Intensive Marine Bivalve Cultivation in a Controlled Recirculating Seawater Prototype System

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College of Marine Studies
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Preface

The mariculture program at the University of Delaware formally began in September 1968, with an award from the Office of Sea Grant. The initiating proposal was based in part on earlier work at the University by C. N. Shuster, Jr. in 1959, T. P. Ritchie in 1962, and D. Maurer in 1966. Maurer was supported jointly by the then Bureau of Commercial Fisheries, now the National Marine Fisheries Service, and the Shellfisheries Commission of the State of Delaware. The state commission also awarded an annual grant for basic shellfisheries research. With the encouragement of Mr. Harold Goodwin, the Associate Director of the National Sea Grant Program at that time, the program was conceived as a multidisciplinary approach to the propagation of the American oyster toward aiding the recovery of the oyster populations in the Delaware Bay region. The industry had been reduced a hundred-fold by an epizootic oyster disease caused by the haplosporidian parasite MSX (Multinucleate Sphere Unknown), now recognized as *Minchinia nelsoni*. With the impetus of such a catastrophe and the model of the poultry industry in the background, a "pilot shellfish factory" was planned where broodstock could be conditioned and spawned at will, larvae and spat reared for field planting, research conducted, and technicians and watermen trained in promising technology. The potential for a multidisciplinary approach presented an exciting prospect. Disease resistant "super" oysters might be bred and established, and sufficient control could be exerted over the whole process to assure reliability and commercial application. Much of the process would be conducted intensively in closed systems and would bring to bear appropriate elements of biology, husbandry, environmental science, chemistry, and engineering, and, when the technology was solid, law and economics.

The intervening years, 1968-80, have been occupied with these areas, adapting and integrating diverse basic knowledge, carrying out basic research where information was lacking, and reexamining the lore of oyster cultivation for application to confined, managed systems. Overviews of these efforts have been presented (Price et al. 1976[1] and W. S. Gaither 1982[2]).

The present contribution is a technical report which describes the production of oysters in an intensive, controlled, recirculated-seawater system, a prototype for the Delaware process. The prototype is an oyster cultivation scheme whose design evolved in 1978-79 from the prior decade and more of investigation. It was constructed in the new Otis H. Smith Laboratory in 1979-80 and operated during 1980-81. The integrated process includes the supporting activities of the

algae seed laboratory, the hatchery production of juvenile oysters, and chemical and microbiological services. The design is due largely to the efforts of Dr. Gary D. Pruder and Hugh L. Fisher. Jeffrey L. Thielker managed the construction and operation of the prototype. N. Dean Dey was responsible for the algae seed laboratory, John Ewart for the hatchery, Anne Baggaley for chemical services, and Kevin P. Smith for microbiological services. Other technical personnel, especially Robert Baggaley, Alva Schmidt, Philip Alatalo, and Richard Brian tended the daily operation of the prototype. Dr. Christopher Langdon carried out bioassays of water quality. The report was compiled and edited by Dr. Ellis T. Bolton. All the writers of this report thank Mary Rae Clark for her contribution throughout the preparation of this document.

We are pleased to acknowledge the generous long-term support of the Office of Sea Grant, National Oceanographic and Atmospheric Administration, Department of Commerce, and the State of Delaware. Industry has made significant contributions through the Industry Partners program (Columbia Gas System Service Corporation, Frederic R. Harris, Inc., Delmarva Power and Light Company, and Gilbert Associates) and the Mariculture Partners program (Campbell Soup Company, Borden, Inc., and Unilever, Ltd.). Special thanks are due the Jessie Smith Noyes Foundation, Inc., for Fellowships in Mariculture. The Otis H. Smith Laboratory was made possible by a grant from The Economic Development Administration, gifts from anonymous donors to the University of Delaware, Mr. Richard Loring of Cultured Clams, Inc., Dennis, Massachusetts, and Mrs. Bobbie Graham Kleinholz of Boothbay Harbor, Maine.

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Abbreviations

aff.	affinity
cal	calories
cm	centimeter(s)
DE	diatomaceous earth
DO	dissolved oxygen
g	grams(s)
GF/C	glass fiber filter; /C indicates coarseness, C being finer than A
hp	horsepower
Kcal	kilocalories
L	liter(s)
M	mole(s)
m	meter(s)
meq	milliequivalent
mg	milligrams(s)
mL	milliter(s)
mm	millimeter(s)
mv	millivolts
nm	nanometer(s)
PE	photosynthetic efficiency
ppm	parts per million
ppt	parts per thousand
v/v	volume to volume
w/v	weight to volume
µg	microgram(s)
µgat	micrograms atomic weight
µm	micrometer(s)
µv	microvolt(s)

Symbols

C	carbon	Mn	manganese
Ca^{++}	calcium ion	Mo	molybdenum
$CaCl_2$	calcium chloride	N_2	nitrogen
Co	cobalt	Na	sodium
CO_2	carbon dioxide	NaCl	sodium chloride (salt)
Cu	copper	NH_3	ammonia
H	hydrogen	$NaHCO_3$	sodium bicarbonate
HCl	hydrochloric acid	NaOH	sodium hydroxide
He	helium	NH_4Cl	ammonium chloride
H_2O	water	O_2	oxygen
Hg	mercury	Zn	zinc
KCl	potassium chloride		

Executive Summary

From its inception the Mariculture Program at the University of Delaware has pursued the objective of developing a commercially viable, intensive, controlled-environment, recirculated-seawater mariculture system for the production of bivalve molluscs, particularly oysters and clams. The minimal requirements are a dependable supply of molluscs including broodstock and their offspring, a nutritionally adequate and ample feed supply, and a seawater medium that is compatible with the life cycle of bivalves and other necessary life forms especially microalgae feed. At the initiation of the program little was known about the fundamental requirements for life support of shellfish and algae in confined, artificial environments. As a result of a sequence of research efforts over the course of more than a decade, ways of meeting the requirements have largely been satisfied. The earlier findings permitted sets of physical, chemical, biological, and engineering specifications to be drawn for intensive bivalve cultivation in a recirculated-seawater system. As a culmination of this work a prototype shellfish cultivation system incorporating the specifications was designed and constructed. Its operation has demonstrated the technical feasibility of 'the Delaware process'.

Results

This report describes in detail the results of a year's intensive effort. In particular:

- A prototype integrated, intensive, controlled-environment, seawater, bivalve-cultivation system was designed, constructed, and operated continuously for one year.

- The design and operation of the system are based on specifications that were derived in large measure from a decade of research at the University of Delaware on the biology of oysters and single-celled algae, the chemistry and microbiology of seawater, and bio-engineering practices.

- Supporting activities, algae seed production, hatchery juvenile oyster production, and chemical and microbiological monitoring and assay were established to serve the prototype and other laboratory functions.

- A filtered and chemically processed recirculated-seawater environment mutually compatible with the growth of oysters and feed microalgae was established and utilized. Three hundred thousand gallons of seawater from the Indian River Inlet, Delaware, was imported during the operational period

in 1980-81 to the Smith Laboratory by tank truck, diluted with tap water, fertilized with mineral nutrients, and recirculated at a daily rate of 77 percent of the prototype volume to conserve energy and nutrients. The seawater quality was maintained throughout the operational period.

- Seed algae and juvenile oysters were dependably and systematically introduced into the prototype.

- Oysters grew on the average in the prototype at a constant rate and reached about one-fifth the size specified. They converted, on a dry weight basis, 3 percent of the algal food that they filtered. Their meat was comprised of typical amounts of water, ash, protein, glycogen, and fat. The mineral and organic content of the shell was also typical, but the shell was not robust.

- Algae *in situ* production met the feed supply specifications, and the photosynthetic efficiency over the course of the production period was 2.1 percent for the food diatom (*Thalassiosira pseudonana*, 3H) and 1.4 percent for the flagellate (*Isochrysis* aff. *galbana*, Tahitian).

Research Needed

These results emphasize the need for additional investigations on the intensive long-term cultivation of bivalve molluscs in controlled-environment systems:

1. The failure of oysters to grow on the average to the size specified, the apparent lack of spawning, the relatively fragile shell, and the low average conversion of algal food to oyster organic material signify a condition(s) of stress under cultivation. Despite the fact that all design and operating specifications were essentially met, the origin of such stress remains unknown. Among the environmental factors that require further research are:

   - Mechanically or physically induced stress such as handling or prolonged cultivation at high temperatures, and

   - Nutritionally induced stress as a result of one or more deficiencies, either of particulate or dissolved forms, either organic or inorganic in nature. Research on the physiological consequences of such stress for the growth of oysters and for oyster confirmation to a desired product specification is needed.

Toward ameliorating some of these factors we are particular interested in developing a more thorough understanding of algal/bacterial/silt systems, for they may contribute substantially to intensive molluscan production. Coupled algal/bacterial/silt systems may substantially reduce the cost of producing oyster feed, allow the recycling of waste organics, and help to resolve the oyster growth-rate problem.

2. The prototype production of algae for bivalve feed met the specifications. Nevertheless, extension of algal culture life is much to be desired to lessen the demands imposed by frequent replenishment of seed for mass culture. Further study of conditions leading to unbalanced algal growth that presages culture failure are needed, and means to ameliorate the condition should be sought. New work is necessary to learn how to provide the carbon dioxide necessary for photo synthesis and to remove the deleterious oxygen generated in-the process in ways which are economical and compatible with the use of algae as bivalve feed.

   Bacteria, with some dependence upon silt, utilizing organic feedstock materials, could provide carbon dioxide, consume excess oxygen, and thereby contribute to the maintenance of environmental conditions suitable for high-rate algal photosynthesis. This would be a biological solution to gas exchange problems which, in the prototype, required purging algal cultures with carbon-dioxide-enriched nitrogen. We have experimental evidence that mixed algal/bacterial/silt cultures provide superior nutritive value, as compared to mixtures of unialgal cultures, for the more rapid growth of oysters.

3. Research is needed to design nutritious diets and rations that can economically serve as alternatives or supplements to microalgal diets for oysters and other commercially interesting bivalves.

4. Dissolved microconstituents, both organic and inorganic, in the seawater milieu need investigation to ascertain their importance for oyster and other bivalve cultivation.

5. A wide range of growth rates was observed among individual oysters cultivated in the prototype. This observation implies that a range of genetic competence exists among the oysters for growth in the artificial prototype environment. Experiments to improve oyster lines and strains for intensive controlled cultivation under artificial conditions would thus appear desirable.

The Question of Commercial Viability

The objective of developing an intensive, controlled-environment, recirculating system for the production of commercially significant bivalve molluscs implicitly includes the potential for economic viability.

The operation and management of the present prototype system cast little light on the question of economic viability. Its essential purpose was to test the technical feasibility of the process over a sufficiently long period of uninterrupted operation. The technical evaluation of such a complex process required personnel, equipment, instrumentation, materials, laboratory services, and operational protocols which would be quite different from those for commercial production operations. Nevertheless information from the prototype and from our auxiliary research, as well as findings of others around the world, sheds some light on the potential for economic viability. Assuming that factors repressing the growth of oysters can and will be resolved without major cost impacts, some pertinent comments may be made.

For the present only certain species of marine microalgae support the growth and development of commercially desirable bivalves throughout their life cycles. The cost of producing these algae is very high; our best estimates place these costs in the range of $20 to $50 per pound, dry weight. It now appears quite certain that we can produce 3 to 5 pounds of oyster live weight, including shell, for each dry-weight pound of algae fed. The cost of oyster production attributable to feed is $4 to $17 per pound of oysters, live weight including shell. Feed costs are reported to represent from 15 to 85 percent of the cost of producing oyster seed. In the current marketplace the retail price for adult oysters ready for human consumption ranges from a few cents up to one dollar per pound, live weight including shell. Therefore, it would appear that any process dependent upon cultured algae as the principal feed has little if any potential of producing *adult* bivalves economically. However, seed oysters are currently sold on a dollars-per-thousand basis. Converting the current retail price per thousand seed oysters to dollars per pound shows that seed oysters retail at between $100 and $300 per pound, live weight including shell. These high prices reflect the cost of production using current methods since most seed hatcheries are only marginally profitable. At this time insufficient information exists concerning the size of the market and

the interstate transport of seed to predict the commercial viability of the intensive, controlled-environment, recirculated seawater system production of seed oysters.

Technology Transfer

The technology and improved technical methods developed during the research efforts over the past 10 years have been reflected in the design and operation of the recirculating system. Improved methods were developed in the following areas:

A. Conditioning and maintaining broodstock,

B. Rearing of larvae through metamorphosis,

C. Rearing of spat and seed,

D. Maintenance of algal species for inoculum,

E. High-rate production of algae at high concentrations under bright light, and

F. Rearing oysters at high temperature by delivering large amounts and high concentrations of algal feed with pulse feeding techniques.

The following commercial enterprises have made use of these improved technical methods and have cooperated importantly in the information exchange necessary for technology transfer:

- System Culture Incorporated, Kahuku, HI (Methods D, E, and F)
- Aquatic Farms, Inc., Kaneohe, HI (Methods D, E, and F)
- Cultured Clams Inc., Dennis, MA (Methods A, B, C, D, and E)
- Indian River Mariculture Corp., Massey's Landing, DE (Methods A, B, and C)
- SATMAR, La Saline, Barfleur, France (Methods A, B, C, D, E, and F)
- International Shellfish Enterprises, Inc., Moss Landing, CA (Methods D, and E).

1. Introduction

The Mariculture Context

Aquaculture is the cultivation of aquatic organisms. It comprises art and mechanics, scientific knowledge, and lore and wisdom accumulated throughout the long period of man's attempt to improve the productivity of natural waters. Mariculture is the marine counterpart of aquaculture. It is concerned with animal, plant, and microbial organisms that can be cultivated in salt-water environments. Aquatic organisms are ordinarily immersed in the aqueous environment from birth to death, generation after generation. To some degree they are physiologically, as well as metaphorically, extensions of the surrounding fluid and are intimately responsive to it. They, in turn, alter the environment in accord with their metabolic activities by releasing metabolic products and new generations of offspring. The organisms, the aqueous medium and the structures which confine it, the atmosphere, diurnal light cycles, temperature regimes, and other organisms, interacting together in complex and subtle ways, form a system of which no part can be wholly ignored when the goal is improved cultivation.

Cultivation and husbandry require that the mariculturist tend to each of these elements as assiduously as the prudent farmer, who tills and fertilizes his fields and nurtures his stock. It is a crucial task of the mariculturist to provide a saline environment that is chemically, physically, biologically, and nutritionally adequate to sustain and increase the organisms he hopes to benefit from. As a consequence, mariculture is inherently interdisciplinary.

There are two basic types of mariculture: extensive and intensive, although the distinction between the two in a particular comparison may not be precise. For example, some aspects of intensive mariculture, such as hatchery operations, may take place as part of an extensive mariculture activity, where seed or juveniles are released to a natural body of water from which adult forms are later harvested. The essential difference lies in the degree of control the mariculturist is able to exert on the cultivation system.

Extensive mariculture is characterized by minimal control of the culture environment and of the genetic composition of the organisms cultivated. Traditional and relatively primitive technological practices are often employed, and low production efficiency results. Usually natural bodies of water are used,

and the system is subject to the vagaries of a particular site and its ecosystem--unpredictable amounts and kinds of feed and nutrients, susceptibility to the local climatology, and varying conditions of the water column all take their toll.

Intensive mariculture is characterized by a relatively high degree of control, especially of environmental factors. It implies the application of technology (and consequently, higher costs) to achieve high production efficiency--usually considered to be enhanced growth toward a maximum amount of product in a minimal volume of seawater. To effect maximum control it is necessary to confine the mariculture system and to minimize or avoid the many uncertainties (such as disease, predation, the effect of storms, and especially the undependability of feed supplies) that are common experiences to those who farm the edges of the sea.

In the late 1960s scientists at the University of Delaware began to envision the possibility of cultivating oysters in a closed system which might be remote from the sea and which might be a marine analog of the intensive poultry cultivation industry. Over a decade of work has been invested to nurture the idea toward fruition and to discover the underlying requirements of biology, chemistry, physics, and engineering that would have to be mastered, assembled, and integrated to create a working model system. A prototype system has been evolved and operated. The primary purpose of this report is to describe the system design, operation, performance, and analysis, and the technical activities necessary for its support.

Intensive cultivation of many marine filter-feeding animals, such as bivalves, depends upon an adequate supply of appropriate single-celled marine algae as a source of feed. The technical problem of supplying algal food is by no means trivial. It stands as the single most demanding aspect of the intensive cultivation of oysters in confined systems. The required algae grow photosynthetically (a process by which carbon dioxide is assimilated and biochemically transformed, along with other nutrient materials, into more new algae, with light, usually sunlight, serving as the source of energy).

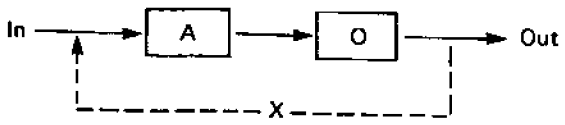
It is possible to estimate the maximum photosynthetic efficiency that can be expected for this process and thereby set a *theoretical* maximum limit to the amount of feed which might be produced in a unit area. From considerations of photo- and biochemical energetics it has been shown (Bassham, 1977)[3] that the theoretical limit to the efficiency of conversion of light absorbed is quite precise: about 12.3 percent of the total solar radiant energy at the surface of the earth can be stored in the form of organic chemical energy or biomass. Conversion of the average annual solar energy at the earth's surface in the U.S. would produce 224 metric tons of single-celled algal dry matter per hectare per year, which is equivalent to 100 tons per acre

per year or 61 grams per square meter per day. Such an astonishing productivity is not achievable in practice for many reasons which involve physical, chemical, and biological parameters of real environments and the particular kind of alga. Nevertheless, it is important to realize what the theoretical limits of such productivity may be in order to design and carry out mariculture schemes which will take optimal advantage of the potential afforded and which can be evaluated and assessed in terms of a nearly universal quality.

Single-celled marine algae are the only feed known for certain to sustain and promote growth of oysters throughout their life cycle. Many and various oyster feeding experiments have been carried out over several years; they indicate a very approximate feed requirement: about 10^{12} algal cells to grow an oyster to market size. If the theoretical photosynthetic productivity level of 61 grams of algal dry matter per square meter per day could be achieved, such an amount ought to support an increase in oyster mass equivalent to the production of 1,700 individual market-size oysters (about 5 bushels) in a year. This numerical projection indicates the biological potential that exists and defines the upper limit of expectations for filter-feeding animal production.

Flow-Through and Recirculating Systems

It is convenient to categorize mariculture schemes based on the pattern of seawater flow; whether a given volume of seawater is introduced into a confinement and allowed to be removed without retracing its path (flow-through), or whether it is overtly caused to retrace its path a few or many times (recirculated). The distinction is not always precise in practice except for laboratory schemes, such as plug-flow reactors, specifically designed to assure minimal circulation and remixing of the bulk of the liquid. The literature is replete with descriptive terms each emphasizing a particular attribute of the flow of water: still-water, running-water, open, semi-open (or semi-closed), closed, multipurpose environmental, flow-through, recirculating systems, etc. Most of the systems, whether natural or artificial, are described by this simple block diagram:



The path of the seawater flow is indicated by the arrows. Thus with the dotted-line loop closed by the valve X, water flows from the input supply through block A (for algae) thence through block O (for oyster) to the exhaust (flow-through system). Alternatively, with the valve X open, an amount of water which has traversed the upper path can be diverted back to the input where it again traverses the original path (recirculating system). The schema can be elaborated to accommodate the essential considerations for controlled-environment mariculture. Figure 1 is a diagram showing several aspects of the recirculating-seawater controlled-environment process developed at the University of Delaware.

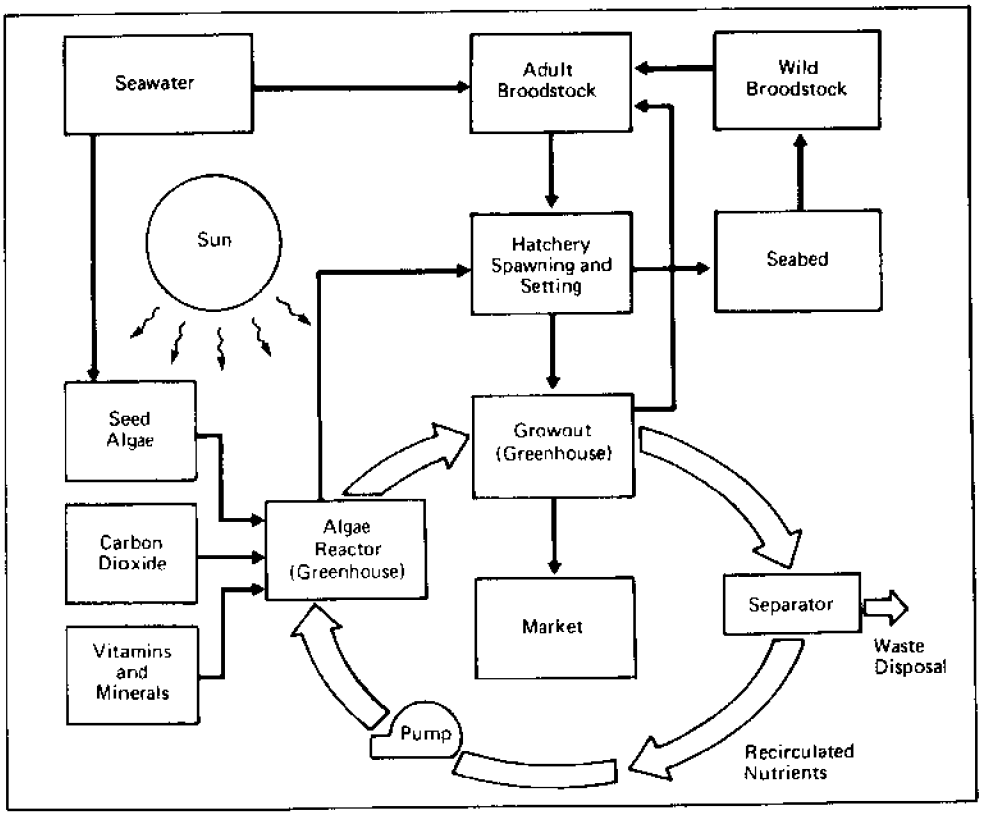


Figure 1. Diagram of a recirculated-seawater controlled-environment cultivation system. The continuous recycling of food- and nutrient-laden seawater to the oyster and back to the algae leads to the designation of the controlled-environment mariculture process at the University of Delaware. Broodstock oysters are spawned and eggs fertilized. Juvenile offspring are placed in a continuous grow-out system that employs recirculating seawater, a waste treatment and disposal subsystem, and an *in situ* algal feed production subsystem. The processed seawater is compatible with algae and oysters.

Basic Elements of the Intensive Controlled Recirculated Seawater Mariculture System

The diagram of the Delaware Process (Figure 1) illustrates the basic elements of an intensive, controlled, recirculated-seawater mariculture system. Such elements have been assessed and specifications put forward for the design and operation of the prototype facility as described in subsequent sections of this report.

Algae and oysters are the organisms of primary concern. Single-celled marine algae are the only kind of feed known for certain to sustain oysters through their life cycle, and they must meet certain minimal requirements: they must be nutritious, be of a fairly narrow size-range (approximately 5 to 25 micrometers in maximum dimension), and be suited to culture in amounts sufficient for useful growth of the oysters. Much prior work at the University of Delaware has shown that a combination of two algal species, the diatom *Thalassiosira pseudonana*, strain 3H, and the flagellate, *Isochrysis galbana*, strain Tahiti, suffice to meet these requirements. Many other algae, or algal combinations, have been studied here and elsewhere, but there is no evidence which suggests that a highly superior species or combination will be found. To grow the algae in sufficient amounts with high photosynthetic efficiency it is necessary to supplement the cultures with carbon dioxide, mineral nutrients, and some vitamins and to provide temperature-controlled seawater in transparent vessels configured and equipped to allow efficient gas exchange and maintenance of specifications for other environmental parameters. The mass cultivation of algae and the operation of the algae supply system are described in sections 2 and 3, respectively.

Oysters (*Crassostrea virginica*) originate in local waters and are presumably resistant to the MSX disease which decimated the major oyster populations of the Delaware estuarine system in the 1950-60 period. They are brought into the laboratory as adults, conditioned, and spawned, and the offspring cultivated under hatchery management to provide juvenile stock for further cultivation. The hatchery activities take place in controlled environments irrespective of natural seasonal cycles; they are described in detail in Section 3.

The algae culture procedures apply to most species of single-celled marine algae of interest as feed or as sources of marine photosynthetic biomass or biochemicals. The hatchery

procedures apply to a great variety of marine filter-feeding animals throughout their life cycle or during particular stages of their life cycle such as larval and juvenile development.

Seawater

The cultivation facility, having no *in situ* supply, uses seawater obtained during the incoming high tide at the ocean inlet to the Indian River Bay, Delaware. The seawater is pumped to a fiberglass tank-truck, which transports it to the facility, where it is passed through a filter to a distribution system. Such a seawater supply, while varying seasonally in temperature, is relatively constant in other important features: salinity, pH, low sediment content, and proportions of ionic materials. Like other natural waters it varies mostly in minor constituents such as dissolved or suspended organic materials, but we have no evidence that these variations present special problems for cultivation of algae or filter-feeding animals. Since the seawater has a relatively high salinity, about 30 parts per thousand (ppt), it is a decided convenience merely to dilute it with freshwater to yield an optimal salinity for the organisms under culture.

Natural seawater is, of course, a highly complex medium subject to many kinds of variations. The mariculturist should be on guard to respond to any change that may affect the cultivation activity. Experience with the seawater available has shown that routine or spot checks of the incoming, stored, or distributed supply by standard physical, chemical, and microbiological procedures are sufficient to assure that it is suitable for use in cultivation. These procedures are described in sections 2 and 3 of this report.

Recirculating System

The prototype oyster growout system is housed in a greenhouse and accepts the major inputs of seawater, sunlight, algae, and juvenile oysters. Algae are mass cultured in specialized reactor vessels and distributed to oysters growing in seawater which flows through flumes in a fashion that assures that all

oysters have ready access to the feed. Seawater containing oyster wastes is processed to condition it for reuse by algae and circulated back into the algae reactor vessels, thus completing the flow cycle. The system has been operated for many months and many physical, chemical, and biological data have been collected to provide information for technical analysis and assessment.

2. Prototype Oyster Cultivation System Design, Operation, Performance and Analysis

Introduction

The University of Delaware has directed its research effort in bivalve mariculture toward the development of a commercially viable, intensive, controlled-environment system for producing the Eastern oyster, *Crassostrea virginica* (Gmelin). This program responded to the decimation in the late 1950s of the oyster population in the Delaware Bay that resulted from the rapid spread of the intracellular protozoan disease MSX (*Minchinia nelsoni*). A shellfish production factory, analogous to the production schemes for the poultry industry, was envisioned as early as 1967 in a proposal submitted to the Office of Sea Grant as a means for circumventing the many environmental uncertainties and dangers that beset the local oyster industry (Price et al. 1976)[1]. After a decade of interdisciplinary research needed to gather basic facts and establish biological, chemical, and engineering specifications and guidelines, the design, construction, and test operation of a prototype oyster cultivation unit was recommended. It would assess the response of oysters to continuous cultivation in an integrated system over an extended growth period which would carry the oysters from seed to market size.

Several experimental schemes were devised and tested (Price et al. 1976[1] and Gaither 1982[2]). Subsystems, such as algal cultivation processes, were set up and analyzed to ascertain the engineering requirements for dependable long-term culture. Oyster growth response to various feed combinations and feeding levels was measured, and various means for treating recirculated seawater were tested. During these investigations it was necessary to conduct a relatively large amount of basic physiological research on algae and oysters in order to quantitate many biological processes so that engineering specifications could be drawn and integrated systems devised which would work harmoniously.

The prototype unit that has evolved from this work takes advantage of the underlying principles of intensive oyster mariculture described in Section 1 as well as the facilities and procedures described in Section 3. It uses all the appropriate specifications that were in hand together with somewhat arbitrary best-judgement criteria to arrive at the physical assembly and mode of operation of an integrated system. It comprises three subsystems: oyster growth, algae production, and water and waste handling, which are connected through the flow of recycled seawater and controlled to optimize productivity and efficiency.

The unit is a working model yielding quantitative information on component performance, material flow, and cost-related factors over a continuous long-term oyster production cycle.

The specific objectives sought by operating and managing the prototype unit were:

- Continuously cultivate oysters over an extended period in an intensive, controlled, recirculating culture system;
- Analyze materials and energy utilization, performance of system components and integrated processes, and quantitative chemical, physical, and biological data;
- Identify and define problems that may arise from continuous long-term operation of the system, including any which may be peculiar to the recycling scheme;
- Provide shellfish for product evaluation, and
- Document the operation and performance of the system.

Prototype Design Considerations

The design features and specifications take into account the integrated system, facilities, and support activities diagrammed in Figures 1, 2, and 3. The prototype provides a means for the continuous, long-term, intensive cultivation of oysters and algal feed in a recirculated-seawater medium. It attempts to provide the algae and oysters with optimal conditions that permit them to express their potential for growth as fully as possible. It is physically housed in the 6,000-square-foot greenhouse at the southern end of the Smith Laboratory (described in Section 3). Its use allows quantification of growth, energy consumption, water conservation, environmental parameters and control methods, and yields shellfish for product evaluation. It is conveniently viewed as comprising three subsystems: oyster cultivation, mass algae cultivation, and water and waste handling.

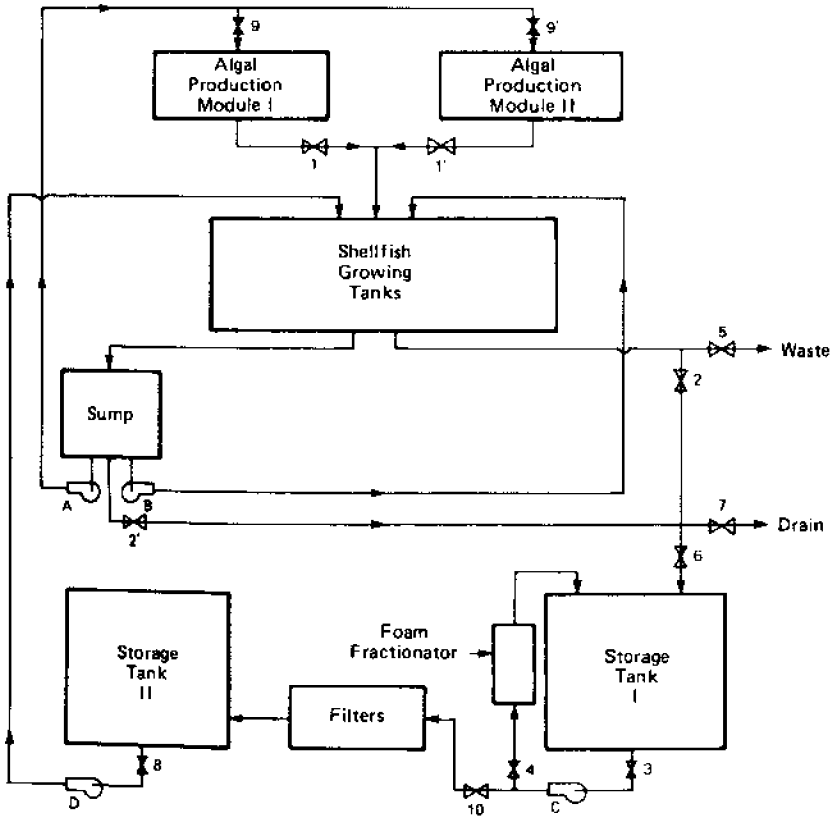


Figure 2. Block diagram of the prototype arrangement of the algae culture subsystem (upper), the oyster cultivation subsystem (middle), and the waste and water processing subsystem (lower).

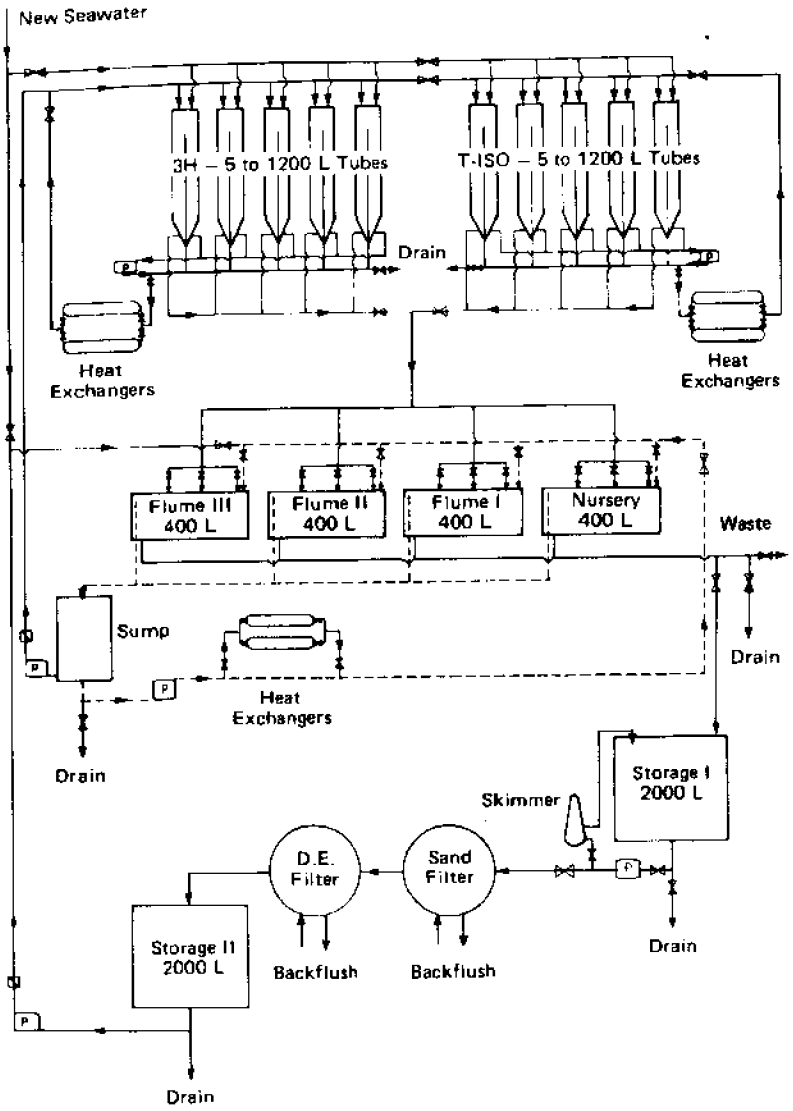


Figure 3. Schematic diagram of the algae culture subsystem (upper), the oyster cultivation subsystem (middle), and the water and waste processing subsystem (lower).

Oyster Cultivation Subsystem

OYSTERS

A production goal of 10 bushels of market-size oysters produced from seed grown through one year of continuous cultivation was the principal design determinant. The size of the effort necessitated by these specifications was judged to be adequate to meet the objectives outlined in the introduction to this section. Since the cultivation process is continuous, oysters are introduced into the cultivation system as seed produced in the hatchery (Section 3) from spawned adult broodstock parents, *Crassostrea virginica* (Gmelin), taken from waters of the Delaware Bay estuarine system at the Broadkill River. The hatchery can spawn oysters at 6-week intervals throughout the year. Thus, the system contains groups of oysters in a series of age classes separated by 6-week sequential intervals. Oyster growth in the prototype was specified to occur in a curvilinear fashion so that on the average oysters would reach a market size of about 80 grams in a year. The specifications for oyster growth were in fact set out as a best judgement based on:

- Laboratory and field experience (particularily the warm-weather growth rates known to occur for oysters maintained in the local Broadkill River, Figure 4),
- The maintenance of an environment sufficient for growth, and
- A relatively homogeneous size population of oysters that would be capable of responding by steadily increasing its growth rate until the oysters reached the 80-gram harvest weight.

These were intentionally demanding specifications to provide a serious test of the prototype system.

A plan for stocking the prototype is given in Table 1. Allowance is made for 20-percent mortality during the projected cultivation period. Each age class of cultchless oyster seed results from the hatchery selection of the offspring of a separate spawning and are kept separated through the prototype growth period in marked containers. The hatchery process involves multiple parent matings of native oysters for each age class to assure that the genetic diversity of the original native broodstock is maintained.

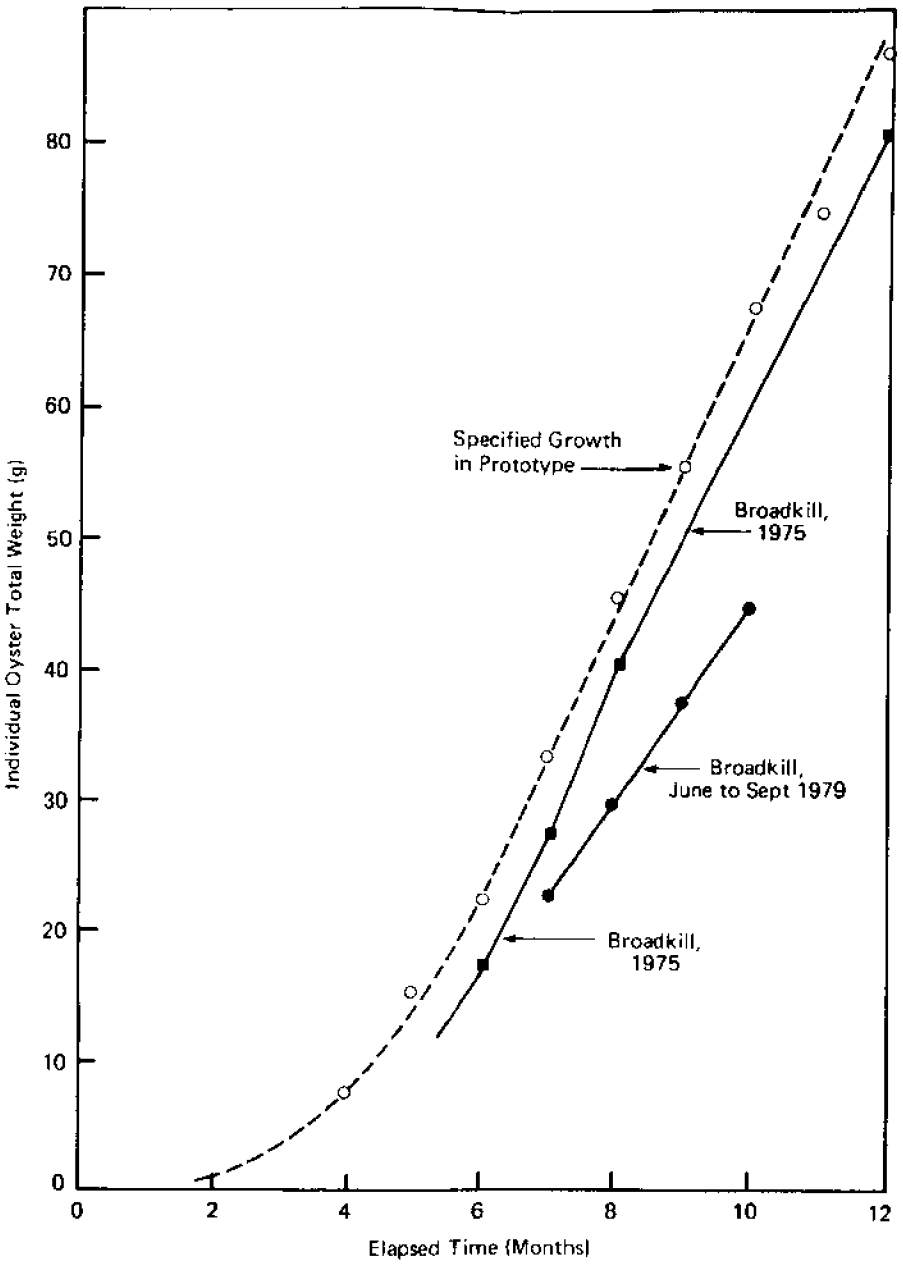


Figure 4. Measured and specified oyster growth. Oysters were suspended in trays in the Broadkill River, and growth was measured over a 6-month period in 1975 (Δ——Δ) and a 3-month period in 1979 (•——•). Oyster growth specified to occur in the prototype system is indicated by o—o.

Table 1. Planned Age-Class Distribution for Prototype Stocking

Age (wk)	Average Whole Weight (g)	Average Shell Height (cm)	No. of Oysters	Biomass (g)
6	0.1	1.0	311	31.1
12	2.5	2.0	303	757
18	8.0	3.5	295	2,360
24	16.0	5.0	287	4,592
30	33.0	6.0	279	9,207
36	50.0	6.8	271	13,550
42	66.0	7.5	264	17,424
48	76.0	8.0	257	19,532
54	85.0	8.5	250	21,250
			2,517	88,703

ENVIRONMENTAL CONSIDERATIONS

The oyster culture subsystem is required to provide physical and chemical conditions conducive to rapid growth. Prior experimentation has shown that the conditions listed in Table 2 should be maintained.

Table 2. Oyster Cultivation Environmental Specifications

Salinity	20 ppt
Temperature	22° to 26°C
pH	7.0 to 8.0
Dissolved Oxygen	4.5 to 7.0 ppm
Ca^{++}	$\geqslant$ 200 ppm
NH_3	$\leqslant$ 1 mg/L $\approx$60 µgat. N/L
Algal Feed Density	5×10^4 to 5×10^5 cells/mL

The American oyster is found in natural seawaters that range widely in salinity, from the brackish waters of rivers (~8 to 10 parts per thousand) to nearly oceanic salinities (~30 ppt) (Galtsoff 1964)[4]. A salinity of 20 ppt is specified since the local oyster grows well in this salt concentration and the seawater imported to the facility by tank truck can readily

be diluted with potable community water. Freshwater is less expensive than seawater, and the intermediate salinity discriminates against unwanted organisms that require higher or lower salt concentrations. This salinity helps to preserve the substantial buffering capacity of seawater in the desired pH range (7.0 to 8.0), and accordingly the alkalinity. The calcium ion (Ca^{++}) concentration does not fall below about 200 parts per million (ppm), a level adequate to sustain shell growth. Temperature is maintained at 22° to 26°C, a range known to permit rapid growth. Dissolved oxygen levels are held in the range 4.5 to 7.0 ppm, the lower limit being required to prevent anoxia and the upper being near the saturation concentration for oxygen in seawater at the temperature and salinity specified. The higher level is not deleterious to oyster growth. Dissolved ammonia (NH_3), a principal waste product of oyster metabolism, is held at 1 milligram per liter (mg/L), or about 60 microgram atoms of nitrogen per liter (~60 µgat N/L), or less, to prevent toxic effects.

Many feeding experiments (Epifanio 1979[5], Epifanio and Ewart 1977[6], Ewart and Epifanio 1981[7]) with the two algal species employed show that feeding densities in the range $5x10^4$ to $5x10^5$ cells/mL suffices to provide high feed levels without stimulating the production of unacceptable levels of pseudofeces by the oysters. It has been assumed that filtration rates of the oysters are adequate to supply their algal cell requirements. Support of this assumption is given by Widdows[8] (1979), Winter[9] (1973), and Walne[10] (1972) who, using a variety of bivalves, showed an inversely proportional relationship between pumping rate and particle density, so that the number of cells filtered during a given period of time is relatively constant. Epifanio and Ewart[6] (1977) showed that oysters in algal suspensions denser than 10 µg/mL (ca. $5x10^5$ cells (3H equivalent)/mL) increased pseudofeces production substantially. It would therefore appear prudent to provide algae at densities below this limit. In a heavily loaded oyster vessel, however, it is advantageous to maintain high algal densities to assure adequate cell distribution to all oysters, thereby reducing requirements for large flow rates. In light of these considerations, a density of $5x10^5$ cells/mL, which approaches the 10 µg/mL limit, is used as the operating concentration in the shellfish tanks. A continuous flow of algae is metered into oyster tanks at a rate approximately equal to the clearance rate of the oysters to maintain the concentration specified.

EQUIPMENT

In a recirculated-seawater system it is desirable to process the least volume of water while maintaining a high oyster biomass loading and meeting the essential physical and chemical environmental specifications, including the provision of food and the removal of wastes. Control of the environmental parameters must

be readily effected within the limits specified. The recirculating seawater as well as the oysters in a prototype system must be easily available for direct visual observation and analysis. These requirements are met by a baffled flume arrangement in which oysters are held in stacked trays. The configuration is shown in longitudinal sections in Figure 5. The oysters are monolayered in 2-foot-square Nestier® trays which are stacked two high. The 12-foot length of the flume can accommodate twelve trays, each stack separated by a baffle, thus creating six chambers. Arrangement of the baffles as shown in Figure 5 induces mixing in each chamber and prevents reverse flow to adjacent upstream chambers. Dye studies in such a flume have shown the flow pattern diagrammed and have provided assurance of adequate water movement for uniform water, algae, and oxygen distribution, and for metabolite removal. Algae are metered into the flumes continuously at three locations.

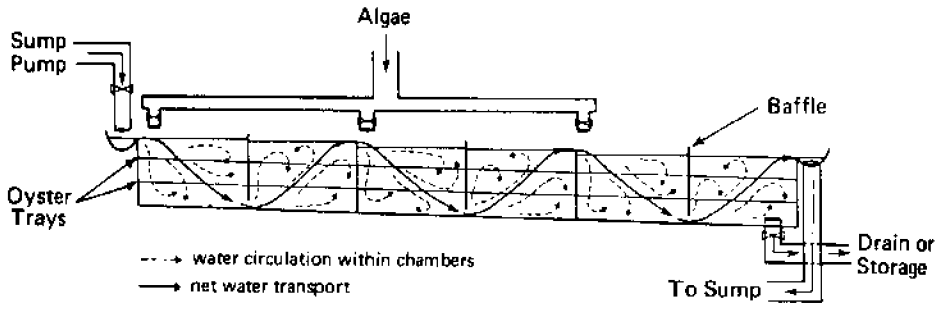


Figure 5. Baffled flume design for oyster cultivation. The flume contains six compartments holding two stacked trays. The compartments are separated by baffles arranged to provide a sinuous seawater flow. Algae are fed at three inlets, and recirculation and drainage is provided.

The amount of food and the retention time of the suspension within the chambers is adjusted by varying the input flow rates, thus providing the control necessary to maintain the specified food levels. The flumes are equipped with a drain and a means for recirculating the seawater via a sump, which contains a heating/cooling unit for temperature control as well as a means for aerating the water. The trays in each chamber of the flume are stocked with oysters according to the schedule in Table 3.

The biomass of the oysters causes a demand for dissolved oxygen which is given by the equation (Epifanio, Srna, and Pruder 1975)[11]:

$$\text{Log } Q_{O_2} = -0.344 \log W - 0.716$$

where Q_{O_2} = the oxygen consumed in mL/g whole oyster weight/hr, and

W = the whole weight of the oyster in grams.

Table 3. Oyster Stocking Schedule

Flume 1					Flume 2				
Chamber	Tray	Size (g)	No. of Oysters	Age	Chamber	Tray	Size (g)	No. of Oysters	Age
1	1	0.1	311	6	1	1	16	58	24
1	2	2.5	101		1	2	33	39	
2	3	2.5	101	12	2	3	33	40	
2	4	2.5	101		2	4	33	40	
3	5	8.0	73		3	5	33	40	30
3	6	8.0	74		3	6	33	40	
4	7	8.0	74	18	4	7	33	40	
4	8	8.0	74		4	8	33	40	
5	9	16	57		5	9	50	38	
5	10	16	57		5	10	50	38	
6	11	16	57	24	6	11	50	39	36
6	12	16	58		6	12	50	39	
		8.6	1138				37.2	491	

Flume 3					Flume 4				
Chamber	Tray	Size (g)	No. of Oysters	Age	Chamber	Tray	Size (g)	No. of Oysters	Age
1	1	50	39		1	1	76	37	
1	2	50	39	36	1	2	76	37	
2	3	50	39		2	3	76	37	48
2	4	66	37		2	4	76	37	
3	5	66	37		3	5	76	37	
3	6	66	38		3	6	85	36	
4	7	66	38	42	4	7	85	36	
4	8	66	38		4	8	85	36	
5	9	66	38		5	9	85	36	
5	10	66	38		5	10	85	36	54
6	11	76	36		6	11	85	35	
6	12	76	36	48	6	12	85	35	
		63.7	453				81.2	435	

Note: The horizontal lines indicate age-class division.

Since the oxygen content of seawater does not exceed 7 mg/L and the volume of seawater per unit biomass of oyster is relatively small, aeration is necessary to replenish the oxygen consumed by the oysters. This is accomplished by impinging the seawater from the flume into the sump volume by means of a bypass in the return flow to the head of the flume. The rate of this recirculating water flow in each flume is thus critical for supplying oxygen to the oysters. The calculated flow rates corresponding to the projected oyster loading are shown in Table 4. This flowing water also carries away the oysters' metabolic products, especially toxic NH_3. The amount of NH_3 generated is given by the equation (Epifanio and Srna 1957)[12]:

$$\text{Log } Q_{NH_3} = -0.344 \log W - 1.596$$

where Q_{NH_3} = the ammonia production in μM NH_3/g whole oyster weight/hour, and

W = the whole weight of the oyster biomass.

The retention time and circulating flow rate of the seawater in the flumes, as needed for oxygen supply, prevent NH_3 buildup beyond the level specified in Table 2.

Water and food demands of the oyster biomass also determine pump and plumbing sizes. Manifolds are used to distribute water and algae uniformly to the oyster trays in the flumes. Valves and flow meters achieve the necessary flexibility and control. Utilization of gravitational head pressure allows drainage of the flumes in the daily operation as well as permitting the oysters to be fed and the water changed in the event of a power or pump failure.

Table 4. Recirculating Water Flow Rate

Flume	Chamber	No. of Oysters	Average Whole Weight (g)	Oxygen Consumption (mg/hr)	Flow Rate (L/min)
1	1	412	1.3	134.5	0.56
	2	202	2.5	101.3	0.42
	3	147	8.0	158.1	0.66
	4	148	8.0	159.1	0.66
	5	114	16.0	193.1	0.80
	6	115	16.0	194.8	0.81
		1138	8.6	940.9	3.91
2	1	97	24.5	217.3	0.91
	2	80	33.0	217.9	0.91
	3	80	33.0	217.9	0.91
	4	80	33.0	217.9	0.91
	5	76	50.0	271.9	1.13
	6	78	50.0	279.1	1.16
		491	37.2	1422.0	5.93
3	1	78	50.0	279.1	1.16
	2	76	56.5	294.6	1.23
	3	75	66.0	321.9	1.34
	4	76	66.0	326.2	1.36
	5	76	66.0	326.2	1.36
	6	72	76.0	339.0	1.41
		453	63.4	1887.0	7.86
4	1	74	76.0	348.4	1.45
	2	74	76.0	348.4	1.45
	3	73	80.5	356.9	1.49
	4	72	85.0	364.9	1.52
	5	72	85.0	364.9	1.52
	6	70	85.0	354.7	1.48
		435	81.25	2138.2	8.91

Oxygen Consumption: Q_{O_2} = mL O_2/g whole weight/hour

$\text{Log } Q_{O_2} = -0.344 \log W - 0.716$

Algae Cultivation Subsystem

ALGAE

For *in situ* mass culturing of single-celled algae in sufficient quantity for the planned oyster cultivation, the algae must be capable of high practical photosynthetic efficiency (cf. Section 1) and be amenable to control to assure dependable production. Two species of single-celled marine algae, the diatom *Thalassiosira pseudonana* ("3H") and the flagellate *Isochrysis* aff. *galbana*, Tahitian strain ("T-ISO"), have proved (Epifanio, Srna, and Pruder 1975[11], Pruder, Bolton, and Epifanio 1977[13], Pruder 1978[14], Pruder and Bolton 1979[15,16]) to be readily mass cultured and serve in combination as a feed which allows oysters to grow through all stages of the life cycle. The diatom 3H is tolerant of a wide range of culture conditions, and the flagellate T-ISO, unlike a number of other highly nutritious flagellates, is tolerant of relatively high temperatures (22° to 30°C). These species can be reliably cultured at high densities to meet the feed production target necessary to support the oysters in the prototype. Furthermore, they can be cultured in a seawater medium that is compatible with the environment specified for *oyster* growth. As a result these algae do not need to be cultured remotely and separated from their culture medium before being fed to the oysters. Rather, the oysters themselves serve directly to harvest the algae from the culture medium, which in fact is the seawater environment in the prototype system.

To feed the oysters in the prototype adequately an amount of algae is required as given by an experimentally determined feeding equation (Pruder, Bolton, and Faunce 1977)[17]:

$$Y = 8.2\ (x)^{-0.21}\ (20° \text{ to } 26°C)$$

where x = whole fresh oyster weight including shell (g), and

Y = algal cells cleared $x10^8$/g whole fresh oyster/day.

This relationship permits the calculation of a daily production target for the algae. The required number of cells is proportioned between the two species in accord with their weight ratios. The feeding rate schedule is given in Table 5. Epifanio[5] (1979) has shown that an algal suspension comprised of these species in a 1:1 ratio on a dry-weight basis provides an excellent ration for oyster growth and development. Using an experimentally determined dry-weight ratio of 1.6:1 (T-ISO:3H, respectively) and the oyster stocking schedule (Table 3), a daily algae production requirement in terms of cell number can be calculated. Cell

counts are preferred because they can be rapidly made by visual examination of a sample with a microscope equipped with a counting chamber. The total requirement (Table 5) for four flumes is 3.2×10^{13} algal cells per day, or 2.0×10^{13} 3H cells per day and 1.2×10^{13} T-ISO cells per day. Two years of testing have shown that 3H and T-ISO cell densities of 5×10^{6} and 3×10^{6}, respectively, can be achieved consistently in mass culture for up to one week before algal reseeding is necessary. To satisfy the feed demand of the oysters, 4,000 liters of culture for each algal species must be supplied.

Table 5. Algal Feed Rate

		Loading		Projected Algae Requirements		
Flume	Chamber	No. of Oysters	Average Whole Weight (g)	No. of cells per day X 10^{12}	3H* (L/min)	T-ISO* (L/min)
1	1	412	1.3	0.42	0.07	0.11
	2	202	2.5	0.34	0.06	0.10
	3	147	8.0	0.62	0.10	0.17
	4	148	8.0	0.63	0.10	0.17
	5	114	16.0	0.84	0.14	0.23
	6	115	16.0	0.84	0.14	0.23
		1138	8.6	3.69	0.61	1.01
2	1	97	24.5	1.00	0.17	0.28
	2	80	33.0	1.04	0.17	0.29
	3	80	33.0	1.04	0.17	0.29
	4	80	33.0	1.04	0.17	0.29
	5	76	50.0	1.40	0.23	0.38
	6	78	50.0	1.41	0.23	0.39
		491	37.2	6.81	1.15	1.92
3	1	78	50.0	1.41	0.23	0.39
	2	76	56.5	1.51	0.25	0.42
	3	75	66.0	1.68	0.28	0.47
	4	76	66.0	1.70	0.28	0.47
	5	76	66.0	1.70	0.28	0.47
	6	72	76.0	1.81	0.30	0.50
		453	63.4	9.81	1.62	2.72
4	1	74	76.0	1.86	0.31	0.52
	2	74	76.0	1.86	0.31	0.52
	3	73	80.5	1.92	0.32	0.53
	4	72	85.0	1.97	0.33	0.55
	5	72	85.0	1.97	0.33	0.55
	6	70	85.0	1.92	0.32	0.53
		435	81.2	11.50	1.92	3.20

*Assuming 3H density: 5×10^{6} c/mL
T-ISO density: 3×10^{6} c/mL

ENVIRONMENTAL CONSIDERATIONS

Control of many environmental parameters is of the utmost importance for successful mass culture of single-celled marine algae. The culture system must be fashioned to respond to the seasonal and diurnal solar cycle since sunlight drives the algal photosynthetic growth process. The rate of algae production thus follows the sunlight pattern. These considerations determine not only the seawater environmental specifications but also the control, operating, and equipment specifications.

Seawater must be supplemented with mineral nutrients and some vitamins to culture the desired algae in the amounts needed for the projected oyster growth. A medium described by Guillard[18] (1975) can support copious algal growth. Importantly, all stages of the oyster life cycle tolerate the seawater-supplement medium well, as indicated by the usual bivalve cultivation practices in these laboratories. The nutrient supplement composition, which is suitable for both 3H and T-ISO in 20 ppt salinity, is given in Table 6 together with the upper and lower limits of nutrient concentration. The upper limit (100%) corresponds to the concentrations given by Guillard. The lower limit (20%) has been selected to assure that the algae do not encounter a nitrogen limiting condition.

Table 6. Nutrient Supplement Composition

Soluble Nutrients	Nutrient Concentration Limits 3H 100% → 20% (μm/L)		T-ISO 100% → 20% (μm/L)	
$NaNO_3$	882.4	176.0	882.4	176.0
NaH_2PO_4	36.2	7.0	36.2	7.0
H_3BO_3	80.9	16.0	80.9	16.0
$Na_2SiO_3 9H_2O$	105.6	21.0	Trace	
$FeCl \cdot 6H_2O$	11.7	2.34	11.7	2.34
$CuSO_4 \cdot 5H_2O$	0.04	0.008	0.04	0.008
$ZnSO_4 \cdot 7H_2O$	0.077	0.0154	0.077	0.0154
$CuCl_2 \cdot 6H_2O$	0.042	0.0084	0.042	0.0084
$MnCl_2 \cdot 6H_2O$	0.091	0.018	0.091	0.018
$NaMoO_4 \cdot 2H_2O$	0.025	0.005	0.025	0.005
Vitamin B_{12}	$3.7 \cdot 10^{-4}$	$0.7 \cdot 10^{-4}$	$3.7 \cdot 10^{-4}$	$0.7 \cdot 10^{-4}$
Biotin	--	--	$2.0 \cdot 10^{-3}$	$0.4 \cdot 10^{-3}$
Thiamine	--	--	$3.0 \cdot 10^{-1}$	$0.6 \cdot 10^{-1}$
EDTA	11.7	2.3	11.7	2.3

Specifications for other environmental parameters are given in Table 7. The algae cultures are illuminated by sunlight and actively fix carbon through photosynthesis. During this process they consume carbon dioxide and release oxygen. At night carbon is not fixed, but heterotrophic metabolism persists and oxygen is consumed. It is critically important for maximum net photosynthetic efficiency, and hence the rate and amount of algal feed production as well as for extended life of the cultures, to control the levels of these gases in solution. Pruder's work[15,16] (1979) has shown that departure from the specifications given in Table 7 is likely to cause unbalanced growth of the algae, a condition which may actually alter their chemical composition so that photosynthesis is repressed and premature death of the culture evoked ("culture crash"). For the algae production rate required under the other conditions specified, the dissolved carbon dioxide concentration must not fall below 16 μm/L nor the oxygen content depart from the range 7.0 to 10.0 ppm. These values apply throughout the seasons over the whole year. Oxygen levels in excess of 10 ppm are generally deleterious to algae irrespective of culture cell density. The concentration of carbon dioxide (16 μm/L) in the temperature range 20° to 28°C at pH 7.0 to 8.0 is the 20-ppt seawater equilibrium concentration with air. The alkalinity is kept within the limits 1.5 to 2.5 millequivalents per milliliter (meq/mL) to assure adequate buffer capacity and ionic composition of the seawater.

Table 7. Algae Cultivation Environmental Specifications

Parameter	3H	T-ISO
Salinity	20 ppt	20 ppt
Temperature	20° to 27°C	21° to 28°C
pH	7.0 to 8.0	7.0 to 8.0
Dissolved Oxygen*	7.0 to 10.0 ppm	8.0 to 10.0 ppm
Total Alkalinity	1.5 to 2.5 meq/L	1.5 to 2.5 meq/L
Illumination	Solar	Solar
CO_2	≥ 16 μm/L	≥ 16 μm/L

*Lower limit may be exceeded during nighttime respiration.

ALGAE CULTURE EQUIPMENT

Specifications for the algae production subsystem promote high-cell-density cultivation. In order to maximize the use of floor space, equipment, and processed recirculated seawater, it is desirable to maximize the photosynthetic efficiency (see Section 1) of the cultivated algae. This requires the optimal use of the incident solar radiation for high-density high-rate cultures. Culturing algae in this intensive fashion places strict constraints on reactor design alternatives. High-yield cultures must be provided with CO_2 above the levels ordinary air can supply, and O_2, which is generated at a high rate, must be extracted. These requirements necessitate efficient gas mass transfer and hydraulic mixing.

A specialized reactor device has been designed and constructed to culture the marine algae. It is illustrated in Figure 6. The design of the reactor takes into account the need for adequate solar illumination, the culture attributes of the algae, and biochemical engineering principles.

To take advantage of the solar radiation while providing for adequate gas exchange of carbon dioxide and oxygen and assuring mixing so that nutrients are distributed to the growing algae, a transparent fiberglass (Kalwall® Sunlight Premium) vertical tube configuration is employed. The tube is 85-percent transparent in the photosynthetically active region (400 to 700 nanometers), mechanically robust, tolerant of the chemical processing and environmental conditions encountered, and may be fashioned into cylinders of the required size.

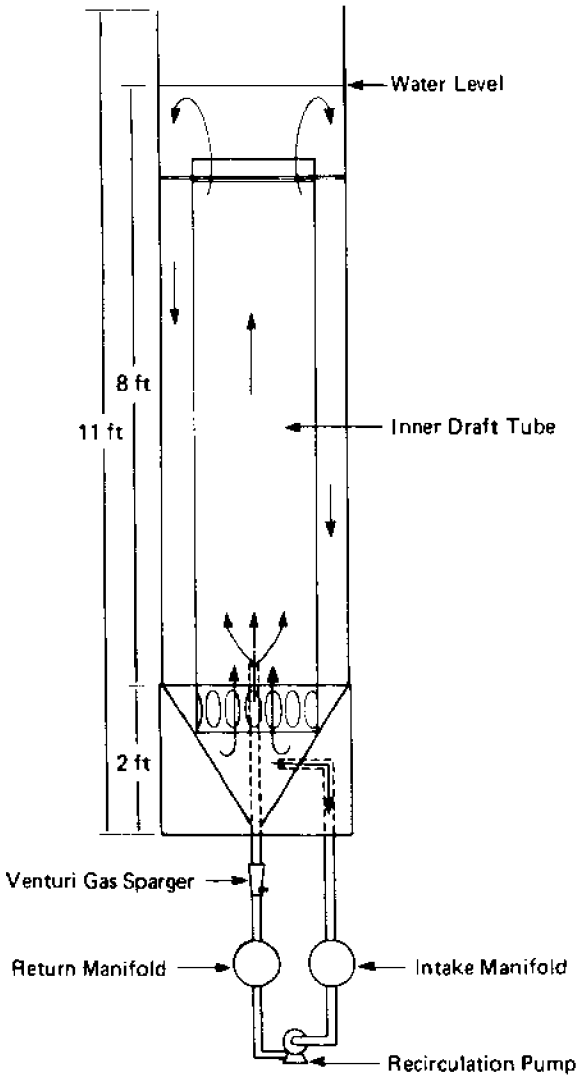


Figure 6. Vertical, fiberglass algae culture reactor. The transparent outer tube is supported on a conical base, and an inner concentric draft tube is provided for mixing. Gas introduction occurs through venturis incorporated in the recirculating lines.

The diameter of the tube is 76 centimeters. This diameter was determined by relating the light flux incident on the surface of the tube during a typical summer day to the requirement for producing a desired increment of algal cells, e.g. $2.5 \times 10^6 \rightarrow 10 \times 10^6$ cells/mL in the seawater culture medium. A polynomial model for tubular reactors (see Appendix A) was used to test the effect of various inputs on cell production. For numerical integration these inputs included the diurnal insolation as a sinusoidal function over a 12-hour day, a time increment, a test reactor radius and radius increment, an initial algal cell concentration, and carbon content per cell. For the solar light flux at Lewes, Delaware and an algal cell increment between 2.5×10^6 and 10×10^6 cells/mL, a radius of 38 centimeters was found to be optimal. The algae production indicated was achieved in an experimental test of the model using a 76-centimeter tube. Consistent, long-term algae growth in a semicontinuous culture regime at the high final cell density of 10×10^6 cells could not be sustained. Consequently, the specification for algal cell density was lowered to 5×10^6 cells/mL (see above) to increase culture life.

Photosynthetically generated oxygen dissolved in the culture medium has been shown to inhibit the rate of carbon fixation in 3H cells and limit the lifetime of rapidly dividing cultures (Pruder and Bolton 1979)[15,16]. In order to purge oxygen from the medium, nitrogen gas can be injected through the venturis. Concentration gradient driven dissolved oxygen is transferred from the medium into the bubbles. It has been found that in high-density cultures of 3H (approaching 1×10^7 cells/mL) oxygen production proceeds at such a rate that even N_2 sparging is technically difficult and certainly economically impracticable. It became apparent that even at lower algal densities extraction of oxygen is of critical consideration for high-performance algal reactors.

In the design of cylindrical reactors for the commercial production of yeast, the mass transfer of O_2 into the medium is a demanding process. As a result, several engineering studies investigating the optimization of this process have been conducted (Tannenbaum and Wang 1975)[19]. Since the basic physical and engineering principles involving dissolved oxygen manipulation are the same for algae and yeast production, results of these studies have led to the incorporation of a concentric draft tube within the reactor. Using a mathematical model (Hatch 1973)[20] a surface-area ratio of 0.8 (outer tube : inner draft tube) was selected to allow the highest performance for oxygen transfer in tubular reactors. This configuration encourages a countercurrent hydraulic flow pattern and utilizes a single-pass gas reaction chamber which releases oxygen to the atmosphere (see Figure 6).

A conical base is fitted to the reactor vessel. This shape allows support of the inner draft tube and provides a convenient means of sparging the vessel contents with gases while inducing a countercurrent flow pattern so that no dead volumes exist. A number of individual reactor tubes can be ganged together by means of a manifold so that the algae culture can be handled as a unit, or portions of it isolated. This arrangement lends itself to modular construction of culture vessel arrays with ready access to each tube of a unit for filling, seeding and harvesting, addition of nutrients, cleaning, plumbing, and equipping with control devices. A manifolded arrangement is shown in Figure 7. A single pump mixes the medium within and among the tubes, and drives venturis through which gas or air is injected. In this way the contents of the five culture tubes can be treated as one culture, and a single continuous sampling-monitor/control system can be employed. Sensors monitor the culture for pH and O_2 content and activate solenoid valves which allow injection of the appropriate gases to maintain specifications. A tank of liquid carbon dioxide supplies gaseous CO_2.

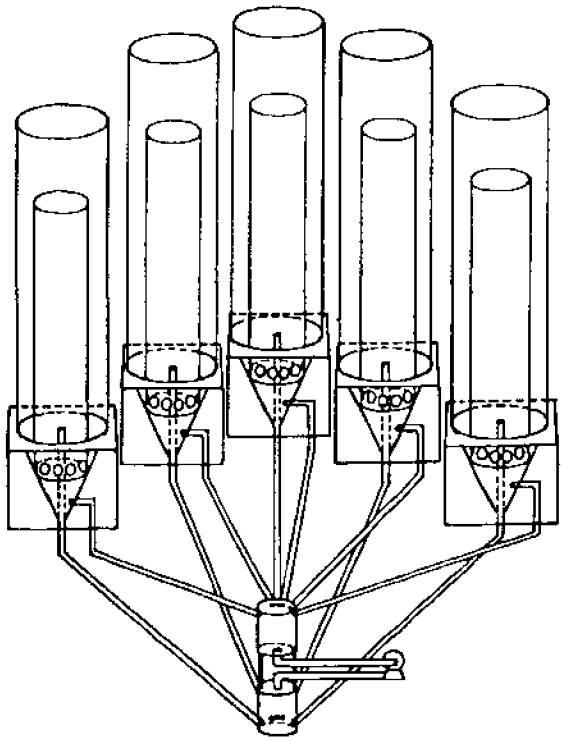


Figure 7. Manifolded arrangement of five algae reactors. The individual reactors can be isolated or manifolded in combinations.

Water and Waste Handling Subsystem

SEAWATER

Seawater trucked to the Smith Laboratory is diluted with tap water to a salinity of 20 ppt, a level compatible with both oyster and algal osmotic requirements. Dilution also conserves seawater. The seawater entering the prototype system meets the environmental specifications listed in Table 8, after being passed through sand and diatomaceous-earth filters and heat exchangers for temperature adjustment. It contains micronutrients, a balanced ionic composition including adequate calcium levels and the carbonate buffer system, and serves as the transport medium for feed and wastes.

Table 8. Water and Waste Handling Environmental Specifications

Salinity	20 ppt ± 2
Temperature	22° to 26°C
pH	7.0 to 8.0 (2.5 to 4.0 for disinfection)
Dissolved Oxygen	6.5 to 7.0 ppm
Total Alkalinity	1.5 to 2.5 meq/L

In the prototype system the water is reconditioned following its use by the algae and oysters to maintain the specifications in Table 7, and any water lost in the process is replenished.

EQUIPMENT

The equipment employed for water circulation and waste removal comprises fiberglass tanks, pumps, plumbing, sand and diatomaceous-earth filters, and a foam fractionating device. These are illustrated in the prototype schematic diagram (Figure 3). Insofar as possible contact between seawater and metallic elements is avoided. Fiberglass 2,000-liter gel-coated tanks, plastic pump housings and impellers, polyvinyl coated (PVC) pipes and plumbing fixtures, plastic filter housings and screens, and a plexiglass foam fractionator are compatible with the organisms and the processing procedures. The equipment is easily cleaned to assure sanitary conditions and prevent the accumulation of unwanted chemicals.

WATER PROCESSING

In order to meet the requirements of the oyster and algae subsystems and work within the limits specified for treating the water (Table 8) an operating procedure was developed to process the seawater in the system. Seawater (20 ppt) is disinfected by addition of 0.15 to 0.30 mL of 10 N HCl to storage tank II (Figure 2). A pH of 3.5 to 4.0 is obtained, and the water is held overnight before being neutralized. Alternatively a pH of 2.5 to 3.0 for time periods up to 2 hours may be used. The water is neutralized with a 50-percent NaOH solution to pH 7.0 to 7.8 and enough $NaHCO_3$ (0.12 g/L) is added to reinstate the carbonate buffer system to the level specified. During the disinfection step the water is circulated through a foam fractionator. This device uses a venturi to create a foam comprised of highly surface active dissolved organic materials and chelated cations. Collection and disposal of the foam prevents accumulation of these materials in the recirculating system. Before neutralization the acidified water is passed through a sand filter and a diatomaceous-earth filter plumbed in series to remove particles $\geq$5 μm. Specifications are given in Table 9.

Table 9. Water Processing Specifications

Sand Filter Pressure	7 to 12 psi
Diatomaceous Earth Filter Media	0.1 lb/sq ft = 2.5 lb (25-sq-ft unit)
Diatomaceous Earth Filter Pressure	7 to 12 psi
Disinfection	10 N HCl pH 3.5 to 4.0 $\geq$ 12 hr pH 2.5 to 3.5 $\geq$ 2 hr
Neutralization	50% NaOH to pH 7.0 to 7.8
Carbonation	$NaHCO_3$ 0.12 g/L

Operational Design Criteria for the Integrated Prototype System

A comprehensive system management program was developed to assure efficient operation and to obtain quantitative data and information that could be used to quantify material flow, to determine algae and oyster productivity, and to provide a basis for estimating costs. Since subsystem incompatibility may be subtle and may remain obscure for an extended length of time, it is imperative to adhere to the specified standard operating procedures to provide a rigid test of the recirculated-seawater prototype concept. Measurements of water chemistry parameters, oyster growth and condition, and algal quality and productivity are used to evaluate performance of the system, identify system problems, and construct guidelines for improved efficiency. The major physical, chemical, and biological specifications are summarized in Table 10. The plan of work for two 10-hour feeding cycles in the daily routine is outlined in Table 11 and Figure 8.

The subsystems are physically connected through the seawater environment as illustrated in Figures 3 and 8 to form the prototype system proper. Various specifications have been listed above which pertain particularly to the subsystems. It is paramount that the specifications and functioning of the individual subsystems be in harmony with one another because they interact intimately in the integrated activity. For example, the seawater in the system is in fact a continuum physically connecting the subsystems, and it must not vary in a detrimental way from one part of the prototype to another or a loss in algae- or oyster-growth efficiency will result. The prototype system recirculates seawater from the algae subsystem through the oyster subsystem and thence through the waste treatment subsystem before being returned to the algae culture tubes.

The operational scheme is to recirculate the seawater in batch sequence rather than continuously in order to avoid cross-contaminating the diatom (3H) culture with the flagellate (T-ISO) culture. To assure this culture isolation two feeding periods are employed, one for each algal species. T-ISO divides more slowly than 3H while growing during the daytime, and hence relatively less control is needed to maintain its feed rate to the oysters. Consequently, during the day the T-ISO culture is metered into the oyster flumes at the specified rate while at the same time an equal volume of the algae-depleted seawater from the sump at the oyster flume is supplied back to the growing T-ISO culture to maintain the flagellate culture volume.

Table 10. Specification Summary for Oyster Cultivation, Algae Cultivation, and Water and Waste Handling Subsystems

Oyster Cultivation	
Salinity	20 ppt ± 2
Temperature	20° to 26°C
Calcium	> 200 ppm
Dissolved Oxygen	4.5 to 7 ppm
Ammonia	< 1 ppm
pH	7.0 to 8.0
Algal Ration	1:1 mixture by dry weight of *Thalassiosira pseudonana* and *Isochrysis* aff. *Galbana*
Algal Cell Density	5×10^4 to 5×10^5 cells/mL
Algal Cell Quantity	According to the feed equation $Y = 8.2 \times 10^{-0.21}$ where Y = the number of algal cells $\times 10^8$ required per gram of oyster live weight per day and X = the mean individual oyster live weight in grams
Feeding Regimen	Combined continuous (night) and pulse feeding (day)
Oyster Growth Rate	84.9 grams mean individual oyster live weight in 54 weeks
Oyster Production Capacity	10 bushels per year
Algae Cultivation	
Salinity	20 ppt ± 2
Temperature	20° to 27°C
Dissolved Oxygen	7 to 10 ppm *(Thalassiosira pseudonana)* 8 to 10 ppm *(Isochrysis* aff. *galbana)*
Total Alkalinity	1.5 to 2.5 meq/L
pH	7.0 to 8.0
Illumination Source	Sunlight
Purging Gas	Carbon dioxide and nitrogen
Culture Density	2×10^6 to 5×10^6 cells/mL
Nutrient Enrichment	Guillard's f/2
Water and Waste Handling	
Salinity	20 ppt ± 2
Temperature	20° to 26°C
Dissolved Oxygen	6.5 to 7.0 ppm
Total Alkalinity	1.5 to 2.5 meq/L
pH	7.0 to 8.0
Sand Filter	Medium sand, 7 to 12 psi
Diatomaceous Earth Filter	25 sq ft, 7 to 12 psi
Water Source	Indian River Inlet, mixed 2:1 with freshwater to yield 20 ppt salinity
Disinfection	10 N HCl to pH 3.5 to 4.0 for 12 hours or pH 2.5 for 2.5 to 3.5 hours
Neutralization	50% NaOH to pH 7.0
Carbonation	$NaHCO_3$ 12 g/L

At the sump a recirculation pump moves water back into the oyster tanks providing flow velocities necessary for algae and oxygen dispersion. A liquid-level limit-switch activates a pump to transfer water from the sump back to the T-ISO culture tubes to maintain the initial volume. Thus, most of the water passing through the four parallel oyster flumes is recycled internally, while a volume equal to that metered into the flumes as algal culture is returned to the tubes. Metabolism and respiration of

Table 11. Daily Work and Data Collection Routine

Time	Task
0600	Feed OFF (3H). Make cell counts of reactors and flumes (Flume Chamber 6). Measure dissolved oxygen in flumes (Chamber 6) and sump. Fill filters with freshwater and precoat diatomaceous earth. Take water quality samples (when indicated).
0700	Drain and flush flumes and lines. Transfer water from Storage Tank I to Storage Tank II. Backflush filters. Adjust water in Storage Tank II (pH, salinity, $NaHCO_3$). *Seed T-ISO reactor; drain 3H reactor.
0800	Refill flumes. Record recirculating water flow rates to flumes. Record algal feed rates. Record seed tube data and make adjustments. Add nutrients for 3H reactor.
0900	Feed ON (T-ISO). Take water quality samples (when indicated).
1000	
1100	Take seed tube data and make adjustments. Seed algae seed tubes.
1200N	
1300	
1400	Record cell counts and dissolved oxygen in each chamber of flumes. Record and adjust feed rates at each input location.
1500	*Seed 3H reactor.
1600	Record seed tube data and make adjustments. *Drain T-ISO reactor.
1700	Fill Storage Tank II – 20 ppt, and acidify – pH 3.5.
1800	Feed OFF (T-ISO). Make cell counts of reactors and flumes (Chamber 6). Record dissolved oxygen in flumes (Chamber 6).
1900	Drain and flush flumes and lines.
2000	Adjust pH in Storage Tank II and refill flumes. Adjust and record flow rates and algal feed rates.
2100	Feed ON (3H). Complete daily data sheets.

*Only on days when cultures are reseeded.

OPERATIONS SEQUENCE BETWEEN FEEDING CYCLES

Hour	Operation	Description
0	Valves 1 or 1' -- CLOSE	Stop algae introduction into oyster flumes. Prevent pump A from activating, as no additional increase in sump volume occurs.
1.0	Pump B -- OFF Valves 2, 2', 6 -- OPEN	Stop water flow through flumes. Drain effluent from flumes and sump into storage tank I.
1.5	Valves 3, 4 -- OPEN Pump C -- ON Valve 2, 2' -- CLOSE Valve 5 -- OPEN	Begin foam fractionator Flush solids to drying bed.
2.0	Valve 5, 6 -- CLOSE Valve 7 -- OPEN Valve 2, 2' -- OPEN	Flush lines with fresh water to drain.
2.5	Valve 7 -- CLOSE Valves 2, 2' -- CLOSE Valve 8 -- OPEN Pump D -- ON	Fill flumes and sump with water from storage tank II.
3.0	Pump D OFF Valve 8 -- CLOSE Valve 1 or 1' -- OPEN Valve 9 or 9' -- OPEN Pump B -- ON	Begin feeding cycle.
3.5	Valve 10 -- OPEN Valve 4 -- CLOSE	Filter water passing from storage tank I to storage tank II.
4.0		Disinfection procedure of water in storage tank II.
12.0	Repeat the above cycle.	

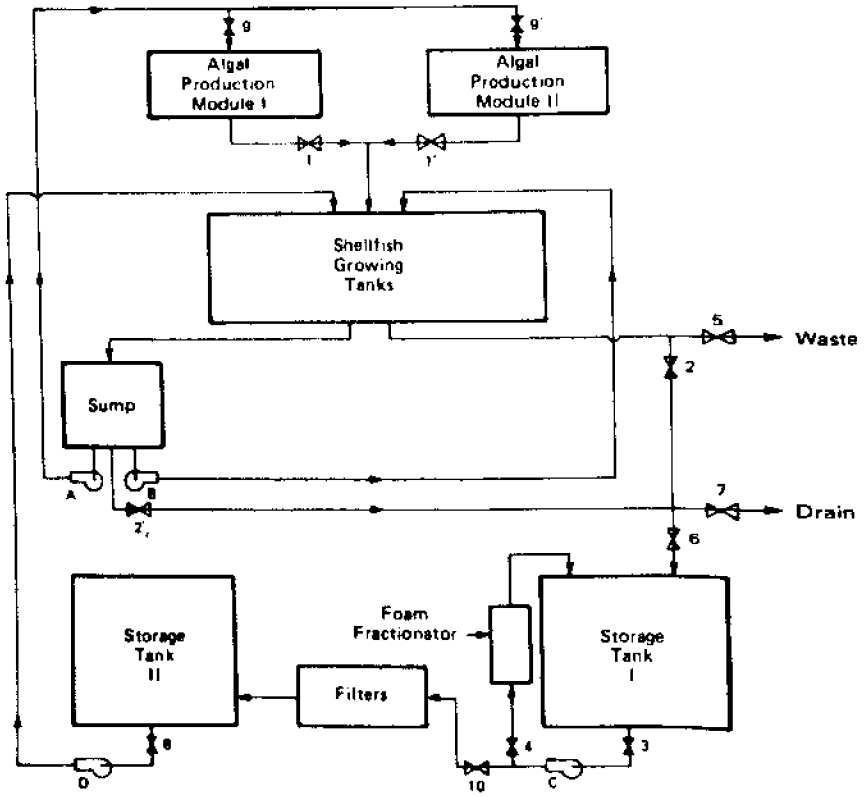


Figure 8. Operations sequence for water processing between feeding cycles. A through D are pumps; 1 through 10 are valves.

the oysters increase NH_3 in solution and reduce levels of dissolved oxygen in the water returning to the algae culture tubes, thus serving to enhance phytoplankton productivity.

At the end of the T-ISO feeding period (cf. Table 11, Figure 8) metering of the culture is halted, and the oysters continue to deplete the residual T-ISO cells in the flumes over the course of one hour. The water is drained from the flumes and held for treatment and reuse. The feces which remain in the bottom of the flumes after draining are flushed from the system. The oysters, flumes, and associated plumbing are then rinsed with freshwater. Disinfected seawater at the specified temperature is next pumped to the flumes, and the 3H culture, which has reached its specified density during daytime growth, is metered to the shellfish for the nighttime feeding period. Water and 3H-algae-culture circulation is accomplished as described for the T-ISO daytime feeding sequence. Seawater that has been collected from the flumes is processed independently for reuse in the subsequent feeding period in the manner described above. Any water that is newly added to the system (makeup water) is also filtered and disinfected before being used. Table 12 shows the water volumes and losses that are expected as a result of the operational sequence. The average daily amount of water recycled is 77 percent of the total prototype system volume.

Table 12. System Water Volumes and Losses

Volumes (liters)

Subsystem	Description	Volume
Algal Production	10 to 1200 L Tubes	12,000
	Manifold	200
Shellfish Life-Support	4 to 400 L Flumes	1,600
	Sump	400
Water and Waste Handling	Storage	2,000
Total:		16,200

Losses (liters)

Description	Daily	Weekly
Filtration	140	980
Evaporation, Cleaning, and Flushing Operations	200	1,400
Discharge from Flumes	1,600	11,200
Replenish Algae Tube Cultures	--	12,000
Total:	1,940	25,580

Daily Percent Recycle = 100 - [(25,580 l/wk ÷ 7 days/wk ÷ 16,200 L/day) X 100] = 77.4%

Prototype Operation

SHAKEDOWN PHASE

Prototype construction was completed in the greenhouse of the Smith Laboratory in February 1980 in accord with the plan outlined above. The system was charged with seawater, and the pumps, plumbing, and control systems were operated. Six thousand oysters (mean live weight ~6 grams) provided an initial biomass loading. They comprised five age classes ranging from 1 to 26 months in age. The average weight of the oysters in a class ranged from about 1 gram to 25 grams. These oysters had been subjected to various laboratory environmental regimes and were held under minimal maintenance conditions for several months during the transition to the new Smith Laboratory. From March through July 1980 personnel were trained in the new procedures, planned operating sequences were adjusted and standardized, juvenile oysters were introduced from the new hatchery, algal seed requirements for the anticipated mass production schedule were met, and the integrated system operated for troubleshooting to optimize system management.

SYSTEM OPERATION

From 29 July 1980 to 2 September 1981 the prototype was operated continuously in general accord with the plan and experience outlined in the design section above. A number of modifications were found to be necessary during the course of this effort. On 23 October 1980 the personnel working shift was changed to 8 hours from 16 hours and the feeding-cycle regime changed to 15.5 hours for 3H, and 4.5 hours for T-ISO. Flow rates were adjusted to maintain the oyster ration. By 19 November 1980 the solar intensity was declining, and the oysters were increasing in biomass and were feeding vigorously. Therefore all 5 tubes of the algae reactors were operated, only 4 tubes for T-ISO and 3 for 3H being previously required. On 12 December 1980, to avoid sporadic seed production, the 250-liter algae seed tubes in the greenhouse were removed to the algae seed production room in the Smith Laboratory to provide better control of pH, temperature, and light. By 13 January 1981 the data collected showed that neither algae production nor oyster growth was satisfactory. The oyster biomass was reduced to match the algae production capability, and another foam fractionator was added to the oyster flumes and continuously run as a precaution to help assure water quality. With the drop in requirement for 3H due to the oyster culling, excess 3H culture

was diverted to a holding tank whence it could be used to supplement the next day's supply if required. A second tank containing disinfected seawater was added for algae culture dilution to increase the flexibility of control of the algal density in the reactors. On 2 March 1981, the 1,000-liter algae seed tubes in the greenhouse were producing heavily under the springtime solar regime. To control the seed production and protect the seed from undergoing unbalanced growth due to increased light and temperature, neutral density screens were used to shade the cultures. The prototype was operated with no further change until the full operation was terminated on 2 September 1981. Figure 9 shows the oyster biomass in the prototype system and indicates the time of new oyster introduction (↑) or oyster removal (↓) for the several age classes, A, B, ... P.

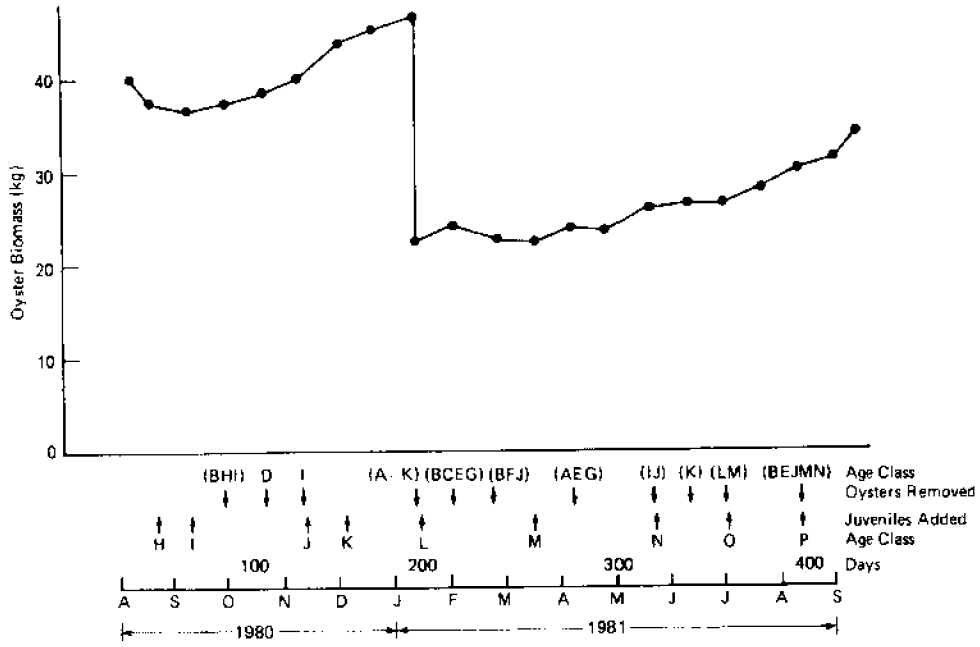


Figure 9. System oyster biomass loading. The ordinate is the total fresh weight of oysters in the prototype. The abscissa is month and year. B through P are oyster age classes. The arrows indicate oyster introduction (upward) and oyster removal (downward). Major culling took place on January 14th 1981. *See Table 13 in Appendix B.*

DATA COLLECTION

During the operating period data were collected daily as indicated by the Daily Work and Data Collection Routine schedule. At 3-week intervals all oysters were removed from the flumes, rinsed, drained, blotted, and weighed. The determinations were used to adjust algal feed rates and water flow rates. At twice-weekly intervals chemical analyses were conducted on samples from several key stations to monitor the seawater condition. Samples were also taken for bacterial assays. At the completion of the full operation oysters were measured and sacrificed for proximate analysis. Seawater and nutrient consumption were logged, and a continuous record of solar insolation was obtained for the entire operation period.

Findings

The principal data compiled over the operating period of the prototype are summarized below.

OYSTER GROWTH

The total system oyster biomass loading is shown in Figure 9, which has been constructed from the entries in Table 13. The oyster load on the system derives from a combination of the introduction of oysters, culling, and oyster growth. Figure 9 also indicates the age class, the time at which juveniles were added to the system, and the time at which oysters were removed.

Note: Table 13, Prototype Oyster Biomass Loading, and Table 14, Oyster Weight Data, are included in Appendix B.

Oysters in each age class were arrayed in trays in the prototype, and each tray of each class was weighed at 3-week intervals. The number of oysters in each tray was counted, and the average live weight of the oysters in each tray was calculated. Table 14 summarizes the weight data for each tray of every age class. Figure 10 shows, as an example, the growth of the oyster biomass in age class E and in trays E_1 and E_2 together with the reduction

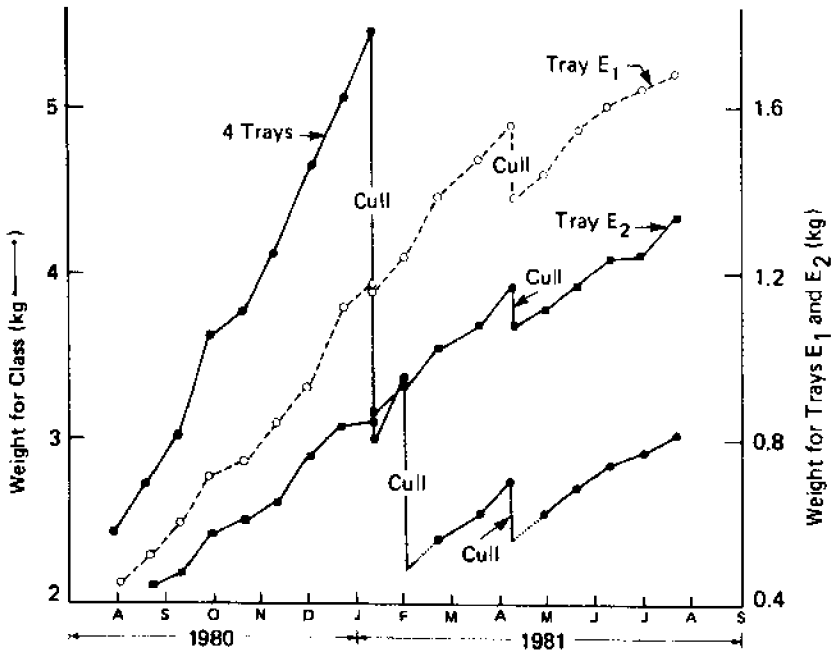


Figure 10. Growth of oysters in the four trays of age class E (•—•) and in trays E_1 (o---o) and E_2 (x---x). The reductions in the total weight of age class E were due to culling in January, February, and April of 1981. The linear course of weight increase is evident.

in biomass as a result of culling. Culling, as determined by visual inspection, generally removed the smaller oysters from a tray. From Figure 10 it is clearly evident that the oyster biomass increased linearly in the class and in the individual trays of the class, and further, that the linear increase in oyster biomass persisted at approximately the same rate after a culling as before. Analysis of variance of the growth data for age classes B through N (Table 14) confirmed the obvious features of the oyster growth patterns for the age classes and for the trays of a class. Further, there is no statistical evidence that the average oyster growth improved or deteriorated with continued cultivation in the prototype.

The linear increase in the production of oysters was observed for all age classes, and for each tray of an age class. It extends also to the 10-best oysters in a tray. The 10-best oysters were judged by visual examination to be the 10 largest individuals in a tray on 4 February 1981 for classes that had previously been introduced to the prototype. The shells of these oysters were indelibly marked so that the group of 10 could be followed and weighed separately from their tray-mates during the subsequent operation of the prototype. Figures 11 and 12 show the increase in mean weight of the oysters in age class E, tray

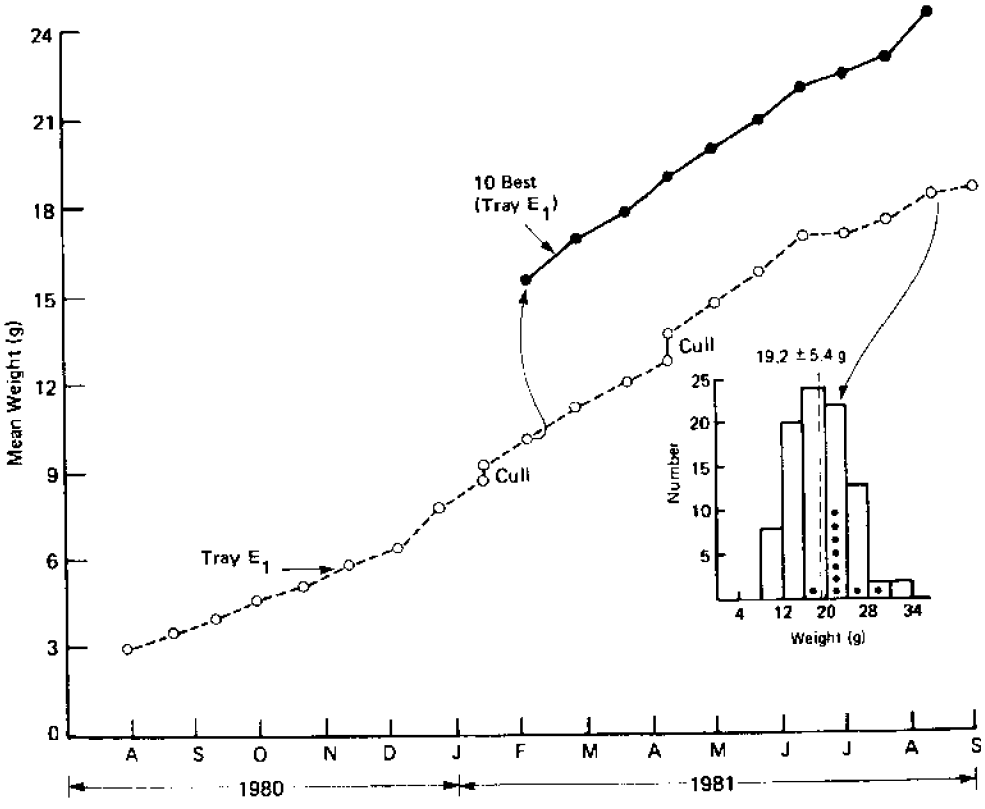


Figure 11. The mean individual weight gain for oysters in tray E_1 of age class E and for the 10-best oysters in tray E_1. At the conclusion of the prototype operation in September 1981, the oysters were individually weighed, and the weight-frequency distribution was determined. The oysters in tray E_1 averaged 19.2 + 5.4 grams (mean deviation). The solid dots on the frequency distribution indicate the location of the 10-best oysters within a size class.

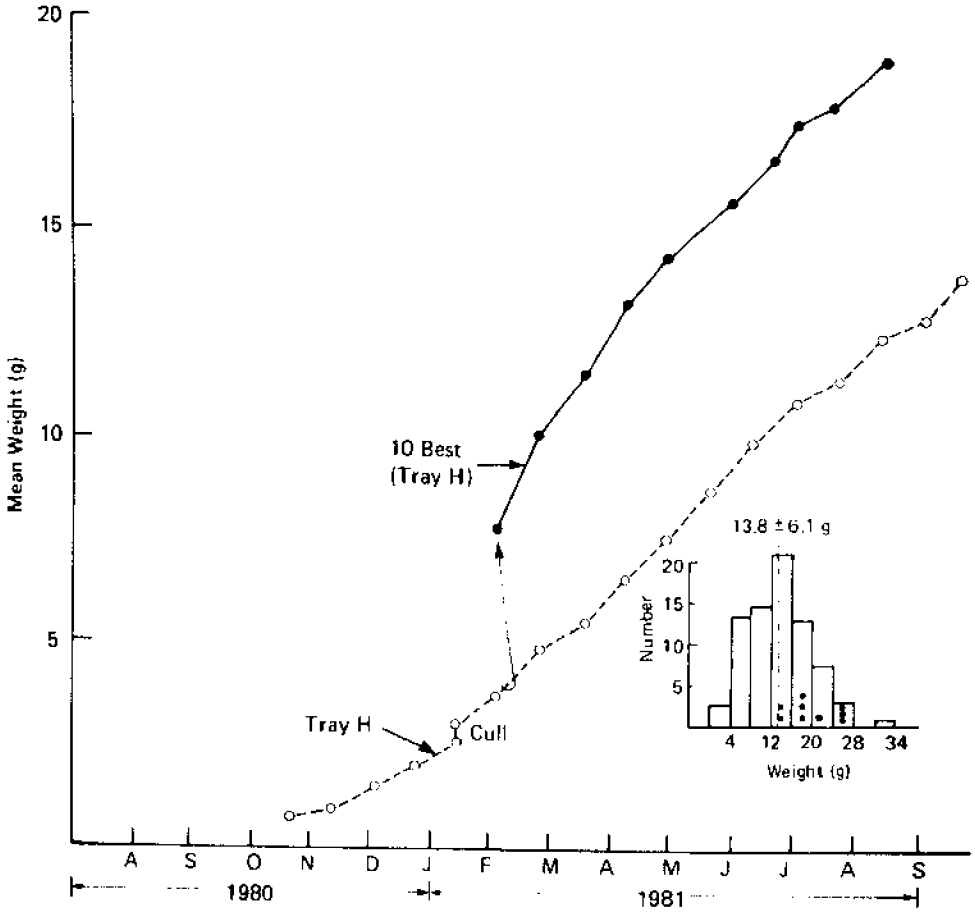


Figure 12. The mean individual weight gain for all oysters and the 10-best oysters in age class H. At the conclusion of growth in the prototype the oysters were individually weighed and a size-frequency distribution constructed. These oysters weighed on the average 13.8 ± 6.1 grams (mean deviation). The weight-class locations of the nine surviving oysters of the 10-best are shown as dots in the bar chart.

E_1, and age class H (where all oysters of the class were held in a single tray), respectively. Culling, of course, increased the mean weight of oysters in the tray and the groups of 10 best obviously weighed more on the average than their tray-mates. Nevertheless, the linear growth response persists.

The data of Table 14 have been analyzed as linear patterns of growth for the trays of the age classes, and the slopes of the lines have been calculated. The average lines of increase in mean weight for oysters in trays which were not culled, for culled trays, and for the group of 10 best are shown in Figure 13, Growth Observed. The bars to the right of these growth responses show the extremes of the spread in the linear growth patterns for each of the three categories. The specified curvilinear growth response is also shown in Figure 13. It is clear that the oysters in the prototype grew on the average to a size only a fraction (about one-sixth) of that specified during a year.

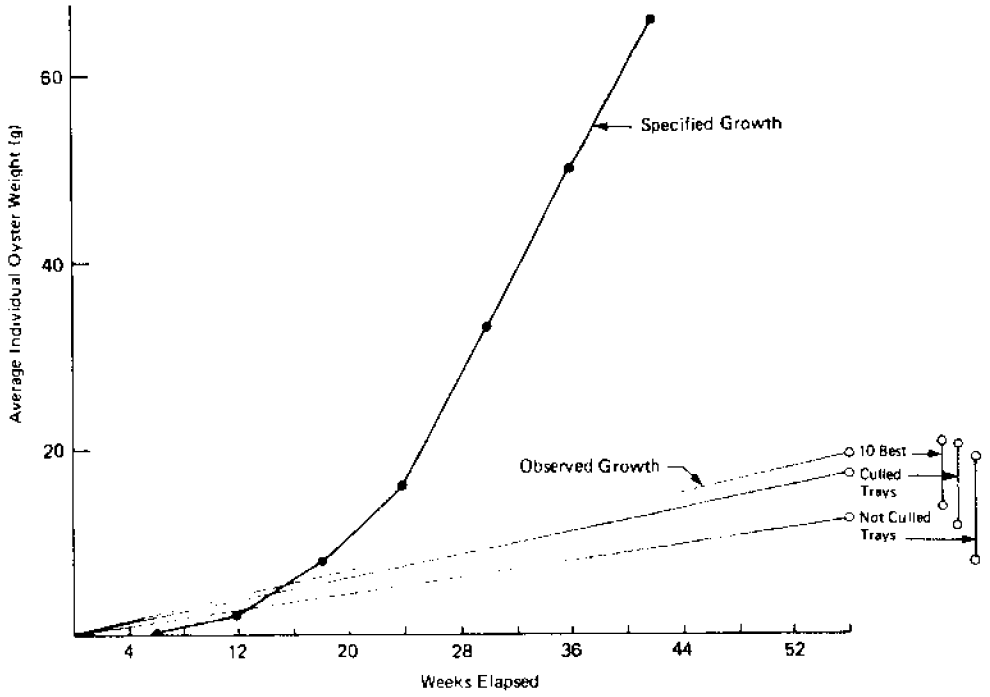


Figure 13. The average oyster growth of all oysters in the prototype. The growth specified for the prototype design is shown in the upper curve (Specified Growth). The growth observed is shown for oysters grouped by culling (culled, not culled) or marking (10 best). The vertical bars to the right indicate the extremes of the average growth of oysters in the threee groups.

At the termination of the prototype operation in September 1981 all oysters (more than 3,300) were individually weighed. The resulting data permitted the construction of a population distribution graph for the oysters in any tray of an age class. Population distributions for tray E_1 of age-class E and for age-class H are shown in Figures 11 and 12, respectively. The population distributions are quite broad. In the example of the tray E_1 group, the individual weights ranged from >8 grams to < 34 grams averaging $\pm$ 5.4 grams (mean deviation), and individuals in age-class H ranged from from >4 grams to < 34 grams, averaging 13.8 grams $\pm$ 6.1 grams. The dots on the bar graphs show the weight class in September 1981 of the oysters judged and marked in February 1981 as being the 10 best. It is evident that individual oyster growth response varies widely even though they were held in close proximity to one another and presumably were exposed to the same environmental and manipulative (washing, drying, weighing) procedures throughout their sojourn in the prototype system.

The efficiency of conversion of algae to oyster meat was determined by relating the overall total of the oyster ash-free meat weight produced to the dry weight of algae filtered (cleared) by the oysters. On this dry-weight basis (oyster dry meat produced ÷ dry weight of algae cleared x 100) the conversion averaged 2.7 percent for the period 29 July 1980 to 13 January 1981 and 3.0 percent for the period 14 January 1980 to 12 August 1981.

COMPOSITION OF OYSTERS AT TERMINATION OF PROTOTYPE OPERATION

At the termination of the prototype operation in September 1981 the oysters in each tray of an age class were arranged in an array of decreasing size as judged by visual inspection. Every third oyster of the array (every sixth one for age classes 0 and P) was individually marked with a code designation and submitted to analysis according to the scheme outline in Figure 14.

Table 15 summarizes size and weight measurements of 980 individual oysters according to means for an age class or class tray. Table 16 expresses the mean values as percentages. The average weight composition for the population as a whole was:

- Shell, 69.9% (68.1% ash, 1.8% organic)
- Meat (free of mantle fluid), 11.6%
- Mantle fluid (liquor), 18.5% (by difference)

The weight of the dry meat compared to the whole fresh weight of the oysters was 2.5 percent; the meat dry weight was composed of 13.0 percent ash. The fresh meat contained 79.6 percent water.

Duplicate lyophilized samples of pooled meats were submitted to a modified proximate analysis (see Appendix C) to determine the approximate proportions of lipid, protein, and glycogen. The results are presented in Table 17. A set of similar analyses (Hicks, 1981)[20] is shown for oysters harvested from the Broadkill River in 1979 and 1980, and for oysters cultivated in a laborator greenhouse while being fed algal rations.

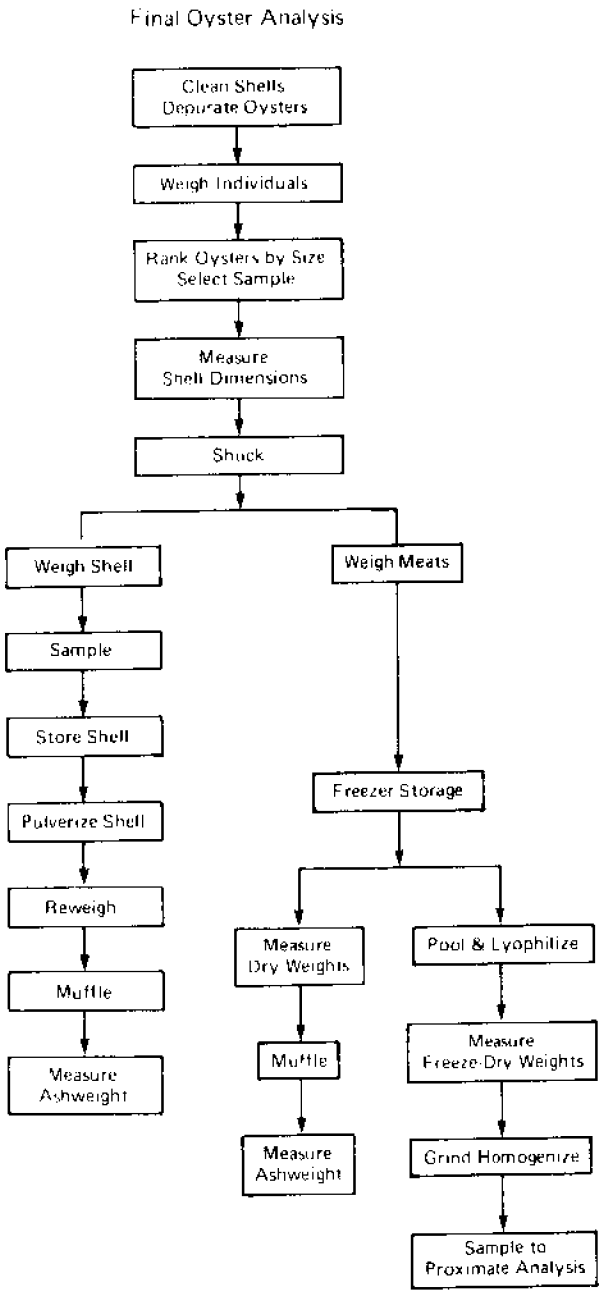


Figure 14. Process diagram for final oyster analysis. The process yields the whole fresh weight, the shell weight and the shell ash and organic content (by difference), the fresh-meat weight and the meat ash and organic content (by difference), and pooled samples for proximate analysis.

Table 15. Final Oyster Weight Measurements

	N*	Shell Diameter** $\bar{x}\ \sigma$ (c)†	Shell Weight‡ $\bar{x}\ \sigma$ (g)	Whole Weight $\bar{x}\ \sigma$ (g)	Meat Weight $\bar{x}\ \sigma$ (g)	Dry Meat $\bar{x}\ \sigma$ (g)	Meat Ash Weight $\bar{x}\ \sigma$ (g)	Meat Ash Free $\bar{x}\ \sigma$ (g)
B_1	21	5.08 ± 0.45	20.99 ± 5.5	27.35 ± 7.2	2.57 ± 0.9	0.532 ± 0.31	0.059 ± 0.02	0.473 ± 0.29
B_2	23	4.79 ± 0.20	18.21 ± 5.6	24.01 ± 7.1	2.36 ± 1.0	0.538 ± 0.27	0.064 ± 0.02	0.474 ± 0.24
C_1	32	4.60 ± 0.76	12.88 ± 5.8	18.26 ± 7.9	2.45 ± 1.1	0.48 ± 0.23	0.058 ± 0.02	0.420 ± 0.21
C_2	30	4.16 ± 0.66	10.74 ± 4.7	15.33 ± 6.8	1.84 ± 1.1	0.41 ± 0.28	0.045 ± 0.02	0.375 ± 0.27
D_1	17	4.00 ± 0.74	10.18 ± 5.5	14.34 ± 7.5	1.76 ± 1.0	0.45 ± 0.34	0.046 ± 0.03	0.372 ± 0.33
D_2	16	3.61 ± 0.83	7.91 ± 5.5	11.35 ± 8.7	1.29 ± 1.1	0.30 ± 0.34	0.029 ± 0.023	0.268 ± 0.032
E_1	31	4.62 ± 0.49	13.57 ± 3.97	19.42 ± 5.9	2.11 ± 0.9	0.45 ± 0.27	0.052 ± .023	0.400 ± 0.252
E_2	28	4.19 ± 0.54	11.56 ± 4.10	16.28 ± 5.5	1.66 ± 0.74	0.363 ± 0.216	0.0408 ± 0.021	0.322 ± 0.196
F_1	32	4.70 ± 0.40	14.13 ± 3.26	20.66 ± 4.5	2.00 ± 0.72	0.429 ± 0.185	0.0515 ± 0.017	0.377 ± 0.174
F_2	29	4.05 ± 0.41	10.28 ± 3.90	14.79 ± 5.5	1.34 ± 0.42	0.258 ± 0.097	0.038 ± 0.012	0.221 ± 0.087
G_1	31	5.22 ± 0.62	16.20 ± 4.76	23.23 ± 6.5	2.39 ± 0.9	0.457 ± 0.203	0.063 ± 0.029	0.395 ± 0.183
G_2	31	5.03 ± 0.51	15.59 ± 5.18	22.2 ± 6.9	2.55 ± 0.8	0.528 ± 0.227	0.0597 ± 0.018	0.468 ± 0.210
H	51	4.19 ± 0.83	9.61 ± 4.6	14.2 ± 6.7	1.59 ± 0.9	0.342 ± 0.226	0.050 ± 0.032	0.292 ± 0.197
I_1	30	3.79 ± 0.51	8.39 ± 3.7	11.67 ± 4.8	1.22 ± 0.5	0.273 ± 0.136	0.033 ± 0.019	0.24 ± 0.188
I_2	27	3.90 ± 0.6	9.28 ± 3.5	12.71 ± 5.1	1.45 ± 0.7	0.312 ± 0.034	0.034 ± 0.017	0.277 ± 0.138
J_1	30	4.48 ± 0.54	9.14 ± 3.6	13.60 ± 5.2	1.64 ± 0.87	0.379 ± 0.25	0.0477 ± 0.02	0.331 ± 0.229
J_2	30	4.45 ± 0.56	10.16 ± 3.6	14.90 ± 5.0	1.64 ± 6.70	0.364 ± 0.172	0.0449 ± 0.02	0.322 ± 0.162
K_1	34	4.93 ± 0.47	12.03 ± 3.5	17.06 ± 5.0	1.76 ± 0.62	0.400 ± 0.164	0.0479 ± 0.018	0.35 ± 0.15
K_2	33	4.74 ± 0.48	10.46 ± 3.2	14.67 ± 4.4	1.60 ± 0.57	0.341 ± 0.129	0.0434 ± 0.014	0.297 ± 0.119
L_1	33	4.42 ± 0.67	7.54 ± 3.8	11.03 ± 5.2	1.42 ± 0.61	0.329 ± 0.173	0.0413 ± 0.016	0.288 ± 0.162
L_2	31	3.36 ± 0.75	3.71 ± 2.6	5.5 ± 3.8	0.67 ± 0.53	0.131 ± 0.111	0.0211 ± 0.013	0.110 ± 0.099
M_1	32	3.53 ± 0.37	5.08 ± 1.7	7.5 ± 2.5	0.88 ± 0.34	0.188 ± 0.08	0.0277 ± 0.011	0.161 ± 0.073
M_2	33	2.97 ± 0.37	2.56 ± 1.0	3.9 ± 1.5	0.55 ± 0.28	0.117 ± 0.057	0.0194 ± 0.009	0.097 ± 0.049
N	62	3.29 ± 0.45	3.67 ± 1.3	5.4 ± 1.9	0.70 ± 0.26	0.147 ± 0.050	0.0265 ± 0.009	0.121 ± 0.043
O	92	2.77 ± 0.51	2.09 ± 0.9	3.1 ± 1.5	0.45 ± 0.22	0.086 ± 0.04	0.0104 ± 0.005	0.075 ± 0.041
P	99	1.95 ± 0.3	0.78 ± 0.3	1.1 ± 0.5	0.17 ± 0.07	0.031 ± 0.013	0.006 ± 0.002	0.025 ± 0.012

*Number of individuals

**Shell diameter = [length + width] /2

†$\bar{x}$, σ = mean, standard deviation

‡The organic content of 217 oyster shells averaged 2.6% of the shell weight (1.8% of the whole oyster weight)

Table 16. Final Oyster Weights (Percent)

Age Class	Shell Wt/ Whole Wt	Fresh Meat Weight/ Whole Wt	Dry Meat Weight/ Whole Wt	Dry Meat Weight/ Fresh Meat Weight	Ash Meat Weight/ Dry Meat Weight
B_1	77	9.4	1.94	20.7	11.1
B_2	76	9.8	2.24	22.8	11.9
C_1	70	13.4	2.63	19.6	12.1
C_2	70	12.0	2.67	22.3	11.0
D_1	71	12.3	3.14	25.6	10.2
D_2	70	11.4	2.64	23.3	9.7
E_1	70	10.9	2.32	21.3	11.5
E_2	71	10.2	2.23	21.9	11.2
F_1	68	9.7	2.08	21.4	12.0
F_2	69	9.1	1.74	19.2	14.7
G_1	70	10.3	1.97	19.1	13.8
G_2	70	11.5	2.38	20.7	11.3
H	68	11.2	2.41	21.5	14.6
I_1	72	10.5	2.34	22.4	12.1
I_2	73	11.4	2.45	21.5	10.9
J_1	67	12.1	2.79	23.1	12.6
J_2	68	11.0	2.44	22.2	12.3
K_1	70	10.3	2.34	22.7	12.0
K_2	71	10.9	2.32	21.3	12.7
L_1	68	12.9	2.98	23.2	12.5
L_2	67	12.2	2.38	19.5	16.1
M_1	68	11.7	2.51	21.4	14.7
M_2	66	14.1	3.00	21.3	16.6
N	68	13.0	2.72	21.0	18.0
O	67	14.5	2.77	19.1	12.1
P	71	15.4	2.82	18.2	19.3
Avg:	69.9%	11.6%	2.5%	21.4%	13.0%

Mantle fluid = 100 − (69.9 + 11.6) = 18.5% (by difference)

Table 17. Proximate Analysis, 100 mg Samples (dry weight)

Artificial Oyster

	Recovered	%
56 mg – Bovine Albumen, Fraction V, (Powder)	50 mg	89
16 mg – Menhaden Oil (Oil)	13 mg	81
28 mg – Oyster Glycogen (Powder)	21 mg	75
100 mg – Freeze-Dried/Ground/Analyzed		

Freeze-Dried Oysters*

Group	Lipid (mg)	Protein (mg)	Glycogen (mg)
B_1	5.0 ± 0.04	42.0 ± 2.6	6.7 ± 3.9
B_2	4.0 ± 1.5	48.4 ± 1.0	10.1 ± 0.3
C_1	6.7 ± 1.0	54.7 ± 6.3	8.3 ± 0.8
C_2	8.2 ± 1.7	52.6 ± 3.6	15.7 ± 0.1
D_1	9.3 ± 1.7	46.6 ± 9.2	22.3 ± 1.0
D_2	11.1 ± 0.3	43.4 ± 15.3	21.7 ± 1.7
E_1	9.7 ± 2.2	44.7 ± 2.1	20.0 ± 2.3
E_2	13.7 ± 0.6	51.3 ± 6.0	17.5 ± 5.7
F_1	8.5 ± 0.3	36.8 ± 1.8	18.0 ± 8.1
F_2	7.5 ± 0.6	46.8 ± 6.4	21.5
G_1	9.6 ± 2.3	40.9 ± 10.2	20.1 ± 7.8
G_2	8.6 ± 4.5	41.9 ± 1.7	21.3 ± 1.2
M_1	9.2 ± 0.6	37.1 ± 3.7	13.8 ± 3.1
N	9.2 ± 0.5	33.8 ± 1.6	11.6 ± 1.9
O	7.9 ± 0.2	31.0 ± 6.1	9.6 ± 2.0
P	8.0 ± 0.4	30.4 ± 6.6	7.9 ± 1.7

*Weights = mean and standard deviation of duplicate samples.

Analysis of Oysters by D. T. Hicks**

Group	Lipid (mg)	Protein (mg)	Glycogen (mg)
BR 79	7.3 ± 2.1	35.2 ± 0.1	28.3 ± 2.6
G 79	6.9 ± 0.9	41.1 ± 0.2	18.9 ± 1.7
BR 79	6.5 ± 0.2	45.6 ± 3.3	20.7 ± 3.6
G 80	6.0 ± 0.8	43.2 ± 2.4	11.2 ± 1.9

**Master's Thesis, 1981, University of Delaware, Newark, DE, Reference 21.
BR – Broadkill River oysters
G – Greenhouse oysters

ALGAE PRODUCTION

The daily production of the algae *Thallasiosira pseudonana* (3H) and *Isochrysis galbana* (T-ISO) is given in Table 18 and expressed as cell number per liter of culture. Prior to the third week in November 1980, four of the five T-ISO reactor vessels were in manifold operation, while three of the five 3H reactor vessels were in production. Full production of the two 5-vessel reactor assemblies was instituted on 19 November 1980 for T-ISO and on 22 November for 3H. The brackets in the table indicate culture lifetimes. Cultures were terminated and restarted either as a result of an algal "crash" or as a result of operational desirability such as the need to clean the vessels, the feed requirements of the oyster biomass, the initial seed availability, or the residual algal content at the end of a particular day which would serve as the new daily starting algal concentration for the next day's production. It is important to emphasize that the algae production described in this report does not characterize the maximum potential of the vertical-tube reactor system to produce single-celled marine algae; rather, it is the actual production that resulted from operating the integrated prototype system according to the criteria described earlier.

Note: Table 18, Daily Algae Production, is included in Appendix B.

The daily concentrations of algae for each of the two species have been averaged for each month of the prototype operation to yield the monthly mean daily algal concentrations in units of 10^9 cells/liter. While the algae were being produced during the course of the prototype operation a continuous record of the daily insolation was obtained by means of a recording pyroheliometer located on the roof of a nearby building in the College of Marine Studies complex at Lewes, Delaware. Monthly mean daily averages of the total insolation were computed from the records. Figure 15 illustrates the insolation cycle and the

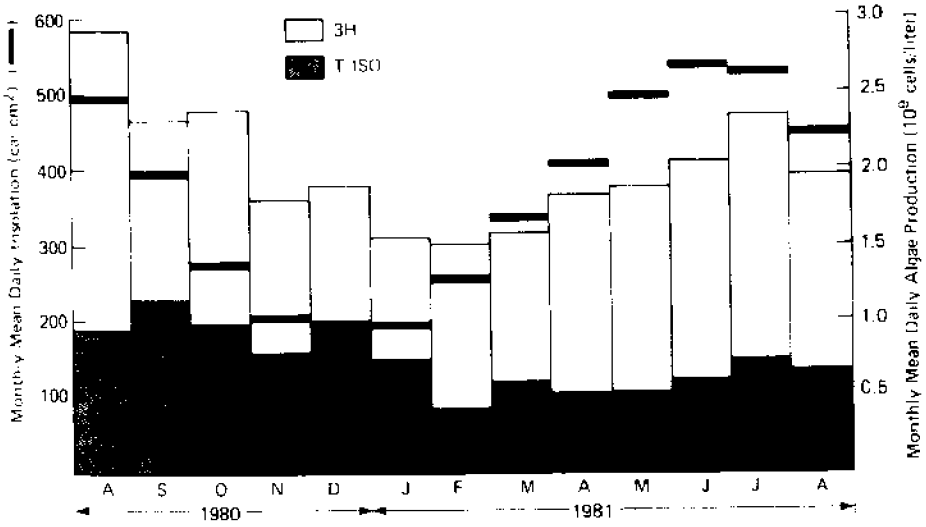


Figure 15. Monthly mean daily insolation (cal/cm^2) at Lewes, Delaware, and monthly mean daily algae production (cells X 10^9/liter) during prototype operation for the species 3H and T-ISO. *See Tables 18 (in Appendix B) and 19.*

algal concentrations over the course of the prototype operation. The algal concentrations are only poorly correlated with the solar cycle. This result clearly illustrates the modulating effect on algae production of the operational and management dictates of the integrated system.

Table 19 is a summary of the solar energy regime experienced by the algae reactors and the algal production expressed as chemical energy for the two species, 3H and T-ISO. The conversion of algal cell number to chemical energy in kilocalories (Kcal) and of the insolation to radiant energy (Kcal) is given as follows:

- Algal Production:

  $(n \times 10^9$ cells/liter/day)

  x (reactor volume = 6,000 liters)

  x (grams of carbon/cell)

  x (12 g/mole of carbon = gram atomic weight of carbon)$^{-1}$

  x (114 Kcal/mole) = chemical energy stored

  = Output, Kcal/day/reactor

  where n = monthly mean daily number of cells produced in a liter calculated from daily measurements

  g carbon/cell = 5×10^{-12} for 3H

  = 8×10^{-12} for T-ISO

- Insolation:

  (monthly mean cal/cm^2/day)

  x (transparent area of the 6,000-L reactor = 3.38×10^5 cm^2)

  x (0.24)

  = Input, Kcal/day/reactor

  where the factor 0.24 is a constant which reduces the insolation values measured by the rooftop pyrheliometer to take into account the geometry of the reactors, shading, sun angle, and reflective and absorptive losses.

- Photosynthetic Efficiency:

  (Output ÷ Input) x 100 = Photosynthetic efficiency in %

It is evident from Table 19 that the photosynthetic efficiencies of the algae in the managed algae reactor production system differ, 3H being more efficient than T-ISO under the conditions employed. The photosynthetic efficiencies vary on a monthly basis.

Table 19. Photosynthetic Efficiency of the Prototype

	3H (Average/day)			T-ISO (Average/day)		
	Output ($\times 10^2$ Kcal) Algae Production	Input ($\times 10^4$ Kcal) Solar Insolation	P.E. (%)	Output ($\times 10^2$ Kcal) Algae Production	Input ($\times 10^4$ Kcal) Solar Insolation	P.E. (%)
1980						
August	4.99	2.41	2.1	3.43	3.21	1.1
September	3.95	1.90	2.1	4.23	2.53	1.7
October	4.10	1.31	3.1	3.61	1.74	2.1
November	3.47	1.15	3.0	3.14	1.45	2.2
December	5.39	1.32	4.1	4.61	1.32	3.5
1981						
January	4.45	1.54	2.9	3.42	1.54	2.2
February	4.28	2.03	2.1	1.96	2.03	1.0
March	4.50	2.73	1.6	2.74	2.73	1.0
April	5.22	3.34	1.6	2.42	3.34	0.7
May	5.33	3.96	1.3	2.42	3.96	0.6
June	5.73	4.32	1.3	2.83	4.32	0.7
July	6.73	4.28	1.6	3.42	4.28	0.8
August	5.59	3.59	1.6	3.01	3.59	0.8
September	4.96	3.32	1.5	3.65	3.32	1.1
October	4.87	2.22	2.2	3.56	2.22	1.6
			$\overline{X} = 2.1$			$\overline{X} = 1.4$

In part this variation results from the modulation of the production system required by management needs and in part by the annual solar regime, both species tending to be more efficient under the reduced solar intensities of the late fall, winter, and early spring months.

The annual mean daily photosynthetic efficiency for 3H and T-ISO in the managed reactor culture system was 2.1 and 1.4 percent, respectively. This is a significant finding since many estimates of algal or terrestrial crop plant photosynthetic efficiences reported in the literature result from projections of relatively short-term measurements, whereas the values above derive from daily measurements made for over a year during the course of the prototype operation.

The necessary management procedures for the integrated prototype system, which primarily require tuning algae production to the shellfish needs, tend to lessen the potential photosynthetic efficiency, and hence the productivity, of the vertical-tube algal-culture reactors. Thus, the annual mean daily photosynthetic efficiency is less than that achievable for algae production by this means were the reactor system to be isolated from the prototype.

WATER QUALITY

Chemical and microbiological analyses of the seawater in the prototype have demonstrated that the prototype operation continuously maintained the measured parameters according to specifications.

Chemical Analyses

Over 6,000 separate chemical analyses have been made on seawater samples from a number of key stations during the operation of the prototype. Nearly 3,000 earlier analyses were made during the operational shakedown phase. In addition to these analyses which were part of the operational routine, hundreds of *ad hoc* spot checks were made to test the adequacy of gas exchange in the algae reactors and oyster flumes and to provide additional monitoring of the water treatment and circulation system. These analyses demonstrated that water quality parameters could be held within specifications (Tables 8 and 9). Departure from the specifications were relatively rare and generally short lived since corrective measures could readily be taken to bring deviations back within specified limits.

Four examples of the biweekly analyses are shown in Tables 20 to 23 for the dates 10 October 1980 and 8 January, 7 April, and 7 July 1981, to illustrate the near monotony among the data irrespective of season. The calcium content was 438 ppm $\pm$ 27 (standard deviation) for the input Indian River seawater, 279 ppm $\pm$ 33 for oyster flume 3 before recharging with makeup water (cf 03B, Tables 20 to 23), and 283 ppm $\pm$ 33 after makeup water recharge (cf 03A, Tables 20 to 23). Since the seawater in the prototype was reduced in salinity from the nominal 30 ppt of the Indian River water to 20 ppt, the calcium content would be expected to be 292 ppm in the oyster flumes, well above the specified minimum of 200 ppm. The pH of the seawater in the prototype remained within or acceptably close to the specified range of 7 to 8. The pH of the input Indian River seawater throughout the year was 7.90 $\pm$ 0.11 (standard deviation). The NH_3 concentrations in oyster flume 03B (just prior to the daily makeup water addition, when NH_3 is expected to be at its highest level as a result of oyster metabolism and excretion) was 20.9 $\pm$ 9.5 µgat N/L over the year of prototype operation; whereas the specification for NH_3 was set as $\leq$ 60 µgat N/L as a conservative safe limit. The silicate, phosphate, and nitrate levels are determined by the mineral nutrient additions to the algae reactor vessels as well as by algae metabolism. These ions are not deleterious to algae or oysters even at levels far higher than those reported, nor does the nitrite ion, which results largely from bacterial nitrification, ever rise to levels that might cause concern. The low levels of nitrite are consistent with low levels of ammonia oxidizing bacteria. The low levels of both ammonia and nitrite are consistent with the function of the algae

Note: Tables 20 through 23 are included in Appendix B.

as a seawater processing agent, as planned in the design of the prototype. The total alkalinity sometimes exceeded the specified upper limit during the first few months of operation. This resulted from the neutralization step following acidification for seawater reprocessing (see Section 2 and Table 9). A change in the proportion of alkalis (NaOH, $NaHCO_3$) thereafter kept the total alkalinity at a satisfactory level.

Microbiological Analyses

Standard microbiological analyses applied for a number of sampling stations throughout the course of the prototype operation showed that bacterial levels were consistently low and that there has been no evidence for human or shellfish pathogens or evidence that bacteria play a determinant role in the ecology of the intensive, controlled mariculture activity as it is practised in this laboratory. We believe that the basis for this situation lies principally in the care to apply 'good housekeeping' procedures and preventative sanitation measures including seawater origin and processing, algae culture methods, animal husbandry throughout the oyster life cycle, and a facilities layout which minimizes airborne contamination as well as human traffic.

Bacterial counts, for four days of summertime operation, observed by direct microscopic examination of fluorescently stained bacteria in algal culture vessels, oyster flumes, and storage and water treatment tanks, revealed that the extreme values ranged from 2.2×10^5 to 6.4×10^6 bacteria per mL (Table 24). The dry biomass of 10^6 bacteria is approximately one-quarter of

Table 24. Four-day Spot Check of Bacteria Levels* (10^6 per mL) in the Prototype

Code	Station		8/13/81	8/14/81	8/15/81	8/16/81	8/17/81
RI	3H Reactor	a.m.	--	2.3	3.4	2.7	3.4
		p.m.	2.3	5.6	3.1	4.3	--
RII	T-ISO Reactor	a.m.	--	--	4.3	4.6	4.3
		p.m.	3.5	1.6	4.4	3.7	0.38
OB	Oyster Flume Before Makeup	a.m.	--	1.2	5.4	6.4	6.1
		p.m.	2.0	2.9	1.4	1.8	3.4
OA	Oyster Flume After Makeup	a.m.	--	--	--	--	--
		p.m.	1.3	1.9	0.22	--	--
ST I	Storage Tank I		--	--	0.94	0.68	0.48
ST II	Storage Tank II		--	--	0.28	0.22	0.52
E	Seed Tube		--	--	--	--	3.1
W	Seed Tube		--	--	--	--	3.5

*Diagnosed by direct counts of Acridine Orange stained particles concentrated on polycarbonate filters. Viable (colony-forming) bacteria would be appreciably fewer than indicated by the spot-check data above.

one microgram, an amount which cannot substantially affect major parameters such as carbon dioxide production or oxygen depletion. Highly specific effects such as disease occurrence have not been observed. Further, the low levels of nitrite indicate low levels of nitrifying bacteria in oyster feces, filter beds, and in suspension.

Water Quality Bioassay Studies

The seawater milieu in the prototype is artificial and perhaps even unique. While enough earlier information was at hand to set reasonable specifications and to devise an operating scheme to assure holding the system to physical, chemical, and microbiological specifications, there was little prior experience for evaluating other features or operating procedures inherent in the prototype design. A series of bioassay experiments was conducted separately from the prototype operation to accomplish the following:

- Evaluate the effect of algal medium and prototype water on the growth of oyster spat;
- Bioassay water quality using clam (*Mercenaria mercenaria*) larval growth as an indicator;
- Evaluate the effect on water quality of adsorbent resins (XAD class of Rohm and Haas) with a larval bioassay;
- Test the effect of treated and untreated holding-tank seawater on oyster spat growth, and
- Test the effect of foam fractionated prototype seawater on oyster spat growth.

Details of these experiments are included as Appendix D.

The results show that:

- High levels of algal medium, which includes the f/2 concentration of Guillard's trace metal mixture and in which the prototype oysters are grown, has a beneficial effect on oyster spat growth (whether there may be long-term effects or a different response by larger oysters is not known);
- The experiments with oyster spat and clam larvae show that the integrated water treatment process (acidification, foam fractionation, and diatomaceous-earth filtration) is effective in improving and maintaining water quality at least equivalent to that of fresh Indian River seawater;

- The complete water treatment process does not operate continuously but is applied discontinuously once a day after feeding the T-ISO algal species; the bioassay results indicate that the prototype water quality may deteriorate between the daily treatment periods; foam fractionation in itself is inadequate to maintain high water quality and may have adverse effects, although larvae and spat are notoriously more sensitive to water quality deterioration than are larger animals, and

- Certain resinous adsorbents of the XAD (Rohm and Haas) series may be beneficial in improving water quality.

COST CONSIDERATIONS

The specific objectives sought by the operation and management of the intensive, controlled, recirculating system were:

- Continuously cultivate oysters over an extended period;

- Analyze materials and energy utilization, performance of system components and integrated processes, and quantitative chemical, physical, and biological data;

- Identify and define problems that may arise from continuous long-term operation of the system including any which may be peculiar to the recycling scheme;

- Provide shellfish for product evaluation, and

- Document the operation and performance of the prototype system.

The costs associated with meeting these objectives were:

Labor--Marine scientists and research technicians	$ 86,451.00
Greenhouse	40,514.00
Utilities--Electricity and propane	11,690.00
Materials and Supplies	5,608.00
Seawater	8,040.00
External Laboratory Fees	7,090.00
Equipment and Instrumentation	*37,487.00
TOTAL	$197,880.00

* (breakdown included below)

Hatchery, algae room, and chemical and microbiological support services were provided by the Center for Mariculture Research of the College of Marine Studies.

The technical evaluation of the system honoring the specific objectives listed above required personnel, equipment and instrumentation, and operational protocols which are not necessarily relevant to commercial production. The cost data cast little light on the question of economic viability.

Equipment Cost Breakdown

Oysters

4	Flumes, 400-L @ $200	$ 800.00
1	Sump (PVC Tank and Control Valve)	436.00
1	Foam Fractionator	150.00
2	Pump, 110V 1/2 hp @ $418	836.00
1	Pump, 220V 3/4 hp	500.00
1	Heating/Cooling Unit for Water	1,820.00
48	Nestier® Trays @ $10.31	495.00
1	Spare Pump	500.00
-	Plumbing and Valves	1,411.00
-	Support Structure	200.00
-	Buckets, Hoses, Transfer Lines, etc.	200.00
-	Fabrication Mats	100.00
		$ 7,448.00

Hatchery

3	Rearing Vessels (40-L fiberglass cones) @ $35	$ 105.00
2	Flumes (broodstock tanks) @ $200	400.00
4	4-Liter Nalgene® Beakers	44.00
6	Sieves, Stainless Steel @ $50	300.00
-	Glassware	50.00
-	Microscope Supplies	50.00
-	Miscellaneous Supplies	886.00
		$ 1,035.00

Algae

10	Reactor Sidewalls (Kalwall®)	$ 800.00
10	Reactor Draft Tubes (Kalwall®)	500.00
10	Reactor Fiberglass Cones @ $72	720.00
10	Reactor Bases (wooden supports) @ $30	300.00
2	Reactor Service Platforms @ $150	300.00
2	Pumps, 1.5 hp (220v) @ $680	1,360.00
1	Pump (Spare)	680.00
2	Glass Shell and Tube Heat Exchangers @ $2,000	4,000.00
2	pH Monitor/Controller System @ $700	1,400.00
2	DO Monitor/Controller System @ $800	1,600.00
6	Seed Tubes, 1000-L @ $100	600.00
6	Seed Tubes, 250-L @ $65	390.00
-	Hoses, Tansfer Equipment	200.00
1	Pressure Sprayer	650.00
-	Fabrication Materials	200.00
-	Miscellaneous Equipment and Glassware	300.00
-	Plumbing and Valves	1,772.00
		$15,772.00

Algae Seed Lab

1	Microscope and Supplies	$ 700.00
1	pH Meter	200.00
1	Pump, Vacuum	170.00
1	Incubator	400.00
-	Miscellaneous	200.00
-	Glassware	450.00
-	Autoclave	520.00
		$ 2,640.00

Lab Equipment

1	pH Meter	$ 200.00
1	Dissolved Oxygen Meter	350.00
1	Microscrope and Supplies	1,500.00
1	Balance	150.00
-	Miscellaneous Instrumentation	2,000.00
		$ 4,200.00

Water and Waste Handling

2	2000-L Fiberglass Tanks @ $325	$ 650.00
2	6000-L Fiberglass Tanks @ $1,250	2,500.00
1	Pump, 1/2 hp 110	190.00
1	Pump, 1/2 hp 110	418.00
1	Pump, 3/4 hp 220	500.00
1	Pump, 1/2 hp (spare)	418.00
1	Foam Fractionator	100.00
1	Sand Filter	400.00
1	Diatomaceous Earth Filter	600.00
-	Plumbing and Valves	616.00
		$ 6,392.00

Utilities

Electricity @ $0.067/kw-hr @ ~335/month	$ 4,355.00
Water (fresh) @ $0.0016/gal	335.00
Propane (heat and hot water) @ $0.0068/gal	7,000.00
Saltwater @ $38/Load and Water Handling Charge	8,040.00
	$19,730.00

Discussion and Conclusions

Oyster growth in the prototype was specified to occur in a curvilinear fashion so that on the average oysters would reach a market size of about 80 grams in one year. The specifications for oyster growth were in fact set out as a best judgement based on:

- Laboratory and field experience (particularily the warm-weather growth rates known to occur for oysters maintained in the local Broadkill River),
- The maintenance of an environment sufficient for growth, and
- A relatively homogeneous size population of oysters that would be capable of responding by steadily increasing its growth rate until the oysters reached the 80-gram harvest weight.

These were intentionally demanding specifications to provide a serious test of the prototype system.

None of the 15 age classes of oysters periodically introduced into the prototype grew according to these specifications: the average weights of the oysters increased linearly with the result that the oysters achieved (or, for lately introduced classes, would achieve) only about 16 grams at the end of a year. All age-class mean weights increased in approximately the same linear fashion. A few individuals in each age class reached (or would reach) a weight of over 30 grams, while others grew relatively little, reaching only 8 grams or less in a year. This spread in growth response is indicated in Figure 13 (vertical bars) and illustrated by the population-frequency histograms for tray E_1 of age-class E and for age-class H (Figures 11 and 12, respectively). As an overall average the oysters converted about 3 percent of the dry weight of algae cleared to ash-free dry oyster meat weight.

The reasons for the low, linear growth response and the population spread of individual performance are not known. A number of conjectures may be put forward:

OYSTER GROWTH

In order to gather the necessary growth data all the oysters were removed from the prototype every 3 weeks, air dried, weighed, and returned. This amount of handling, in spite of reasonable care, caused obvious damage to newly grown shell at the margin of the bill of many oysters at each handling, and subjected the oysters to temperature changes (from seawater at 22° to 26°C to the ambient greenhouse and Smith Laboratory temperatures), and drying. Shell-margin damage is known to cause growth interruption in bivalves. It is possible that this operational exigency caused a repression in growth-rate increase and artifically forced a linear pattern to occur.

The shape of the shell was generally symmetrical, as expected for cultchless oysters, and relatively deeply cupped with a flat upper valve. The shell was less robust than that of comparably sized oysters harvested from local waters. Whether this condition reflects an inappropriate specification for cultivation is not known.

The prototype environment may have been deficient in one or more nutrient elements. It is well known that inadequate levels of nutrients required to be supplied exogenously can limit the growth rate of diverse organisms. The principal sources of organic nutrients in the prototype are the algal species *Thalassiosira pseudonana* and the Tahitian strain of *Isochrysis galbana*. This feed was supplied in accord with a well established empirical relationship to assure that the bulk of the feed was adequate. It is possible that, in fact, these species may be deficient in a micronutrient or a class of nutrients necessary for long-term oyster cultivation under the more or less constant year-round temperature regime employed. Juvenile oysters and other juvenile bivalves very recently have been shown to take up dissolved organic materials such as amino acids. Other molluscs are now known to respond to dissolved peptide growth factors at very low concentrations. Such factors may have been deficient or even removed, if present, by the water treatment process. This process removes suspended inorganic particulate matter as well. Inorganic particles may be beneficial for oyster growth.

In spite of the warm temperatures and rich algae feeding regime employed in the prototype, spawning was not observed. This fact may indicate a deficiency in the accumulation of tissue reserves (such as glycogen) or in biochemical composition related to gonad development and maturation. However, proximate analyses (Table 17) do not indicate that the prototype oysters suffered from gross disparities in protein, glycogen, or lipid content.

Precautions were taken in the choice of broodstock and in the hatchery procedures to insure against the introduction of oysters into the prototype which were not genetically representative of the native oyster stocks. Such oysters may be genetically unsuited to prototype cultivation. The local oysters are a temperate variety subjected to annual seasonal cycles, whereas the prototype environment is relatively invariant seasonally. Prototype oysters were not subjected to the lower temperature regimes wherein oysters naturally "fatten", i.e. accumulate tissue reserves such as glycogen. To achieve better growth over prolonged periods of cultivation it may be necessary to "program" the local oysters with a different set of environmental specifications that more closely mimic the natural cycle or to improve oysters genetically specifically for growth in the artificial prototype regime.

ALGAE PRODUCTION

Mass algal feed production of the two species, *Thalassiosira pseudonana* (3H) and *Isochrysis* aff. *galbana* (T-ISO), was carried out essentially as specified. The algae reactor devices performed adequately to provide the feed required by the oyster biomass. The overall algae production was photosynthetically efficient, the mean efficiency for 3H being 2.1 percent and for T-ISO, 1.4 percent. These values compare well with those for intensively cultivated terrestial crops such as corn and soybeans. The efficiencies for microalgae cultivation could be significantly higher were the reactors not modulated by the demands of the integrated prototype oyster cultivation process.

To maintain consistent productivity of the algae reactors a dependable algal seed culture supply is necessary. The supply from the algae seed laboratory proved adequate to replenish the reactors when the mass cultures began to lose productivity or when the operational scheme demanded a new seed charge.

SEAWATER

During the operation period of the prototype 1.7×10^6 liters (450,000 gallons) of seawater adjusted to 20 ppt salinity were used for the recirculation process. Three hundred thousand gallons at about 30 ppt were imported by tank truck from the Indian River Inlet. The daily proportion of water recirculated was about 77 percent of the total volume of the system. The seawater met the specifications for water quality as a result of the operating, control, and processing procedures employed. Aside from the fact that the chemical parameters measured almost invariably were in accord with specifications, recirculation of the processed oyster wastewater back into the algae reactors gave no indication that algae production was adversely affected. In addition, the filtration (clearing) of algae by the oysters was consistently about 90 percent for every feeding cycle. The acidification process for seawater regeneration appears advantageous for keeping unwanted microbial flora and fauna at low levels. Continuous operation of the foam fractionator is of questionable value for water processing. It removes about 7 percent of the algae and may be deleterious, as bioassays with very young bivalves suggest. Recirculation of seawater conserves considerable heating and cooling energy, and the dilution of seawater with ambient tap water to meet the salinity requirement conserves seawater itself.

Research Needed

The discussion and conclusions point to a number of areas in which additional investigations are needed:

OYSTER GROWTH

The relatively poor oyster growth as compared to the specification, the apparent lack of spawning, the relatively fragile shell, and the low average conversion of algal food to oyster organic material signify a condition(s) of stress under cultivation. The origin of such stress is unknown. Among the environmental considerations are:

- Mechanically induced stress as a result of handling,
- Physically induced stress such as prolonged cultivation at high temperatures, and
- Nutritionally induced stress as a result of one or more deficiencies, either of particulate or dissolved forms, either organic or inorganic in nature. Research is needed on the physiological consequences of such stress for the growth of oysters and for oyster confirmation to a desired product specification.

Hatchery procedures were designed to provide juvenile oysters representative of the local population and capable of growing at similar rates. Precaution was taken to expose all oysters to the same environment during cultivation in the prototype. Nevertheless, a wide range of growth rates was observed among individuals. This observation implies that a range of genetic competence exists among the oysters for growth in the artificial prototype environment. Experiments to improve oyster lines and strains for intensive controlled cultivation under artificial conditions would thus appear desirable.

ALGAE PRODUCTION

The prototype production of algae for bivalve feed met the specifications. Nevertheless, extension of algal culture life is highly desirable to lessen the demands imposed by frequent replenishment of seed for mass culture. Further study of conditions leading to unbalanced algal growth which presages culture failure are needed, and means to ameliorate the condition should be sought. New work is necessary to learn how to provide the carbon dioxide necessary for photosynthesis and to remove the deleterious oxygen generated in the process in ways which are economical and compatible with the use of algae as bivalve feed.

DIETS AND MICRONUTRIENTS

Research is needed to design nutritious diets and rations which can economically serve as alternatives or supplements to microalgae diets for oysters and other commercially interesting bivalves.

Dissolved microconstituents, both organic and inorganic, in the seawater milieu need investigation to ascertain their importance for oyster and other bivalve cultivation.

Summary

- A prototype integrated, intensive, controlled, recirculating-seawater, bivalve-cultivation system was designed, constructed, and operated continuously for one year.

- The design and operation of the system are based on specifications that were derived from nearly a decade of research on the biology of oysters and single-celled algae, the chemistry and microbiology of seawater, and bio-engineering practices.

- Supporting activities, algal seed production, hatchery juvenile oyster production, and chemical and microbiological monitoring and assay were established and conducted to serve the prototype and other laboratory functions.

- A filtered and chemically processed recirculating seawater environment mutually compatible with the growth of oysters and feed microalgae was established and utilized. Three hundred thousand gallons of seawater from the Indian River Inlet, Delaware, were imported to the Smith Laboratory by tank truck, diluted with tap water, fertilized with mineral nutrients, and recirculated at a daily rate of 77 percent of the prototype volume to conserve energy and nutrients. The seawater quality specified was maintained throughout the operational period.

- Seed algae and juvenile oysters were dependably and systematically introduced into the prototype.

- Oysters grew, on the average, in the prototype at a constant rate and reached about one-fifth of the size specified. They converted on a dry-weight basis, 3 percent of the algal food which they filtered. Their meat was comprised of typical amounts of water, ash, protein, glycogen, and fat. The mineral and organic content of the shell was also typical, but the shell was not robust.

- Algae production met specifications, and the photosynthetic efficiency over the course of the production period was 2.1 percent for the food diatom (*Thalassiosira pseudonana*, 3H) and 1.4 percent for the flagellate (*Isochrysis* aff. *galbana*, Tahitian).

3. Support Activities

Facility

The intensive, environmentally controlled mariculture activities are housed in the Otis H. Smith Laboratory at the University of Delaware, College of Marine Studies complex in Lewes, Delaware. The laboratory, illustrated in Figures 16 and 17, was completed in late 1979. The building is L-shaped and accommodates east-west running greenhouses attached to the main structure. The greenhouses are constructed of modular aluminum framing and are enclosed in plastic sheeting. They are ventilated and equipped with utilities including a gaseous carbon dioxide supply and hot, cold, and ambient-temperature seawater. The plan of the principal structure is indicated in Figure 17. The east-west wing contains research and analytical laboratories and the halophyte activities. The north-south wing contains offices, wet laboratories, chemical and microbiological support laboratories, the algae seed rooms, and hatchery. In addition to ordinary utilities the building also has hot, cold, and ambient-temperature seawater available throughout the laboratories. The heating/ventilating/air-conditioning system employs 14 heat-pump units strategically placed to permit temperature and air-circulation control of various room combinations.

Seawater is brought to the facility in a fiberglass tank truck from an ocean inlet to the Indian River Bay. It is chilled, heated, or allowed to remain ambient before distribution by gravity feed from an elevated head-tank. This arrangement permits the use of noncorrosive materials throughout the sytem to minimize maintenance and contamination.

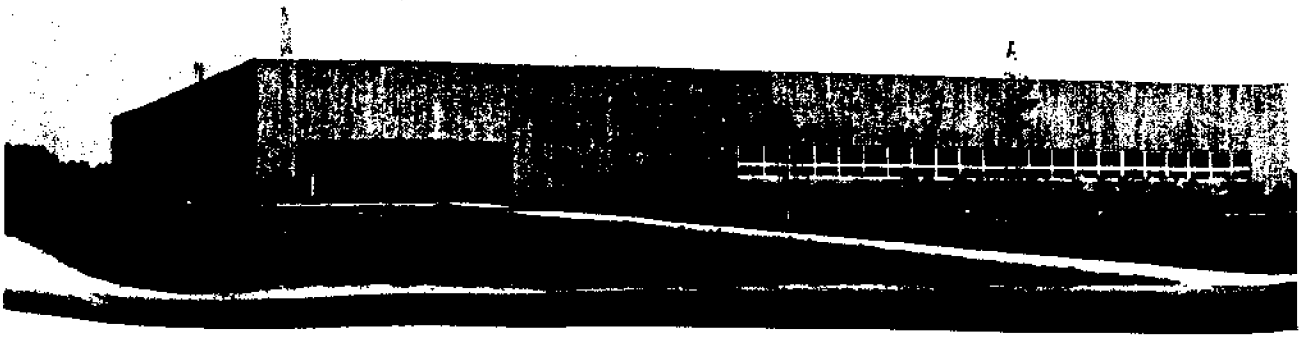


Figure 16. The Otis H. Smith Laboratory at the University of Delaware College of Marine Studies in Lewes, Delaware.

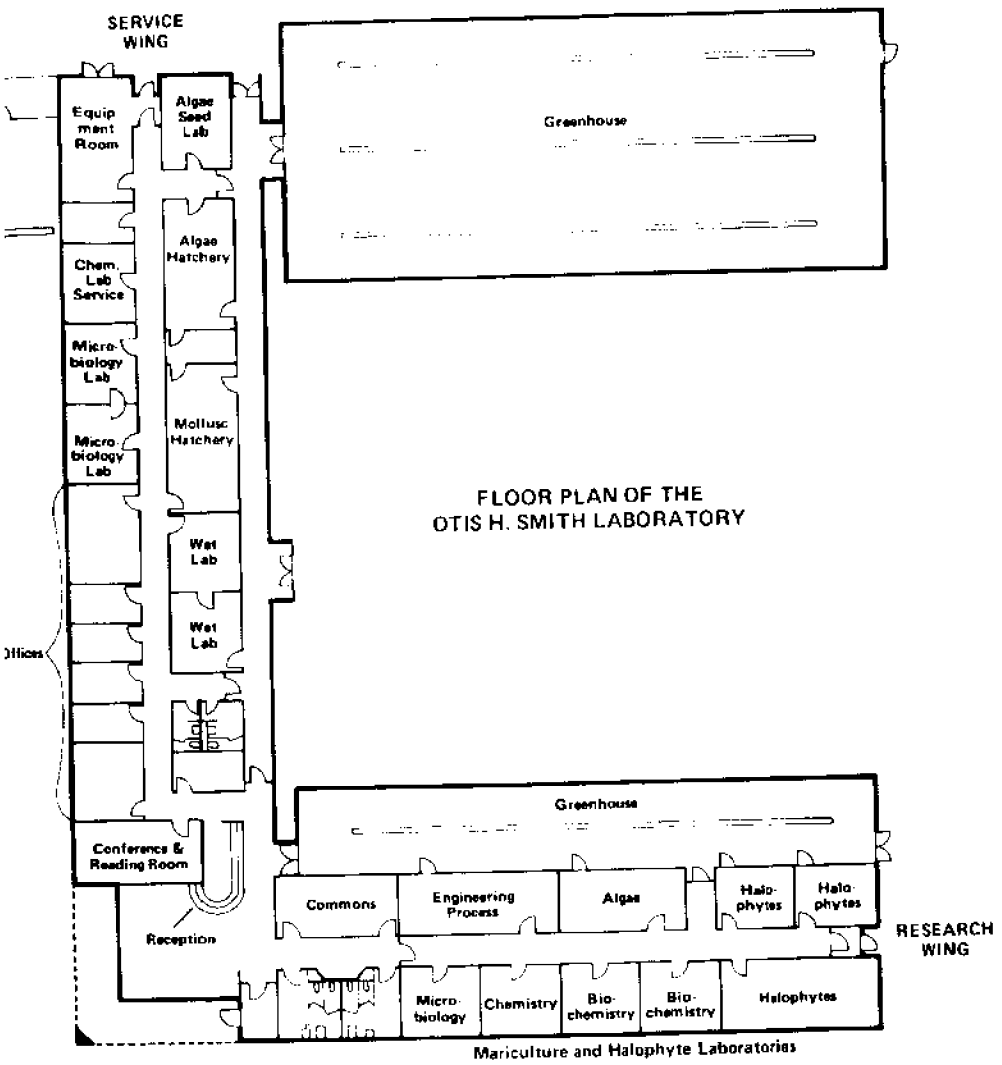


Figure 17. Plan of the Otis H. Smith Laboratory. The prototype is located in the 6,000-square-foot greenhouse. The algal, hatchery, chemical, microbiological, and research activities are housed in this L-shaped building.

Algae Laboratory

Phytoplankton have been known as a major source of bivalve nutrition since at least the turn of the century. As the University of Delaware's research on bivalve culture began to expand, it was recognized that routine algae production would be a necessary component for the maintenance of year-round research stock. Early in the program, modest quantities of algae were produced using techniques suited for small-scale research operations. When an old building was refitted for mariculture research in the early 1970's, an algae production room was an integral part of the plan. The operation of the production room followed the techniques described by R. R. L. Guillard[18] (1975) to produce algae up to the 20-liter culture size. As knowledge of feed requirements expanded and the need to maintain more research animals increased, larger algal supplies had to be provided. By 1976, 5-liter sealed carboys had been replaced by 150-liter open glass aquaria, and in 1978 the aquaria were superceded by 250-liter fiberglass (Kalwall®) tubes. The change in culture vessels, along with procedural changes made in Guillard protocol, presently allows one operator to routinely produce 1,500 to 3,000 liters of high quality algae per day. The cultures produced in this fashion may be any of several dozen species of interest and can be fed to a wide variety of filter feeders.

The role of the algae laboratory has thus evolved from a producer of small quantities of food stock for research animals to its current role as producer of larger quantities of high quality algae as prototype seed, for the hatchery, and for experimental work. Accordingly, the laboratory:

- Provides high quality seed cultures to support the prototype system;
- Provides nutrients to the prototype system;
- Provides algae of sufficient quantity and quality to support broodstock conditioning, larvae culture, nursery grow-out, and other hatchery operations;
- Maintains a reference collection of 22 algal species of potential interest to researchers (see addenda 1 and 2, at the end of this subsection);

- Provides algae, of sufficient quality and quantity, to CMS researchers for their experiments, and provides small stock seed cultures to researchers (University and commercial) who wish to initiate their own cultures, and

- Provides information and advisory services to those requesting assistance.

The art of algae production is well established, and its roots incorporate a good deal of scientific knowledge. One should periodically critically examine all procedures, whether in established protocols or shortcuts adopted consciously or even unwittingly. No procedure should be taken for granted. Many modifications may initially seem acceptable, but lead to subtle changes that affect the long-term quality of the algae. As new knowledge is gained about algal physiology, these techniques may be added to the routine, but only after replicated, controlled experiments on the scale where the changes will be made. Trials should extend over several culture cycles before final acceptance. Changes such as modifications to nutrient level, new types of nutrients, or percentage of harvest would fall into this class. To meet the need that a certain level of algae production be maintained (usually specified as a given number of cells of each species), it is generally necessary to allow for certain trade-offs. For instance, when large quantities of algae need to be produced in a semi-batch culture system, the cultures will not be axenic although levels of bacteria and contaminating algae will be acceptably low. However, when a given experiment protocol requires axenic cultures, extra precautions and time can be taken for the specific cultures.

FACILITIES

The algae production facilities are physically separated based on function. The seed laboratory was designed for small-scale seed production, transfers, and other routine maintenance work. The production laboratory was designed for large-scale production of algae.

The seed laboratory is located at the south end of the north-south wing of the Smith Lab (Figure 17). This location allows sunlight exposure similar to that in the greenhouse while isolating the room from casual traffic which may bring in unwanted contaminants. Ideally, the precautions taken in the operation of this room would be similar to those commonly practiced in microbiology laboratories.

The laboratory is designed with adequate bench space around the perimeter for work space. Specific space is included for a microscope, lighted incubator, laminar flow hood, sink, and desk. The remaining space allows for holding various pieces of equipment, for handling glassware, and setting up experiments.

Benches are covered with plastic laminate to allow ease of cleaning and disinfection. Cabinets are provided for storage of extra supplies. Four shelves line one wall over the bench. These plastic laminated shelves are backed by fluorescent lights so that flasks and test tubes of algae may be grown on them. A controlled-temperature, lighted incubator is used to hold stock tube cultures. Transfers are made from old cultures to fresh media in a laminar flow hood to lessen the possibility of airborne contamination. Greenhouse windows in the south wall allow natural photoperiod experiments to be conducted on a small scale. The sink, in addition to being used to wash glassware, also has a saltwater tap, a convenience in making media.

The production room is designed specifically for the production of large-scale algal cultures in fiberglass Kalwall® tubes. The laboratory is provided with two 8-foot shelves to hold 8-liter carboy seed cultures. The remainder of the laboratory has a 5-inch high platform to raise large tubes off the floor.

The laboratory is lined with six banks of dual 8-foot flourescent light fixtures horizontally mounted on 1-foot centers and encased behind sliding plexiglass doors to enable heat removal and protect the electrical components from the saltwater. Heated air from the lighting system is recirculated into the building. Lights in both labs are 40-watt cool-white fluorescent tubes. These lights put out >800 $\mu w/cm^2$ at the light surface and provide 450 to 600 $\mu w/cm^2$ over the entire culture surface. This arrangement provides enough light for cultures in 18-inch-diameter tubes. Special-spectrum lights do not offset their higher cost with apparent improved algae production. The lights and other electrical components are provided with ground fault interruptors to prevent electrical shock in a wet, highly conductive environment.

Both the seed and the production laboratories are provided with similar utilities. The laboratories have hot, cold, and ambient seawater. The hot seawater is supplied at 40°C and the cold at 10°C. This allows mixing of seawater to enable a wide variety of algae to be grown. Domestic freshwater is used for cleaning and washing tubes and floors. All seawater plumbing is polyvinyl coated (PVC) to prevent corrosion and allow easy modification. Room temperature control is provided by adjustments to the three heat-pump units for the two labs. Temperatures are kept at 18° to 20°C throughout the year. A high-temperature cutoff is not provided, as the labs are in constant use, and abnormal temperatures would be detected before the water mass temperature contained in the tubes became critical. Air and carbon dioxide are provided and plumbed in such a way as to provide a convenient outlet near every culture over 1 liter in volume. Carbon dioxide is mixed into the air supply at about 0.1 to 0.3 percent. The exact amount of CO_2 is determined by a flowmeter which is adjusted to keep cultures within the pH range of 6.6 to 7.9. The use of PVC pipes for the air system within the labs allows easy modification by laboratory personnel.

EQUIPMENT

The permanent equipment provided for the algae production laboratories has proven useful for the dual research and support activities.

The microscope is a basic part of the research and production equipment. Many types of microscopes are available, the most convenient being those with binocular eyepieces, 200x magnification, substage illuminator, and mechanical stage. The microscope used in the algae production lab is an Olympus KHC with a phase contrast attachment. This attachment provides a dark background with the cells standing out and brightly lit.

Algae cells are counted on an American Optical Improved Neubauer Hemocytometer, with a 0.1-mm-deep chamber. The hemocytometer is designed for enumerating blood samples, and has a total of nine 1-mm-square grids, each variously subdivided. Generally, one square millimeter, or a subsample of 300 to 400 cells, is counted. The number of cells per square millimeter is then multiplied by 10,000 to give the number of algae cells per milliliter. The hemocytometer is used in preference to an electronic particle counter. Hemocytometer counts take less than 5 minutes per sample, while providing visual information about culture health and contamination level. Counting algae cells is greatly assisted with the use of some type of tallying device. A hand tally with four digits has proven to be convenient.

A method for monitoring the pH of cultures is necessary for success in growing algae cultures. The preferred pH range is 6.5 to 8.5. The algae laboratory utilizes a Fisher Accumet model 520 pH meter with a single probe.

Sterile media are necessary for small cultures such as stock tubes. Autoclaving containers with up to one liter of media requires 250°F and 15 psi for 25 minutes. The autoclave used at the algae lab is an AMSCO, General Purpose, Model LB-20AS. This model has a large chamber capacity allowing the simultaneous processing of up to six 8-liter carboys.

A place to transfer algal cultures is necessary and should be as free from airborne contamination as possible. While small single-purpose rooms have been commonly used, the use of laminar flow hoods has proven to be an improvement. A Laminaire® 36-inch hood has proven to be convenient and to provide a sterile air flow. Media exposed to the flow for 20 minutes showed no sign of contamination.

A lighted incubator is used for holding stock seed cultures. These cultures are held at about 18°C under constant illumination. A Precision Scientific Company Freas 818 Dual Program illuminated incubator and a Precision Scientific model 805 retrofitted with three 2-foot cool-white fluorescent lights are used. Both allow the holding of stock cultures while buffering against changes in

the surrounding environment. Magnetic stirrers and teflon-coated stirring bars have proven necessary to rapidly dissolve large quantities of nutrients during their preparation. Stirrers are capable of mixing 20-liter containers. An assortment of stirring bars is useful in order to size the bar to the container. Several balances are available for the weighing of various salts used in the preparation of nutrient stocks. The most common balance used is a Mettler with a range of 0 to 1,000 grams, accuracy to 0.1 gram, and a tare of up to 300 grams. Another Mettler model is used to weigh vitamins to 0.01 gram and is accurate to 0.00001 gram. A small desk-top centrifuge has proven useful in concentrating algae samples. The centrifuge is a Clay-Adams DYNAC with horizontal trunnion cups for 8- to 15-mL test tubes. A tabletop vacuum pump made by Millipore Filter Corporation and capable of maintaining 38 centimeters of mercury is used to speed filtration of nutrients and media. A cart is used for moving material around the laboratory. The cart is inert to seawater and unaffected by hot glassware and media. Its weight capacity is on the order of 100 kg. Algae are grown in 300-liter fiberglass tubes made by Kalwall® Solar Corporation, Manchester, New Hampshire. These tubes are 18 inches (46 cm) in diameter, 6 feet high (1.83 m) and transmit about 90 percent of the light. These tubes may be purchased or constructed on site using fiberglass sheeting by Kalwall® or a similar product. The side seams are sealed with resin and held by clamps until dry. The bottom seam is then sealed with resin and fiberglass using nitrogen microbubbles as a filler in the resin to thicken it. Tubes have been in daily service for more than 5 years. Occasional minor damage caused by carelessness is easily repaired with fiberglass cloth and resin. A 1/4-horsepower magnetic-drive pump is used to distribute the algae to various holding tanks through 75 feet of 3/4-inch PVC feed line.

Screw-top test tubes (13 x 125 mm) are used for keeping stock seed cultures. These tubes, while disposable, may be reused if desired, although they are difficult to clean. Large standard test tubes (25 x 150 mm) are used for weekly culture transfers. These tubes contain 25 mL of culture, a quantity sufficient to seed larger vessels. They are lipless and easy to clean. One-liter Erlenmeyer or Florence flasks are used for the next step in culture size increase. These flasks contain 500 mL of media; to avoid confusion, each type of flask is used exclusively for one type of culture: Erlenmeyer flasks for diatoms, Florence flasks for flagellates. The largest glass culture vessels used are 9- and 20-liter Pyrex® carboys. These are used for producing seed for larger vessels. Normal culture volumes are 8 and 16 liters, respectively. Foam stoppers are used for plugging test tubes and flasks during autoclaving, and they remain through the culture phase. For the 9- and 20-liter carboys the foam stoppers are replaced at the culture phase by two-hole hard-rubber stoppers and glass tubing so that the carboys may be aerated. An assortment of pipettes is used for routine transfers. Pipettes may be disposable or reuseable, the latter requiring pipette cans. Sizes found

convenient range from 0.1 to 25 mL. Disposable petri dishes (100 x 15 mm) are used for solid or semi-solid substrate culture of algae. Thirty-milliliter plastic medicine cups are used for routine sampling of cultures. These low-cost disposable cups provide enough volume for both pH measurements and counting. They are available from medical supply catalogues. Also useful are microhematocrit capillary tubes. These are used for one-time sampling of cultures and loading hemocytometer cells for counting. Graduated cylinders of various sizes are used to measure nutrients. These may be of glass or unbreakable plastic. Vacuum flasks are used, along with a Buchner funnel, to filter media and nutrients. The flasks used have a 4-liter capacity with a 1-liter vacuum flask used as a water trap to protect the pump. Other laboratory ware includes test tube racks, plastic funnels, thermometers, wash bottles, assorted Tygon® tubing, hose clamps, screw clamps, dish pans, dropping bottles, marking tape, and indelible-ink pens.

CHEMICALS

A number of chemicals, mostly technical-grade salts, are used for algae culture. For ease, nutrient stock solutions are prepared to last about one week. This period minimizes contamination of the nutrients by bacteria and algae. Nutrients may be autoclaved and added aseptically to sterile media or to open culture vessels. Sufficient nutrients to fertilize 10,000 liters daily are kept on hand. Approximately 20 liters of each major nutrient stock solution are made weekly. The nutrients used are modified slightly from Guillard's media mainly in that the stock solutions are more concentrated. The quantities used and chemical makeup of the nutrients are still open to experimentation. Actual media levels of nutrients should be adjusted to suit the organisms under culture. Table 25 shows the nutrients used and the stock and final concentration of each in the media.

Two vitamin solutions are used: a combination of biotin, thiamine, and B_{12}, and a B_{12} solution. The B_{12} solution is used for diatom cultures while the vitamin combination is used for all other algae. Both solutions can be made more stable by slightly acidifying them with HCl, especially during autoclaving. Normally, enough stock solution is made up for one week. The B_{12} and biotin salt stock bottles are kept refrigerated in a desiccator. Thiamine need not be refrigerated.

Sodium nitrate is the usual nitrogen source although ammonium chloride and urea may also be used for some species. Sodium nitrate dissolves readily with mixing in 20 liters of distilled water. Sodium phosphate is readily soluble in distilled water, but the solution is susceptible to a slow-growing green algal contamination. The stock container should be washed between batches. This green algal contaminant does not grow well in seawater. Boric acid is used, although it is probably not required as a supplement to undiluted seawater. Boric acid use

Table 25. Phytoplankton Nutrient Media (Identified from Guillard's f/2 Formulation, 1975)

Components	Stock Solution		Algae		Final Media Concentration	
			Flagellates (mL Used)	Diatoms (mL Used)		
	(g/L)	(M/L)			(mg/L)	(μm/L)
Vitamins						
B_{12}	0.001	7.4×10^{-7}	--	--	0.5 μg	3.7×10^{-4}
Biotin	0.001	4.1×10^{-6}	0.5	--	0.5 μg	2.0×10^{-3}
Thiamine	0.2	5.9×10^{-4}	--	--	0.1 mg	0.30
B_{12} alone	0.002	1.48×10^{-6}	--	0.25	0.5 μg	3.7×10^{-4}
$NaNO_3$	150	1.76	0.5	0.5	75 mg	882.4
NH_4Cl*	106	1.98	--	0.25	26.5	495
Trace Metals						
Na_2·EDTA	8.7	0.0233	--	--	4.35	11.7
$FeCl \cdot 6H_2O$	6.3	0.0233	--	--	3.15	11.7 (0.65 mg Fe)
$CuSO_4 \cdot 5H_2O$	0.0196	7.85×10^{-5}	--	--	0.01	0.040 (2.5 μg Cu)
$ZnSO_4 \cdot 7H_2O$	0.044	1.53×10^{-4}	0.5	0.5	0.022	0.077 (5 μg Zn)
$CoCl_2 \cdot 6H_2O$	0.020	8.4×10^{-5}	--	--	0.01	0.042 (2.5 μg Co)
$MnCl_2 \cdot 6H_2O$	0.360	1.8×10^{-3}	--	--	0.18	0.091 (0.05 μg Mn)
$Na_2MoO_4 \cdot 2H_2O$	0.0126	5.2×10^{-5}	--	--	0.006	0.025 (2.5 μg Mo)
$NaH_2 \cdot PO_4$	20	0.145	0.25	0.25	5	36.23
H_3BO_3+	24	0.388	0.25	0.25	6	80.87
$Na_2S: O_3 \cdot 9H_2O$	60	0.211	--	0.5	30	105.6
TRIS**	121.14	1	4	4	484	400

*NH_4Cl is sometimes used in place of $NaNO_3$ for *Thalassiosira pseudonana*.

**Used only in media for stock cultures which are autoclaved pH adjusted to 7.6 to 7.8 with HCl. 500 mg of Tris are used /L of enriched seawater.

+H_3BO_3 is present in seawater, and probably not needed at higher salinities.

is limited to diatom cultures and all stock and seed cultures. Like phosphate, boric acid may become contaminated with a green alga, so frequent washings of the stock-solution container or initially autoclaving the solution is necessary. Sodium metasilicate is used in diatom cultures. Silicate salts dissolve only slowly in distilled water even when mixed vigorously. When the stock solution is added to seawater, a temporary precipitate

forms but disperses when the suspension is agitated. TRIS (trishydroxymethylaminomethane) is used as a pH buffer when autoclaving media. If omitted, a phosphate-silicate deposit forms in larger vessels, but it is apparently harmless to algae.

The trace-metal solution is the most complicated to mix. For convenience, the trace-metal mix is routinely made up 1.5 times as concentrated as the final stock solution, then diluted to the final stock solution concentration. The disodium-EDTA (ethylenediaminetetraacetate) is first dissolved in several liters of water, while the iron chloride is dissolved separately. The relatively insoluble acid form of EDTA may also be used if it is converted to disodium EDTA by adding 2 moles NaOH for each mole EDTA. This method may provide significant cost savings. Once both salts are in solution, the iron solution is added to the Na_2 EDTA solution. Once mixed, the highly concentrated metal stock solutions are added. The final metal solutions may be added separately or added as a mixture. To avoid precipitation they should be mixed in the following order: Cu, Co, Mn, Mo, Zn. These metal stock solutions are made up 1,000 times more concentrated than the major nutrient stock solutions for convenience.

Table 26 shows the amount of each salt used in making up each stock solution.

MEDIA PREPARATION

All media up to 8 liters in volume are autoclaved. Containers 500 mL and smaller have nutrients added prior to autoclaving. The 8-liter carboys have nutrients added just prior to seeding. The medium most frequently used is 30 ppt seawater with Guillard's f/2 enrichment for each type of algae; however, the f/2 recipe has been modified slightly for biological and economic reasons. Guillard[18] recommends the following nutrients: all vitamins (biotin, thiamine, B_{12}), nitrates, trace metals, phosphates, borates, and silicates. For flagellates, we omit the silicates in all cultures and the borates in cultures 8 liters and above. For diatoms we do not add biotin and thiamine. For the reference diatom, *Thalassiosira pseudonana* (3H), nitrates have been found to be too high, and phosphates and silicates too low. Thus, in the 250-liter production tubes, 10 to 20 percent extra phosphates and silicates are used, while nitrates are reduced by 50 to 75 percent. Nitrate reduction also seems to counteract the clumping often seen in 3H cultures.

Volumes up to 50 mL are autoclaved for 15 minutes. Flask containers 50 mL to 2 liters are autoclaved for 25 minutes, while 8-liter carboys are treated for 60 minutes. Prior to autoclaving, media to which nutrients and TRIS have been added are quickly vacuum-filtered through a coarse paper filter to remove any particulate matter.

Table 26. Mixing Directions for Preparation of Stock Solutions

Nutrient Solutions		
Nutrient	g/8L	g/20L
All Vitamins	0.008 g B_{12} 0.008 g Biotin 1.6 g Thiamin HCl	0.020 0.020 4.0
Vitamin B_{12}	0.016 g	0.04
$NaNO_3$	1200 g	3000
NH_4Cl	848	2120
Trace Elements	69.8 g EDTA 50.4 g $FeCl_3$	174.4 126
$CuSO_4$ $CoCl_2$	"	"
$ZnSO_4$ $MnCl_2$ $NaMoO_4$	8 mL of each stock solution from below	20 mL each stock
NaH_2PO_4	160 g	400
H_3BO_3	192 g	480
Na_2SiO_3	480 g	1200

Trace Element Stock Solutions		
Element	g/L	g/250 mL
$CuSO_4 \cdot 5H_2O$	19.6	4.9
$ZnSO_4 \cdot 7H_2O$	44.0	11.0
$COCl_2 \cdot 6H_2O$	20.0	5.0
$MnCl_2 \cdot 4H_2O$	360.0	90.0
$Na_2MoO_4 \cdot 2H_2O$	12.6	3.2

Media for use in 10-, 25-, and 500-mL cultures are prepared in 4-liter batches. Seawater is drawn, appropriate nutrients added, 484 mg TRIS added per liter, and the media mixed on a magnetic stirrer. The media are then filtered through a coarse paper filter (Whatman No. 4, 12.5 cm, or similar) and the filtrate collected. Aliquots are then added to test tubes or flasks and closed with screw caps, put on loosely, or foam stoppers. The media are then autoclaved at 121°C, 15 to 17 psi for 25 minutes. Once cooled the media may be used for 1 to 2 weeks.

The media for the 250-liter tubes are prepared by drawing 250 liters of seawater from the laboratory service lines. This water has been previously sand- and diatomaceous-earth filtered. Appropriate nutrients are added and an air line is installed. The air line consists of a 3-meter plastic air tube attached at one end to a PVC pipe fitting which serves as an anchor. The other end is hooked to an air valve in the CO_2-enriched air manifold.

Agar plates are sometimes used for maintaining seed cultures. These plates are prepared and handled exactly as plates are in microbiology laboratories. Seawater is drawn and the appropriate nutrients are added, with or without TRIS. The media is not filtered once mixed; 1 percent agar is added, and the mixture is heated and stirred until the agar is dissolved. When the agar is dissolved, the solution is poured into a flask (no more than half-filled), foam stoppered, and autoclaved. After autoclaving, the agar medium is cooled in a water bath to 55°C, and then poured into petri dishes. When gelled, the dishes are refrigerated until use. One liter is sufficient to fill about forty 100- x 15-mm petri dishes.

TRANSFERS AND SEEDING

Transfers and seeding are best done in a clean environment such as a laminar flow hood. The stock test-tube cultures are transferred monthly. As in all transfers, the procedures require the use of aseptic techniques. Stock transfers are made by removing 0.1-mL aliquots from the 1-month-old 10-mL stock culture and placing the aliquot in 10 mL of fresh media (see Table 27). Tubes are labeled prior to transfers to avoid confusion. The 25-mL cultures (working tubes) are transferred weekly. In this case, a 2.0-mL aliquot is removed from the week-old culture and placed in the fresh media. After being used as a source for the following week's transfers, the tubes may then be used for seeding flasks. The flasks may be the recipients of the 25-mL working-tube cultures or may constitute a separate seed line with flask-to-flask transfers. In the latter case, 25 mL of algae are removed from a flask and placed in a flask of fresh media. Such transfers take place every 2 to 5 days depending on species and culture conditions.

Table 27. Volumes of Algal Cultures Needed for Transfers and Seeding

Vessel Size	Transfer Volume to Start Culture of Similar Size	Seed Volume to Start Larger Culture	Time Until Ready to Use
10 mL	0.1 mL	--	2 to 4 weeks
10 mL	--	2 mL (to 25 mL)	1 week
25 mL	2 mL	--	1 week
25 mL	--	25 mL (to 500 mL)	2 to 4 days
500 mL	25 mL	--	2 to 4 days
500 mL	--	500 mL (to 8 L)	2 to 4 days
8 L	--	4 to 8 L (to 250 L)	2 to 4 days
250 L	--	250 L (to 1,000 L)	2 to 4 days

Agar plates may be used to maintain stock-grade algal cultures. These cultures are best initiated by beginning with a concentrated algal culture. Concentration is typically carried out by centrifugation. A portion of the pellet may then be transferred to the agar plate with the aid of a flamed inoculating loop. The plate is placed on a turntable, the loop placed in the center of the agar surface and moved readily outward as the turntable is slowly spun. This produces a spiral seeding pattern on the agar surface if the loop is not lifted. The plate is then turned upsidedown and sealed shut with parafilm or specially designed rubber bands. Plates seeded in this manner are capable of maintaining cultures for 4 to 8 weeks when held in a manner similar to the stock tubes.

HARVESTING

For the routine production of algae for feeding and experimental purposes, 250-liter tubes are used. The amount to be harvested is calculated from hemocytometer counts. When the cell concentration is known, the volume of algae to be harvested from the 250-liter tubes may be looked up in a table such as Table 28. The harvest values for each cell concentration are determined depending on the species, growth rate, and growth-curve shape as determined for a given laboratory and set of culture conditions. The cell concentration at which a species enters stationary stage growth is determined from growth curves for several cultures. Moving back along the exponential portion of the growth curve, the cell concentration 24 hours before stationary phase was reached is determined. This is the initial production concentration. The harvest volume chart may then be calculated so that after harvest and refill the initial production concentration is reestablished. For example, T-ISO (*Isochrysis* aff. *galbana*) grown at 20°C indoors is expected to double once each day, and reach a stationary phase, or at least a very reduced growth rate, at above 4×10^6 cells/mL. Thus, a 50-percent harvest is indicated to produce an initial production concentration of 2×10^6 cells/mL. The amount to be harvested for several species can be determined from Table 28. Tube cultures (250-liter) can generally be harvested seven or more times over a 7- to 10-day useful culture life. By the end of the period, a reduced growth rate becomes evident along with increased levels of contamination by algae and bacteria.

CLEANING PROCEDURES

Screw-top test tubes are considered disposable and are generally discarded after use. The 150- x 25-mm test tubes (that contain the 25-mL cultures) are always reused, and are quite easy to clean. To reuse these tubes, the contents are drained, and the tubes are soaked in soapy freshwater for a period of time. The tubes are then rinsed in tap water, brushed out, rinsed again, and soaked in 10-percent HCl. The tubes are then rinsed thoroughly

Table 28. Harvest Schedule — Liters to be Harvested

Cell Count X 10^6	Actin	*Dunaliella Chroomonas*	*3H Chaetoceras*	*Skeletonema*	T-ISO	ISO
0.1	100	--	--	--	--	--
0.15	125	--	--	--	--	--
0.2	150	--	--	--	--	--
0.25	160	--	--	--	--	--
0.3	180	--	--	--	--	--
0.35	--	--	--	--	--	--
0.4	--	0	--	--	--	--
0.45	--	30	--	--	--	--
0.5	--	50	--	--	--	--
0.55	--	70	--	--	--	--
0.6	--	80	--	--	--	--
0.65	--	90	--	--	--	--
0.7	--	110	--	0	--	--
0.75	--	120	--	50	--	--
0.8	--	125	--	50	--	--
0.9	--	140	--	80	--	--
1.0	--	150	--	80	--	--
1.1	--	--	--	80	--	--
1.2	--	--	--	100	--	--
1.3	--	--	--	100	--	0
1.4	--	--	--	100	--	50
1.5	--	--	0	125	--	60
1.6	--	--	80	140	--	70
1.7	--	--	90	150	--	75
1.8	--	--	100	160	--	80
1.9	--	--	110	160	--	90
2.0	--	--	125	170	0	100
2.1	--	--	130	180	50	110
2.2-2.3	--	--	140	190	50	120
2.4-2.6	--	--	150	190	80	130
2.7-2.9	--	--	160	190	100	140
3.0-3.2	--	--	170	200	100	150
3.3-3.8	--	--	180	200	125	160
3.9-4.1	--	--	190	200	125	170
4.2-4.5	--	--	190	200	130	180
4.6-4.8	--	--	200	220	140	180
4.9-5.2	--	--	200	220	150	190
5.3-5.9	--	--	210	--	160	190

Standard Harvest: 100 L — *Isochrysis* (ISO, T-ISO), *Chroomonas, Dunaliella*
125 L — *Thalassiosira weisflogii* (Actin), *Chaetoceras*
150 L — *Skeletonema*
170 L — *Thalassiosira pseudonana* (3H)

in tap water followed by a distilled water rinse. The test tubes are then placed upsidedown in a test tube rack to dry before use. Flasks and carboys are cleaned in a manner similar to test tubes, except that instead of soaking them in soapy or acidified water, the vessels are filled with the solution.

The 250-liter test tubes are cleaned by first emptying the contents then laying the tube on its side and hosing out the interior to remove loose algae. Next the tube is set upright and brushed out with soapy water. Then it is hosed out with freshwater. If necessary the tube may then be placed back in service. However, it is best to fill the tube with freshwater, or acidified (100 mL HCl per 250 L) freshwater and allow it to stand until needed. When needed, the tubes are drained, rinsed, and filled with seawater.

The feed line is flushed daily with hot freshwater. Periodically, a 5-percent hypochlorite solution is pumped into the line to remove organic matter. The feed line is then flushed thoroughly with hot freshwater.

ADDENDUM 1--ALGAE COLLECTION

	Obtained from:
Division Haptophyta	
Class Haptophyceae	
Order Isochrysidales	
Family Isochrysidaceae	
Isochrysis galbana Parke	Guillard, WHOI (1976, 1977, 1978, 1979, 1981)
(strain G, strain O, strain N, strain A, strain B)	Syd Kraul, Kahuka Farms, HI
Isochrysis aff. *galbana* (Green)	G. Krantz, 1977
(T-ISO, high temperature)	
(T-ISO, strain B axenic)	Guillard, 1981
Order Pavlovales	
Family Pavlovaceae	
Monochrysis lutheri Droop	Guillard, WHOI (1974)

Division Chlorophyta	
Class Prasinophyceae	
Order Pyramimonadales	
Family Pyramimonadaceae	
Pyramimonas sp. (Pyr-1)	Guillard, WHOI (1976)
Pyramimonas sp. (Pyr-2)	
Order Prasinocladales	
Family Prasinocladaceae	
Tetraselmis suecica	Guillard, WHOI (1975)
Tetraselmis tetrahelí	Martin, Tahiti (1979)
Order Volvocales	
Family Dunaliellaceae	
Dunaliella tertiolecta (strain A, strain B)	Guillard, WHOI (1977)
Dunaliella bardawil (high salinity)	ATCC, 1981
Division Chrysophyta	
Class Bacillariophyceae	
Order Bacillariales	
Family Thalassiosiraceae	
Skeletonema costatum	Guillard, WHOI (1978)
Thalassiosira pseudonana (3H) (strain 3HF, strain G 3H, strain N3H, strain A, strain B)	Guillard, WHOI (1974, 1977, 1979, 1981)
Thalassiosira pseudonana (13-1)	
Thalassiosira weisflogii (Acti)	Guillard, WHOI (1979)
Family Cymbellaceae	
Phaeodactylum tricornutum (mixed strain)	L. Provasoli, (1972)
(fusiform strain)	Guillard WHOI (1978)
Family Chaetoceroceae	
Chaetoceros calcitrans	
(strain VI)	K. Haines, St. Croix (1978)
(strain ISE)	International Shellfish Enterprises (1978)
Chaetoceros gracile	Law, Univ. Of Hawaii, 1981
Division Pyrophyta	
Class Cryptophyceae	
Order Crytomonadales	
Rhodomonas spp.	Guillard, 1980
Chroomonas salina (strain A, strain B)	Guillard, WHOI, 1980, 1981

Unidentified Species

Marine chlorella	Persoone, Belgium, 1977
Va-12 *pseudo-isochrysis*	Sutton, VIMS, 1976
Va-52 small green flagellate	Sutton, VIMS, 1976
Chaetoceros CH-4	Syd Kraul, Hawaii, 1979
Chaetoceros CH-1	Syd Kraul, Hawaii, 1979

The classification system used was taken from: Parke, Mary, and Peter S. Dixon (1976). Checklist of British Marine Algae - Third Revision. Journal of Marine Biological Assoc. of the United Kingdom, 56: 527-594.

Strains listed are not true strains, but rather are laboratory designations for isolates of the original clone which have been received by the laboratory at various times.

All cell lines are maintained in 10-mL liquid cultures. Most, but not all, cell lines are axenic. The media is 30-ppt seawater, with Guillard's f/2 enrichment.

ADDENDUM 2--ALGAE WEIGHT, CARBON, NITROGEN, AND C/N RATIO

Algae	Dry Weight per Cell (g)	Carbon per Cell (g)	Nitrogen per Cell (g)	C/N Ratio
3H	1.32×10^{-11}	0.829×10^{-11}	1.51×10^{-12}	5.49
	"	0.924×10^{-11}	1.54×10^{-12}	6.00
	"	0.932×10^{-11}	1.56×10^{-12}	5.97
Isochrysis (used for T-ISO)	2.35×10^{-11}	1.41×10^{-11}	1.69×10^{-12}	8.34
Tetraselmis suecica	1.066×10^{-10}	8.46×10^{-11}	6.85×10^{-12}	12.35
Pyramimonas	"	10.0×10^{-11}	1.33×10^{-11}	7.54
	"	9.12×10^{-11}	1.12×10^{-11}	8.14
Skelatonema costatum	"	1.09×10^{-11}	1.92×10^{-12}	5.67
	"	0.97×10^{-11}	1.41×10^{-12}	6.87
Chaetoceras calcitrans	"	4.35×10^{-12}	0.648×10^{-12}	6.71
	"	2.99×10^{-12}	0.56×10^{-12}	5.34

Hatchery

The hatchery in Smith Laboratory is used to cultivate oyster (*Crassostrea virginica* Gmelin) and clam (*Mercenaria mercenaria*) seed throughout the year and is active in various aspects of the reproduction and propagation of these and other bivalve species.

The principal responsibility of the hatchery is the production of oyster spat of approximately 10 mm in shell height (dorsal-ventral axis) which are used to stock the prototype shellfish culture system. Hatchery equipment, facilities, and technical assistance are also available to staff and students at the University of Delaware and on a limited basis to students and staff at other academic institutions with programs of study in marine science. In addition the hatchery participates in research directed toward improving existing hatchery technology and methods for larval and post-larval cultivation of molluscs.

DESIGN AND FUNCTION

The hatchery occupies 825 square feet of laboratory space and can accommodate all phases of bivalve reproduction ranging from the conditioning of broodstock to post-larval/nursery culture. Figure 17 shows the hatchery area. Figure 18 illustrates the major components of the hatchery process. Oysters and clams used for broodstock or for experimental purposes are maintained in holding tanks in two rooms located adjacent to the hatchery. Seawater temperature in these rooms is kept at 21°C and 15°C in order to encourage conditioning and post-spawning rehabilitation, respectively. All other aspects of bivalve propagation take place in the hatchery, which has sufficient space available for spawning, larval development, setting, and post-larval culture. Seawater in the hatchery can be delivered at temperatures between 20° and 30°C and is routinely maintained at 26° to 28°C for larval and spat culture. The hatchery is set up with a minimum amount of fixed or permanently mounted equipment. The portable nature of most of the tanks, stands, and bench/storage areas permits considerable flexibility in the use of limited laboratory floor space for a variety of hatchery-related activities.

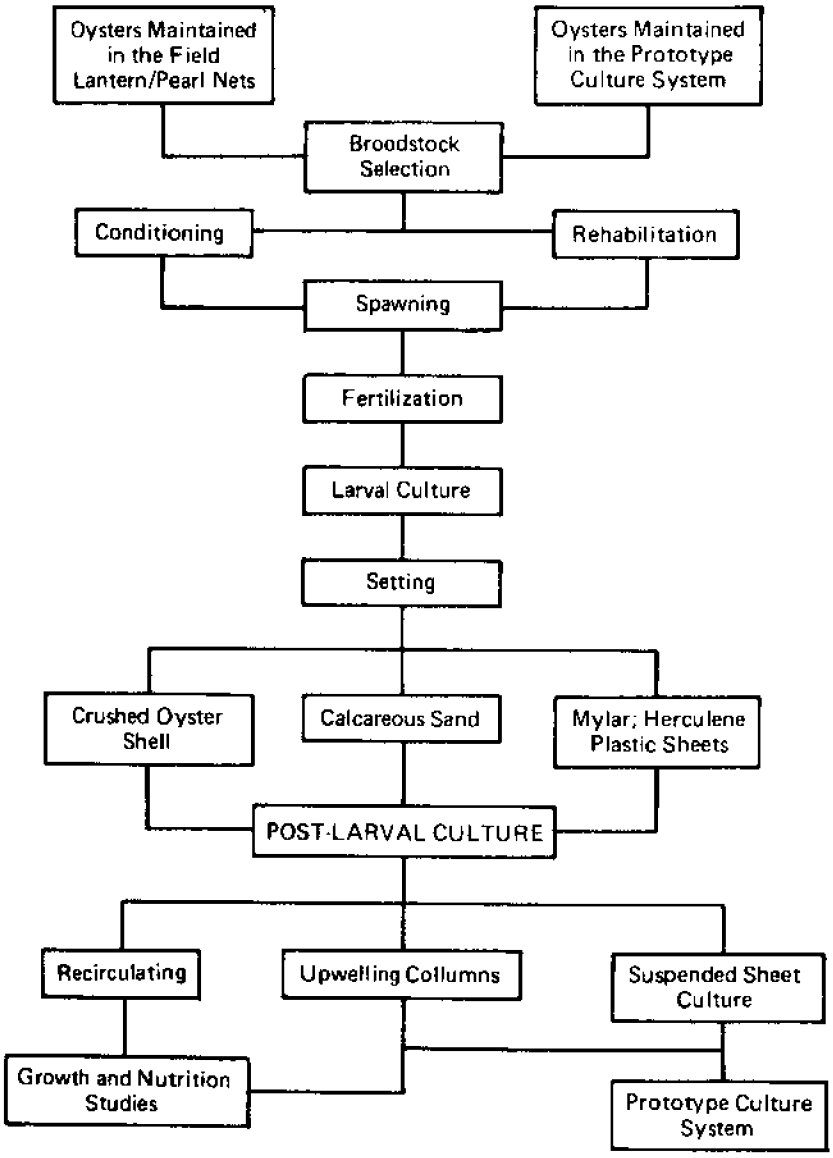


Figure 18. Flow diagram of the hatchery processes.

BROODSTOCK MANAGEMENT

The propagation of oyster seed in the hatchery occurs throughout the year with new broods of larvae produced at approximately 6-week intervals. Oysters used as broodstock are obtained from local oyster beds and periodically from the prototype culture system. Smaller sized sexually mature oysters (≅ 45 grams, wet weight) are preferred as broodstock because a larger number of individuals can be maintained within a limited holding tank space and can be provided an adequate algal food supply.

Broodstock animals are maintained in two separate holding tanks that differ only in the temperature of the seawater. Oysters that are to be conditioned (ripened to produce eggs and sperm) are held at a temperature of 21°C. This temperature encourages the development of gonads and the formation of gametes, and artificially simulates the temperature conditions characteristic of the natural reproductive season in the Mid-Atlantic region (late spring and summer). Oysters held at this temperature and supplemented with an algal diet produce viable gametes in approximately 8 weeks (Price and Maurer, 1971)[22]. An additional broodstock tank is maintained at 13° to 15°C; it serves a dual function. The cooler water simulates conditions in the field characteristic of late fall and winter. It provides an environment for recently spawned oysters to complete the reproductive cycle by readsorbing gonadal material and accumulating energy reserves (glycogen). Broodstock oysters are generally transferred from the conditioning tank to the rehabilitation tank after a maximum period of 12 weeks in order to provide an optimum conditioning period (Lannan et al. 1980)[23]. These oysters are allowed a period of 12 to 15 weeks to fatten (develop glycogen reserves) prior to reconditioning.

The additional advantage of holding broodstock animals at 13° to 15°C is that the development of gametes of unripened or partially ripened oysters can be delayed during the normal field reproductive season. Oysters maintained in this way can then be conditioned at a later date for spawning throughout the fall and winter months.

Broodstock oysters in both the conditioning (21°C) and rehabilitation (13° to 15°C) tanks are fed an algal diet consisting of equal quantities by cell count of the diatom *Thalassiosira pseudonana*, clone 3H, and a tropical flagellate *Isochrysis* aff. *galbana*, clone T-ISO. The oysters are fed on the basis of the number of cells that they are capable of clearing per day. In order to avoid underfeeding, the biomass of the broodstock (grams live whole weight) is periodically determined, and the daily quantity of food needed is calculated from algal feed ration equations. These expressions relate the number or total weight of algal cells cleared each day per gram of live

oyster whole weight and serve as a reference or guideline in determining the approximate quantity of algal cells required for maintenance and growth. Two useful ration equations which are based on laboratory feeding experiments are:

$$Y = 8.2\ (x)^{-0.21}$$

where Y = number of algal cells x 10^8/gram live oyster whole weight/day and

X = individual oyster live whole weight in grams (Pruder, Bolton, and Faunce 1977),[17] and

$$R/W = 0.01W^{-0.41}$$

where R = daily algal ration (grams dry weight) and

W = oyster live whole weight in grams (Epifanio and Ewart 1977)[6]

Algal food is delivered to the oysters through a combination of batch and continuous feeding methods. Each day a storage container adjacent to the tank is filled with a sufficient volume of algae necessary to satisfy the estimated daily ration requirement. Algal cells are added to the holding tank at an initial concentration of 2.0×10^5 to 3.0×10^5 cells/mL. Additional quantities of algae are added to the tank periodically throughout the day as the cells are cleared from suspension. At the end of the work day the remaining ration is metered into the tank by gravity feed, thus providing a constant supply of food throughout the evening.

The seawater used in the hatchery and broodstock areas is passed through sand- and diatomaceous-earth filters prior to use. Seawater in the holding tanks is recirculated by a 1/4-horsepower electric pump which produces a flow rate of 20 liters/minute (Figure 19). On alternate days the water in the tanks is drained, and the animals and tanks are scrubbed clean of algae, fecal material, and epifauna. The tanks are then refilled with clean filtered seawater.

In addition to laboratory culture facilities oysters are also held in the natural environment (Broadkill River estuary) in lantern nets and pearl nets (Figure 20). During the natural reproductive season (late spring and summer), some oysters are transferred from the lantern nets to the conditioning tank for eventual spawning while others are held in cooler water (13° to 15°C) to delay gametogenesis and to provide ripe individuals for subsequent spawning in the fall and winter months.

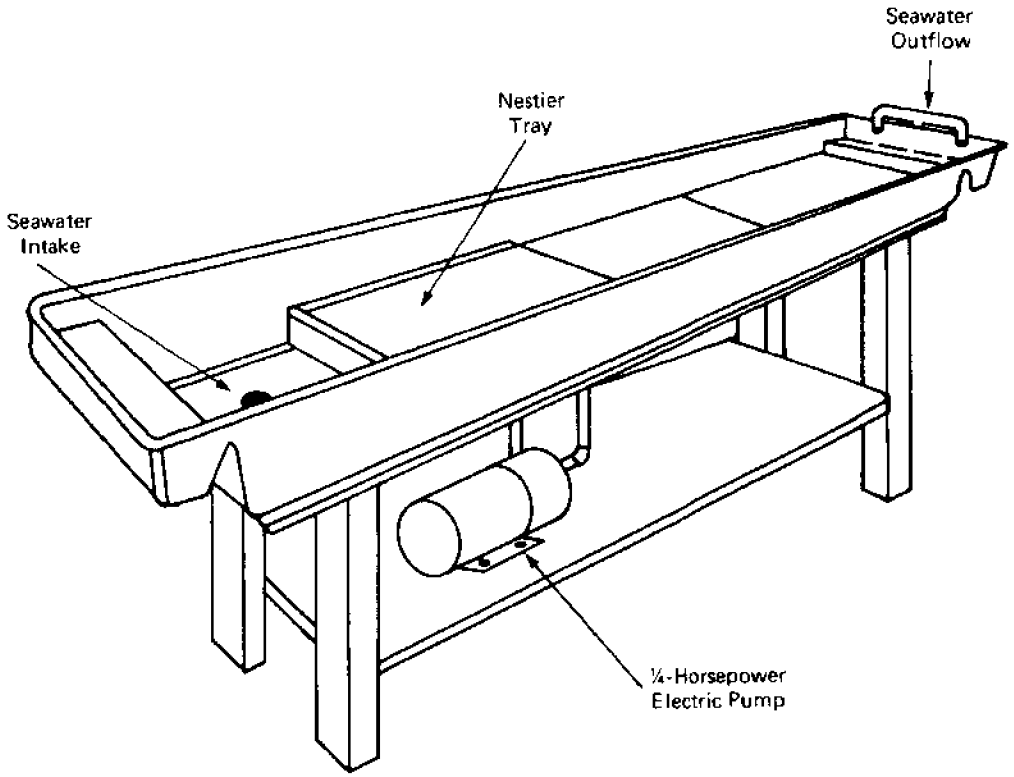


Figure 19. Typical flume arrangement for hatchery cultivation of bivalves.

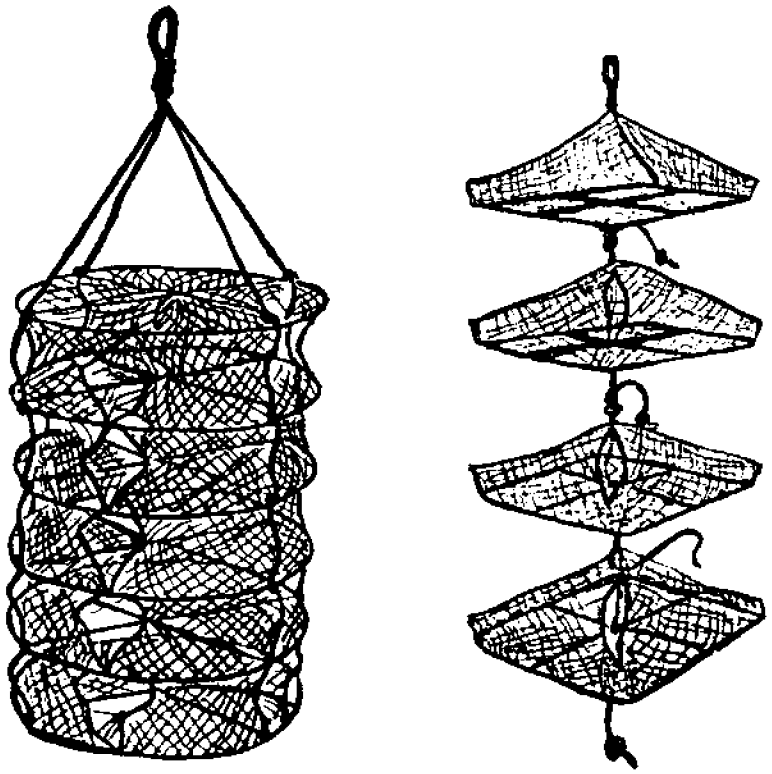


Figure 20. Lantern (left) and pearl (right) nets for field rearing of bivalves.

SPAWNING

Oysters selected for spawning are removed from the conditioning tank (21°C) and placed in 25°C seawater for an hour. A very small amount of algal cells is added to help encourage pumping. After it has been determined that most of the oysters are actively pumping, the seawater is drained from the tank and replaced with clean filtered seawater which has been heated to a temperature of 29° to 30°C. Seawater temperatures in excess of 30°C are avoided because of potential adverse effects on eggs and sperm viability as well as physiological strain to the broodstock oysters. The shocking effect of the increase in water temperature on the oyster often initiates spawning.

It is usually necessary to drain the water in the spawning tank when it cools to 27°C and to refill it two or three times with heated water before spawning is initiated. The cyclic nature of the drain refill process appears to enhance the temperature-shock technique. In addition, small amounts of sperm obtained by stripping ripe individuals may be added to the water to further encourage spawning. Once an oyster begins to release gametes it is transferred to a finger bowl filled with filtered seawater and allowed to spawn to completion. Generally all eggs are combined and kept separate from similarly treated sperm until fertilization is desired. Once enough gametes have been obtained, the spawning tank is drained, and all the broodstock animals are thoroughly washed with freshwater to remove any discharged gametes. These animals are also quarantined overnight in a separate holding tank prior to return to either the conditioning tank (21°C) or the rehabilitation tank (15°C) as an additional precaution against inadvertent spawning in the holding tanks.

FERTILIZATION

Released eggs are combined in a 20-liter battery jar and diluted with filtered seawater. Fertilization is accomplished by adding small quantities of sperm (5 to 10 mL of sperm per 100 liters of egg suspension; $\simeq$ 25 sperm/ egg) to the egg suspension. Subsamples of the fertilized eggs are then observed for the first hour after fertilization and the percentage of fertilized eggs is noted. If there is evidence of incomplete fertilization, additional small quantities of sperm are added to the egg suspension. Care is taken not to add excessive amounts of sperm in order to avoid polyspermy. Additional examinations of fertilized eggs are made periodically to check for polar body formation and the early stages of cleavage. Once it is determined that early development is proceeding normally, the embryos are counted and moved to the larval rearing vessels at an initial stocking concentration of 30 larvae per milliliter. The suspension is counted by drawing replicate 1-mL subsamples from a uniformly distributed volume of eggs with an automatic or blow-out-type pipette. The total

number of fertilized eggs can then be determined, and the dilutions required to attain initial stocking densities in the larval rearing vessels are made accordingly. No algal food is added during the first 24 hours of development as the embryos undergo the transition from trochophore to veliger larvae.

LARVAL CULTURE

In the 24-hour period following fertilization the oyster embryos develop into straight hinge or D-stage larvae. As the larvae grow they pass through five stages of development in 14 to 18 days culminating in mature pediveliger larvae which make the transition from a planktonic to a sedentary mode. Seawater temperature during the 2-week larval period is maintained at 26° to 28°C. Initial larval stocking densities of 30 larvae per milliliter are gradually reduced to 1 to 3 larvae per milliliter at setting. Larval numbers are reduced by a process of size selection. Stainless and Nitex® sieves of several different mesh sizes are used to separate larvae into arbitrary size classes. The largest or fastest growing larvae are retained, and the slower growing larvae are discarded. Adjustments to the stocking density and/or size frequency distribution of the larval population are made on alternate days during water changes.

The larvae are fed the same algal diet as the broodstock. A diet consisting of 3H and T-ISO are batch fed daily at cell concentrations which generally increase from 3.0×10^4 to 4.0×10^4 cells/mL 24 hours after fertilization to 1.0×10^5 to 1.5×10^5 cells/mL at setting. Care is taken to ensure that the larvae are not underfed by monitoring the rate at which the cells are removed from suspension as well as observing larval vigor and the quantity of food material present in the gut.

SETTING

Approximately 2 weeks after fertilization the oyster larvae attain a size of 250 to 300 µm and are often observed grouped together in swirling aggregates at the surface of the rearing vessel. Mature pediveliger larvae, characterized by the presence of an eye spot and a foot, are transferred from the larval rearing vessels to setting tanks. Nearly all oyster larvae produced in the hatchery are set using cultchless techniques. This involves the use of substrates such as plastic sheeting (Mylar®; Herculene®) or small particles of crushed shell and calcareous sand. In general, the crushed-shell or calcareous-sand method is used when large numbers of spat are required. Crushed oyster shell of approximately 1 mm in size is evenly distributed on the bottom of a holding tank or other suitable container. Oyster larvae attach to the individual shell chips and eventually grow larger than the substrate thus becoming an

individual or cultchless juvenile oyster. Calcareous sand grains (calcium carbonate) ranging in size from 500 µm to 1 mm are also used as a substitute for crushed shell.

Smaller quantities of oyster spat are set using a technique developed by Dupuy and Rivkin[24] (1972). Eyed larvae are transferred to setting trays which measure 45 x 57 x 10 centimeters deep. Plastic sheets of drafting paper (Mylar®; Herculene®) are placed in the bottom of the trays and serve as the substrate for larval settlement.

In general, the process of metamorphosis or setting for most oyster larvae occurs over a relatively short period of time (24 to 48 hours) after the eyespot has developed. Setting larvae are fed algae at the same level as during the latter stages of larval development. 3H and T-ISO are batch fed in equal amounts at a cell concentration of 1.0×10^5 to 1.5×10^5 cells/mL. Seawater in the tanks or containers used for setting is replaced daily. Larvae that have set within the first 48 to 72 hours are retained for nursery culture. Seawater temperature during setting is kept at 26° to 28°C.

POST-LARVAL CULTURE

Three different methods are used in the hatchery for the post-larval or nursery culture of oyster spat. Although differences exist in the individual ways the spat are handled, the algal diet and methods of administering it to each culture scheme are the same. A two-part diet of 3H and T-ISO is gradually increased in the nursery tank from 2.0×10^5 to 5.0×10^5 cells/mL during the 4- to 5-week post-larval growth period. Batch feeding is eventually switched over to continuous gravity feed as the biomass and consequently the demand for food in the nursery tank increases.

The three post-larval culture methods used in the hatchery include: a recirculating system used in association with crushed shell or calcareous sand as cultch, spat culture on plastic sheets, and spat culture in upwelling columns.

Recirculating System

Oyster larvae that have settled onto crushed shell or calcareous sand are nursery cultured in 400-liter fiberglass tanks. Seawater is recirculated within the tank once setting is completed by a small 1/20-horsepower electric pump. The newly settled spat remain in the recirculating tank for 4 to 5 weeks until they have grown to a shell height of 3 to 5 mm and are larger than the cultch material (0.5 to 1.0 mm). At this point a separation process is initiated whereby all of the cultch is passed through an appropriately sized sieve. Oyster spat larger than the cultch material are retained on the sieve while smaller

spat pass through the sieve and are returned to the setting/nursery tank for an additional period of growth. Spat are separated out from the cultch material weekly and are transferred to Nestier® trays.

Plastic Sheets

Oyster larvae that have set on plastic sheets are nursery cultured *in situ* on the sheets or are removed to upwelling columns. The former approach follows the technique developed by Dupuy and Rivkin[24] (1972) in which the sheets with newly settled spat are mounted in plexiglass frames and suspended vertically in a square 400-liter fiberglass tank. The spat are maintained in the tank for a period of 4 weeks during which time they increase in size from approximately 350 µm to 5 to 10 mm in shell height (hinge to ventral margin). At this size they are easily removed from the plastic substrate and are transferred to Nestier® trays for growout. Spat cultured by this method are supplied as seed oysters to the prototype culture system and are also used as experimental animals in various kinds of feeding and growth studies.

Upwelling Columns

The third post-larval culture method uses upwelling columns to maintain recently settled larvae until they are large enough to be handled and transferred to Nestier® trays. Oyster spat which have set on plastic sheets are scraped off with a razor or other sharp blade within 24 hours after settlement or before permanent attachment to the substrate has occurred. These very small spat (≅ 300 to 350 µm) are then transferred to upwelling columns (Figure 21) made of acrylic tubing sealed off at the bottom by Nitex® screen of various mesh sizes. Seawater is recirculated between a reservoir and the column tank by a small-capacity electric pump. An overflow line in each column or tube returns seawater to the reservoir at a lower level than the column tank overflow. This small hydrostatic pressure difference is sufficient to cause the seawater to flow from the column tank up through the plastic tube or column before it is returned to the reservoir, creating a gentle vertical flow. Newly settled spat are gently suspended in the upwelling current; they feed upon algae which is added to the reservoir using batch and continuous-drip feed methods. Flow rates in the upwelling columns are regulated by the diameter of the plastic tubing used and by clamping the overflow line which returns seawater to the reservoir.

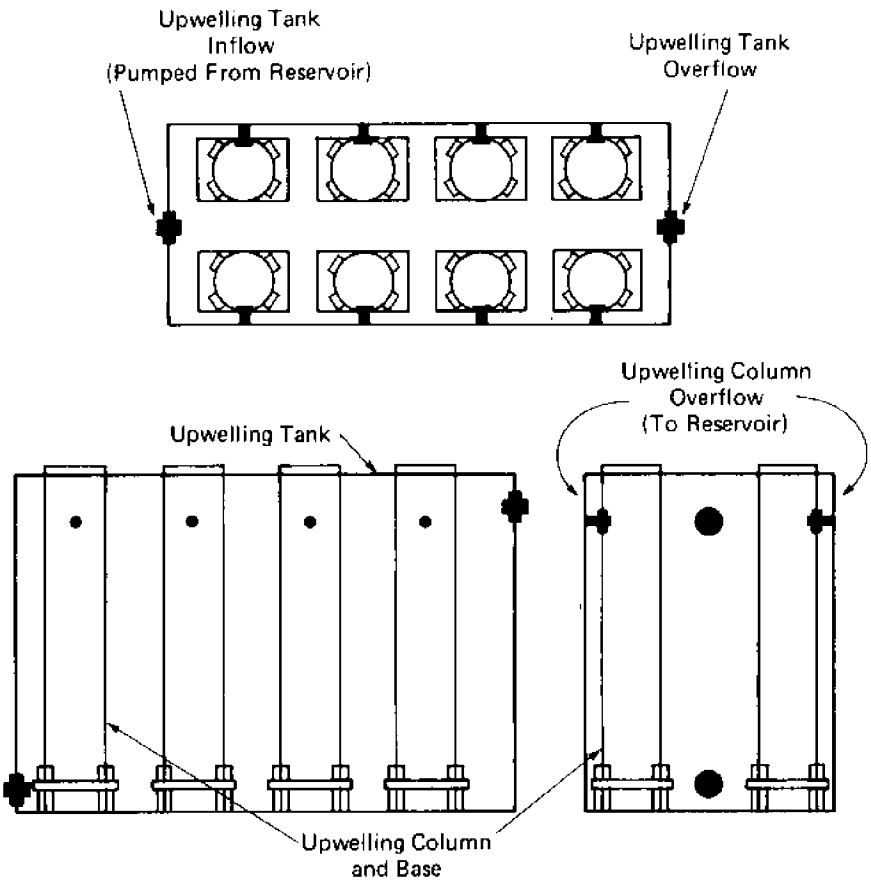


Figure 21. Upwelling device for laboratory rearing of juvenile bivalves.

Chemistry

Natural seawater varies in chemical composition, and such variations may influence the well-being of organisms being cultured in it. While we have attempted to assure minimal variation by using an ocean inlet source, we have also chemically monitored the seawater quality throughout the Otis H. Smith Laboratory and the mariculture activity routinely, as well as by request.

ROUTINE ANALYSES

Routine analyses follow the procedures in Strickland and Parsons' A Practical Handbook of Seawater Analyses, 1972, Fisheries Research Board of Canada, Second Edition. This manual, which is the standard for seawater analyses, describes each method in detail with precision and accuracy figures, method interferences, helpful hints, data calculations, temperature requirements, etc. The nitrite, nitrate, phosphate, and silicate methods are standard wet chemical analyses. These methods are mainly for the open ocean water samples in which the levels of nutrients are very low. Since our nutrient levels are much higher than those described in the Strickland and Parsons Handbook, we have developed and tested our own dilution techniques for each nutrient. Nitrite and silicate are salinity sensitive. Therefore all dilutions and blanks for these are made with the artificial seawater mixes specified in the handbook for standards and substandards. The nitrite and phosphate methods are not affected by salinity, and samples, standards, and blanks are made with distilled water. Samples analyzed for these four parameters are colorimetrically measured on the Bausch and Lomb Spectronic 20. The pH and total alkalinity methods are taken directly from the handbook, and there are no deviations from the standard method.

Ammonia is determined by the "standard addition method" with an Orion ammonia electrode. The method was developed here at the College of Marine Studies in 1973 and is discussed in detail in The Use of Ion Specific Electrodes for Chemical Monitoring of Marine Systems - Part I by Srna et al. (1973). The electrode is used with a Fisher Accumet millivolt meter that can be read to $\pm$ 0.1 mv. Samples are analyzed in a 25°C water bath and slowly stirred with magnetic stirrers. Ten-molar sodium hydroxide for adjusting pH to >11 is dispensed with an automatic pipette. The ammonium chloride used for standards is dried and stored in a

dessicator. The 0.1 M NH_4Cl standard is made fresh weekly in distilled water. The substandards, usually 10^{-3} M and 10^{-4} M are made daily. Before each daily run, an electrode check procedure is performed to insure that the electrode is operating properly. After the check, samples are run immediately to avoid any possible changes in concentration. Sample size can range from 2.5 to 100 mL, and volatile amines are the only known interference. The time required for each sample is dependent upon the sample concentration. Low levels of ammonia ($<1x10^{-5}$ M) may take as long as 20 to 30 minutes, whereas high concentrations may only take several minutes. The only maintenance required on the electrode is the changing of the hydrophobic membrane and addition of fresh internal filling solution. The cost of the electrode is under $300 and each part (membranes, O-rings, filling solution, internal probe) is available separately.

Calcium is determined with an ion exchange type electrode from Orion. The method, using standard addition techniques, is one developed by Orion for specific use in seawater. It is used with a single junction electrode filled with 4 M KCl, and both are connected to a Fisher Accumet millivolt meter. This calcium method, like the ammonia method, is temperature sensitive. The samples are run in a 25°C water bath and slowly stirred so that no vortex is created. The reagents required are a standard 0.1 M $CaCl_2$ solution, which is available from Orion, and ISA (ionic strength adjustor - 4 M KCl). A daily electrode check is performed on an artificial seawater sample that is prepared with a concentration of calcium approximately equal to that of the samples to be analyzed. Samples, which can range in size from 3 to 100 milliliters, are run immediately after the performance check. Since we work in the 10^{-2} M to 10^{-1} M range, the time required for each sample is usually 1 to 2 minutes. The only maintenance required on these probes is the internal rinsing of the reference electrode. The calcium module life is about 6 months, although we have used one as long as 18 months with no problems. The cost of the calcium electrode is approximately $300 and the reference electrode $90. The module is available separately for $100.

Data from the routine analyses are tabulated, logged, and distributed to the appropriate staff at the end of each analysis day. A notebook of graphs on all the parameters is maintained for use by principal investigators.

OTHER ANALYSES

The chemical service laboratory is also equipped to determine carbonate alkalinity, total carbon dioxide, bicarbonate, carbonate, dissolved oxygen, salinity, sulphide, copper, magnesium, chlorine, amino acids, suspended solids, and boron. These are handled on a request basis. Specialized chemical analyses are also available upon request and are thoroughly tested with standards similar to the expected levels of the chemical being tested. A Perkin-Elmer

elemental analyzer is used to determine carbon, hydrogen, and nitrogen on lyophilized seawater, particulates, liquids, and dried materials. It is described in detail in the addendum at the end of this subsection.

FACILITIES

All of the above analyses with the exception of those requiring the elemental analyzer are performed in the 225-square-foot chemical service laboratory. The work area is U-shaped with cabinets and drawers underneath the countertops providing storage space. There are wall-hung cabinets along the two sides of the U with one large cabinet for chemical storage. One area under the countertop contains a small refrigerator for chemical and reagent storage and another is open for sitting at the work bench. Electrical outlets (110 volt) are located every 2-1/2 feet along the work area. A 220-volt outlet is provided for special equipment or instrumentation. The plumbing consists of air and gas outlets in three different locations and two double sinks with cold and hot tap water and seawater. Two large drying racks are hung above the sinks for drying glassware.

EQUIPMENT

The equipment and instrumentation requirements for the chemical service laboratory are minimal. Several magnetic stirrers used in pH and ammonia determinations are also used in preparation of stock solutions, reagents, titrations, etc. A temperature-controlled water bath provides a 25°C environment for the temperature sensitive analyses (pH, ammonia, and calcium). Other miscellaneous equipment used are pipette washers, hot plate, pipette racks, dessicators, automatic pipettes, and Millipore® filtering apparatus. A portable eye wash station is maintained for emergency use. Chemicals are weighed on Mettler balances located in other laboratories in the building. A Bausch and Lomb Spectronic 20 is used for colorimetric analyses. Its simplicity, stability, and low cost (under $800) makes it an ideal instrument for routine testing. Its spectral range is from 340 to 625 nm and to 950 nm with a quick-change phototube and infrared filter. The sample holder can accommodate several sizes of round cuvettes and a 1/2-inch-square cuvette. Two Fisher Scientific Accumet meters provide pH readings to $\pm$ 0.003 units and millivolt readings to $\pm$ 0.1 mv. Each meter is equipped with slope and temperature corrections and a test function for checking the instrument. The cost is approximately $1500 each. The Accumets have proven to be extremely reliable, trouble free, and easy to maintain.

CHEMICALS AND GLASSWARE

A general stock of chemicals and glassware is maintained in the laboratory. Included in the chemical stock are chemicals for reagents used in the analyses, standards and buffers for electrodes and pH probes, chemicals for equipment and instrument calibration and cleaning, various acids and bases, and chemicals for various artificial seawater mixes. Glassware stock consists of various sizes of volumetric and Erlenmeyer flasks, beakers, graduated cylinders, and pipettes. Nalgene® plastic ware is used whenever possible. It is chemically resistant, autoclavable, and unbreakable. Glassware and plastic ware are cleaned with a laboratory detergent, tap water rinsed, and triple rinsed with distilled water. If necessary a 10-percent HCl or cleaning solution (chromic-sulfuric acid) rinse or soak will be added to the cleaning procedure. Distilled water from an adjacent laboratory is stored in 5 and 10 gallon Nalgene® tanks with spigots. Sample and reagent bottles in various sizes are available in plastic and glass. Dissolved oxygen bottles with known capacities are required for Winkler titrations. Miscellaneous accessories include rubber stoppers, aluminum foil, parafilm, wash bottles, Sparkleen® (laboratory detergent), spatulas, glass stirring rods, and forceps.

ADDENDUM--ELEMENTAL ANALYSIS

The Model 240 Elemental Analyzer determines nitrogen, carbon, and hydrogen by measuring their combustion products - CO_2, H_2O, and N_2. Several different types of samples can be analyzed: solids, liquids, lyophilized seawater, and particulates. With careful weighing and sample handling techniques, an accuracy of 0.3 percent absolute may be obtained. Ultra-high-purity oxygen is used for the sample combustion and ultra-high-purity helium is the carrier gas used to transport the sample through the system. Gas flows are controlled by a series of seven solenoid valves. A cam-type programmer automatically operates the instrument after initial sample injection. There are three systems in the analyzer: the combustion train, the analytical system, and the electronic system. Included in the combustion train are independently heated combustion and reduction furnaces and their separate reaction tubes. The sample fitting plug assembly at the end of the combustion tube is for ladle and sample insertion. The analytical system contains the mixing and sample volumes, pressure switch, detectors, and adsorption traps. There are three sets of thermal conductivity cells for measurement of H_2O, CO_2, and N_2. For detection of H_2O, an adsorption trap of magnesium perchlorate is located between the two cells. As the gas moves through the trap the water is adsorbed, and an electrical signal proportional to the amount removed is produced. The CO_2 is similarly measured by using an adsorption trap of colorcarb between the pair of thermal conductivity cells. The nitrogen signal is produced by comparing the thermal conductivity

of the remaining sample gas with pure helium. These output signals are recorded on a 1-mv recorder. The electronic system contains two power supplies, thermostat, programmer, and detector safety circuit. The detector safety circuit prevents damage to the detectors by shutting off power to the detectors whenever the air concentration is greater than ten percent. A pilot lamp remains lit until the analytical system has been purged.

The elemental analyzer is located in its own temperature controlled laboratory. This allows small separate areas for sample preparation, maintenance work, sample weighing, actual analyses, and data tabulation. There is storage space for spare parts, maintenance equipment, gas cylinders, and chemicals. The analyzer, balance, and recorder are located in a row on the bench top. It is convenient for the area under the bench to be open so that the operator can sit at the analyzer.

The recorder used in conjunction with the Model 240 is a Perkin-Elmer Model 56. It is a 1-mv flatbed type with an accuracy of $\pm$ 0.2 percent of the span and a sensitivity less than 0.1 percent of span. The chart speed can be set at six different speeds which range from 5 to 160 mm/min. Chart paper is easily installed in the front part of the recorder, and the roll has red margins for chart depletion warning. There is a recorder case angle for chart cutting directly under the edge of the paper as it rolls out of the recorder. The pen assembly is located on top of the recorder. Its parts are separate which allows for quick and easy cleaning. The ink reservoir system which is connected to the pen assembly with vinyl tubing is located under the hinged top cover of the recorder. The ink from the reservoir can be transported directly to the pen assembly by pressing the compression bulb on the ink reservoir. Maintenance requirements for the Model 56 recorder are minimal. The recorder gain and zero settings are checked weekly. The pully bearings should be lubricated with a drop of instrument oil approximately every 6 months. The pen usually needs cleaning at least once a week because the ink tends to solidify in the tip. This can be done with warm water and a piece of fine wire. The driving cable should be replaced when decreased sensitivity is noted. The precut cable is wound clockwise and counterclockwise around the motor pully and connected to the pen assembly with tension springs. An extra pair of hands to help to maintain tension on the cable is a great help when winding the cable.

The balance used for sample and standard weighings is a Perkin-Elmer microbalance Model AM-2. It has a digital type meter which reads either in the milligram or microgram range. A convenient and useful item is the filter selector. It can be set at the LO position for most weighing purposes or at the HI position when working in low ranges or in noisy environments. There are fine and coarse controls for zeroing and calibration. Calibration is checked weekly with a good Class M or better

weight. The AM-2 is designed to hold calibration from one range to another. When weighing standards and solid samples, platinum boats are used. A 1-mg tare weight in a boat is placed on the tare tray and an empty boat on the sample tray. With the fine and coarse controls the meter can be set at zero or any number. The sample or standard is added to the boat and the weight recorded. The balance should be free of infractional effects in the surroundings and should be located on a sturdy table on a sturdy floor. It should not be located near heating and air conditioning ducts, heavy traffic, or in direct sunlight. The room temperature should be controlled to within 5°C. Failure to comply with these requirements could lead to erroneous readings.

Also located in the analyzer room are equipment and apparatus associated with sample preparation. There is a two-rack drying oven for drying sample and wet filter pads and a muffle furnace for firing filter pads and aluminum foil. Several dessicators are used to store samples before analysis and standards and chemicals for the combustion and reduction tubes. Vacuum pumps and filtering apparatus are used for preparing particulate samples of algae.

Dry, homogeneous samples require no special treatment and can be weighed in platinum boats and placed directly in the analyzer. Lyophilized seawater samples are handled in a similar manner except for the weighing. Samples must be weighed with extreme care just before insertion into the analyzer. With extra accessories available from Perkin-Elmer, noncorrosive and corrosive liquid samples may be prepared in ampoules for analysis. The ampoules must be wrapped in platinum gauze to catch the exploding glass particles during the high heat of combustion. The most extensive and time consuming is preparation of the algal particulate sample. The filters are Whatman glass microfiber GF/C, 2.1-cm in diameter. To minimize blank values, the filter pads are placed in aluminum foil trays and fired in a 400°C muffle furnace for two hours. After cooling, each is placed in and individual petri dish and stored in a dessicator until sample time. The filtering apparatus, forceps, glassware, and pipettes are cleaned with 10 percent HCl and triple rinsed with distilled water. Filter pads are always handled on their edge with forceps. At sampling time each fiter pad is placed on the filtering apparatus; duplicate volumes of algae are pipetted onto the pads, and the vacuum pump is run until all the liquid has been filtered. The pads are then returned to the petri dish, labelled with a permanent marker, and placed in the dessicator. Blank values on the filter pads are obtained by treating pads in exactly the same manner as samples, except that no sample is filtered onto the pad. The dessicator filled with samples and blanks is placed in the freezer until analysis time. On the day of analysis the dessicator is removed from the freezer and the samples to be run are placed in the drying oven for 1 to 2 hours at 45°C. While the analyzer is being standardized, each pad is placed on a

1-1/4-inch square of aluminum foil and rolled into a tube. It is again placed in its petri dish and stored in the dessicator until actual injection. The aluminum foil squares are cut from household aluminum foil and fired for 2 to 3 hours at 400°C. These are kept in groups in large petri dishes and stored in a dessicator. The square of foil prevents the glass fiber filter pad from adhering to the quartz ladle during the high heating cycle of combustion. During the high heating the aluminum can create some problems in the combustion train. Very small balls of aluminum form in the combustion tube and prevent smooth ladle injection. Very small particles can work their way under and around the sample fitting plug O-ring and surrounding area. This can cause erratic and unpredictable gas leaks. These fine particles can also clog the incoming gas lines to the combustion train. At the end of the day the aluminum dust in the combustion tube is dusted out with cotton swabs. The O-ring is cleaned and inspected daily for embedded particles. If necessary a new O-ring is inserted daily.

A typical daily run begins with the startup procedure. Furnace temperatures are set, and the oxygen cylinder valve is turned on slowly. The helium pressure is set at the normal level determined during previous helium blank runs. An unweighed standard is run to condition the instrument followed by a series of standards and blanks. The standard should be similar in nitrogen, carbon, and hydrogen composition to the samples to be run. Actual analysis of the sample takes approximately 14 minutes. The sample plug is removed and the sample is placed on a magnetically operated ladle and returned to the combusion tube. The start button is pushed and the cycle begins. The entire system is flushed with helium at a high flow rate for 90 seconds. The combustion train is then flushed with pure oxygen for 30 seconds. The program now stops; the red inject light comes on, and the instrument is ready for sample combustion. Nothing occurs until the operator moves the sample into the combustion area and pushes the combust button. The analysis is now fully automatic except for possible attenuation on the final readout. Combustion occurs in an excess of oxygen at 950°C for approximately 2 minutes. The reduction furnace, operated at 680°C, reduces to nitrogen any oxides of nitrogen. Now the combustion products are flushed into a glass mixing volume for 90 seconds where they become a homogeneous mixture. While the sample gases are mixing, helium is flowing through the detectors. This output on the recorder is called "zero" and is actually the instrument background with no sample in the detectors. After mixing the sample gases, the H_2O, CO_2, and N_2 are measured by the detectors for 30 seconds each and displaced into the atmosphere by the carrier gas helium. The signal output is called the "read" and is recorded in bar-graph form. The difference in microvolts between the zero and the "read" value is proportional to the concentration. The percent of N, C, and H or actual weights can then be computed from the use of the K-factor (μg/μv). The K-factor is determined daily after the running of known standards

and blanks. During short periods of nonuse and on weekends, the instrument is left in the standby condition. The oxygen cylinder is shut off, and both furnace temperatures are lowered to 100°C. The program wheel is set at 9, and the helium pressure is lowered.

An analyzer log book is maintained daily by the operator. The operating conditions, weighings of standards and samples, and computations of each sample are recorded. Balance calibration, maintenance work, and background information on the samples are noted. The chart recordings are dated and kept on file. A maintenance sheet listing replacement of gas tanks, traps, scrubbers, and combustion and reduction tubes is posted above the analyzer for quick reference. A general preventive maintenance schedule suggested by Perkin-Elmer is strictly followed to insure optimum operating conditions and long instrument life.

Microbiology

The principal activities for which a microbiology laboratory should be available to a mariculture research facility are to protect against microbial pathogens which might affect eventual human shellfish consumers and to assure the freedom of the shellfish from microbial disease.

In addition to these basic purposes, the mariculture microbiology laboratory at the College of Marine Studies has conducted the following activities:

- Development of techniques for carrying out the principal activities,
- Investigation of microbial activities within the facility with regard to their effect on other areas of research,
- Provision of laboratory services to investigators involved in related research, and
- Provision of information and professional advice to other investigators and the interested public.

Some of the organisms encountered in this work are classified in the federal Center for Disease Control Biosafety Level 2 (sometimes referred to as P2), for which the laboratory conforms to the standards for a containment laboratory. The manuals listed as References 25 through 27 describe the laboratory and procedural requirements. Routine safety procedures are employed, and most work can be done on open benches.

The primary sources of unwanted bacterial contamination in an intensive, controlled mariculture acitivity are water sources, algal food stocks, and introduced bivalves. We have developed relatively straightforward procedures adapted from standard microbiological practices to monitor these primary sources. Much experience has shown that bacterial levels are consistently low and that there has been no evidence for human or shellfish pathogens or evidence that bacteria play a determinant role in the ecology of the intensive, controlled mariculture activity as it is practised in this laboratory. We believe that the basis for this felicitous situation lies principally in the care to apply 'good housekeeping' procedures and preventative sanitation measures including seawater origin and processing, algae culture methods, animal husbandry throughout the oyster life cycle, and a facilities layout which minimizes airborne contamination as well as human traffic.

EQUIPMENT AND PROCEDURES

The microbiology laboratory in the Otis H. Smith Laboratory provides space for storage and media preparation separate from perimeter and island benches. Storage of prepared media and some chemicals make it necessary for the microbiology lab to have a fairly large refrigerator/freezer. A frost-free model is preferable, since some of the products are sensitive to the temperature changes that can occur during manual defrosting.

Culture of coliform bacteria and suspected human pathogens requires an incubator capable of providing a temperature of 35° ± 0.5°C and a water bath at 44.5° ± 0.2°C. Bacteria and fungi in seawater and shellfish samples which do not belong to these groups are best cultured at 20° to 25°C, requiring a refrigerated incubator. A water bath in the temperature range 45° to 50°C is needed to keep agar-containing media liquid until they can be poured.

A small lyophilization apparatus (Virtis) is used in this laboratory to preserve bacterial isolates for further tests and to establish a reference culture.

A compound microscope, equipped with objective lenses of 4, 10, and 40 power (air) and 100 power (oil) and a substage condenser, is used to examine the cells of a bacterial isolate. An ocular micrometer is used to determine the dimensions of the cells. A microscope equipped for ultraviolet epi-illumination is used to count the cells in a sample of water. This instrument allows us to make some real-time decisions rather than waiting for colonies to grow on plated media.

Microorganisms to be disposed of should be killed by sterilization prior to disposal.

For media preparation, a good quality pH meter, a balance accurate to at least 0.1 gram, a magnetic stirrer with a heating element, a colony counter, and a bunsen burner or electrical device that minimizes splatter are used.

A monitoring program requires the repetitive sampling of a source according to a predetermined schedule. In monitoring for the presence of coliform bacteria and human enteric pathogens, it is necessary only to sample from sources of newly introduced water. In a reasonably clean mariculture operation, contamination of in-house water is highly unlikely. Occasional samples of shellfish from the operation should also be taken. Their propensity for accumulating microorganisms makes them an ideal sample-concentrating device.

A program of sampling to monitor the normal indigenous flora of the seawater and shellfish is established according to the information requirements of the laboratory. Once an algal culture or oyster tank has become established, its bacterial flora tends to remain relatively constant in number and in the identity and relative number of constituent genera. Using direct counting techniques, changes in the numbers of bacteria in a source may be detected within hours, but changes in genera can only be detected after culture and isolation procedures which require at least a week to be completed.

The best method for monitoring coliforms is the most probable number technique. This simple technique is the method upon which quality standards for water and shellfish are based.

There is no established procedure for the detection and identification of noncoliform enteric bacteria (which may be pathogens) from seawater and shellfish. We use media which are selective for the genera *Salmonella, Shigella, Vibrio*, and *Enterococcus (Streptococcus fecalis)*. A variety of such media are commercially available.

The culture of indigenous marine bacteria requires a medium which provides a mineral composition similar to that of their native environment. The only commercially available medium which does this is Difco Bacto - Marine Agar (2216)®. There are some legitimate objections to this medium from scientists interested in the native flora of the nutrient-poor open oceans, but they are not really applicable to the mariculture situation.

For plated media, an aliquot of the sample or dilution, usually about 0.1 ml, is deposited on the surface of the agar medium. A bent glass rod, sterilized by immersion in 70 percent ethanol and flamed, is used to spread this over the entire surface of the medium. We prepare 3 to 5 decimal dilutions and replicate each dilution. Plates are then incubated at a temperature appropriate to the bacterial group under consideration.

The direct counting of bacteria requires some means of concentrating them and making them more readily visible. Bacteria are concentrated on the surface of a Nuclepore® polycarbonate membrane filter (25 mm, 0.20 µm pore) after which they are stained with the fluorescent dye Acridine Orange. When observed microscopically under ultraviolet epi-illumination, the bacterial cells fluoresce and are readily counted. A sample can be processed and counted, using this method, in about 15 minutes.

Taxonomic identification of bacteria requires that they be cultured, then isolated so that a pure culture is obtained. Careful transfer of colonies from mixed culture plates to clean plates of marine agar, one colony to a plate, is used to assure purity and to provide a good supply of the isolate for future

work. Colonies from this plate are used to inoculate other media and for the performance of some tests. Isolates are characterized according to physical and biochemical criteria, and these are used to assign each to a particular genus.

There are almost as many schemes of bacterial taxonomy and keys for the identification of marine bacteria as there have been people who have studied the group. These range from the simple scheme of Shewan et al.[28,29] (1954, 1960) to the comprehensive work of Lewis[30] (1973). In an effort to provide a means by which the small lab can identify bacterial isolates at least to the level of genus, a dichotomous key based on the 8th edition of Bergey's Manual of Determinative Bacteriology[31] is presented in the addenda to this subsection.

A related problem for the small laboratory is the necessity to perform a great many biochemical tests in order to properly characterize each bacterial isolate. The preparation of media and chemicals quickly becomes a full-time occupation, and the space required by the multitude of tubes involved is likewise prohibitive. The Mini-Tek® System of miniaturized biochemical tests, developed by BBL, has proved to be most effective. It is not restricted to a specific group of bacteria, as most other systems are, and offers a broad variety of tests. However, a medium containing marine salts is needed for the growth of many marine isolates, and the preparation of this is time consuming. If sufficiently widespread interest in this system were to develop among marine labs, the manufacturer would be able to formulate and supply the appropriate medium.

The use of key tests to "identify" bacteria will always be open to question. The most logical alternative to this is "numerical taxonomy", in which all characteristics are given equal weight and overall similarities of isolates determined. In order to be valid, such comparisons must include a large number of characteristics, and are best carried out by computers. As these devices become more prevalent and inter-computer links become more common, even a small lab may be able to process its data in this fashion and rely on a considerably larger data bank than it alone can generate. The University of Delaware computer facility has been used for organizing and keeping records on bacteria isolated in this lab. Using a library program, the similarities of the isolates have been evaluated in numerical fashion and by comparison with five different dichotomous keys. The key given in the addenda results in neither excessive lumping nor splitting.

Since 1976 the size of our reference culture collection of marine bacterial isolates has grown considerably. Isolates have been provided to other investigators within the University, in other universities and to the Sloan-Kettering Center Research Institute.

ADDENDUM 1--PROTOCOL FOR TOTAL VIABLE PLATE COUNT OF MARINE HETROTROPHIC BACTERIA

1. Inoculate each of two marine agar (MA) plates with 0.1 mL from each of 3 successive decimal dilutions of sample, in the range appropriate to the bacterial density of the sample. Spread the inoculum with an alcohol-flamed "hockey stick" over the entire surface of each plate. Incubate at 25°C.

2. After 48 hours $\pm$ 3, count all colonies larger than 0.8 mm on the agar surface of plates containing between 30 and 300 such colonies. Return plates containing fewer than 300 colonies to the incubator after marking colony locations. Dispose of all other plates in an approved manner.

3. After a total of 7 days incubation, count all colonies on the agar surface of plates containing between 30 and 300 colonies. Proceed to isolation and identification of predominant organisms.

ADDENDUM 2--PROTOCOL FOR IDENTIFICATION OF ISOLATES

1. Pick a colony from the marine agar (MA) plate culture with a sterile, cooled inoculating wire, and transfer it to a sterile MA plate. Streak for isolation of colonies. Incubate at 25°C for 7 days.

2. Pick an isolated colony from the new MA plate culture, as above, and transfer it to a sterile marine broth (MB) tube. Incubate at 25°C until slightly turbid.

3. Note characteristics of an isolated colony on the MA plate culture (e.g. diameter, elevation, edge, opacity, PIGMENT).

4. Place a BBL N Taxo® Disc near an area of heavy growth. Moisten the disc with sterile distilled water. Return it to the incubator. Observe periodically for blackening of the colony (not medium), indicating the presence of OXIDASE.

5. Add a small drop of hydrogen peroxide to an area of dense growth on the MA plate culture. Observe any formation of bubbles, which indicate the presence of CATALASE.

6. When the MB culture (Step 2) is slightly turbid, prepare a wet-mount slide. Observe for MOTILITY. Note the cell characteristics (SHAPE, length, width, end form, ARRANGEMENT).

7. Prepare a smear from the MB culture for GRAM STAIN.

8. Heat two MOF tubes in boiling water for 10 minutes. Cool to room temperature and inoculate both tubes with an inoculating needle flamed, cooled, and immersed in the MB culture. Overlay one tube with melted sterile vaseline. Cap both

tubes *loosely* and incubate at 25°C until a color change is noted (pink → yellow = acid, pink → purple = alkaline) to a maximum of 2 weeks. Acid production in both tubes indicates FERMENTATION, and acid production only in the tube without vaseline indicates OXIDATION. Observe also for bubbles in the medium and/or upward movement of the vaseline plug, indicating GAS production.

9. Withdraw ~1 ml of the MB culture for steps 10 and 11, using a sterile pasteur pipet.

10. Prepare a slightly opalescent suspension of the MB culture in 3 mL of sterile salt solution. Immerse a sterile cotton swab in the suspension, express excess liquid on the side of the tube and spread the swab over the entire surface of an MA plate. Rotate the plate ~120 degrees and spread again. Rotate the plate ~120 degrees and spread a third time. Dispense antibiotic discs. Add *pteridine* (0/129) *disc*. Incubate the plate at 25°C until heavy growth is observed. Record antibiotic sensitivities.

11. Allow approximately 0.5 mL to run over the surface of an MA slant. Incubate at 25°C until growth is apparent. Withdraw a portion of culture and prepare for FLAGELLA STAIN. Retain the slant for stock culture by sealing the top with parafilm.

12. Aseptically transfer 0.5 mL of the MB culture to each of three sterile ampules for freeze-drying.

13. Refer to the appropriate key (Addendum 3 or 4) and record the tentative genus (or genus group) of the isolate.

ADDENDUM 3--TENTATIVE KEY TO GRAM-POSITIVE MARINE GENERA

1.	COCCI.....2.	CATALASE
	RODS.....5.	CATALASE
2.	CATALASE positive.....3.	MOF
	negative.....	*Streptococcus*
3.	MOF fermentative.....4.	TETRADS
	nonfermentative.....	*Micrococcus*
4.	Tetrads.....	*Gaffkya*
	CLUSTERS.....	*Staphylococcus*
5.	CATALASE positive.....6.	SHORT COCCOBACILLI
	negative.....	Coryneform bacteria
6.	SHORT COCCOBACILLI.....	*Brevibacterium*
	THICK SPOREFORMERS.....	*Bacillus*

ADDENDUM 4--DICHOTOMOUS KEY TO GENERA OF GRAM-NEGATIVE, AEROBIC, HETEROTROPHIC BACTERIA ISOLATED FROM MARINE ENVIRONMENTS

	1.	Swimming motility in broth medium.	2
	1.	No swimming motility	.12
(1)[a]	2.	Cells helical (crokscrew-shaped)	3
	2.	Cells not helical.	4
(2)	3.	Acid produced in covered MOF-Glucose	*Spirochaeta*
	3.	No acid produced in covered MOF-Glucose.	*Spirillum*
(2)	4.	Flagella polar	5
	4.	Flagella peritrichous.	9
(4)	5.	Acid produced in covered MOF-Glucose	6
	5.	No acid produced in covered MOF-Glucose.	8
(5)	6.	Luminescent.	*Photobacterium*
	6.	NonLuminescent	7
(6)	7.	Sensitive to Vibriostat 0/129 (Pteridine).	*Vibrio*
	7.	Not sensitive to Vibriostat 0/129.	*Aeromonas*
(5)	8.	Flocculent growth in high-C:N broth.	*Zoogloea*
	8.	Even growth in high-C:N broth.	*Pseudomonas*
(4)	9.	Luminescent.	*Lucibacterium*
	9.	Nonluminescent	.10
(9)	10.	Colonies yellow, orange, brown, or red . . .	*Flavobacterium*
	10.	Coloniies otherwise.	.11
(10)	11.	Acid produced in covered MOF-Glucose .	*Enterobacteriaceae*
	11.	No acid produced in covered MOF-Glucose. . . .	*Alcaligenes*
(1)	12.	Gliding or flexing on solid media.	.13
	12.	No movement on solid media	.18
(12)	13.	Colonies yellow, orange, brown, or red	.14
	13.	Colonies otherwise	*Beggiatoa*
(13)	14.	Cells helical.	*Saprospira*
	14.	Cells not helical.	.15
(14)	15.	Some cells enclosed in sheath.	.16
	15.	No cells enclosed in sheath.	.17

[a] Number in parenthesis refers to step from which this step is reached.

(15)16. Cell diameter < 0.6 µm. . . . . *Flexithrix*
16. Cell diameter >0.6 µm. . . . . *Herpetosiphon*

(16)17. Cellulose, agar, or chitin attacked. . . . . *Cytophaga*
17. Cellulose, agar, and chitin not attacked . . . *Flexibacter*

(12)18. Colonies yellow, orange, brown, or red . . .*Flavobacterium*
18. Colonies otherwise 19

(18)19. Acid produced in covered MOF-Glucose . .*Enterobacteriaceae*
19. No acid produced in covered MOF-Glucose. 20

(19)20. Oxidase positive . . . . . *Moraxella*
20. Oxidase negative . . . . . *Acinetobacter*

ADDENDUM 5--SUMMARY OF CHARACTERISTICS OBSERVED ON MEDIA USED FOR ISOLATION AND IDENTIFICATION

1. Marine Broth 2216 (MB) culture (from MA)
   a. Turbidity (light, moderate, or heavy)
   b. Distribution (even or flocculent)
   c. Pellicle or Ring

2. Wet Mount Preparation (from MB)
   a. Motility (1)*
   b. Cell shape (2, 14)
   c. Cell length
   d. Cell width (16)
   e. Cell arrangement (15)
   f. Cell end form

3. Gram Stain (from MB)
   Positive, Negative or Variable

4. Flagellar Stain (from MA slant prepared from MB) (4)
   a. Polar (at cell ends only)
      i. Monotrichous
      ii. Multitrichous
   b. Peritrichous (over entire cell surface)

5. MOF-Dextrose (from MB)
   a. Acid, alkaline or no change
   b. Gas production

6. MOF-Dextrose, Vaseline Covered (from MB)
   a. Acid, alkaline, or no change (3, 5, 11, 19)
   b. Gas production

7. MB-Cellulose (from MB)
   Liquefaction positive or negative (17)

8. MA-Chitin (from MB)
   Clearing or no clearing (17)

* Number in parenthesis refers to step in key (Addendum 4) where information is applied.

9. High C:N Broth (from MB)
   Dendritic, flocculent growth, or even growth (8)

10. Vibriostat 0/129
    Sensitive or resistant (7)

11. Marine Agar 2216 (MA) Plate Culture (typical isolated colony)

    a. Diameter
    b. Elevation
    c. Edge
    d. Opacity
    e. Color (10, 13, 18)
    f. Diffusible pigment
    g. Fluorescence
    h. Pitting (17)
    i. Luminescence (6, 9)
    j. Gliding or flexing cells (12)
    k. Cytochrome oxidase (20)
    l. Catalase

ADDENDUM 6--AODC-INT PROCEDURES

Filter Preparation

1. From each pack of 25-mm-diameter, 0.1-μm pore Nuclepore® polycarbonate membrane filters, select two filters at random. Place each in a filter holder above a clean 25-mm fiber filter. Filter 1 mL 0.1% Acridine Orange (Eastman Organic Chemicals No. 1757) through each filter set, using 15 cm Hg vacuum. Examine fiber filters. If no detectable white areas are found, Nuclepore® filters are good for use in the counting procedure.

2. Pour approximately 75 mL of Sudan Black B solution into a clean 15 x 150 mm plastic petri dish. Transfer 24 Nuclepore® filters into this solution, one at a time, spreading them so as to minimize overlapping of filters. Let stand approximately 30 hr. Remove filters one at a time, passing each through 3 rinses of 0.2-um-filtered distilled water. Place filters, *shiny side down*, on clean paper towelling and cover with another piece of the same towelling. Let dry overnight.

3. Transfer dried, stained filters to box, interleaving with paper discs. Store filters *shiny side down*.

Sample Collection

Using a sterile pipet, transfer a 5.0-mL sample to a sterile 13- x 100-mm screw-cap test tube. Add 0.5 mL INT dye (Eastman Organic Chemicals No. 9110). Mix well. Incubate, in dark, at ambient temperature for 20 minutes. Add 0.3 mL of 37% formaldehyde. Store at 4°C in the dark. (If necessary, double volumes may be used in a 16- x 100-mm test tube).

Filtration and Counting

Place a 25-mm diameter Nitex® membrane on holder. Apply 5 drops of 0.5% Wayfos 6-TD detergent. After 1 min, apply vacuum (20 to 30 cm Hg) briefly. This filter may be used throughout the remaining procedure.

Place a stained Nuclepore® filter on the fiber filter, shiny side up. Apply vacuum as before. If bubbles are observed under the filter, release the vacuum, lift the edge of the filter to release trapped air and reset the filter. When the filter surface is smooth, tighten the funnel in place.

Put 1 mL of 0.2-μm-filtered artificial seawater on filter. To this, add the desired volume of sample. Apply 15 ± 1 cm Hg vacuum. When the filter is dry, release the vacuum.

Put 1 mL of Acridine Orange on filter. After 80 sec apply 15 cm vacuum. When filter is dry, rinse with 2 mL 0.2-μm-filtered distilled water, maintaining vacuum. When the filter is again dry, release the vacuum.

Transfer the filter to a microscope slide taking care that it does not fold. Allow it to air dry while setting up the next sample. Apply 3 drops of immersion oil (Cargille Type A) from a tuberculin syringe. Deposit a cover slip on the filter, and press gently to remove air bubbles. Allow oil to permeate the filter while continuing work on subsequent samples.

Count the cells fluorescing green to yellow-green under ultraviolet epi-illumination (Osram HBO 200 lamp, BG-12 exciter, DM 500 dichroic mirror, barrier filters at 515 and 530 nm) and those which contain reddish-brown formazan deposits under transmitted light using a green filter. Compare positions of cells using both techniques.

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Glossary

adult oyster--a commercially marketable oyster, having a shell length of at least 3 inches and a weight of at least 80 grams

agar--gelatin-like product of certain seaweeds that is used for solidifying microbiological culture media

aliquot--in chemistry or biochemistry, comprising a definite part of the whole: an aliquot quantity of a solution for analysis

anoxia--a condition characterized by an abnormally low amount of dissolved oxygen

aquaculture--the cultivation of aquatic organisms

axenic--uncontaminated culture (microbiology); germ-free animals or plants

"bill"--the edge of the shell opposite the hinge

bioassay--a test or evaluation that employs a response of a living organism

biomass--a quantity of living matter

biotin--a vitamin of the B-complex

bivalve mollusc--an animal of the phylum *Mollusca* having two shells or valves hinged together, such as the oyster, clam, or scallop

calcareous--containing or like calcium carbonate

chelated cations--positively charged metal ions located within and attached by covalent bonds to two or more nonmetallic atoms of a complex organic compound

coliform bacteria--any of several species of bacteria, especiall of the genera *Escherichia* and *Aerogenes*, whose presence in water indicates fecal pollution from man or animals

culch--solid materials upon which larval oysters settle, attach, and develop

Delaware process--An intensive, controlled-environment, recirculated-seawater, mariculture system developed at the University of Delaware for producing bivalve molluscs

diatom--a member of the single-celled algae group whose cell walls contain silica

diatomaceous-earth--a particulate filtering agent consisting of fossil siliceous shells of diatoms

ecology--the biological science which deals with the relations between organisms and their environment

ecosystem--a system formed by the interaction of a community of organisms with their environment

EDTA--ethylenediaminetetraacetate, a metal ion chelator

enteric--referring to the intestines

epifauna--Animals that occur on the surfaces of other animals or of plants

epizootic--a disease temporarily prevalent among animals

exogenous--derived externally

flagellate--any of a group of one-celled organisms that have flagella, whip-like appendages of locomotion

flume--a trough for conducting water

gamete--a mature sexual reproductive cell that unites with another cell to form a new organism

glycogen--an animal storage polysaccharide composed of very long chains of glucose residues

gonad--the sex organ that produces gametes

halophyte--a salt-tolerant plant

haplosporidian--a protozoan parasite having a single spore

heterotrophic--obtaining food from organic material

hydrophobic--having little or no affinity for water

larva--the early form of an animal that changes radically in appearance as it matures; bivalve mollusc larvae are microscopic and free-swimming

inoculum--the substance, usually microorganisms such as bacteria or algae, used to initiate growth of those organisms in a culture medium

lipid--a class of water-insoluble organic compounds consisting of fats, oil, fatty acids, steroids, and similar substances

lyophilization--drying from the frozen state usually under reduced pressure

macroreticular--the relatively porous three-dimensional network of the gel structures of certain artificial resins

mariculture--the cultivation of marine organisms; the marine branch of aquaculture

metamorphosis--the successive changes in form as an organism develops to maturity

microbiology--the study of microscopic organisms

Minchinia nelsoni--a parasitic protozoan causing an oyster disease

MSX--multinucleate sphere previously unknown; later identified as a parasite, *Minchinia nelsoni*

opalescent--resembling an opal in play of colors; iridescent

osmosis--the tendency of a fluid to pass through a semipermeable membrane like the surface of a cell

pathogen--a disease-causing microorganism

pediveliger--a mollusc larval stage in which the animal has a foot and a velum

peptide--a compound consisting of two or more amino-acid residues joined by a specific chemical bond arising from the amino group of one amino acid and the carboxyl group of another

pH--the acidity of a solution expressed as the negative logarithm of the hydrogen ion concentration; the values range from 1 to 14, 7 being neutral, and lower numbers indicating greater acidity

photosynthesis--the conversion of light energy by plants to organic matter and oxygen from water and carbon dioxide

phytoplankton--the aggregate of microscopic plants suspended in a body of water

protozoa--single-celled animals belonging to the lowest division of the animal kingdom

proximate analysis--a standardized chemical analysis as used in the food industry to determine protein, carbohydrate, fat and ash content of a food sample

pseudofeces--particulate matter that is caught in mucous on the gills of the oyster but does not pass through the digestive tract, instead it is deposited outside the shell

seed oysters--young oysters used for transplanting to growout facilities, usually in natural waters or impoundments; once the juvenile oyster attaches (spat) it can be sold as seed; seed oysters often are 1 to 4 cm in length and sell for $3 to $15 per thousand (1982 dollars)

sparge--to disperse gas bubbles in a liquid

spat--the attached young of the oyster

substrate--an under layer

thiamine--a vitamin of the B-complex; vitamin B_1

TRIS--trishydroxymethylaminomethane, used as a buffering agent

trochophore--an early larval stage of molluscs in which the animals are free-swimming

veliger--a mollusc larval stage more advanced than the trochophore

Appendixes

Appendix A.

Derivation of Algae Reactor Diameter

The diameter of the algae reactor tube (Figure 6) was arrived at by employing a tubular model differential equation as given in Applied Numerical Methods; Carnahan, Luther, and Wilkes; John Wiley and Sons, NY; 1969 (pp. 77-79 and 361-365). The model chosen is a fourth-order Runge-Kutta equation using Gill's constants to solve for concentration of a component and a composite trapezoidal integration with one Richardson extrapolation to solve the integral of the radius of the reactor.

A computer program was set up to derive an optimal reactor radius by testing various combinations of solar input at Lewes, initial algal cell concentrations, carbon fixation rates, and a 12-hour photosynthesis period for an illuminated tubular reactor. For the diatom *Thalassiosira pseudonana* growing to 10 million cells per milliliter in 12 hours under the annual daily mean solar regime at Lewes, Delaware, the optimal diameter was found to be 76 centimeters.

Appendix B. Oyster and Algae Growth Data

Table 13. Prototype Oyster Biomass Loading

Class	7/29/80	8/19/80	9/9/80	9/30/80	10/21/80	11/12/80	12/3/80
A	*10,342 26.3/393	9,124 26.6/343	7,639 26.0/294	6,324 26.1/242	5,139 25.3/203	4,362 23.2/188	4,479 25.7/174
B	20,420 7.7/2648	17,695 7.2/2472	16,459 7.6/2167	16,655 8.0/2092	** 19,257 10.2/1888	19,359 10.2/1892	20,198 10.9/1851
C	2,373 3.0/791	2,490 3.3/744	2,552 3.6/698	2,847 4.2/673	2,855 4.3/668	2,907 4.6/637	3,176 5.1/626
D	1,250 2.0/610	1,316 2.2/608	1,339 2.4/567	1,445 2.7/538	1,355 2.6/538	** 1,163 4.0/292	1,279 4.4/289
E	2,426 1.7/1427	2,734 1.9/1403	3,031 2.3/1325	3,669 2.6/1427	3,785 2.8/1359	4,129 3.1/1336	4,661 3.7/1273
F	1,185 1.7/689	1,361 2.0/665	1,586 2.4/654	1,969 3.0/658	1,998 3.1/641	2,162 3.5/621	2,395 3.9/608
G	2,225 1.5/1480	2,179 1.5/1439	3,051 2.1/1423	3,266 2.3/1437	3,473 2.5/1396	3,720 2.8/1303	4,214 3.3/1272
H		91 0.3/326	214 0.3/695	272 0.4/670	** 205 0.7/304	283 0.9/314	442 1.5/295
I			482 0.2/2404	1,007 0.4/2371	** 285 0.9/302	339 1.1/300	** 999 0.7/1353
J						1,644 0.2/~9126	2,039 0.2/~8530
K							373 0.2/~1900
L							
M							
N							
O							
P							
Totals:							
Biomass	40,221	36,990	36,353	37,454	38,352	40,068	44,255
Number	8,038	8,000	10,227	10,108	7,299	16,009	18,171

Notes:

*Data given are: weight
mean wt/number

**Indicates culling

Table 13. Prototype Oyster Biomass Loading (continued)

Class	12/23/80	1/13/81		1/14/81	2/4/81	2/25/81		3/18/81	4/8/81
A	4,410 25.6/172	4,308 25.8/167	**	725 32.9/22	720 32.7/22	695 33.1/21		614 34.1/18	477 34.1/14
B	20,461 11.3/1813	20,473 11.3/1813	**	6,335 16.2/391	6,492 16.8/386	5,062 20.0/253	**	4,787 20.4/235	4,561 21.1/216
C	3,293 5.3/616	3,340 5.7/582	**	2,808 7.1/395	2,988 7.8/381	2,226 10.1/220	**	2,288 10.4/219	2,322 11.2/208
D	1,146 4.6/250	1,105 4.6/239	**	979 5.9/166	1,005 6.1/165	1,013 6.6/154		1,009 6.8/149	1,018 7.2/141
E	5,077 3.9/1284	5,271 4.2/1248	**	2,999 7.4/404	3,384 8.3/407	2,434 10.0/244	**	2,553 10.5/244	2,750 11.3/243
F	2,490 4.4/567	2,566 4.5/565	**	2,365 5.0/474	2,785 5.9/474	3,135 6.8/458	**	2,045 10.3/199	2,194 11.4/193
G	4,510 3.6/1239	4,875 4.0/1210	**	3,311 6.3/525	3,777 7.3/519	3,565 9.5/375	**	3,894 10.4/374	4,233 11.6/366
H	544 2.0/276	700 2.6/270	**	600 3.0/200	729 3.7/199	961 4.9/197		1,080 5.5/195	1,247 6.6/190
I	1,047 0.9/1186	1,089 1.0/1045	**	826 1.8/450	962 2.2/438	1,198 2.8/424		1,270 3.2/392	1,480 3.9/378
J	2,245 0.3/~8000	2,262 0.3/~8000	**	521 0.7/713	708 1.0/708	1,138 1.7/683	**	961 2.2/437	1,290 3.0/424
K	485 0.3/1580	540 0.4/~1500	**	270 0.4/600	463 0.8/600	767 1.3/597		953 1.6/590	1,356 2.3/584
L				221 0.4/600	395 0.7/600	443 1.0/452		508 1.1/443	636 1.5/414
M								174 0.3/596	414 0.7/584
N									
O									
P									
Totals: Biomass Number	45,708 16,983	46,529 16,639		21,960 4,940	24,408 4,899	22,637 4,078		22,136 4,091	23,978 3,955

Table 13. Prototype Oyster Biomass Loading (continued)

Class	4/29/81	5/20/81	6/10/81	7/1/81	7/22/81	8/12/81	9/2/81
A	**						
B	4,485 21.8/206	4,598 22.5/204	4,446 23.3/191	4,180 23.8/176	4,157 ** 24.2/172	4,016 ** 24.9/161	3,730 25.2/148
C	2,384 11.7/203	2,449 12.1/202	2,486 12.7/196	2,529 13.2/191	2,666 14.0/190	2,763 14.8/187	2,882 15.7/183
D	1,058 7.8/136	1,098 8.5/129	1,141 9.1/126	1,145 9.7/118	1,168 10.6/110	1,188 11.2/106	1,191 11.9/100
E	** 2,565 13.0/197	2,717 13.8/197	2,846 14.7/193	2,919 15.1/193	3,015 15.5/194	2,996 ** 16.1/186	2,998 16.8/178
F	2,330 11.9/195	2,512 13.0/193	2,668 13.9/192	2,723 14.6/187	2,845 15.1/188	2,998 15.9/188	3,054 16.4/186
G	** 2,939 14.8/198	3,145 16.0/197	3,259 17.1/191	3,389 17.7/191	3,528 18.6/190	3,705 19.7/188	3,882 20.5/189
H	1,418 7.6/187	1,631 8.7/187	1,758 9.9/177	1,780 10.8/165	1,880 11.3/166	1,965 12.3/159	2,009 12.7/158
I	1,686 4.5/372	1,893 ** 5.2/362	1,526 7.6/201	1,627 8.6/188	1,723 9.4/183	1,823 10.0/182	1,930 10.8/178
J	1,637 4.2/391	2,008 ** 5.2/387	1,652 8.5/194	1,805 9.1/199	2,015 10.3/196	2,280 ** 11.8/193	2,298 12.2/188
K	1,725 3.0/576	2,208 3.9/560	2,630 ** 4.7/560	1,620 8.1/199	1,955 9.7/202	2,333 11.7/200	2,705 13.5/200
L	783 1.9/402	935 2.7/348	1,050 3.1/334	1,170 ** 3.6/324	1,068 5.3/201	1,189 6.0/199	1,341 6.8/198
M	705 1.2/582	882 1.6/554	941 1.8/510	1,014 ** 2.1/482	1,065 2.7/388	1,211 ** 3.1/384	908 4.6/196
N		77 0.1/600	284 0.5/587	489 0.8/574	718 1.3/559	982 ** 1.8/541	725 3.7/194
O				141 0.2/600	496 0.8/593	859 1.5/588	1,148 2.0/568
P						119 0.2/600	368 0.6/596
Totals:							
Biomass	23,715	26,153	26,687	26,531	28,299	30,427	31,169
Number	3,645	4,120	3,652	3,787	3,532	4,062	3,460

Table 13. Prototype Oyster Biomass Loading (continued)

Class	9/14/81 → Pre-cull	→ Sample	→ 9/24/81 Post-cull	9/24/81	10/1/81		10/8/81	10/15/81
A								
B	3,766 25.8/146	1,126 25.6/44	2,640 25.9/102	2,674 26.2/102	2,688 26.3/102	**	2,438 26.5/92	2,464 27.1/91
C	3,040 16.7/182	1,044 16.8/62	1,996 16.6/120	2,046 17.2/119	2,067 17.5/118		2,118 17.9/118	2,175 18.4/118
D	1,192 12.3/97	425 12.8/33	767 12.0/64	772 12.3/63	777 12.7/61		775 12.7/61	790 12.9/61
E	3,062 17.7/173	1,058 17.9/59	2,004 17.6/114	2,044 18.1/113	2,050 18.1/113		2,076 18.7/111	2,122 19.1/111
F	3,159 17.4/181	1,090 17.8/61	2,069 17.2/120	2,115 17.6/120	2,092 17.9/117		2,130 18.2/117	2,168 18.5/117
G	4,051 22.0/184	1,408 22.7/62	2,643 21.7/122	2,710 22.4/121	2,740 22.6/121		2,799 23.1/121	2,858 23.6/121
H	2,090 13.7/152	724 14.2/51	1,366 13.5/101	1,383 13.7/101	1,390 13.9/100		1,417 14.2/100	1,449 14.5/100
I	2,024 11.9/170	693 12.2/57	1,331 11.8/113	1,343 11.9/113	1,365 12.2/112		1,402 12.5/112	1,449 12.9/112
J	2,501 14.0/178	855 14.2/60	1,646 13.9/118	1,660 14.1/118	1,692 14.5/117		1,746 14.9/117	1,812 15.5/117
K	3,100 15.6/198	1,064 15.9/67	2,036 15.5/131	2,045 15.6/131	2,094 16.1/130		2,173 16.7/130	2,253 17.3/130
L	1,550 8.1/191	534 8.3/64	1,016 8.0/127	1,019 8.1/126	1,039 8.3/125		1,079 8.7/124	1,137 9.2/124
M	1,097 5.6/194	370 5.7/65	727 5.6/129	729 5.6/128	741 5.9/126		772 6.1/126	820 6.5/126
N	987 5.2/188	333 5.4/62	654 5.2/126	659 5.2/126	690 5.6/123		744 6.0/123	816 6.6/123
O	1,622 2.9/549	288 3.1/92	1,334 2.9/457	1,334 2.9/457	1,417 3.1/455		1,499 3.3/448	1,633 3.7/444
P	602 1.0/593	110 1.1/99	492 1.0/494	492 1.0/494	565 1.2/489		626 1.3/485	714 1.5/479
Totals:								
Biomass	33,843	11,122	22,721	23,025	23,407		23,794	24,660
Number	3,376	938	2,438	2,432	2,409		2,385	2,374

Table 14. Oyster Weight Data

GROUP B

Spawn Date – 4/17/79

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
7/08/80	1	100	1583	15.8	--
	2	100	1370	13.7	--
	3	100	1262	12.6	--
7/29/80	1	99	1602	16.2	--
	2	100	1394	13.9	--
	3	100	1270	12.7	--
8/19/80	1	93	1653	17.7	--
	2	97	1377	14.1	--
	3	98	1398	14.2	--
9/09/80	1	91	1557	17.1	--
	2	96	1401	14.6	--
	3	90	1230	13.7	--
9/30/80	1	87	1549	17.8	--
	2	93	1444	15.5	--
	3	89	1261	14.1	--
10/21/80	1	87	1561	17.9	--
	2	93	1439	15.4	--
	3	87	1266	14.5	--
11/12/80	1	88	1612	18.3	--
	2	94	1476	15.7	--
	3	86	1311	15.2	--
12/03/80	1	85	1696	19.9	--
	2	92	1545	16.8	--
	3	84	1389	16.5	--
12/23/80	1	85	1734	20.4	--
	2	92	1596	17.4	--
	3	85	1455	17.1	--
1/13/81	1	85	1760	20.7	--
	2	91	1580	17.4	--
	3	85	1502	17.7	--
2/04/81	1	85	1812	21.3	27.5
	2	87	1581	18.2	22.4
	3	84	1548	18.4	25.1
2/25/81	1	85	1880	22.1	28.9
	2	85	1573	18.5	23.2
	3	83	1609	19.4	26.4
3/18/81	1	82	1854	22.6	29.2
	2	82	1549	18.9	23.6
	3	71	1384	19.5	26.9

Table 14. Oyster Weight Data (continued)

GROUP B (continued)

Spawn Date – 4/17/79

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
4/08/81	1	81	1870	23.1	30.1
	2	80	1570	19.6	24.5
	3	55	1121	20.4	27.9
4/29/81	1	80	1890	23.6	30.5
	2	80	1608	20.1	25.3
	3	46	987	21.5	28.4
5/20/81	1	79	1931	24.4	31.1
	2	79	1648	20.9	26.1
	3	46	1019	22.2	29.7
6/10/81	1	77	1933	25.1	31.7
	2	77	1668	21.7	27.0
	3	37	845	22.8	30.9
7/01/81	1	74	1915	25.9	32.1
	2	75	1622	21.6	27.3
	3	27	643	23.8	32.2
7/22/81	1	72	1885	26.2	33.2
	2	73	1611	22.1	27.7
	3	27	661	24.5	33.6
8/12/81	1	71	1893	26.7	34.0
	2	72	1659	22.8	28.0
	3	18	479	26.6	34.7
9/02/81	1	62	1682	27.1	34.7
	2	70	1596	22.8	28.5
	3	16	452	28.2	35.6

Table 14. Oyster Weight Data (continued)

GROUP C

Spawn Date – 9/19/79

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
7/08/80	1	150	647	4.3	--
	2	150	498	3.3	--
7/29/80	1	148	701	4.7	--
	2	146	536	3.7	--
8/19/80	1	144	760	5.2	--
	2	142	568	4.0	--
9/09/80	1	138	785	5.7	--
	2	136	614	4.5	--
9/30/80	1	139	904	6.5	--
	2	127	682	5.3	--
10/21/80	1	139	924	6.6	--
	2	126	674	5.3	--
11/12/80	1	134	962	7.2	--
	2	117	670	5.7	--
12/03/80	1	133	1056	7.9	--
	2	115	749	6.5	--
12/23/80	1	130	1098	8.4	--
	2	115	795	6.9	--
1/13/81	1	131	1141	8.7	--
	2	114	828	7.3	--
2/04/81	1	119	1178	9.9	18.4
	2	111	888	8.0	14.8
2/25/81	1	114	1275	11.2	20.1
	2	106	951	9.0	16.5
3/18/81	1	115	1318	11.5	20.9
	2	104	970	9.3	17.3
4/08/81	1	107	1320	12.3	22.1
	2	101	1002	9.9	18.4
4/29/81	1	106	1356	12.8	23.1
	2	97	1027	10.6	19.0
5/20/81	1	104	1385	13.3	24.0
	2	98	1063	10.8	19.8
6/10/81	1	100	1396	14.0	24.7
	2	96	1090	11.4	21.6
7/01/81	1	98	1411	14.4	25.3
	2	93	1118	12.0	22.5
7/22/81	1	97	1500	15.5	22.6
	2	93	1166	12.5	23.8
8/12/81	1	96	1553	16.2	27.3
	2	91	1210	13.3	25.0
9/02/81	1	95	1630	17.2	28.1
	2	88	1252	14.2	26.3

Table 14. Oyster Weight Data (continued)

GROUP D

Spawn Date 10/31/79

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
7/08/80	1	150	394	2.6	--
	2	150	302	2.0	--
7/29/80	1	145	469	3.2	--
	2	148	334	2.3	--
8/19/80	1	138	528	3.8	--
	2	139	358	2.5	--
9/09/80	1	129	556	4.3	--
	2	134	379	2.8	--
9/30/80	1	123	621	5.0	--
	2	127	409	3.2	--
10/21/80	1	120	605	5.0	-
	2	120	386	3.2	--
11/12/80	1	112	600	5.4	--
	2	112	397	3.5	--
12/03/80	1	115	703	6.1	--
	2	108	408	3.8	--
12/23/80	1	106	592	5.6	--
	2	81	390	4.8	--
1/13/81	1	104	601	5.8	--
	2	74	336	4.5	--
1/14/81	1	73	548	7.5	--
	2	93	430	4.6	--
2/04/81	1	73	566	7.7	13.1
	2	92	439	4.8	11.4
2/25/81	1	73	572	7.8	14.0
	2	81	441	5.4	12.7
3/18/81	1	70	562	8.0	14.9
	2	79	447	5.7	13.4
4/08/81	1	67	559	8.3	16.0
	2	74	460	6.2	14.5
4/29/81	1	64	591	9.2	16.7
	2	72	467	6.5	15.2
5/20/81	1	61	613	10.1	17.9
	2	68	485	7.1	17.0
6/10/81	1	59	633	10.6	18.9
	2	67	508	7.6	18.1
7/01/81	1	54	632	11.7	19.5
	2	64	513	8.0	18.7
7/22/81	1	53	656	12.4	20.3
	2	57	512	9.0	19.6
8/12/81	1	52	674	13.0	21.3
	2	54	513	9.5	20.4
9/02/81	1	49	678	13.8	22.1
	2	51	513	10.0	20.7

Table 14. Oyster Weight Data (continued)

GROUP E

Spawn Date - 1/3/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
7/08/80	1	150	354	2.4	--
	2	150	290	1.9	--
7/29/80	1	150	445	3.0	--
	2	146	363	2.5	--
8/19/80	1	150	524	3.4	--
	2	145	429	2.9	--
9/09/80	1	150	592	3.9	--
	2	144	475	3.3	--
9/30/80	1	150	717	4.7	--
	2	142	576	4.0	--
10/21/80	1	145	745	5.1	--
	2	137	601	4.3	--
11/12/80	1	145	857	5.9	--
	2	135	646	4.8	--
12/03/80	1	142	927	6.5	--
	2	134	768	5.7	--
12/23/80	1	138	1122	8.1	--
	2	133	834	6.3	--
1/13/81	1	135	1175	8.7	--
	2	129	830	6.4	--
1/14/81	1	127	1151	9.1	--
	2	127	866	6.8	--
2/04/81	1	127	1283	10.1	15.5
	2	124	934	7.5	13.9
2/25/81	1	124	1408	11.3	16.9
	2	120	1026	8.5	15.2
3/18/81	1	124	1472	11.9	17.7
	2	120	1081	9.0	15.9
4/08/81	1	123	1573	12.8	19.1
	2	120	1177	9.8	17.1
4/29/81	1	99	1449	14.6	19.9
	2	98	1116	11.4	17.4
5/20/81	1	99	1540	15.6	20.8
	2	98	1177	12.0	18.1
6/10/81	1	95	1604	16.9	21.9
	2	98	1242	12.7	18.7
7/01/81	1	95	1643	16.9	22.3
	2	98	1243	13.0	19.0
7/22/81	1	97	1677	17.3	22.9
	2	97	1338	13.8	19.7
8/12/81	1	94	1715	18.3	23.5
	2	92	1346	14.6	20.4
9/02/81	1	93	1724	18.5	23.0
	2	85	1273	15.0	22.0

Table 14. Oyster Weight Data (continued)

GROUP F

Spawn Date – 2/12/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
7/08/80	1	303	544	1.8	--
	2	388	360	0.9	--
7/29/80	1	303	723	2.4	--
	2	383	462	1.2	--
8/19/80	1	289	811	2.7	--
	2	376	550	1.4	--
9/09/80	1	283	945	3.3	--
	2	371	641	1.7	--
9/30/80	1	283	1165	3.9	--
	2	365	804	2.2	--
10/21/80	1	283	1184	4.1	--
	2	357	813	2.2	--
11/12/80	1	283	1280	4.5	--
	2	335	883	2.6	--
12/03/80	1	282	1396	4.9	--
	2	326	999	3.1	--
12/23/80	1	282	1435	5.1	--
	2	284	1055	3.6	--
1/12/81	1	281	1438	5.1	--
	2	284	1128	4.0	--
1/14/81	1	239	1358	5.7	--
	2	235	1007	4.3	--
2/04/81	1	238	1598	6.7	13.5
	2	235	1187	5.0	13.9
2/25/81	1	225	1826.8	8.1	15.6
	2	233	1308.6	5.6	15.2
3/18/81	1	100	1158	11.5	16.8
	2	98	887	9.1	15.7
4/08/81	1	99	1259	12.7	18.2
	2	94	935	9.9	16.4
4/29/81	1	99	1344	13.4	19.1
	2	94	986	10.4	17.2
5/20/81	1	99	1460	14.6	20.6
	2	93	1052	11.3	18.7
6/10/81	1	99	1554	15.7	22.1
	2	93	1114	12.0	19.4
7/01/81	1	94	1576	16.8	22.2
	2	93	1147	12.3	19.8
7/22/81	1	96	1677	17.5	22.9
	2	92	1168	12.7	20.0
8/12/81	1	96	1783	18.6	23.6
	2	92	1215	13.2	20.7
9/02/81	1	96	1835	19.1	24.5
	2	90	1219	13.6	21.4

Table 14. Oyster Weight Data (continued)

GROUP G

Spawn Date – 4/7/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
7/08/80	1	311	309	1.0	--
	2	751	530	0.7	--
7/29/80	1	306	482	1.6	--
	2	758	859	1.1	--
8/18/80	1	299	776	2.5	--
	2	743	923	1.2	--
9/09/80	1	303	963	3.2	--
	2	781	964	1.2	--
9/30/80	1	295	1299	4.4	--
	2	765	1306	1.7	--
10/21/80	1	285	1343	4.7	--
	2	735	1418	1.9	--
11/12/80	1	275	1440	5.2	--
	2	667	1476	2.2	--
12/03/80	1	272	1703	6.3	--
	2	648	1610	2.5	--
12/23/80	1	264	1865	7.1	--
	2	621	1677	2.7	--
1/13/81	1	264	2052	7.7	--
	2	586	1794	3.1	--
1/14/81	1	200	1774	8.9	--
	2	175	886	5.1	--
2/04/81	1	199	1918	9.6	18.4
	2	175	1081	6.3	10.2
2/25/81	1	199	2210	11.0	21.3
	2	175	1355	7.7	12.0
3/18/81	1	198	2336	11.8	22.7
	2	175	1558	8.9	14.1
4/08/81	1	194	2454	12.7	24.1
	2	173	1779	10.3	16.3
4/29/81	1	98	1644	16.8	25.0
	2	100	1295	13.0	17.6
5/20/81	1	97	1724	17.8	26.5
	2	100	1421	14.2	19.3
6/10/81	1	95	1776	18.7	27.9
	2	97	1483	15.4	19.9
7/01/81	1	94	1821	19.4	28.8
	2	97	1568	16.2	20.7
7/22/81	1	94	1863	19.8	29.5
	2	96	1665	17.3	21.7
8/12/81	1	93	1944	20.9	31.5
	2	96	1760	18.5	23.4
9/02/81	1	93	2026	21.8	32.8
	2	96	1856	19.3	24.5

Table 14. Oyster Weight Data (continued)

GROUP H

Spawn Date – 5/22/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
7/08/80	1	~500	--	--	--
	2	~10,000	--	--	--
7/29/80	1	~500	--	--	--
	2	~10,000	--	--	--
8/19/80	1	84	40	0.5	--
	2	242	51	0.2	--
9/09/80	1	173	57	0.3	--
	2	195	90	0.4	--
9/30/80	1	169	70	0.4	--
	2	300	82	0.2	--
10/21/80	1	304	205	0.7	--
11/12/80	1	304	283	0.9	--
12/03/80	1	295	442	1.5	--
12/23/80	1	276	544	2.0	--
1/13/81	1	270	700	2.6	--
1/14/81	1	200	600	3.0	--
2/04/81	1	199	729	3.7	7.8
2/25/81	1	197	961	4.9	10.1
3/18/81	1	195	1080	5.5	11.5
4/08/81	1	190	1247	6.6	13.2
4/29/81	1	187	1418	7.6	14.3
5/20/81	1	188	1631	8.7	15.6
6/10/81	1	177	1758	9.9	16.7
7/01/81	1	165	1780	10.8	17.5
7/22/81	1	166	1880	11.3	17.9
8/12/81	1	159	1965	12.4	18.5
9/02/81	1	158	2009	12.7	18.9

Table 14. Oyster Weight Data (continued)

GROUP I

Spawn Date -- 7/3/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
9/09/80	1	2400	482	0.2	--
9/30/80	1	2370	1007	0.4	--
10/21/80	1	300	285	0.9	--
11/12/80	1	300	339	1.1	--
	2	1170	424	0.4	--
12/03/80	1	287	435	1.5	--
	2	1066	564	0.5	--
12/23/80	1	263	474	1.8	--
	2	923	573	0.6	--
1/13/81	1	255	534	2.1	--
	2	790	555	0.7	--
1/14/81	1	200	499	2.5	--
	2	250	327	1.3	--
2/04/81	1	194	544	2.8	5.2
	2	244	418	1.7	3.1
2/25/81	1	182	639	3.5	6.3
	2	242	559	2.3	4.4
3/18/81	1	169	663	3.9	6.9
	2	223	607	2.7	5.1
4/08/81	1	163	742	4.5	7.7
	2	215	738	2.9	6.2
4/29/81	1	157	815	5.2	8.5
	2	215	871	4.1	7.1
5/20/81	1	151	893	5.9	9.4
	2	211	1000	4.7	7.7
6/10/81	1	100	824	8.1	10.1
	2	100	702	7.1	8.8
7/01/81	1	99	878.5	8.9	10.6
	2	99	749	7.6	9.6
7/22/81	1	93	911	9.8	11.1
	2	90	811	9.0	10.4
8/12/81	1	93	955	10.3	11.7
	2	89	867	9.7	11.4
9/02/81	1	92	1013	11.0	12.2
	2	86	917	10.7	12.6

Table 14. Oyster Weight Data (continued)

GROUP J

Spawn Date – 8/19/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
10/21/80	1	300	65	0.2	--
	2	~2700	274	0.1	--
11/12/80	1	260	90	0.3	--
	2	1970	318	0.2	--
12/03/80	1	250	127	0.5	--
	2	1630	449	0.3	--
12/23/80	1	247	180	0.7	--
	2	1600	532	0.3	--
1/13/81	1	243	212	0.9	--
	2	1600	537	0.3	--
1/14/81	1	213	203	0.9	--
	2	250	171	0.7	--
2/04/81	1	210	265	1.3	3.1
	2	248	245	1.0	2.2
2/25/81	1	203	409	2.0	4.7
	2	240	388	1.6	3.6
3/18/81	1	199	493	2.5	5.6
	2	238	468	2.0	4.8
4/08/81	1	192	666	3.5	7.1
	2	232	624	2.7	6.8
4/29/81	1	173	810	4.7	8.5
	2	218	827	3.8	7.7
5/20/81	1	171	945	5.5	10.1
	2	216	1063	4.9	9.2
6/10/81	1	100	796	8.0	11.2
	2	94	856	9.1	11.3
7/01/81	1	100	850	8.5	12.0
	2	99	955	9.6	12.7
7/22/81	1	98	923	9.5	12.3
	2	98	1081	11.0	14.4
8/12/81	1	97	1115	11.5	12.8
	2	96	1165	12.1	15.4
9/02/81	1	95	1094	11.5	13.6
	2	93	1204	12.9	16.1

Table 14. Oyster Weight Data (continued)

GROUP K

Spawn Date – 10/1/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
12/03/80	1	~1900	373	0.2	--
12/23/80	1	1580	485	0.3	--
1/13/81	1	1500	540	0.4	--
1/14/81	1	300	160	0.5	--
	2	300	110	0.4	--
2/04/81	1	300	278	0.9	1.6
	2	300	186	0.6	0.8
2/25/81	1	300	454	1.5	2.5
	2	297	313	1.1	1.4
3/18/81	1	297	571	1.9	3.6
	2	294	382	1.3	2.3
4/08/81	1	290	811	2.8	5.2
	2	294	545	1.9	2.9
4/29/81	1	288	1028	3.6	6.9
	2	288	697	2.4	3.6
5/20/81	1	288	1305	4.5	8.2
	2	272	903	3.3	4.6
6/10/81	1	284	1553	5.5	9.8
	2	268	1076	4.0	5.6
7/01/81	1	100	925	9.3	10.8
	2	99	694	7.0	6.4
7/22/81	1	102	1097	10.8	12.3
	2	100	858	8.6	7.9
8/12/81	1	101	1290	12.8	14.0
	2	99	1044	10.5	9.7
9/02/81	1	101	1470	14.4	16.0
	2	98	1235	12.6	11.8

Table 14. Oyster Weight Data (continued)

GROUP L

Spawn Date – 11/11/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
1/14/81	1	300	134	0.4	--
	2	300	87	0.3	--
2/04/81	1	300	242	0.8	1.2
	2	300	152	0.5	0.6
2/25/81	1	300	346	1.1	1.8
	2	150	97	0.7	1.2
3/18/81	1	298	391	1.3	2.2
	2	145	117	0.8	1.6
4/08/81	1	276	479	1.7	2.9
	2	138	157	1.1	2.5
4/29/81	1	266	586	2.2	3.5
	2	136	197	1.5	3.4
5/20/81	1	227	691	3.0	4.6
	2	121	243	2.0	4.5
6/10/81	1	214	772	3.6	5.3
	2	120	278	2.3	5.5
7/01/81	1	210	857	4.1	6.1
	2	114	312	2.7	6.2
7/22/81	1	99	709	7.2	7.4
	2	102	359	3.5	7.1
8/12/81	1	99	794	8.0	8.2
	2	100	395	3.9	8.0
9/02/81	1	99	904	9.1	9.2
	2	99	436	4.4	8.8

Table 14. Oyster Weight Data (continued)

GROUP M

Spawn Date – 1/12/81

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
3/18/81	1	596	174	0.3	--
4/08/81	1	584	414	0.7	--
4/29/81	1	582	705	1.2	2.4
5/20/81	1	554	882	1.6	3.2
6/10/81	1	510	941	1.8	3.8
7/01/81	1	482	1014	2.1	4.1
7/22/81	1	193	716	3.7	4.6
	2	195	349	1.8	2.7
8/12/81	1	192	816	4.3	5.2
	2	192	395	1.0	3.3
9/02/81	1	97	600	6.2	6.2
	2	99	307	3.1	4.1

GROUP N

Spawn Date – 3/17/81

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
5/20/81	1	600	77	0.1	--
6/10/81	1	587	284	0.5	0.8
7/01/81	1	574	489	0.85	1.5
7/22/81	1	559	718	1.3	2.1
8/12/81	1	541	982	1.8	2.5
9/02/81	1	194	725	3.7	3.1

Table 14. Oyster Weight Data (continued)

GROUP O

Spawn Date - 4/29/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
5/20/81	1	600	77	0.1	--
7/01/81	1	600	141	0.24	--
7/22/81	1	593	496	0.8	1.2
8/12/81	1	588	859	1.5	2.2
9/02/81	1	568	1148	2.0	3.1

GROUP P

Spawn Date – ~6/15/80

Date	Tray No.	Number	Total Weight (g)	Mean Weight (g)	10-Best Mean Weight (g)
8/12/81	1	600	119	0.2	--
9/02/81	1	596	368	0.6	--

Table 18. Daily Algae Production*

Date (1980)	T-ISO	3H	Date (1980)	T-ISO	3H	Date (1980)	T-ISO	3H
7/29	0.21	2.39	9/1	1.21	4.14	10/1	1.35	0.31
7/30	1.35	5.22	9/2	0.71	1.08	10/2	0.58	3.61
7/31	1.17	5.97	9/3	0.79	1.69	10/3	0	2.03
			9/4	0.48	2.17	10/4	0.65	3.72
8/1	0.92	2.06	9/5	0.50	--	10/5	0.52	3.92
8/2	0.50	2.25	9/6	1.56	2.17	10/6	1.21	2.56
8/3	0.77	0.47	9/7	1.71	3.25	10/7	1.50	0.81
8/4	1.79	2.89	9/8	1.54	3.44	10/8	1.29	1.58
8/5	1.77	4.92	9/9	1.21	1.69	10/9	0.77	4.14
8/6	1.94	3.75	9/10	2.21	2.69	10/10	0.71	4.14
8/7	1.65	1.56	9/11	1.81	1.36	10/11	1.67	3.14
8/8	1.06	1.39	9/12	1.17	5.25	10/12	1.52	3.92
8/9	0.44	--	9/13	0.87	0.97	10/13	1.10	3.08
8/10	0.71	--	9/14	0.21	0.31	10/14	--	0.69
8/11	1.23	2.03	9/15	0.75	2.61	10/15	0.10	4.72
8/12	0.68	3.56	9/16	0.77	2.28	10/16	1.00	4.97
8/13	0.94	2.86	9/17	0.92	4.19	10/17	1.94	3.83
8/14	0.81	2.08	9/18	0.58	1.36	10/18	1.79	0.05
8/15	0.54	1.03	9/19	1.08	1.94	10/19	1.54	2.50
8/16	0.10	--	9/20	1.33	5.30	10/20	1.23	5.06
8/17	0.96	2.61	9/21	2.10	2.22	10/21	0.03	3.83
8/18	0.90	2.25	9/22	2.31	5.50	10/22	1.96	2.96
8/19	0.10	1.56	9/23	2.04	2.81	10/23	2.12	--
8/20	1.10	4.83	9/24	1.17	1.31	10/24	1.48	0.01
8/21	0.65	3.11	9/25	0.48	0	10/25	1.29	0.01
8/22	0.50	2.53	9/26	0.79	1.42	10/26	0.40	3.19
8/23	0.73	--	9/27	0.81	5.39	10/27	--	2.0
8/24	1.17	1.11	9/28	1.91	1.33	10/28	0.39	1.92
8/25	1.21	5.39	9/29	1.58	1.03	10/29	1.15	1.89
8/26	1.40	7.50	9/30	0.90	0.50	10/30	0.94	0
8/27	0.87	4.83				10/31	0.60	--
8/28	0	6.61						
8/29	1.02	1.50						
8/30	1.06	3.22						
8/31	1.69	7.83						
Aug $\overline{X}$ =	0.94	2.92	Sept $\overline{X}$ =	1.16	2.31	Oct $\overline{X}$ =	0.99	2.40

Notes:

*10^9 cells/liter

Brackets indicate culture lifetimes.

Table 18. Daily Algae Production* (continued)

Date (1980)	T-ISO	3H	Date (1980)	T-ISO	3H	Date (1981)	T-ISO	3H
11/1	0.85	--	12/1	0.30	3.37	1/1	1.15	1.15
11/2	0.54	--	12/2	1.0	0.93	1/2	0.60	2.77
11/3	0.01	4.36	12/3	1.30	-	1/3	0.43	0.20
11/4	1.25	2.22	12/4	2.40	3.63	1/4	0.50	2.33
11/5	0.50	3.81	12/5	2.45	3.75	1/5	0.70	3.97
11/6	1.27	3.47	12/6	3.10	3.08	1/6	0.70	3.63
11/7	0	3.31	12/7	1.33	3.00	1/7	0.10	1.17
11/8	1.56	2.36	12/8	1.13	3.78	1/8	1.00	0
11/9	1.58	--	12/9	0.20	1.18	1/9	0	--
11/10	0.27	2.44	12/10	0	--	1/10	0.7	--
11/11	1.52	3.69	12/11	1.0	0.80	1/11	1.00	2.10
11/12	--	3.64	12/12	1.73	3.25	1/12	2.30	4.17
11/13	0.39	4.67	12/13	2.28	3.70	1/13	0.20	--
11/14	1.44	0.50	12/14	1.53	2.37	1/14	0.50	1.50
11/15	0	0.64	12/15	1.07	0.90	1/15	0.70	1.07
11/16	1.12	1.19	12/16	0.60	0.28	1/16	0.30	0.67
11/17	0.87	3.00	12/17	0	1.50	1/17	1.20	2.22
11/18	0.39	1.36	12/18	--	3.47	1/18	0.70	1.37
11/19	0.50	0.31	12/19	--	1.92	1/19	1.10	1.63
11/20	1.62	3.78	12/20	--	1.35	1/20	0.30	1.75
11/21	1.87	0.25	12/21	1.30	1.38	1/21	0	1.17
11/22	2.15	0.50	12/22	1.45	1.30	1/22	0.33	3.52
11/23	1.52	3.25	12/23	0.20	0.98	1/23	0.80	0.92
11/24	0.67	0.73	12/24	1.45	1.52	1/24	1.20	0.38
11/25	--	0.30	12/25	1.0	1.50	1/25	1.30	2.20
11/26	0.20	0.43	12/26	1.23	2.52	1/26	0.20	1.20
11/27	0.30	0.40	12/27	--	0.48	1/27	0	1.00
11/28	0.68	0.98	12/28	0.30	--	1/28	0	0.87
11/29	0	1.38	12/29	0	0.20	1/29	2.10	0.60
11/30	0.30	1.52	12/30	1.20	3.43	1/30	2.10	2.30
			12/31	1.83	2.95	1/31	1.20	2.58
Nov $\bar{X}$ =	0.78	1.82	Dec $\bar{X}$ =	1.01	1.89	Jan $\bar{X}$ =	0.75	1.56

Notes:

*10^9 cells/liter

Brackets indicate culture lifetimes.

Table 18. Daily Algae Production* (continued)

Date (1981)	T-ISO	3H	Date (1981)	T-ISO	3H	Date (1981)	T-ISO	3H
2/1	1.30	4.13	3/1	1.40	1.35	4/1	0.60	3.20
2/2	0	0.37	3/2	0.50	0	4/2	1.70	4.45
2/3	--	1.00	3/3	0.30	1.05	4/3	0.20	0.63
2/4	1.20	4.27	3/4	0	0.75	4/4	0.50	1.08
2/5	1.20	2.87	3/5	0.20	0.40	4/5	0	--
2/6	0.50	1.28	3/6	0.60	3.57	4/6	0.80	0.40
2/7	1.40	0.50	3/7	0.20	3.22	4/7	0.30	3.22
2/8	0	0.73	3/8	0.20	2.68	4/8	0.50	2.40
2/9	0	2.18	3/9	--	1.02	4/9	0.40	0
2/10	0.20	1.60	3/10	0.20	2.15	4/10	0.80	1.70
2/11	0.40	2.10	3/11	1.00	1.92	4/11	0.20	1.43
2/12	1.30	--	3/12	1.20	0.47	4/12	0	0.98
2/13	0.50	1.52	3/13	1.70	--	4/13	0.63	1.30
2/14	0.70	3.67	3/14	1.30	0.90	4/14	0.90	0.92
2/15	0	1.95	3/15	1.30	4.30	4/15	1.30	1.78
2/16	0.10	1.43	3/16	0	1.18	4/16	0.90	0.60
2/17	0	0	3/17	0.60	4.40	4/17	0.20	2.02
2/18	0.60	0	3/18	0.40	0.38	4/18	1.10	3.68
2/19	0.40	2.07	3/19	0.10	0.83	4/19	0.80	1.63
2/20	0.30	1.88	3/20	0.50	2.62	4/20	0.17	0.53
2/21	0	0	3/21	0.10	0.87	4/21	0.40	1.88
2/22	0	--	3/22	0	0.73	4/22	1.00	4.30
2/23	0.10	1.77	3/23	--	0.80	4/23	0.10	2.17
2/24	0.30	3.00	3/24	1.30	2.85	4/24	0.10	1.98
2/25	0.60	1.97	3/25	1.20	1.87	4/25	0.40	1.70
2/26	0.30	0.88	3/26	0.40	1.00	4/26	0.50	1.08
2/27	0	0.23	3/27	0	1.07	4/27	0.63	4.08
2/28	0.70	0.50	3/28	0.60	3.60	4/28	0	2.87
			3/29	1.50	1.67	4/29	0.50	2.15
			3/30	0.30	0	4/30	0.20	0.72
			3/31	1.40	1.40			
Feb $\bar{X}$ =	0.43	1.50	March $\bar{X}$ =	0.60	1.58	April $\bar{X}$ =	0.53	1.83

Notes:

*10^9 cells/liter

Brackets indicate culture lifetimes.

Table 18. Daily Algae Production* (continued)

Date (1981)	T-ISO	3H	Date (1981)	T-ISO	3H	Date (1981)	T-ISO	3H
5/1	0.30	0.27	6/1	0.30	1.27	7/1	0.30	2.82
5/2	0.60	--	6/2	0	--	7/2	0.60	3.28
5/3	1.00	2.80	6/3	0.57	1.80	7/3	0.10	0.78
5/4	0.60	3.68	6/4	0.80	2.47	7/4	--	1.03
5/5	0.20	1.97	6/5	1.10	2.15	7/5	0.70	1.98
5/6	0.60	2.37	6/6	0.70	2.98	7/6	0.80	0.65
5/7	0.70	0.48	6/7	1.40	3.83	7/7	0	3.35
5/8	1.10	1.20	6/8	0	2.53	7/8	1.10	3.73
5/9	0.80	4.37	6/9	0	1.48	7/9	2.00	2.07
5/10	0	2.38	6/10	--	2.18	7/10	0.70	1.45
5/11	0.20	1.40	6/11	1.00	0.40	7/11	1.30	5.25
5/12	0.70	1.28	6/12	1.10	--	7/12	1.00	3.07
5/13	0.70	1.33	6/13	0.80	0.80	7/13	1.10	1.53
5/14	0.70	1.35	6/14	0.60	2.50	7/14	0.80	0
5/15	0.50	2.50	6/15	1.20	2.92	7/15	0.20	2.03
5/16	0	1.90	6/16	0.90	1.15	7/16	0.40	3.57
5/17	0.40	2.00	6/17	0.60	0.77	7/17	0.80	4.60
5/18	0.40	1.40	6/18	0.08	3.03	7/18	2.00	4.22
5/19	0	1.00	6/19	0.30	1.13	7/19	0.90	4.13
5/20	0.70	2.63	6/20	0.10	0.28	7/20	0.10	2.32
5/21	0.30	2.62	6/21	1.20	--	7/21	0	3.53
5/22	0.08	1.48	6/22	0.50	1.55	7/22	1.70	3.03
5/23	0	--	6/23	0.30	4.15	7/23	1.00	3.47
5/24	0.70	2.80	6/24	1.20	3.15	7/24	1.10	0.52
5/25	1.20	2.68	6/25	0.90	3.70	7/25	0.30	1.23
5/26	1.00	3.90	6/26	0.60	2.17	7/26	1.10	1.33
5/27	1.30	1.63	6/27	1.30	1.83	7/27	0	2.23
5/28	0	0.33	6/28	0.60	2.30	7/28	1.00	0
5/29	0.48	0.70	6/29	0.30	3.50	7/29	0.30	2.13
5/30	0	2.72	6/30	0.30	4.37	7/30	1.20	3.22
5/31	1.00	2.67				7/31	0.80	0.60
May $\bar{X}$ =	0.53	1.87	June $\bar{X}$ =	0.62	2.01	July $\bar{X}$ =	0.75	2.36

Notes:

*10^9 cells/liter

Brackets indicate culture lifetimes.

Table 18. Daily Algae Production* (continued)

Date (1981)	T-ISO	3H	Date (1981)	T-ISO	3H	Date (1981)	T-ISO	3H
8/1	2.30	1.90	9/1	0.80	0.82	10/1	0.20	2.47
8/2	0.30	3.80	9/2	0.80	0	10/2	0.60	0.50
8/3	0.60	3.15	9/3	1.40	2.42	10/3	0.45	0
8/4	1.00	2.73	9/4	1.23	2.72	10/4	0	2.38
8/5	0.90	2.08	9/5	1.23	3.98	10/5	0.40	2.48
8/6	0.50	0	9/6	1.10	2.40	10/6	0.88	2.38
8/7	0.50	3.12	9/7	1.00	0.68	10/7	0	0.68
8/8	0.12	1.93	9/8	1.25	1.37	10/8	0.60	1.17
8/9	1.10	3.40	9/9	1.13	2.88	10/9	1.47	2.45
8/10	0.80	3.60	9/10	1.80	2.05	10/10	1.82	2.55
8/11	0.60	2.77	9/11	0	1.27	10/11	1.45	3.77
8/12	0.20	0.67	9/12	0.50	2.17	10/12	1.10	2.20
8/13	0.80	1.40	9/13	1.10	2.05	10/13	1.42	0.82
8/14	0.70	2.15	9/14	1.77	1.78	10/14	1.27	0.12
8/15	0.80	2.77	9/15	0.98	0			
8/16	0.90	1.48	9/16	0.62	0.30			
8/17	1.50	0	9/17	0	1.68			
8/18	0.30	1.68	9/18	0.10	1.70			
8/19	0	1.78	9/19	0.85	2.77			
8/20	0.80	1.95	9/20	1.28	3.23			
8/21	0.90	3.63	9/21	0.90	0.78			
8/22	0.20	1.50	9/22	0.11	1.58			
8/23	0.10	0.33	9/23	0.70	0.12			
8/24	0.61	0	9/24	0.30	0			
8/25	0	3.12	9/25	1.30	0.90			
8/26	0.90	2.48	9/26	0.75	2.72			
8/27	1.10	1.88	9/27	0.30	4.28			
8/28	0.90	0.47	9/28	0	0.60			
8/29	0.70	0.78	9/29	0	2.50			
8/30	0	1.80	9/30	0.77	2.37			
8/31	0.20	2.58						
Aug $\bar{X}$ =	0.66	1.96	Sept $\bar{X}$ =	0.80	1.74	Oct $\bar{X}$ =	0.78	1.71

Notes:

*10^9 cells/liter

Brackets indicate culture lifetimes.

Table 20. Water Quality Analysis (Friday, 10 October 1980)

Sample	NH_3 (μgat/L)	NO_2 (μgat/L)	NO_3 (μgat/L)	PO_4 (μgat/L)	Si (μgat/L)	pH	Total Alkalinity (meq/L)	Ca (ppm)
Tap	6.25	0.02	301.72	0.32	320.33	7.526	<0.84	--
IR	1.55	0.00	12.20	1.30	10.89	7.948	2.11	433
01B	33.30	6.33	217.71	12.57	13.39	7.522	1.50	--
01A	18.40	3.95	272.61	14.19	109.38	7.999	1.53	--
02B	35.10	7.71	214.52	12.57	34.60	7.470	1.53	--
02A	21.90	4.95	294.21	14.59	97.10	7.853	1.53	--
03B	25.00	6.70	223.00	13.58	42.41	7.474	1.50	273
03A	23.10	4.40	262.78	14.39	137.28	7.907	1.53	249
04B	26.00	6.51	196.85	13.18	53.57	7.514	1.50	287
04A	23.70	4.68	260.63	14.19	123.89	7.882	1.50	255
R1-b	1.50	4.99	224.62	11.27	64.29	7.977	2.11	--
R1-a	1.48	5.01	350.56	29.68	57.41	7.395	1.57	--
R2	1.42	0.83	448.54	31.82	232.69	7.342	1.06	--

IR = Indian River seawater
01 = Oyster flume 1
B = Before adding makeup seawater
A = After adding makeup seawater
R1-b = Before nutrient addition to reactor 1
R1-a = After nutrient addition

Table 21. Water Quality Analysis (Thursday, 8 January 1981)

Sample	NH_3 (μgat/L)	NO_2 (μgat/L)	NO_3 (μgat/L)	PO_4 (μgat/L)	Si (μgat/L)	pH	Total Alkalinity (meq/L)	Ca (ppm)
Tap	3.01	0.13	295.34	0.23	313.58	7.048	<0.84	--
IR	1.14	0.00	5.27	0.23	1.20	7.687	2.19	461
01B	16.50	13.76	407.40	32.41	56.66	7.514	2.40	--
01A	1.39	5.87	835.36	50.57	147.52	6.975	2.33	--
02B	32.70	14.68	380.00	30.86	51.76	7.516	2.26	--
02A	1.67	5.76	960.47	50.38	114.77	7.086	2.59	--
03B	18.60	15.41	428.56	31.87	65.76	7.514	2.29	295
03A	1.19	5.60	918.95	52.83	136.01	7.057	2.45	330
04B	23.70	14.50	478.67	30.70	54.24	7.497	2.70	316
04A	1.18	5.73	905.57	51.43	158.01	7.035	2.50	303
R1-b	20.90	10.50	463.53	32.73	93.03	7.403	2.47	--
R1-a	5.80	9.87	520.94	42.62	83.31	7.458	2.49	--
R2	1.69	5.19	896.61	53.84	125.22	7.347	2.43	--

IR = Indian River seawater
01 = Oyster flume 1
B = Before adding makeup seawater
A = After adding makeup seawater
R1-b = Before nutrient addition to reactor 1
R1-a = After nutrient addition

Table 22. Water Quality Analysis (Tuesday, 7 April 1981)

Sample	NH_3 (μgat/L)	NO_2 (μgat/L)	NO_3 (μgat/L)	PO_4 (μgat/L)	Si (μgat/L)	pH	Total Alkalinity (meq/L)	Ca (ppm)
Tap	< 1.01	0.00	349.69	0.12	276.45	7.454	< 0.84	--
IR	< 1.01	0.00	6.47	0.55	5.69	7.878	2.17	421
03B	8.97	9.90	216.01	8.09	73.12	7.652	1.35	308
04B	10.90	9.22	213.77	8.29	78.13	7.626	1.35	303
02A	< 1.01	6.72	924.20	38.40	113.46	8.264	2.00	--
03A	< 1.01	6.47	904.21	37.14	107.37	8.207	2.03	295
04A	3.16	6.80	918.34	37.97	119.55	8.175	2.05	280
R1-b	2.15	7.52	484.16	29.88	149.84	7.592	2.12	--
R1-a	1.21	8.78	456.95	24.59	96.48	7.972	2.16	--
R2-b	1.21	6.84	889.41	40.10	106.85	8.071	2.22	--
R2-a	1.08	7.11	796.67	41.33	128.69	8.424	1.98	--

IR = Indian River seawater
03 = Oyster flume 3
B = Before adding makeup seawater
A = After adding makeup seawater
R1-b = Before nutrient addition to reactor 1
R1-a = After nutrient addition

Table 23. Water Quality Analysis (Tuesday, 7 July 1981)

Sample	NH_3 (μgat/L)	NO_2 (μgat/L)	NO_3 (μgat/L)	PO_4 (μgat/L)	Si (μgat/L)	pH	Total Alkalinity (meq/L)	Ca (ppm)
Tap	1.05	0.04	267.76	0.24	281.37	7.671	< 0.84	--
IR	3.92	0.30	7.36	0.87	15.12	7.920	2.07	441
02B	20.90	8.59	323.11	28.81	114.21	7.784	1.61	--
02A	< 1.01	4.79	438.18	29.48	101.08	7.948	1.81	--
03B	20.20	8.69	298.25	28.42	118.36	7.762	1.59	280
03A	1.27	4.16	469.74	30.91	94.96	7.897	1.81	321
04B	21.20	8.59	318.47	28.81	134.97	7.598	1.61	255
04A	1.41	4.36	435.48	30.59	100.05	7.894	1.89	265
R1-b	1.12	4.61	328.44	28.81	123.43	8.277	2.16	--
R1-a	7.26	7.33	345.97	31.85	45.02	8.818	1.89	--
R2-b	4.01	5.37	465.49	35.21	126.50	8.335	2.04	--

IR = Indian River seawater
02 = Oyster flume 2
B = Before adding makeup seawater
A = After adding makeup seawater
R1-b = Before nutrient addition to reactor 1
R1-a = After nutrient addition

Appendix C.
Modified Proximate Analysis

One hundred milligrams of freeze dried meats were slurried with 1.0 milliliter of distilled water. Chloroform (1.25 mL) and methyl alcohol (2.5 mL) were added; the suspension was agitated for 1 minute and then centrifuged. The dense lower layer (chloroform) was separated and reextracted with methanol (2.5 mL) and water (1.5 mL). The lighter upper layer and sediment was reextracted with chloroform. The chloroform extracts were pooled and the chloroform evaporated from a preweighed vial. The vial and residue were weighed to give the amount of lipid in the sample.

The methanol-water extract and suspension were treated with 10 mL of water and 1 mL of 0.15% sodium deoxycholate at 20°C for 10 minutes; 50% trichloracetic acid (1.25 mL) was added and the precipitate removed by centrifugation. The precipitate was washed with ether: ethanol (1:1, v/v) and weighed to give the approximate amount of protein in the sample.

An aliquot of the trichloracetic acid containing supernatant was adjusted to contain 30% (w/v) potassium hydroxide and incubated at 90°C for 30 minutes. After cooling the digest to 4°C, two volumes of 95% ethanol were added and glycogen precipitation allowed to occur overnight. The glycogen precipitate was recovered by centrifugation, resuspended in 1 mL of water and 0.5 mL of 5% phenol, and heated at 90°C for 20 minutes. After cooling the solution to room temperature in an ice-bath, the color that developed was measured at 620 nm in a spectrophotomer. The glycogen content was estimated from a standard absorbancy curve determined for known quantities of glycogen.

An "artificial oyster" was contrived from known weights of a mixture of glycogen, menhaden oil, and beef albumen. This mixture was analyzed as described above. The actual oyster analyses were corrected for the recoveries obtained.

Appendix D.
Water Quality Bioassay Experiments

In the experiments reported here an attempt was made to test the prototype water quality and its effect on spat and larval growth by means of simple bioassay growth experiments. This kind of work had not been done before at this site.

There is surprisingly little information in the literature about the optimum conditions for the rapid growth of oysters over 1 gram, wet weight. Most workers in the past have worked with larvae and spat. Prototype oysters do not grow as rapidly as the wild animals in the Broadkill River. Possible nutritional limitations of the algal diets employed in the prototype are being examined as part of another University of Delaware Sea Grant research project.

The following experiments are described in this section:

I. The effect of algal culture medium and prototype water on the growth of *C. virginica* spat.

II. The bioassay of water quality using bivalve larvae.

III. Larval bioassay of the effect of XAD resins (Rohm and Haas) on water quality.

IV. The growth of *C. virginica* spat in treated and nontreated holding tank seawater.

V. The growth of *C. virginica* spat in foam fractionated prototype water.

EXPERIMENT I--THE EFFECT OF ALGAL CULTURE MEDIUM AND PROTOTYPE WATER ON THE GROWTH OF *C. virginica* SPAT

The feeding of oysters on algal diets often involves the addition of large volumes of algal culture medium. Indeed, prototype water largely consists of Guillard's f/2 algal culture enrichment medium. Thus, artificially cultured oysters may be exposed to much higher trace-mineral and B-vitamin concentrations than occur in the natural state. An experiment was therefore designed to examine the effect of growing oyster spat in algal culture medium.

Experimental Design and Conditions

Dates:	17 October to 6 November 1980
Mean initial individual spat wet weight:	50 mg
No. of spat per 4-L beaker:	8
Initial biomass per liter:	100 mg
Diet:	3H
Ration:	100 cells/µL/100 mg spat wet weight/day
Culture vessels:	aerated, 4-L glass beakers
Duration of experiment:	3 weeks
Temperature:	27°C
Salinity:	30 to 32 ppt

Water changed every other day

The experimental design and results are given in Table 29.

Table 29. Growth of *C. virginica* Spat in Different Types of Seawater

Treatment	Percent Increase in Spat Wet Weight After 3 Weeks		
	Beakers		
	1	2	Mean
1. 3H and Medium	265.2	256.2	261.0
2. Centrifuged 3H Minus Medium	196.5	191.1	193.8
3. Centrifuged 3H with Medium Added Separately	254.2	218.2	236.2
4. 3H + 4 L of 3H Medium[4]	350.4	329.4	339.8
5. 3H + 4 L of Holding Tank Water[1]	103.3	137.5	120.4
6. 3H + 4 L Guillard's f/4 Medium	244.0	344.8	294.4
7. 3H + 4 L Guillard's f/2 Medium	231.7	342.5	287.1
8. 3H + 4 L Guillard's f Medium	359.0	309.5	334.2
9. 3H + 4 L Guillard's 2f Medium	266.7	276.2	271.4
10. 3H + 4 L Guillard's 10f Medium	0	0	0
11. 3H + 4 L Pre-Treatment Prototype Water[2]	193.4	203.7	198.5
12. 3H + 4 L Post-Treatment Prototype Water[3]	249.3	294.1	271.7

Notes:

1. The holding tank (400 L) had a high biomass loading (> 10 g/L) of a wide variety of bivalve species. The water was changed every other day, and the animals were drip fed on approximately 300 L of algae (3H, T-ISO) a day. Water used in this study was collected every other day, GF/F filtered, and then added to the beakers.
2. Pre-treatment prototype water was collected from the prototype each evening after the day feeding of T-ISO. The water was stored overnight at 5°C, then warmed to 27°C and GF/F filtered before being added to the beakers.
3. Post-treatment prototype water was water that had been acidified with HCl (pH 3.5 to 4.0), foam fractionated, and then filtered with diatomaceous earth.
4. The 3H medium was prepared by GF/C filtration of a 3H culture.

Results

1. Additions of both the algal culture medium and Guillard's medium up to a 2f concentration improved spat growth compared with the growth of spat in natural seawater. At 10f concentration, however, spat growth was greatly suppressed.

2. Pretreatment prototype water was of poor quality, but the full water treatment process restored its quality to a level that was slightly better than that of seawater.

3. Shellfish holding tank water did not support good spat growth.

EXPERIMENT II--BIOASSAY OF WATER QUALITY USING BIVALVE LARVAE

The oyster spat experiments (I, above) lasted 3 weeks, and the number of treatments which were possible to test was limited because of the volumes of water and the size of the vessels required. As an alternative, the feasibility of using *Mercenaria mercenaria* larvae for quick and more convenient bioassays of seawater quality was investigated. Various seawater types were treated with different concentrations of EDTA and metasilicate. Larval growth was measured in each of the seawater types after a 6-day period.

Experimental Design and Conditions

Dates:	30 October to 5 November 1980
Animals:	2-day-old *M. mercenaria* larvae Initial mean shell length 125 µm
No. of larvae/100 mL:	500
Diet:	3H
Ration:	100 cells/µL/day
Culture vessels:	125-mL flat-bottomed flasks agitated on an orbital shaker
Temperature:	28°C
Salinity:	prototype water 20 ppt other seawater types 30 ppt
Duration of experiment:	6 days

Water changed every other day

The experimental design and results are given in Table 30.

Table 30. Growth of *M. mercenaria* Larvae in Various Types of Seawater Treated with EDTA and Metasilicate

Treatment		Mean Larval Shell Length (μm) After 6 Days ± Standard Deviation					
EDTA (ppm)	Metasilicate[1] (ppm)	Seawater	Holding Tank Water[2]	Algal Medium[3]	Prototype Water[4] Untreated	Prototype Water[4] Treated	Mean ± Std D
1	20	202.3 ± 21.4	187.8 ± 18.3	204.7 ± 18.5	203.3 ± 11.5	174.1 ± 10.0	195.9 ± 21.8
1	20	213.5 ± 12.6	145.5 ± 13.4	227.0 ± 13.7	190.9 ± 10.0	209.2 ± 14.4	
2	40	214.2 ± 15.4	172.5 ± 13.3	217.5 ± 13.3	190.9 ± 15.3	198.6 ± 16.8	202.8 ± 14.9
2	40	213.7 ± 15.1	--	215.1 ± 17.5	189.2 ± 14.1	214.0 ± 11.9	
5	100	207.0 ± 15.7	170.0 ± 19.1	213.6 ± 16.9	188.9 ± 16.9	189.7 ± 20.2	196.0 ± 17.1
5	100	210.0 ± 08.2	164.7 ± 19.0	219.0 ± 12.0	197.9 ± 13.8	199.7 ± 16.1	
0	20	200.3 ± 15.5	171.9 ± 25.7	211.3 ± 13.6	188.6 ± 13.7	197.5 ± 15.2	194.2 ± 11.3
0	20	203.0 ± 14.0	176.9 ± 14.9	199.0 ± 15.5	197.0 ± 11.6	196.7 ± 14.3	
0	40	200.7 ± 13.0	175.9 ± 18.4	232.8 ± 12.1	185.1 ± 14.9	193.6 ± 14.3	198.5 ± 18.2
0	40	209.0 ± 13.4	168.8 ± 14.7	216.6 ± 17.4	194.6 ± 13.0	208.0 ± 17.8	
0	100	216.7 ± 11.5	167.7 ± 20.1	214.6 ± 18.7	202.7 ± 12.7	193.5 ± 16.0	198.0 ± 17.2
0	100	206.7 ± 20.8	167.7 ± 14.8	216.7 ± 12.7	201.5 ± 12.3	192.2 ± 12.6	
0	0	204.2 ± 13.3	155.6 ± 13.6	214.4 ± 14.4	188.3 ± 11.5	196.1 ± 12.8	191.9 ± 17.8
0	0	204.4 ± 14.9	166.6 ± 14.1	210.0 ± 07.7	186.9 ± 18.8	192.4 ± 10.2	
Mean ± Std Dev		207.6 ± 05.2	168.8 ± 09.8	214.7 ± 09.1	193.3 ± 05.9	196.8 ± 09.3	--

Notes:

1. EDTA and Na metasilicate added to flasks from stocks dissolved in distilled water.
2. The holding tank contained > 10 g oyster biomass/L (see Note 1 to Table 29).
3. Algal medium was collected by GF/C filtering a culture of 3H.
4. Prototype water was 20 ppt saline, while that of other seawaters tested was 30 ppt.

Results

1. Larval growth rates showed the same trends as those of spat in Experiment I in that growth in the holding tank water was poorest and growth in the algal medium was best.

2. Neither EDTA nor metasilicate improved or inhibited larval growth at the concentrations tested. A similar result was obtained with oyster spat in an experiment designed to test the effects of EDTA and metasilicate on the quality of prototype water (personal communication, J. Ewart, College of Marine Studies, University of Delaware).

EXPERIMENT III--LARVAL BIOASSAY OF THE EFFECT OF XAD RESINS (ROHM & HAAS) ON WATER QUALITY

From the results of Experiments I and II, larval growth showed the same trends as spat growth with seawaters of different quality. In Experiment III, the potential usefulness of XAD resins in improving water quality was tested. XAD resins are macroreticular resins that have been developed for the removal of dissolved organic material from wastewater by adsorption. It was thought possible that seawater quality, as measured by the described bioassays, could be associated with high levels of dissolved organic material that accumulated during the oyster's feeding activities. The use of such resins might therefore reduce the level of dissolved organic material in the seawater and possibly improve its quality.

Experimental Design and Conditions

Dates:	13 December to 19 December 1980
Animals:	2-day-old *M. mercenaria* larvae Initial mean shell length 125 µm
No. of larvae/100 mL/flask:	500
Diet:	first 3 days 50 3H + 50 T-ISO cells/µL/day; second 3 days 100 3H/µL/day
Culture vessels:	125 mL flat bottomed flasks agitated on an orbital shaker

1 ppm EDTA and 20 ppm metasilicate added to all flasks

Temperature:	28°C
Salinity:	30 ppt

Water changed every other day

Duration of experiment: 6 days

The XAD resins were prepared as follows:

1. Resins washed with 4 bed-volumes of methanol
2. Left in methanol for 30 minutes
3. Washed with 20 bed-volumes of distilled water
4. Washed with 2 bed-volumes of 1 mole NaCl
5. Stored at 5°C in 1 mole NaCl

The washed resins were added to the seawaters at the required concentrations and agitated by aeration for 12 hours. The seawaters were then GF/C filtered to remove the resins, and their temperatures and salinities were adjusted.

Preliminary experiments demonstrated that XAD-2,-7, and -8 resins had no adverse effects on the development of fertilized *M. mercenaria* eggs to the D-stage at concentrations as high as 10 g/L. Indeed, the proportion of eggs that successfully developed

to normal D-stage larvae was significantly (at the 99% confidence level) improved when holding tank water was treated with the XAD-2 resin at 100 mg/L and the XAD-7 and -8 resins at 1 g/L.

The experimental design and results for the larval growth experiments in XAD-treated seawaters are given in Table 31.

Table 31. Effects of Seawater Treated with XAD Resins on the Growth of *Mercenaria mercenaria* Larvae

Treatment		Mean Larval Shell Length (μm) After 6 Days ± Standard Deviation[1]	
Resin	Conc mg/L	Seawater	Holding Tank Water
None	--	194.7 ± 11.1	188.0 ± 18.5
XAD2	100	--	194.2 ± 19.6
	1,000	--	193.2 ± 16.2
	[2]10,000	175.6 ± 17.9	183.3 ± 19.8
XAD4	100	--	191.6 ± 14.9
	1,000	--	189.9 ± 16.5
	10,000	190.6 ± 12.5	188.1 ± 16.2
XAD7	100	--	190.4 ± 18.5
	1,000	--	182.4 ± 14.4
	10,000	189.2 ± 13.0	184.4 ± 20.2
XAD8	100	--	192.8 ± 16.6
	1,000	--	189.1 ± 15.0
	[2]10,000	192.7 ± 11.4	196.6 ± 17.0

Notes:

1. Each mean and standard deviation value is based on the results of triplicate larval cultures. Approximately 100 larvae were measured from each culture when possible.
2. High mortality (> 50%) occurred with the XAD2 and XAD8 resins at 10,000 mg/L and holding tank water.

Results

1. Holding tank water was significantly improved (>99% confidence level) by treatment with XAD-2,-7, and -8 resins at 100 mg/L.

2. Duncan's multiple-range test indicated that there was no statistically significant difference between the growth of larvae in seawater and that in XAD-2 and -8 treated holding tank water. Thus, treatment of holding tank water restored its quality to that of seawater.

3. A concentration of 100 mg/L of the XAD resins was found to be the optimum concentration; larval growth was less at higher concentrations. High larval mortality occurred with 10 g/L of the XAD-2 and -8 resins.

EXPERIMENT IV--THE GROWTH OF *C. virginica* SPAT IN TREATED AND NONTREATED HOLDING TANK SEAWATER

In Experiment III, XAD resins were shown to be effective in restoring the quality of holding tank water with *M. mercenaria* larvae. In this experiment the most effective resins, XAD-2 and XAD-8 at 100 mg/L, were used to treat holding tank water, and the effect of these treatments on spat growth was measured.

In Experiment I it was observed that the prototype water treatment process was effective in restoring the quality of the recycled water. The treatment system comprised acidification, foam fractionation, and filtration with diatomaceous earth filters. In this experiment the separate effects of foam fractionation and filtration treatments on seawater quality were measured. Holding tank water was also treated with suspended Broadkill River silt, since this had been shown to improve water quality in growth experiments with spat (personal communication, John Ewart, College of Marine Studies, Lewes, Delaware).

Experimental Design and Conditions

Dates:	11 May to 4 June 1981
Mean initial individual spat wet weight:	20 mg
No. of spat per 4-L beaker:	20
Initial biomass per liter:	100 mg
Diet:	3H
Ration:	100 cells/µL/100 mg, spat wet weight/day
Culture vessels:	aerated, 4-L glass beakers
Temperature:	24°C to 25°C
Salinity:	30 ppt

Water changed every other day

Duration of experiment: 3 weeks

Preliminary filtration-rate studies indicated that XAD-2 and -8 resins have no adverse effect on the filtration rate of *C. virginica* spat at concentrations as high as 1,000 mg/liter. The resins were prepared as in Experiment III.

The experimental design and results are given in Tables 32 and 33.

Table 32. Growth of *C. virginica* Spat in Holding Tank Water Treated in Various Ways

Treatment	% Increase in Wet Weight (3 weeks)		
	Beaker 1	Beaker 2	Mean
Fresh Seawater	838.5	786.8	812.6
Holding Tank Water (HTW), Untreated[1]	915.6	548.2	731.9
HTW Foam Fractionated[2]	606.2	637.8	622.0
HTW + XAD8 Resin (100 mg/L)[3]	878.7	570.4	724.5
HTW + XAD2 Resin (100 mg/L)	698.5	734.2	716.4
HTW + Broadkill Silt (50 mg/L)[4]	686.7	852.3	769.5
HTW + Diatomaceous Earth (1 g/L)[5]	907.4	768.3	837.8

Notes:

1. Holding tank water was taken from a holding tank stocked with > 10 g oysters/L which were fed on 300 L of algae/day. Tank water was changed every other day and was collected on the second day after to remove uneaten algal cells, and part was treated in various ways (see below).
2. Eight liters of holding tank water was collected and foam fractionated for 12 hours. The foamed water was added to the beakers.
3. Resins were added to the collected holding tank water and agitated by aeration for 12 hours. The resins were then removed by GF/C filtration.
4. Broadkill silt was collected and added to the HTW and agitated by aeration for 12 hours. A silt concentration of 50 mg/L was chosen because this concentration was reported to have an improving effect on oyster growth when presented to oysters mixed with the food (personal communication Sayed Ali, College of Marine Studies, Lewes, Delaware). In this experiment the silt was removed from the treated water by GF/C filtration before being added to the beakers.
5. In this experiment diatomaceous earth was added to the holding tank water at 1 g/L, agitated for 12 hours, then removed by GF/C filtration. This concentration was chosen because it was equivalent to the levels used in the prototype water treatment system.

Table 33. Mean Percent of Algal Cells Consumed in 24 Hours by *C. virginica* Spat Fed on 3H in Various Seawater Types

Treatment	Percent of Algal Cells Consumed in 24 hr (Mean Value for 3-Week Period Based on Determinations for 20 Days)		
	Beaker 1	Beaker 2	Mean
Fresh Seawater	87.7	78.3	83.0
Holding Tank Water (HTW), Untreated	92.0	82.3	87.1
HTW Foam Fractionated	81.7	77.0	79.3
HTW + XAD8 (100 mg/L)	87.0	82.3	84.6
HTW + XAD2 (100 mg/L)	86.6	80.0	83.3
HTW + Silt (50 mg/L)	86.7	90.3	88.5
HTW + Diatomaceous Earth (1 g/L)	90.0	90.0	90.0

Results

1. The growth of spat in the duplicate beakers with treated and nontreated holding tank water was generally much more variable than that of spat in fresh seawater. This makes comparisons between treatments difficult.

2. The diatomaceous earth treatment appears to have the greatest beneficial effect on water quality. Growth in this treatment was comparable with that occurring in fresh seawater.

3. The growth of spat of both the duplicate cultures with foam fractionated water was poor. Foam fractionation appeared to further reduce the quality of the holding tank water.

4. Spat fed in the foam fractionated holding tank water did not appear to clear the algal cells as rapidly over a 24-hour period as spat fed in the other seawater types (Table 33). The reduction in the clearing rate of spat in the foam fractionated water compared with the clearing rate of spat in the untreated holding tank water was statistically significant at the 99% level of confidence ($t = 2.8418$, 78 d.f.) for the combined beaker 1 and 2 data. There is no significant improvement in the clearing rate of spat grown in the diatomaceous earth treated holding tank water and spat in the untreated water at the 95% level of confidence ($P = 94.1\%$, $t = 1.58$, 78 d.f.)

EXPERIMENT V--THE GROWTH OF *C. virginica* SPAT IN FOAM FRACTIONATED PROTOTYPE WATER

The results of Experiment IV indicated that foam fractionated water may have an adverse effect on spat growth, possibly reducing their filtration rates. The foam fractionator used in Experiment IV was not the same as that used in the prototype, and furthermore, holding tank water and not prototype water was used in Experiment IV.

On 13 January 1981 a foam fractionator was incorporated into the prototype system and used continuously to treat the water from the oyster flumes. By this means it was hoped to reduce the levels of dissolved organic matter. Experiment V was designated to test the effect of this treatment on water quality and spat growth.

Experimental Design and Conditions

Dates:	19 February to 4 March 1981
Animals:	6.9 mg *C. virginica* spat
No. of spat per 4-L beaker:	22
Initial biomass per liter:	12.5 mg
Diet:	3H
Ration:	100 cells/µL/100 mg, wet weight/day
Culture vessels:	aerated, 4-L glass beakers
Duration of experiment:	2 weeks

For the purpose of this experiment the foam fractionator on the prototype was turned on or off for 24-hour periods of time. Water was collected from the prototype after 5 hours and 17 hours of feeding the oysters on 3H. Fully treated prototype water (acidified, foam fractionated, and filtered) was also collected after treatment was complete. This fully treated water was from the T-ISO feeding period. Water from the prototype was collected once every 4 days. After the water was collected, uneaten algal cells were removed by continuous centrifugation and the water stored in 16-liter glass carboys at 5°C until required. Non-foamed water was stored for a maximum of 3-1/2 days and foamed water for 2-1/2 days at 5°C before being used for the experiment. The water was warmed to 23°C before being added to the beakers.

To test whether or not the foam removed from the prototype water had an adverse effect on spat growth, foam was collected after 17 hours of continuous treatment, centrifuged to remove algal cells, and stored at 5°C for a maximum period of 2 days. The total volume of foam produced by the fractionator was measured and, knowing the total volume of water that had been treated over the 17 hour period, it was possible to calculate the

volume of foam produced per liter of prototype water. This concentration of foam (X mL/L) or multiples of it were then added to fresh seawater (20 ppt) and the effect on spat growth measured.

The experimental design and results are given in Table 34.

Table 34. Growth of *C. virginica* Spat in Foam Fractionated Prototype Water

Treatment	Percent Increase in Wet Weight of Spat (2 weeks)		
	Beaker 1	Beaker 2	Mean
Fresh Seawater	823.8	790.2	807.0
Prototype Water - 5 hr, Fractionator OFF	811.4	658.2	734.8
Prototype Water - 5 hr, Fractionator ON	717.9	743.2	730.5
Prototype Water - 17 hr, Fractionator OFF	745.2	802.1	773.6
Prototype Water - 17 hr, Fractionator ON	700.6	687.1	693.8
Fully Treated Prototype Water	843.3	807.9	825.6
Seawater + 1/2 X mL Foam per liter	798.1	891.0	844.5
Seawater + X mL Foam per liter	906.5	865.1	885.8
Seawater + 2 X mL Foam per liter	927.4	865.8	896.6
Seawater + 10 X mL Foam per liter	886.4	905.5	895.9
Prototype Water - 17 hr, Fractionator ON + X mL Foam per liter	687.3	695.5	691.4

Results

1. After 5 and 17 hours of the 3H feeding period in the prototype there was a reduction in water quality.

2. Foam fractionation of prototype water did not restore its quality. Indeed, in this experiment and in Experiment IV, foam fractionation appeared to have had an adverse effect on water quality.

3. The complete water treatment of the prototype (acidification, foam fractionation, and filtration) restored prototype water quality to that of seawater. Since foam fractionation alone did not have this positive effect, diatomaceous earth filtration and/or acidification are clearly a necessary part of the process.

4. The foam removed by the foam fractionator did not have an adverse effect on spat growth when added to either seawater or prototype water, even at high concentrations. Indeed the addition of foam to seawater improved spat growth. Interestingly, no such improvement was observed when it was added to the 17-hour foam-fractionated prototype water. This indicates that other factors associated with foam fractionation, apart from removal of possible growth enhancing constituents present in the foam, were responsible for the poor spat growth in this water.

SUMMARY OF CONCLUSIONS

The simple bioassay experiments described in this report are important in that no other oyster growth tests of the prototype water quality or effectiveness of the water treatment systems have been carried out before. Experiment I shows that the high levels of algal medium, which include an f/2 concentration of Guillard's trace metal mix, have a positive effect on spat growth. This result is important since prototype oysters are grown essentially in f/2 algal medium. Whether there are long-term effects of such exposures on oyster growth or whether large animals respond differently to algal medium than small spat was not investigated.

The results of Experiments I and II indicate that the complete water treatment process (acidification, foam fractionation, and filtration with diatomaceous earth) is effective in improving prototype water quality as compared to fresh seawater. Experiment V indicates, however, that water quality deteriorates in as little as 5 hours after the commencement of feeding in the prototype. Oysters in the prototype may therefore be subjected to reduced water quality for some of the time. Continuous use of the foam fractionator is not effective in improving water quality (Experiment V), and in Experiment IV it was shown to have an adverse effect on oyster growth, perhaps by reducing the filtration rate of the oysters (Table 33). The foam removed from the prototype water in Experiment V improved the growth of spat when it was added to fresh seawater, so foam fractionation may have the effect of removing growth promoting substance(s) from seawater. Carriker et al.[37] (1980) found that the foam contained high levels of cadmium, copper, and zinc. Adding foam to foam-fractionated prototype water (Experiment V) did not improve its quality, and so other adverse effects of foam fractionation, apart from the removal of growth promoting substances, may be involved.

Experiments II and III illustrate the usefulness of larval bioassays in the testing of seawaters of different types and treatments. Larval bioassays are quick, and because only small volumes of seawater are required, large numbers of flasks, i.e. treatments and replicates, can be treated at once. Experiment II indicates that the responses of larvae to seawater types are similar to those of spat. Experiment III indicates the potential usefulness of XAD resins in the treatment of fouled seawater. If further experimentation shows that resins consistently improve water quality for oysters, then the resins could easily be incorporated in a prototype design system. This approach may be very effective in the continuous treatment of prototype water during oyster feeding activity.