated, interdisciplinary effort towards developing a closed system. Few organizations have approached shellfish production, or any other problem, from an integrated, multidisciplinary point of view, and even fewer people are concerned with a closed system . . . It may not be possible to design systems to commercially produce oysters in a controlled environment in a few years, but the real value of the work lies in the future.

That was a fair statement in 1970 and, in spite of substantial progress during the intervening years, it is a reasonable statement to repeat in March 1982. Rather than carry you through a chronological recitation of the successes and failures during nearly 14 years of research, I will address a few selected topics that are relevant to why and how we attacked this problem and where we see shellfish aquaculture developing in the future.

Why Recirculating System Mariculture?

The first issue was to define the problem and the most promising approach to solving the problem as clearly as possible. The problem was the uncertainty of culturing shellfish successfully in the uncontrolled, natural environment, which is subject to the vagaries of nature as well as to the carelessness of mankind. The chosen approach to solving the problem was to emulate agricultural production systems where domesticated plants and animals, bred to possess desirable characteristics, are provided with optimum food and environmental conditions consistent with the price consumers are willing to pay for the final product.

Why Should the University of Delaware Undertake This Research?

The second question that needed to be answered was why should the University of Delaware undertake this line of research? In answering that question we typically apply a three point test.

1. Is the problem or opportunity one of substantial significance to the nation and mankind?
2. Is the problem one to which others are as yet giving inadequate attention?
3. Is the problem one for which the University of Delaware has a unique advantage–or at the very least–no significant disadvantage?

Our response to each of these three questions was positive.

Can Bivalves be Reared in Captivity?

To begin the research program we first had to answer the question: "Can bivalves be grown in captivity?" In a 1969 Sea Grant conference organized by Drs. Price and Maurer addressing the issue of the *Artificial Propagation of Commercially Valuable Shellfish* (Ref. 3), it became clear that a great deal was already known about holding, conditioning, and spawning adult oysters as well as culturing the larvae through settlement to metamorphosis, into juvenile stages. What was not known, or at least demonstrated, was how to put all of the knowledge together into a dependable operating system from which a specified output rate and quality could be guaranteed.

In spite of the large amount of information available on the general biology of bivalves, the literature was unable to provide fundamental information necessary to specify 1) the rate of consumption of food, oxygen, and dissolved chemicals by bivalves, 2) the rate of production of wastes by the animals, and 3) the tolerance of the animals to various water quality conditions, particularly those resulting from accumulation of their own waste products.

Our first step in quantifying information necessary for engineering design was to develop mathematical models relating bivalve intakes and outputs. From these relationships system design specifications were developed (Ref. 4). In addition, we conducted extensive experiments on the tolerance of bivalves to their own waste products and were able to define safe levels of compounds such as ammonia, nitrites, nitrates, and orthophosphates (Ref. 5).

By 1973 our researchers were able to spawn and grow both oysters and clams in the laboratory. Annual growth rates that were equal to or greater than those found in nature were achieved (Ref. 6). By 1975 we had successfully grown clams and oysters to market size in a recirculating system, although the number of animals reared in this way were few, due to difficulties in providing a dependable source of nutritious food. In a few cases growth rates were spectacular and we were optimistic that progress would be rapid. By 1978 some of our brood stock comprised animals which had been spawned and cultured in captivity for several generations.

What Should Bivalves Be Fed?

From the outset of our research we knew that bivalves would grow if fed an algal diet. What was not known were the bivalve requirements for (1) quality, (2) quantity, and (3) supplements. In our research program the problem of bivalve nutrition has been a most difficult and perplexing area. The primary component of the problem has been the basic question of understanding the nutritional requirements of bivalves. The other component of the problem was providing enough algal food for the animals in a recirculating system.

The nutrition question has been addressed two ways. The first has been to determine which algal diets are most suitable for bivalves and to try to understand the underlying reasons for differences in algal food quality. The second approach has been to create a complete diet from defined dietary components so that classical deletion experiments may be conducted through the manipulation and the successive withdrawal of components of the diet so as to examine the nutritional requirements of the animals.

Since algal diets were examined initially and algae is the food produced in our most sophisticated recirculating system, I will summarize that line of research first. Fortunately for the Delaware team, much work on algal diets for larvae had been undertaken in other laboratories and research stations. The principal emphasis of the Delaware algal research has been focused on the food value of various species and combinations of species for bivalves. Apparent differences in the digestibility of algal food species has been found. This has been attributed mainly to the ability of the bivalve to break down the algal cell wall. A second important finding of this research has been that a mixture of two or more algal species generally results in more rapid bivalve growth than when either species is fed alone (Ref. 7).

The second part of our algal research focused on ration size. In other words, what is the amount of food an animal requires to achieve the maximum efficiency of growth? Several approaches to this problem were undertaken including investigation of

cell density effects, continuous versus discontinuous feeding, and absolute requirements for food in a fixed time period (Refs. 8 and 9). The outcome of this research, together with other results, has been an empirical equation that predicts the maximum daily algae requirement of oysters of any particular size (Ref. 10).

Another component of our nutrition research was to correlate food ingestion and growth with temperature (Ref. 11). One of the most interesting findings of this research was that growth was highly dependent on both ration and temperature. Under high temperature (28°C) and low ration conditions, shell growth was enhanced while meat growth was retarded. Whereas with a high ration, at the same high temperature (28°C), the ratio of meat to shell was enhanced.

At the low end of the temperature range tested (18°C), a low ration resulted in modest growth, and the highest ration produced only a slightly greater growth rate than with the lowest ration. This finding is obviously important to the operation of a recirculating system where it is necessary to maximize food-utilization efficiency by selecting the most appropriate temperature and ration size.

The potential use of non-algal diets was examined. These studies included various formulated feeds, several types of starch, ground whole cereals, and Torulopsis yeast. The yeast proved to be the only promising non-algal food tested (Ref. 12).

In addition to the algae studies, a more fundamental approach has been taken to the question of bivalve nutrition. The research program consists of two complementary efforts, the first, now largely completed, and the second just underway (Ref. 13). The objective of this research program is to design and test artificial diets for bivalves so that all the essential constituents and the optimum dietary levels of these constituents can be determined.

To succeed in this approach the first step is to obtain a dependable supply of axenic oyster larvae. It is important that these larvae be available for nutrition experiments without the presence of any other living organisms, which may act as an undefined food source, and without the use of antibiotics, which may alter the oyster nutritional requirements. This has been accomplished (Ref. 13).

The second step, now well underway, is to develop artificial diets that will support oyster growth. Several microencapsulation techniques have been developed and tested with satisfactory results. Dr. Langdon observed in a recent paper (Ref. 13):

> It is now technically possible to encapsulate all the potentially important constituents of artificial diets, namely, high molecular weight proteins and starches within nylon-protein walled capsules; lipids and lipid soluble components within gelatin-acacia walled capsules and low molecular weight water soluble components such as amino acids, minerals and vitamins within lipid walled capsules. Such capsules are especially useful for nutrition studies with filter-feeders since particle breakdown and nutrient leaching are major problems with micro-sized particles. Instability and leaching are also problems with feeds for large particle feeders such as shrimp and lobsters and encapsulation techniques may prove equally useful in this area.

It is our hope and expectation that this line of research will permit us to pursue a more fundamental and rewarding line of bivalve-nutrition research. This is the kernel of the problem now. The question is, however, whether microencapsulation diets will be economically feasible in the long term. The most promising application at the moment would be their use in providing the oysters with dietary supplements in addition to the algal diets.

Recent experiments indicate silt particles play an important role in sustained rapid growth of oysters (Ref. 14 and 15).

What is the Best Configuration for a Recirculating System for Growing Bivalves?

Up to this point I have addressed only the essential husbandry questions that must be satisfactorily answered if a recirculating aquaculture system for growing bivalves is to be successfully constructed and operated. Let me review. First, can bivalves be reared in captivity? The answer is yes. Second, what should bivalves be fed? The answer is algae will do, but there are probably better and cheaper non-algal feeds that we have not yet identified.

The question of microbial diseases was addressed and found to be a non-problem in the areas investigated though it may be in the future when commercial systems are operated at even higher densities and for long periods of continuous production. For example, it is possible that bacteria plays a role in the instability of larger algae cultures. Also, the question of accelerated growth through species selection and hybridization was recognized as a place where refinements could be made but it was not considered central to the question of building and operating a recirculating aquaculture system for growing bivalves.

The first efforts to construct a laboratory-scale system employed aquaria for the shellfish and flasks,

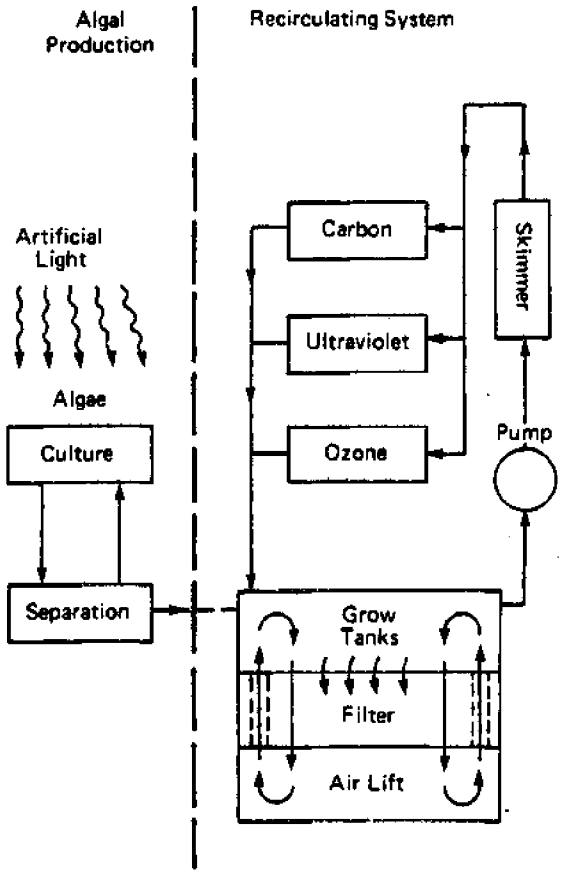

Figure 1. Configuration I

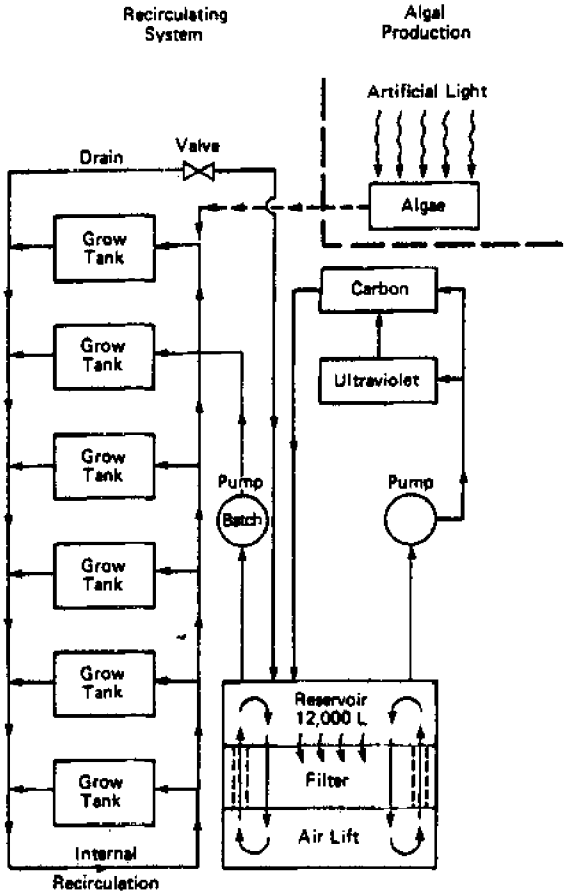

Figure 2. Configuration II

and later carboys, for growing algae. After evaluating these early crude experiments conducted in the late 1960's and early 1970's we set out to develop better system components before putting together the next generation recirculating system.

During the period between 1972 and 1975 three successive small-scale laboratory systems were constructed and operated. The configuration of the first system is shown in Figure 1 (Ref. 16). The primary purpose of this system was to operate and understand the function of a biological filter employed in a recirculating system. The growing tank contained 6000 liters of water and was provided with a false bottom on which rested a sand filter. Water from the oyster growing tanks containing animal wastes and excess food was pumped through a protein skimmer and then alternatively treated with (1) activated carbon, or (2) ultraviolet light. Ozone treatment was considered but not used. Algae for food was grown in a separate system using artificial light and then transferred in batches to the growing tanks. The combined growing tank-biological filter was found to be unsuitable for the mariculture of filter feeders. Attempts to harvest algal cells intact from the algal media

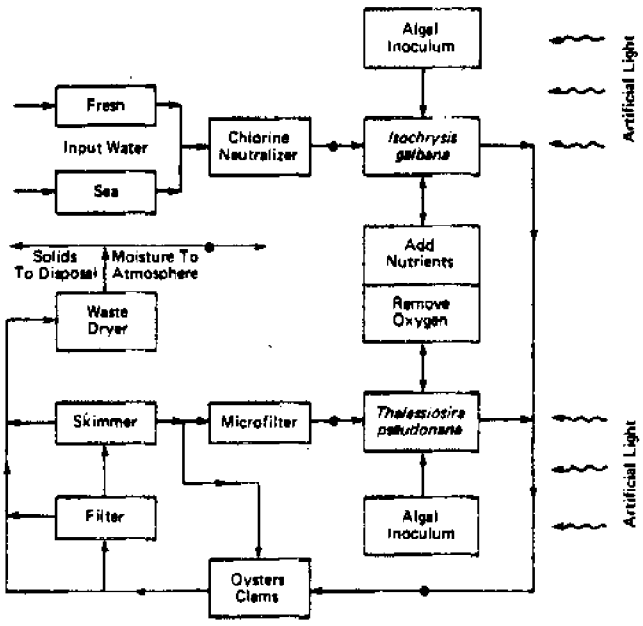

Figure 3. Configuration III

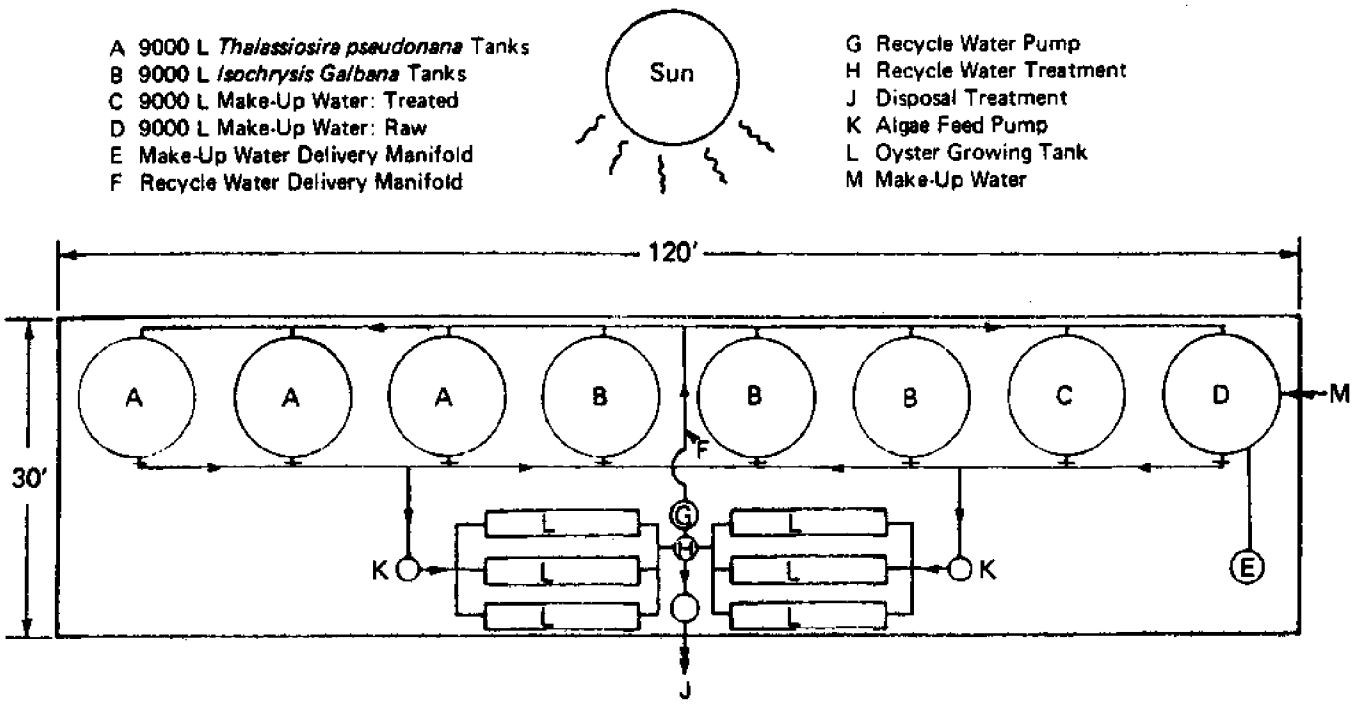

Figure 4. First Generation Configuration IV Greenhouse

efficiently and reproducibly were unsuccessful. The protein skimmer, while apparently useful, was not adequately evaluated in this system.

The second-generation system shown in Figure 2 was designed to simultaneously conduct a series of parallel nutritional studies on both oysters and clams. It featured separation of the filter and growing tanks. Algae were still grown in a separate subsystem using artificial light and batch fed to the oysters. The principal use of this system was for comparative studies of algal nutritive value. Ozone treatment of recirculating water was omitted but activated carbon and ultraviolet light were retained. The waste treatment systems were operated on an intermittent basis controlled by a timer.

The third configuration is shown schematically in Figure 3 (Refs. 10 and 16). It was designed to support approximately four bushels of bivalves. This system was the first designed to provide algae continuously to the oysters in amounts sufficient to meet their nutritional needs. In this system the inputs to the algae and cultures included artificial light, CO_2, calcium, and micronutrients (Refs. 17 and 18). Make-up water was added as needed when settled wastes from the oyster tanks were removed. The use of artificial lights was found to be too costly for scale-up and the system was redesigned to use sunlight.

The matter of shell development in oysters growing in recirculating systems was also addressed, but since shells from recirculating systems were, in all regards except size, normal in comparison with shells of naturally grown oysters, and there were no obvious deformities or anomalies associated with culturing, the matter was not pursued further (Refs. 19, 20, and 21).

The tastes of both oysters and clams produced in a recirculating system were tested. Both oysters *Crassostrea virginica* and clams *Mercenaria mercenaria* harvested from the controlled-environment system were organoleptically compared to their wild counterparts. Sensory evaluations considered appearance, texture, flavor, aroma, and overall acceptance. In the case of oysters, the greenhouse samples were preferred in all categories over their wild counterparts. In the case of clams, there were no significant differences found between greenhouse and wild animals although there was a slight preference for the flavor of clams produced in the natural environment (Refs. 22 and 23).

The fourth generation (or greenhouse) system was the so called "50-bushel system" since it was designed to support the growth of that quantity of oysters on an algal diet. This was put into operation during the spring of 1976 (Ref. 10). Due to the large quantities of algae required, the system was designed to be housed in a dual-skinned vinyl-covered greenhouse shown in Figure 4. Algae were grown in 3.6-meter diameter by 1-meter deep pools. Two species of

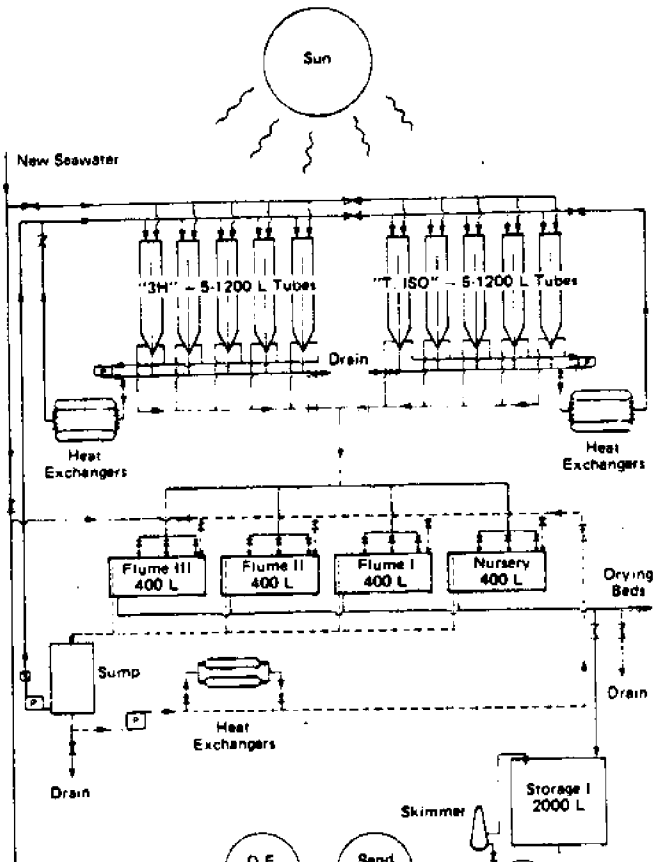

Figure 5. Second Generation Configuration V Laboratory Scale Greenhouse Prototype

algae were grown, *Thalassiosira pseudonana* (3H) and *Isochrysis galbana*, in separate sets of pools. These were the first cultures of this size (9000 liters each) and the first to rely on sunlight instead of artificial light. In this environment, with normal day-night cycles, new problems developed with frequent "crashes" or death of the majority of algae in the pools. These problems led to the study of the biochemistry of photorespiration and the development of equipment to automatically monitor and control CO_2, O_2, and pH (Ref. 24). The objective was to control photosynthesis and photorespiration to (1) prevent "crashes" of the large cultures and (2) prevent the loss of fixed carbon in the algae. Algae were fed directly from the growing pools into the oyster growing tanks. After water passed out of the growing tanks it contained dissolved oyster wastes and some

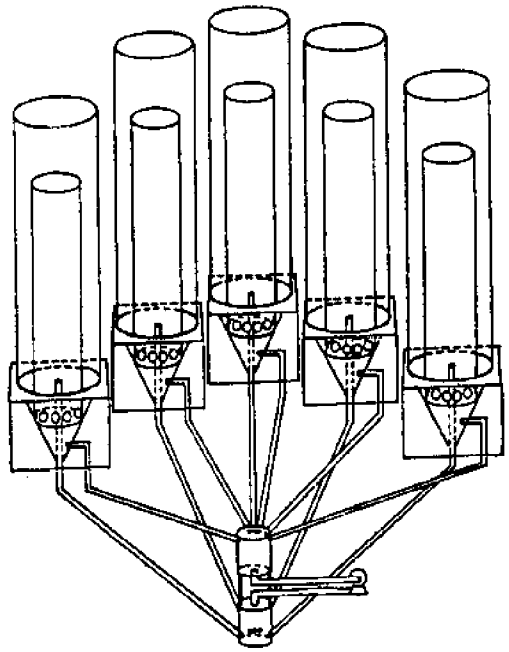

Figure 6. Algae Production Tube Module

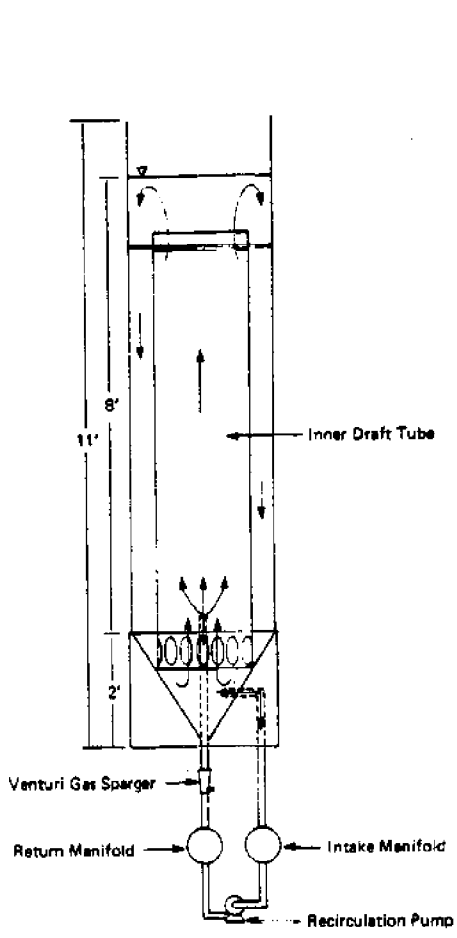

Figure 7. Algae Production Reactor

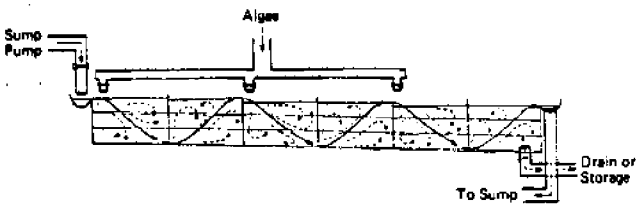

Figure 8. Baffled Flume Design Flow Characteristics

algae not removed by the animals in the growing tanks. These waste materials were removed by a protein skimmer and the water was recirculated directly to the algae growing pools.

The fifth-generation system is shown in Figure 5. This system was designed by a chemical process engineer using design data developed from the previous systems. Two species of algae were grown simultaneously in two sets of five-tube reactor modules. The details of the modules are shown in Figure 6. The details of an individual algal production reactor are shown in Figure 7. The procedure followed was to seed all five tubes from laboratory grown cultures and to provide dissolved CO_2 and sunlight. Two or three doublings of cells occurred each day. Algae were blended and fed to the oysters in the growing tanks at night, holding one full tube to restock the other four tubes each morning.

Oysters were grown in 400-liter baffled flumes as shown in Figure 8. Water, after passing through the growing flumes, was first passed through a protein skimmer, next a sand filter, and finally a diatomaceous earth filter before being returned to recharge the algae reactor modules. Heat exchangers permitted the reactor temperature to be controlled. Automatic controls maintained the desired pH and concentrations of CO_2 and O_2 (Ref. 25).

This system was operated for over a year on a continuous basis during 1980 and 1981. Data from this system is now being analyzed and a report will be completed in the near future.

Conclusions reached from constructing and operating these laboratory systems include

1. It is technically feasible to grow algae and bivalves in a recirculating system from nursery to adult size.
2. The nutritional requirements of bivalves are not yet well understood.
3. A recirculating system is an important experimental tool to permit isolation of important problems and to obtain useful solutions to those problems.

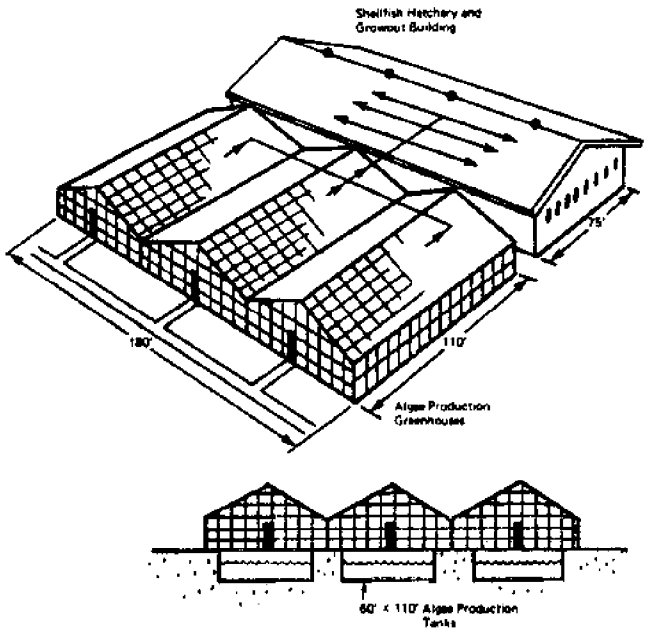

Figure 9. Commercial Concept I

Economics

Thus far in this paper nothing has been said about economics except that the eventual goal of the research is to produce animals in captivity, with that degree of environmental control necessary to insure a uniform output of high quality disease-free animals at a competitive price. At various points in the research, commercial feasibility was estimated by applying the "state-of-existing-understanding" to the design of a hypothetical commercial production system, or "bivalve factory." One such design is shown in Figure 9. The cost of constructing and operating these systems was estimated so that a price per bushel of oysters produced could be estimated. In the 1976-77 period we estimated that (Figure 10) oysters produced in such a facility could be produced at competitive prices with "wild" oysters harvested from boats (Ref. 26). Our optimism proved to be premature, however, as the complexity and cost of producing adequate food for the animals became more evident.

We are now confident that using a recirculating system oysters can be spawned and grown as "singles" up to a size of approximately 15mm diameter. Above that size the cost of algal foods becomes uneconomical for continued growth in a recirculating system as configured in our laboratory. What we now have is a system that offers several options as shown in Figure 11.

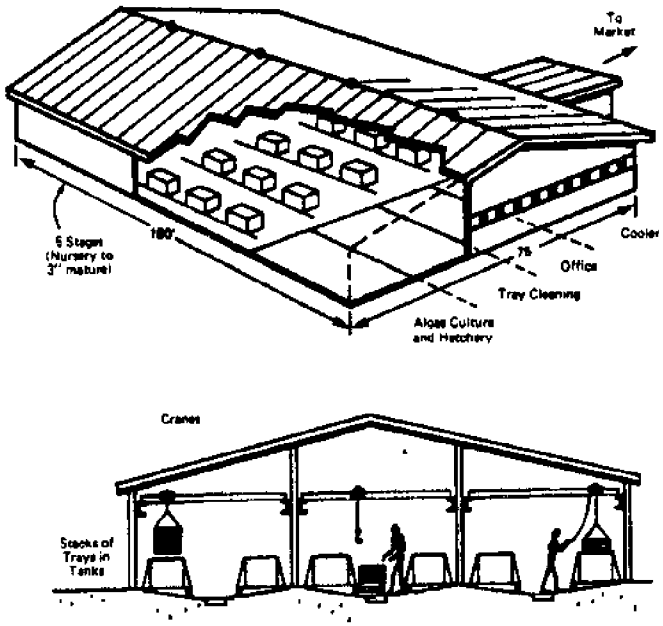

Figure 10. Shellfish Building Commercial Concept I

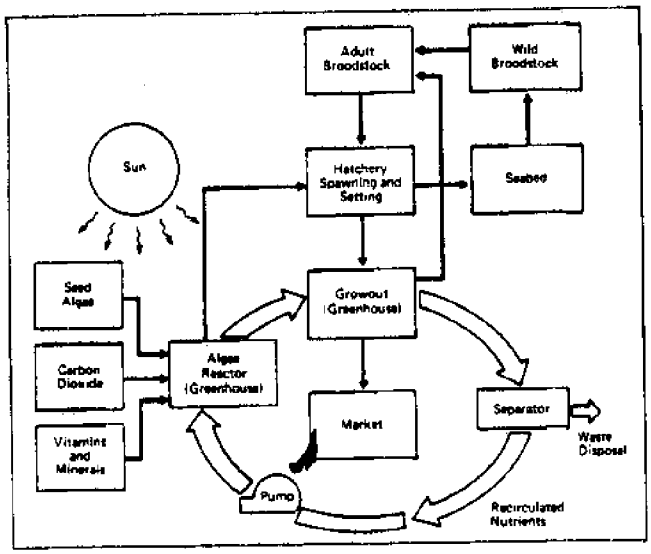

Figure 11. The Delaware Process

Conclusions

I want to conclude by observing that great progress has been made through this research which can be applied to a variety of problems. While we are not yet to the point that investors and entrepreneurs are building and operating production systems with the degree of process control we have achieved in our laboratory, what we have learned has been applied in several commercial enterprises. Research that is yet needed to make the growing of marine bivalves in controlled environment systems an attractive commercial possibility includes

1. Nutrition studies to understand the dietary requirements for both algae and artificial feeds to provide low-cost food for bivalves.
2. Selective breeding and hybridization of suitable species.
3. Resolution of gas exchange problems in aquatic systems (Refs. 27, 28, and 29).
4. Genetic engineering applied to species improvement.

Attractive applications of information derived from this research include

1. Bivalve hatcheries employing recirculating systems and grow-out in semi-controlled environments.
2. Waste treatment systems employing aquaculture techniques that supply the biological oxygen demand and CO_2 for growing algae and bivalves, at least through the seed stage.
3. Polyculture of marine animals and plants.
4. Systems to produce biomass, particularly algae, for end uses of its own.
5. Artificial food encapsulation techniques which can be employed for a variety of commercially important marine species.

Like all research, the outcome cannot be predicted. We have however, through this approach to an important problem, made significant progress and the direction for further research seems clear.

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References

1. Haskin, H.H., L.A. Stauber and J.P. Nackin. 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): causitive agent of the Delaware Bay oyster epizootic. *Science* 153: 1414-1416.
2. New Engineering Approaches for the Production of Connecticut Oysters, American Cyanamid Company, Stanford, Connecticut. June 1968.
3. Price, K.S. and D.L. Maurer (Ed.). 1971. Artificial propagation of commercially valuable shellfish, Proceedings of a Sea Grant Conference, October 22-23, 1969. College of Marine Studies, University of Delaware, Newark, DE. 212 p.
4. Epifanio, C.E., R.F. Srna, and G.D. Pruder. 1975. Mariculture of shellfish in controlled environments: A Prognosis. *Aquaculture* 5: 227-241. Elsiver.
5. Epifanio, C.E. and R.F. Srna. 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. *Marine Biology*. 33: 241-246. Springer-Verlag.
6. Epifanio, C.E., C.M. Logan, and C.L. Turk. 1975. Culture of six species of bivalves in a recirculating seawater system. DEL-SG-1-76, College of Marine Studies, University of Delaware. 13 p.
7. Epifanio, C.E. Phytoplankton and yeast as foods for juvenile bivalves. A review of research at the University of Delaware, Proceedings of Second International Conference on Aquaculture Nutrition: October 27-29, 1981. Lewes, DE. (In press).
8. Epifanio, C.E. and J. Ewart. 1977. Maximum ration of four algal diets for the oyster *Crassostrea virginica* (Gmelin). *Aquaculture* 11: 13-29.
9. Romberger, H.P. and C.E. Epifanio. 1981. Comparative effects of diets consisting of one or two algal species upon assimilation efficiencies and growth of juvenile oysters, *Crassostrea virginica* (Gmelin). *Aquaculture* 25: 89-94.
10. Pruder, G.D., E.T. Bolton, and S.F. Faunce. 1977. System configuration and performance bivalve molluscan mariculture, DEL-SG-1-77, College of Marine Studies, University of Delaware. 21 p.
11. Muller, G.T. 1978. Effects of ration and temperature on growth rates and growth efficiency in the cultured oyster, *Crassostrea virginica*. M.S. Thesis. University of Delaware, Newark, DE. 96 p.
12. Epifanio, C.E. 1979. Comparison of yeast and algal diets for bivalve molluscs. *Aquaculture*. V. 16: 187-192 p.
13. Langdon, C.J. New techniques and their application to studies of bivalve nutrition. Proceedings of Second International Conference on Aquaculture Nutrition, Lewes, DE. October 27-29, 1981. (In press).
14. Ali, Sayed M. 1981. Effects of inorganic particles on the growth of oysters. M.S. Thesis. University of Delaware, Newark, DE.
15. Pruder, G.D. and J. Ewart. 1982. Role of organically coated silt on the growth of oysters. (In press).
16. Pruder, G.D. 1976. Engineering Aspects of Bivalve Molluscan Mariculture: Culture System Configuration. DEL-SG-9-76, College of Marine Studies, University of Delaware, 13 p.
17. Sick, L.V. and C.C. Johnson. 1977. Nutrient and trace metal concentrations in the abiotic and biotic components of a recirculating seawater culture system for oysters. In *American Zoologist*, 17(4): 977-78.
18. Sick, L.V. Effects of the ambient environment in metabolic regulation of shell biosynthesis in marine bivalve molluscs. In Proceedings of the Second International Conference on Aquaculture Nutrition, Rehoboth Beach, DE, October 1981.
19. Palmer, R.E. and M.R. Carriker. 1979. Effects of cultural conditions on morphology of the shell of the oyster *Crassostrea virginica*. Proceedings of Natural Shellfish. Assoc. 69: 57-72 p.
20. Carriker, M.R. and R.E. Palmer. 1979. Ultrastructural morphogenesis of prodissoconch and early dissoconch valves of the oyster *Crassostrea virginica*. Proceedings of Natural Shellfish. Assoc. 69: 102-128 p.
21. Carriker, M.R., R.E. Palmer, and R.S. Prezant. 1980. Functional ultramorphology of the dissoconch valves of the oyster *Crassostrea virginica*. Proceedings of Natural Shellfish. Assoc. 70: 139-183 p.
22. Thoroughgood, C.A. Evaluation of palatability and selected nutrients of bivalve molluscs grown in controlled mariculture and natural systems. Proc. World Mariculture Soc., Honolulu, HA. January 1979.
23. Hicks, D. 1981. Sensory evaluation and selected chemical attributes of cultured and wild oysters, *Crassostrea virginica*. M.S. Thesis, University of Delaware, June 1981.
24. Pruder, G.D. Effect of pH, carbon dioxide, and light on the growth of *Thalassiosira pseudonana* (Hustedt) Hasle and Heimodal clone 3H, an important food for bivalve molluscan mariculture. Ph.D. Dissertation, University of Delaware. June 1978.
25. Thielker, J.L. Design and test operation of an intensive controlled-environment oyster production system. DEL-SG-07-81, College of Marine Studies, University of Delaware, April 1981. 28 p.

26. Gaither, W.S. Testimony before Joint Subcommittee on Fisheries and Wildlife Conservation and the Environment and Subcommittee on Oceanography of the Committee on Merchant Marine and Fisheries, U.S. House of Representatives. 94th Congress, First and Second Sessions, 1976. p. 77-82; 95-102; 233-244.
27. Pruder, G.D. and E.T. Bolton. 1979. The role of CO_2 enrichment of aerating gas in the growth of an estuarine diatom. *Aquaculture*, 17: 1-15 p.
28. Pruder, G.D. and E.T. Bolton. 1979. Differences between cell division and carbon fixation rates associated with light intensity and oxygen concentration: implications in the cultivation of an estuarine diatom. *Marine Biology*, 59: 1-6 p.
29. Pruder, G.D. Aquatic production systems: algae. Proceedings, International workshop on Nursery Culturing of Bivalve Molluscs. State of University Ghent, Ghent, Belgium. February 24-26, 1981. 219-226 p.