COLLEGE OF MARINE STUDIES
UNIVERSITY OF DELAWARE
NEWARK, DELAWARE

ARTIFICIAL PROPAGATION OF COMMERCIALLY VALUABLE SHELLFISH

PROCEEDINGS
OF THE CONFERENCE ON

ARTIFICIAL PROPAGATION OF COMMERCIALLY VALUABLE SHELLFISH

- OYSTERS -

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EDITED BY
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PREFACE

In the late 1950's the oyster industry in the Delaware Bay region suffered a catastrophic collapse due to a parasitic protozoan, familiarly known as MSX (*Minchinia nelsoni* Haskin, Stauber, Mackin). In Delaware the value of oyster landings declined from almost $3 million to less than $40 thousand in a few years.

Several states, including Delaware, with the support of the Bureau of Commercial Fisheries, initiated research directed toward rehabilitation of their oyster industries. Since the late fifties, the University of Delaware Marine Laboratories (UDML) has been attempting to select a disease resistant oyster from the Delaware Bay region by means of field mortality studies.

In March 1966, UDML research took a new tack. Based on previous mortality studies, and using advanced aquacultural techniques, the laboratory initiated studies to produce a disease-resistant oyster that also demonstrated fast growth and good market qualities. The procedure we are following is 1) to spawn known resistant stocks artificially in the laboratory, 2) to rear and set the larvae under hatchery conditions, and 3) to test the progeny for the desired disease and market characteristics under field and laboratory conditions. By means of a carefully planned and controlled selective breeding program, we hope to produce a "super" oyster. However, the ultimate contribution from these efforts may be aquacultural "spin-off" that will allow modernization of oyster production to include the automated culture of oysters under highly refined and controlled environmental conditions; i.e., oyster factories.

Initially, the problem was to develop the necessary techniques for holding and conditioning Delaware Bay brood stock for controlled spawning; to spawn these oysters on demand; to successfully rear and set larvae; and to place spat in the field for convenient, safe monitoring and retrieval. In 1967, we achieved a significant breakthrough in conditioning and spawning Delaware Bay oysters out of season. However, it became readily apparent that the oyster industry's problem in Delaware was of such magnitude and scope that biologists alone would never completely resolve it. Coincident with our successful spawning work, the University of Delaware's administration began to encourage its faculty to develop a multidisciplined marine research project proposal suitable for submission to the National Science Foundation Sea Grant Program. The oyster problem in Delaware captured our interest because it is regional in scope, requires a task force or multidisciplined approach, and the investigation of the problem is consistent with the philosophy of the Sea Grant Program.

Therefore, in October 1967, the senior author with the help of the junior author fostered the idea of a multidisciplined oyster project by writing a tentative research proposal that was submitted to our University colleagues with the plea for support in the form of appropriate subproject proposals. At first the idea of working on oysters was somewhat foreign to nonbiology disciplines. But we received sufficient help to fashion an informal proposal that was submitted to NSF Sea Grant in November 1967. Encouragement from Mr. Harold Goodwin of the Sea Grant Program resulted in the submission (Spring 1968) of a formal proposal by the University with the help of its Marine Science Coordinating Committee. The total project involved three colleges, two divisions and five departments. The project funds were awarded and work was begun in September 1968.

Almost immediately we began planning for a conference on the *Artificial Propagation of Commercially Valuable Shellfish*. We felt that the conference would allow us to present brief progress reports on this Sea Grant project's first full year of oyster research (the introductory speaker's remarks) while at the same time provide a truly interdisciplinary forum for the review and discussion of oyster culture problems from the viewpoints of industry and federal and state scientists (principal speaker's remarks and discussion following). Therefore, the conference was structured on the basis of our Sea Grant Project involving principal investigators of the project and subprojects as introductory speakers. Principal speakers were selected on the basis of their stature as leaders in their fields of endeavor and on their qualification to speak to their topic as related to our Sea Grant subprojects. We definitely feel that the conference was a success in this regard as is evidenced by the quality of the papers herein and the scope of the coverage of these proceedings.

Eleven major papers were presented with their respective introductions. Questions and answers following the presentations were also taped and these have been included in the proceedings. Because participants in the question and answer sessions did not always direct their remarks to the microphone, some interesting commentary was lost. This has obliged us to omit incomplete statements and sources and to indulge in some creative writing for purposes of reconstruction. We hope the poetic license retains the spirit and substance of the original remarks. Insofar as information available permits, titles and addresses of contributors have been updated to reflect their present status.

ACKNOWLEGMENTS

Appreciation and thanks are expressed to Dr. Franklin C. Daiber, Director of the Marine Laboratories for support of the conference and to the principal speakers who brought with them a vast range of knowledge. Without the generous cooperation of the Marine Science Coordinating Committee and the Sea Grant Project team from the Departments of Agricultural Engineering, Biological Sciences, Civil Engineering, Geography and the Divisions of Technical Services and University Extension, the conference would not have been conducted in a manner so exemplary of genuine interdisciplinary spirit and effort. Mr. Donald Bard, Supervisor of Conferences, and his staff efficiently executed the plans and program for the conference. To our colleagues and associates who kindly suffered our excesses of enthusiasm, concern, and occasional doubts, we offer our sincere thanks. Acknowledgments are due to the Bureau of Commercial Fisheries, Delaware Commission of Shellfisheries, the Delaware River Basin Commission and the University of Delaware Research Foundation for making the conference possible through their support of our shellfish research and related projects. And finally, we are very much indebted to the Sea Grant Program for their generous and timely support of this endeavor that is so important to the future of the shellfish industry.

We sincerely hope that this conference and the research described within these proceedings will mark a significant milestone in man's endeavor to farm the sea.

K. S. Price Jr. and D. L. Maurer
Conference Coordinators and Editors of the Proceedings
September 1970

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OPENING COMMENTS

FRANKLIN C. DAIBER
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What is aquaculture? There is a small, scattered, but growing effort in the United States to produce aquatic plants and animals in a controlled environment or a manipulated ecological system. The effort is much greater elsewhere than in this country. We are all greatly impressed by the tons of blue mussels that can be produced per acre in Spain or the quantity of shellfish that can be grown on rafts and other devices in Japanese and Australian waters.

There are certain advantages to aquatic culture over terrestrial production. Many aquatic organisms do not need to expend much energy searching for food; there is no need for temperature regulation or heavy supporting skeletons as required for land existence. We can also make use of the third dimension in the water world.

The purposes of aquaculture are quite different for the United States and for other regions of the world. Outside the United States, the need is to raise an inexpensive source of protein for many hungry people. In this country we are primarily concerned with developing a specialty market for the gourmet trade, and we are interested in meeting the increased demand for marine colloids derived from aquatic plants.

There are certain constraints on the development of aquaculture in this country. Labor costs are high. Incentive is lacking. We do not need to meet the demands for low-cost protein. There is only a demand for luxury items. Legal impediments have been induced when entrepreneurs want to transfer coastal public lands to private management. Restraints have also been applied by competitive interests such as boating or recreational fishing. Technical problems and lack of risk capital also have a fettering effect on the growth of aquaculture.

The technical problems and needs of aquaculture in the United States are centered around the fact that we have insufficient information about the complex ecological interactions that confront the organisms we wish to culture. We still do not know all of the basic parameters that influence a particular organism, nor do we have any real grasp of the situation involving synergistic influences upon the life of a particular species. It is only in recent years that we have begun to appreciate the magnitude of this complexity. We need more information about the biology and genetics of the species we wish to culture. There is increased need for nutritional

studies and growth requirements. We are woefully unprepared to deal with the diseases and parasites of marine organisms when large numbers are placed in restricted situations. Ecological parameters, both of the physical environment and of population structure and growth, need to be examined in the light of the demands being placed upon this kind of information by aquacultural practices. Such information has a profound influence on engineering design.

These kinds of information are expensive to get. It requires a great deal of time, and the equipment needed to gather this information is usually much more expensive than one encounters for land investigations. This is compounded by the fact that we are never sure of the kinds of commercial returns that one might expect from such an investigation. The hazards are great, and industry quite often is unprepared to take the risk that seems to be demanded. It is becoming increasingly clear that greater levels of funding are needed to carry out research programs and it seems desirable to establish a broader base of funding for various pilot studies, including private sources and governmental support.

There is need for ecological engineering design studies concerned with environmental control, predator control, and metabolic control. These designs should be based on the findings of the biologist and carried out in conjunction with the ecologist. These involve equipment, plant design, and techniques dealing with feeding rates and methods, and food additives. They have to deal with larval, juvenile and adult restraint and growth, water quality control, and the problems associated with selective breeding to develop appropriate strains.

There are several benefits that can be readily derived from the establishment of aquacultural procedures. One of these is the development of a brand new marine food industry. Jobs would be created in terms of biological production, the design, manufacture and maintenance of appropriate kinds of equipment for culture, harvesting, processing and packaging. New techniques for advertising and marketing would be developed and would need staffing. Liaison needs to be established between the technical community and industry. The evidence suggests that for the foreseeable future, aquaculture should not be oriented toward bulk production of cheap protein food in this country. This kind of protein probably could best be obtained through the proper management of our coastal fisheries. The evidence also suggests that emphasis should be placed on freshwater and brackish water areas so far as aquaculture is concerned. The greater abundance of nutrients in the shallow coastal waters and the engineering problems associated with the open ocean are such that the demands for development of aquaculture in this part of the marine environment are some way down the road.

Another real benefit is the establishment of a stable and reliable source of selected marine products. These species and the products derived from them could be produced and harvested at times other than under natural conditions. The production of these marine species and any byproducts should be removed from the common property resource concept to that of private property control and management. The evidence certainly suggests that this would be a much more efficient and therefore more profitable route to travel. It would also provide a more efficient use of the water column, so long as there is proper interaction with the other usages that would be applied to that particular marine locale.

Another real benefit would be better quality control with enhanced production through the establishment of new strains, nutrition studies, and environmental control.

Our meeting today and tomorrow has to deal with a particular facet of aquaculture– that concerned with the propagation of shellfish and particularly the oyster. There is the realization of the need for a philosophical change from the long established hunter method for a common resource to agricultural manipulation. We are concerned with the depletion of a resource brought about by poor management, by degradation of the environment, and by disease. The shellfish industry is faced with a low economic return, and deals with a labor pool that is getting old. It is not attractive for economic investment or for bringing new and young men into the labor pool. Further, we are recognizing that single interest groups cannot solve the problems, i.e., the biologist cannot go it alone any more than an engineer or an economist. It is becoming increasingly evident that we need to develop an overall approach drawing on the expertise of many disciplines to evaluate the input at various levels in developing a new industry from the old that will be economically attractive and still function as a part of the ecological whole.

The program that you have in your hand reflects the interaction of these various disciplines on the University of Delaware campus. The marine biologists have been concerned with ecological problems dealing with culturing, breeding, predation, disease, and the general environmental parameters to which an oyster is subjected. The agricultural engineers have been taking advantage of this ecological information and designing refrigerating and heating systems, tanks, culture chambers, etc. to establish controlled environments or to help the biologist gather information useful to understanding the ecological demands that are placed upon the oyster. Our geographers have concerned themselves with the water balance of the region as it is going to affect the salinity regimes of our estuarine areas. The agricultural engineers have also been concerned with the problems of shucking an oyster; problems that are receiving greater impetus because of increased labor demands and a reduced labor pool, making it imperative that we find a more economical method for getting into the oyster. The systems engineers have been taking these various bits and pieces of information and attempting to establish the various kinds of interactions, their intensities, and where they best might enter the entire system to have an appropriate influence on the growth of the shellfish industry from spawning to market. While these kinds of activities are going on, our agricultural extension people have been working on an educational program among the local watermen. It is only too clear that the scientist and engineer can work very hard, but it would come to naught if their findings can not be transmitted to the people who would have greatest economic use for this kind of information. Therefore, we feel our extension program is an extremely important facet to the total project.

Aquaculture is beginning to make some forward steps and for this reason I would like to have this program considered as a progress report, not a final one, and a review session of work accomplished to date.

Introduction to DEVELOPMENT OF CULTURE TECHNIQUES FOR A PILOT SHELLFISH HATCHERY

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As a basis for developing techniques and facilities for oyster culture our operational phases selected were patterned after those practiced by private shellfish hatcheries. These phases involve: 1) conditioning of oyster brood stock for reliable out-of-season spawning, 2) rearing of larvae, 3) setting of larvae, and 4) field and laboratory maintenance of spat. The major objective relative to these phases is to define the variables necessary to develop a shellfish hatchery with a completely controlled system.

Conditioning of Oyster Brood Stock

In the past three years, many (200+) spawning experiments were conducted to determine the temperature-time schedule necessary for command spawning of local oysters. In earlier experiments, oysters were held in the laboratory under a variety of temperature regimes. For example, one group was held 118–147 days at an average temperature of 16.1° C; whereas another group was held under similar conditions with an additional period of 8 to 15 days at 18.0° C. Results of these experiments showed that the average delay between initial stimulation and spawning in these experiments was extremely long, suggesting that these oysters were not conditioned adequately for reliable laboratory spawning. An additional period of conditioning at higher temperatures would allow more precise control of laboratory spawning of Delaware Bay oysters.

In an effort to refine a temperature-time schedule required for command spawning, new spawning experiments were conducted. Oysters were obtained from natural rocks early in February (water temperature of 3.0° to 5.0° C). Histological sections were taken to determine the condition of their gonads. Oysters were divided into three groups; one group was placed in the field as a control, while the other two groups were held in the laboratory and gradually acclimated to 15.0° C and 23.0° C. Histological samples from oyster gonads from each group were taken every two weeks. At the same time, oysters were artificially induced to spawn.

Several points emerged from the spawning data: 1) oysters did not resorb gonads even when held for periods up to eight months in the laboratory if proper temperature and quantities of water are provided for the brood stock; 2) a

temperature-time schedule was developed to predict when Delaware oysters would spawn following a given temperature conditioning period. Our data support results of other workers who found it necessary to determine the conditioning and spawning requirements of the various physiological races of the American oyster before a reliable spawning time could be predicted for a given area.

Rearing of Larvae

Larvae are cultered in natural filtered seawater which has been incubated in a greenhouse for 24–36 hours, to promote phytoplankton densities and heated to 24–26.0° C for optimal larval growth. From May through September this method works, but a hatchery can not be exclusively dependent on natural phytoplankton because it can be unreliable. To this purpose an algal culture facility was constructed. Algal rearing methods follow those practiced at the Marine Biological Laboratory, Milford, Connecticut. Our present algal rearing facility will require additional work before it is a reliable food source.

A promising development in larval rearing has recently emerged. Over two hundred million larvae were reared to setting size in a constant running, seawater tank in a greenhouse. This experiment was performed in July when seawater was approximately 25° C. If this method can be refined, the mass culture of larvae can be accommodated without costly handling expense.

Setting of Larvae

Considerable research has been expended on factors influencing setting and the development of cultchless oysters. In laboratory experiments light, temperature, nutrition, and substrate have been recognized as important factors. We found that setting larvae preferred dark surfaces to light ones, and grooved surfaces to smooth ones. These experiments were coupled with experiments involving natural and artificial chemical attractants. Treated shells yielded higher spat counts than control shells for oyster larvae. By controlling light, substrate and chemical attractants, a means to control setting pattern may be developed. In turn this would reduce wastage due to death by overcrowding.

As an intermittent step to producing cultchless oysters, crushed surf clam shell was used as a substrate. Larvae had no difficulty in setting on shell fragments. Moreover, larvae also set on small pieces of cork, and grew much faster than larvae set on plastic netting which were then removed as cultchless oysters. Suspension in the water column promoted rapid growth of those spat set on cork.

Laboratory and Field Maintenance of Spat

In a hatchery, a growth period of three to four years (the time it requires local oysters to attain market size) would not be commercially feasible. To accelerate the growth rate of lab-reared spat, they were held in running seawater at 25.0° C for over six months. They grew at a rate that, extrapolated to a year, would produce an oyster exceeding minimum market size. This experiment demonstrated that the use of heated water for growth of hatchery spat would permit rapid recycling of oyster generations under mariculture conditions. Pilot studies of spat

placed in the discharge of thermal effluent of a local power plant indicated that growth occurred throughout the winter while spat held in natural growing areas ceased growing.

By inducing artificial spawning in April in oysters that normally spawn in the middle of July, the initial growth period of the resultant spat was increased as much as ten weeks. Further these spat were gradually acclimated in the laboratory to cool spring water temperatures and then placed in the field. The combination of spring spawning with acclimation treatments allows for early growth and rapid turnover of costly hatchery facilities.

Thus far, our research has shown that it should be possible to develop the technology necessary to produce oysters commercially using artificial culture techniques.

DEVELOPMENT OF SHELLFISH CULTURE TECHNIQUES

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INTRODUCTION

Since I am the first speaker on this program, I have a number of advantages; I can select what to discuss, emphasizing any points I choose, and go into the details of any of the special fields to be considered at this conference. I shall confine myself principally, however, to a general review of the history and progress of development of methods for culturing larvae of commercially important mollusks, and leave the details for the speakers who follow me. Several of them, including Messrs. Davis and Engle and Drs. Ukeles and Hidu, were my associates at Milford; others, like Dr. Menzel, followed me in my work as oyster biologist in the State of Virginia and later cooperated with my group and me in studies of the hybrids of *Mercenaria*. I also include in this group Mr. George Vanderborgh Jr., my old friend and associate, because he spent considerable time at the Milford Laboratory studying our methods and approaches. All these people are now recognized as experts in their respective fields. They will do a much better job than I could do in discussing their specialties.

Even before the development of the microscope and the discovery of oyster gametes, some of the men engaged in raising oysters and other bivalves in Europe and Asia almost certainly entertained the idea of increasing production by rearing new generations of these mollusks in confined areas. Similar thoughts undoubtedly prompted biologists during the second half of the last century, when gametes and zygotes of several species of mollusks were already known, to study the development of artificial methods for producing sets of oysters. These studies consisted principally of attempts to grow oysters, clams, or mussels in small artificial or natural ponds either from spawn that was released normally by the parent mollusks, by inducing them to spawn artificially, or by introducing spawn obtained by stripping their gonads.

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EARLY EXPERIMENTS IN CULTURE

Early Pond Experiments in U.S.A.

Literature on breeding oysters in ponds contains many articles in which the possibilities of artificial cultivation of these mollusks are discussed and evaluated (Baughman, 1948). To review all these articles would require a great deal of time; therefore, I shall confine myself to a brief discussion of a few papers that should be of general interest.

The first paper is that by Moore (1898), who expressed his faith in use of small natural or artificial ponds by stating on p. 323 that "the culturists of Europe have shown that a very considerable control can be exercized over the conditions in parks used for growing oysters from seed, and with proper modifications the same success could doubtless be obtained with breeding ponds for raising seed." Moore, who at that time was one of the leading oyster biologists of the United States, discussed spawning ponds, their design, and their use. Part of his discussion concerned the work of Dr. J. A. Ryder, who devised a new method of spat culture that called for a comparatively large pond, 40 × 60 feet, connected with open water by a series of narrow canals about 3 feet wide. At the proper time of the year approximately 100 bushels of ripe oysters were placed in the spawning pond. At the same time special collectors were suspended in the canals in such a manner that they practically blocked them. These collectors were baskets made of galvanized wire netting of 1½-inch mesh, each filled with about 3 bushels of clean oyster shells. Since the width of the baskets was almost equal to that of the canals, it was hoped that the water currents during the rise and fall of the tide would pass through the baskets, thereby keeping the shells clean of any sediment that would interfere with attachment of metamorphosing oyster larvae. The main idea, however, was that a large percentage of the larvae, developing from the spawn released by the parent oysters, would remain in the spawning pond until ready to set and then attach themselves to the shells used as collectors. Unfortunately, this method did not work, because heavy silting rendered the collectors virtually useless, and, chiefly, because most of the larvae were flushed out before they were ready to set.

The other experiment described by Moore consisted of attempts to rear oyster spat from artificially fertilized eggs in practically closed ponds. This experiment, again under the direction of Dr. Ryder, was conducted on the salt marshes of Chincoteague Bay, Virginia. The pond was about 20 feet square and 3½ feet deep. It was connected with the bay by a canal, 10 feet long and 2 feet wide. The mouth of the canal was closed with a filter made of "gunny cloth or bagging material." The water in the pond remained at the same temperature and salinity as that in the open bay. During the spawning season, artificially fertilized eggs were introduced in the pond. Forty-five days after the beginning of the experiment spat ranging from 1/4 to 3/4 inch were found attached to the shells placed in the pond as collectors. Again, the experimenters encountered difficulties because of the heavy sedimentation but, nevertheless, they demonstrated that spat can be raised in ponds from artificially fertilized eggs. Thus, even though this experiment ended in the production of only a small number of oyster spat, it was the first successful attempt at producing seed from artificially fertilized eggs released in a closed pond.

The second reference that I include is unique. It is entitled, "Hibbert patent oyster bed and method of propagating therein." It is a pamphlet of some 13 pages and contains a detailed drawing of Hibbert's patented seed collecting bed (Hibbert, 1897). This document, which I have in my possession, contains no indication as to where and when it was published, although I would assume that it was in 1897 because the pamphlet states that a patent was granted to Mr. Hibbert on March 5, 1895, and December 3, 1896.

Judging by the description of the method, Mr. Hibbert had a rather limited knowledge of the biology and physiology of oysters. This deficiency is borne out by the letterhead of the letter I found in the pamphlet, which stated that Mr. Hibbert was an architect and engineer engaged in, among other things, mortgages and business loans. He was apparently a very capable businessman, nevertheless, and certainly an able advocate of his method. To demonstrate his faith in the possibility of the method, which apparently has never been tried, I would like to quote a portion of the last paragraph of his pamphlet, on p. 13. "To recapitulate: with an original expenditure of $6,000 for one acre of propagating tanks, and $20,000 for ten acres of oyster-raising tanks, altogether $26,000 at the end of the second year will return a profit of at least $120,000, and every succeeding year will show an equal profit. In other words, the investment will show an annual return of something like 500 percent above the original investment. These figures are not imaginary, but are carefully and mathematically calculated." Obviously, even in the days of Mr. Hibbert people wanted to believe that in the hands of capable persons the oyster business was a gold mine.

Early Laboratory-Hatchery Experiments in U.S.A.

Although it may be feasible, under certain conditions, to grow oyster larvae in small ponds, it is almost impossible to control many conditions in these small bodies of water, especially the quality and quantity of food and often even temperature, salinity, pH. It is understandable that because of these difficulties the people concerned with oyster propagation early thought in terms of growing oyster larvae in special containers and under controlled conditions. Strangely enough, little if any progress was made in this field for several decades, regardless of the interesting work of Brooks (1880) on the development of eggs and early larval stages of the American oyster, *Crassostrea virginica*, and the unsuccessful attempts by Ryder (1883) and Winslow (1884) to bring oyster larvae to metamorphosis under laboratory conditions. Unquestionably, many other investigators, whose efforts remained unreported and unknown, met with the same lack of success. Because of these failures even as recently as 1920, Churchill (who was regarded as one of the leading oyster experts of the United States at that time) concluded that even though some of the investigators who repeated Brooks' experiments had managed to raise the larvae to be four or five days old, no one had succeeded in rearing any of them to the setting stage because of immense practical difficulties—chiefly those of providing the organisms with proper food and change of water (Churchill, 1920). He concluded (p. 26), "It is impossible to do this on a scale large enough to be of practical application to the oyster industry and the method in itself is not functional." He also

thought that the same statement was applicable to adaptation of the method in which attempts could be made to substitute artificial ponds for tanks and have these ponds connected by narrow canals in open water. Churchill finally stated (p. 26), "While it cannot be said that the problem of so-called artificial propagation may not be solved at some future time, for the present it must be emphasized that the oyster culturists should base no false hopes on the practical application of this method." He continued further on the same page, "In view of the barren results of 40 years' experiments in this line, it is best to devote attention to the modification and perfection of the methods, which have proved to have a certain measure of success and which are applicable to the industry as carried out on such a vast scale in the United States."

By a strange coincidence, while Churchill was publicly discrediting artificial rearing of oysters and showed his complete distrust in its possibility, a young biologist, W. F. Wells, was preparing to publish his first report on the successful rearing of larvae of the American oyster from fertilized egg to the setting stage under laboratory conditions (Wells, 1920). Wells obtained eggs by stripping ripe oysters. He opened the oysters, dissected away their mantles, and examined a small quantity of spawning material under a microscope to determine the sex. Females that appeared to be ripe were stripped and their eggs placed in a quart jar of water to which a small quantity of sperm from several males was added. Wells "clarified" the water to remove coarse suspended material that could interfere with the larvae.

Approximately two hours later, when the developing eggs were still lying on the bottom of the jars, the supernatant water was siphoned out and the vessels were refilled with new filtered seawater. This step was repeated once more before the eggs developed into swimming embryos and rose to the surface. By this method Wells eliminated the largest portion of unused sperm, blood cells, and other impurities. After the larvae became motile they were gently siphoned into large hatching jars which, in the latter stages of Wells' work, were as large as 50 gallons each and had an outlet at the center of the conical bottom that permitted withdrawal of the water.

Wells used a milk separator to remove the larvae from the water of his hatching vessels every two days and then returned them to a new supply of water. It is clear to me that his experiments were successful chiefly because he was able to change the water in which the larvae were kept and because the seawater, in the area where he was working, contained many food organisms even after being coarsely filtered or, as Wells called it, clarified.

Wells' achievement marked the successful culmination of the effort initiated by Brooks 41 years previously. Wells continued his studies after the original success. In 1922 he established a small laboratory at Cold Spring Harbor but because of the poor "quality" of the water, which probably contained only a few good microorganisms utilizable by larvae, that season was a failure. In 1923 he moved to a new location at Oyster Bay and had a successful season. Within a few years he managed to rear larvae of not only *C. virginica* but also of the common mussel, *Mytilus edulis;* quahog, *Mercenaria mercenaria;* soft clam, *Mya arenaria;* and the scallop, *Pecten irradians.*

It should be emphasized that in all these experiments Wells relied exclusively

on the food naturally present in the seawater. Because he practiced dividing each culture of larvae into two portions after every change of water this measure helped him to provide the larvae with enough food. Wells was able to obtain ripe spawn only from early June until the middle or latter part of August. Since he was not aware of the possibilities of having ripe oysters outside their natural propagating period (Loosanoff, 1945), his season for working with oysters and their larvae was only about 2½ months long.

Wells (1926, 1927) roughly divided his method into the following basic steps: (1) obtaining eggs artificially and fertilizing them; (2) waiting until they developed to the straight-hinge stage; (3) concentrating the larvae by means of centrifuges at two-day intervals, transferring them into new seawater, and dividing the population of larvae of each jar at each change of water; (4) rearing the larvae in the jars, using the centrifuge method until the larvae were large enough to be retained by fine screens; (5) collecting spat on artificial collectors.

Wells reported very few observations on the physiological requirements of larvae of oysters, or other bivalves with which he had been working, and almost nothing on the quality and quantity of food needed by these organisms. Neither does it appear, from his articles, that he realized, as was later found by Milford investigators and European workers, that bivalve larvae are often subjected to diseases caused by microorganisms (Davis, 1953; Loosanoff, 1954; Walne, 1956a).

As many biologists have found from their own sad experiences when they wanted to branch out into a new field of research, Wells' achievements were not widely acclaimed by biologists or by members of the industry. In some quarters, nevertheless, his experiments on rearing oyster larvae were considered promising. Among those who supported Wells was the Bluepoint Oyster Company of West Sayville, New York, which offered to establish an experimental laboratory-hatchery on its premises for his work. The skeptics were, however, much more numerous than those with some measure of faith. Among those who were very critical of the practical value of Wells' contribution was Thurlow Nelson (1921) who in Bulletin 351 of the New Jersey Agricultural Experimental Station entitled *Aids to Successful Oyster Culture*, stated on p. 45, "I would wish in no sense to discourage the development of artificial oyster propagation. The study of scientific oyster farming is still in its infancy. We know, as yet, practically nothing of the relations which exist between the oyster larva and its surroundings. Further investigations may unfold to unthought-of possibilities. With our present knowledge, however, it is only just to the oyster growers to point out that until oysters become a far more expensive commodity than they are at present, the artificial rearing of the larvae could hardly be made profitable.

"Furthermore, I would voice concurrence in the view now held by several oyster investigators, that the more rational method of seed production, for the present at least, is that presented in this bulletin, namely, develop the spawning and setting grounds not available, through intelligent cooperation with natural forces."

Virtually simultaneously with Wells, and only a few miles away from his field laboratory, another American investigator was trying to rear oyster larvae. This investigator was Herbert Prytherch of the former U.S. Bureau of Fisheries, who was spending his summers at Milford, Connecticut, working on spawning and

setting of oysters in Long Island Sound. Unlike Wells, Prytherch did not strip the oysters to obtain the eggs but induced spawning by increasing the water temperature (Prytherch, 1924). After the eggs developed into swimming embryos the latter were transferred to the rearing tanks which were supplied with slowly running seawater. This procedure was a radical departure from Wells' method, which consisted of growing larvae in standing water.

To retain the embryos Prytherch used filters of fine sand and also a material known as "filtrose," which was made in the form of blocks of various porosities. After the larvae reached the age of 10 days, they were retained in the tanks by means of fine monel metal screens which, presumably, permitted a good flow of seawater but yet retained the larvae. When the larvae were ready to metamorphose, which in Prytherch's experiments required between 15 and 20 days, collectors made of various materials and also old oyster and clam shells were placed in the tanks to catch the set. These experiments were relatively successful because in one of the last tests Prytherch collected over 1,000 spat. Like Wells, Prytherch gave no supplementary food to the larvae; nevertheless, he reported some interesting observations on their growth and on some aspects of their metamorphosis which he recorded, in 1925, on 16-mm film. I still have this excellent visual record which Prytherch gave me and which is now about 45 years old, and I hope to show it during this meeting.

I tested the retaining power of the "filtrose" blocks, which I inherited from Prytherch, and found that contrary to his assumption none of these filters retained small oyster larvae. Later on, together with Harry Davis, I repeated the experiment of pouring through the blocks water containing oyster larvae two to four days old, and found again that most of them passed through the block. Therefore, even though Prytherch's idea of using running water for raising larvae was theoretically good, his experiments were mechanically defective because most of the larvae could escape through the blocks. Moreover, the fact that Prytherch used monel metal screens to retain the larvae in the later stages of their development could be responsible for the mortality of these organisms. In our early experiments at Milford we also used monel metal screens for a while but soon found that they were toxic to larvae of all stages. As a result, we eliminated monel metal from contact with the larvae, preferring instead to use articles made of stainless steel.

Like Wells, Prytherch depended upon natural food present in the water and he was also apparently unaware of larval diseases. He excluded most larval competitors and predators, however, by filtering the seawater through fine bolting silk. Thus, regardless of many imperfections of the two methods, the first successful attempts to rear bivalve larvae to metamorphosis under laboratory conditions in both standing and running seawater were made in the United States (Wells, 1920, 1926, 1927; Prytherch, 1924).

Early Pond and Laboratory Experiments in Europe

In Europe, especially in Great Britain, efforts to grow larvae of the European flat oyster, *Ostrea edulis,* were also undertaken. *O. edulis,* as is well known, is larvi-

parous, i.e., it retains recently discharged eggs and holds larvae within the mantle cavity until they are approximately eight days old and measure about 170μ; thus they are considerably larger and much more fitted for survival in open waters than larvae of *C. virginica,* which at the early straight-hinge stage are only about 75μ. Again, as with American investigators, larval rearing experiments in Europe were of two kinds, those which were undertaken in large tanks or small ponds, and those conducted under laboratory conditions where experimenters could exercise much more control than in outdoor tanks. Cole (1937, 1939), probably one of the most successful workers, reared a large number of oyster larvae to metamorphosis in large outdoor tanks. He was working in Conway, North Wales, where he used two large tanks holding about 180,000 liters of water. The method was to fill the tanks with new seawater early in May and stock the tanks with 300–600 spawners. To provide the larvae with enough phytoplankton the water was fertilized with finely ground crab meat, which was found to be much more useful for that purpose than inorganic fertilizers. In some experiments the set of oysters was relatively good but, in general, the results were highly variable and some rearing attempts were almost complete failures.

Cole's attempts to raise larvae in comparatively deep outdoor pools (tanks) were later continued by Hughes (1940) and Wilson (1941), who also tried to increase the number of small naked flagellates in the tanks by enriching the water with crab meat. The results of these experiments also varied greatly. They demonstrated, nevertheless, that failure to obtain set depended not only on variation of such physical and chemical factors as temperature, salinity, hydrogen-ion concentration, and amount of dissolved oxygen, but also (and principally) on quality and quantity of food available to larvae. Because neither of these two factors could be controlled by any of the experimenters so far mentioned, there was still no reliable method for producing abundant oyster set in outdoor tanks or small natural or artificial ponds even in the early 1940's.

Bruce et al. (1940) were probably the first to develop functional laboratory methods for rearing larvae of *O. edulis*. They used 16-liter jars provided with special plungers. The water in the jars was changed by continuous dripping of new water. Loss of larvae was prevented by covering the outflow tubes with bolting silk of a mesh size that would retain the larvae. The larvae were fed cultures of flagellates. These flagellates, which ranged from 1.5 to 7.0 μ in size, were not clearly identified but merely labeled by letters. The authors thought that some species of flagellates were much more useful than others as larval food.

In some of the larval cultures of Bruce et al. (1940) the percentage that reached the stage of metamorphosis exceeded 90; in one lot a high of 99 percent of the larvae metamorphosed. Although Bruce and his colleagues had succeeded in rearing larvae, their results, nevertheless, showed many inconsistencies, thus reflecting the undependability of their method. The inconsistency of their results was probably due chiefly to the differences in the quality and quantity of the food cultures used in their experiments.

Efforts of Scandinavian biologists to rear oyster larvae are discussed in greater detail under the section devoted to larval food. We may add here, nevertheless, that a few years after Bruce et al. (1940), Dannevig (1945) also succeeded in

rearing oyster larvae in the laboratory on a diet of flagellates, whereas his attempts to rear them by using *Chlorella*-like nonmotile algae were unsuccessful.

Experiments in Japan

In Japan, biologists were also concerned with the possibility of developing a method by which bivalve larvae could be reared in the laboratory and in outdoor ponds. Hori and Kusakabe (1927) were among the first in that country to succeed in rearing a few larvae of the Japanese oyster, *Crassostrea gigas*, to metamorphosis. In their experiments these investigators used, as a food, a culture of nonmotile algae, *Chlorella pacifica*.

Artificial breeding of oysters and other bivalves in tanks was successfully accomplished on numerous occasions by Imai and his associates. Their efforts are presented in part in publications by Imai et al. (1950, 1954). In the early efforts of Imai and his colleagues the larvae were fed a noncolored naked flagellate, *Monas* sp., which they grew in tanks fertilized with decomposing organic matter. The numbers of *Monas* were controlled by the amount of glucose added to the organic enrichment used to fertilize the water in which *Monas* were grown. Although Imai and his associates considered *Monas* to be an important larval food, it is possible that other flagellates, as well as other types of phytoplankton, were present in their growing tanks and, therefore, it was not the *Monas* itself but these other forms that were responsible for good growth of larvae. This possibility was supported by our experiments at Milford (Loosanoff and Davis, 1963a). When we used cultures of *Monas* sent to us by Dr. Imai, we were unable to raise oyster larvae on a diet of these flagellates alone. Our experience was shared by biologists of the State of Washington, who were also unable to grow oyster larvae on *Monas* which they received from Dr. Imai's laboratory (C. E. Lindsay, personal communication).

An interesting finding by Imai was that a large number of the oyster larvae in the outdoor tanks were eaten by larvae of mosquitoes. By introducing mosquito-eating fish in their tanks, they were able to control this unusual predator.

Summary of Early Experiments

The above review of the efforts of biologists of several countries to rear artificially the larvae of several species of oysters and other bivalves under laboratory conditions or in small ponds or tanks has, of necessity, been brief. This review is, obviously, far from complete because to make it so would require much more time than I have been given for this presentation. Persons interested in a more extensive review of this subject should consult Baughman (1948), Loosanoff and Davis (1963a), and Galtsoff (1964). To summarize the situation, it may be said, nevertheless, that during the first four decades of this century no reliable, generally acceptable methods for rearing bivalve larvae had been developed. Even though several American, British, and Japanese biologists succeeded in raising these larvae both in standing and in slowly running water, their results were inconsistent and, as a rule, could not be repeated by other workers. These failures are understandable in light of our present knowledge because, for example, neither Wells nor

Prytherch gave the larvae supplementary food nor attempted to control their diseases. In Europe, too, food questions remained unsolved.

I do not recall any published work on rearing of larvae of *C. virginica*, after the initial efforts of Wells and Prytherch, until the staff of Milford Laboratory began to report on their new studies in this field. This lack of interest was well attested by the fact that during the 1930's virtually no papers on cultivation of oyster larvae were presented at any of the meetings of the National Shellfisheries Association. Moreover, neither Korringa (1952) nor Galtsoff (1964) mentioned any investigators in America who, in the 1930's, followed the steps of Wells and Prytherch. It certainly should not be assumed that no one tried to rear larvae during that period. In my own experience, as a graduate student at Yale University, I reared to setting the larvae of mussels, *M. edulis*, and *C. virginica*, on one occasion. The larvae were grown in MacDonald jars. The water was never changed but the larvae were given, every day, a few cubic centimeters of a mixed culture of algae grown in "Erdschreiber" medium. After about 20 days a few young mussels were found attached by the byssus to the walls of the culture vessel, and several days later I found, in another jar, recently set oysters attached to fragments of soft clam shells which I had placed on the bottom of the vessel for that purpose. I repeated the experiment that season but all larvae of both species died within a few days. No doubt a number of other biologists went through the same disheartening experience.

In Great Britain the interesting work of Cole (1937, 1939) was soon relatively forgotten and the studies of Bruce et al. (1940) had been severely criticized by Gross (1947) because of the inconsistencies of the results. Moreover, World War II arrested most of the research work in Europe and Asia not directly connected with problems of national defense.

DEVELOPMENT OF THE RECENT CULTURE METHODS

Need for a Functional Method of Culture

Possibly because I entered into marine biology, particularly the study of marine mollusks, at the time when the experiments of Wells and Prytherch were just being reported, I continued to be deeply interested in the possibilities of artificial rearing of larvae, even after the experiments of the original investigators were nearly forgotten by most people engaged in aquatic sciences and those representing the shellfish industry. My interest in the possibility of rearing larvae became more and more acute because of my close association with, and good knowledge of, the oyster industry of New England and New York, after I was appointed to take the place of Prytherch who had earlier conducted his studies in the summer in this area. From the very beginning of my contact with the oyster industry of Long Island Sound it was clear to me that the oyster industry was gradually declining and could not remain successful because of the rapid reduction of natural spawning and setting beds and because of the infrequency of commercially important sets (Loosanoff and Engle, 1940). Moreover, even during years when setting of oysters was comparatively good, the majority of recently set spat perished during the first few weeks of their existence because of the predatory activities of their enemies, such as oyster

drills and starfish (Loosanoff, 1961a). Obviously, new approaches for supplying the industry with sufficient numbers of high quality seed oysters were urgently needed.

Development of hatchery methods for cultivation of bivalve larvae and juveniles was thought, by a few of us, to be one of the most reliable ways by which the oyster industry could be assured of some seed each year. Unfortunately, our ignorance of how to rear oysters artificially was profound because, regardless of the already mentioned experiments, we had little knowledge of the factors such as optimum temperature and salinity and, especially, quality and quantity of food required for development and growth of larvae and juveniles. We also had very little knowledge about diseases of larvae and their control.

Induction of Precocious Development of Gonads

One of the most serious handicaps for study of the requirements of larvae and development of hatchery methods was the fact that the natural spawning period of *C. virginica* in northern waters is only about 8–10 weeks (Loosanoff, 1942, 1965). Therefore, we had only this short time within the year when oyster spawn was available for general experimentation and, especially, for rearing larvae to study their behavior and ecological and physiological requirements. I remember those days and the frustrations of the biologists, including myself, who never knew whether the oysters they intended to spawn would respond. Fortunately, in the early 1940's, while conducting a series of experiments having no direct relation to spawning of *C. virginica*, we observed that keeping oysters in warm water in the middle of winter caused rapid development of gonads and that oysters could even be induced to spawn outside of their normal spawning period (Loosanoff, 1945). This discovery of how to obtain ripe oysters, even in the middle of winter, was the first major breakthrough in a series of steps leading to development of the "Loosanoff-Davis" method of rearing bivalve larvae on the year-around basis that contributed so much toward the development of commercial hatcheries.

Realizing the advantages of having warm water in the laboratory to induce gonad development of a large variety of marine invertebrates outside of their normal spawning season, we quickly developed a highly functional method to have warm running seawater for this purpose (Loosanoff, 1949). This new facility offered the possibility of conducting throughout the largest part of the year numerous studies which ordinarily could be done only during the short summer period. In other words, artificial conditioning of the northern oyster extended their spawning season to include the period from the middle of November until the end of May, or possibly during a cold spring, even to early June. After that date the oysters that ripen under natural conditions in Long Island Sound become available until the middle or end of August.

The method for conditioning bivalves for spawning in winter and spring is very simple (Loosanoff, 1945). Adult oysters or clams may be brought to the laboratory from natural beds regardless of the temperature existing there. This transfer is feasible throughout the winter even when the water temperature over the natural beds is near zero. The mollusks are placed in trays with running seawater at low temperature, only a few degrees higher than that of their natural environment from

which they just came. Later the temperature is gradually increased, sometimes as much as five or six degrees per day. We found that the gradual approach is usually the best; in other words, it should take several days before the water temperature is increased from that over natural beds to approximately 20° or 25° C at which the actual conditioning is normally done. On some occasions, however, we brought oysters from their beds in the middle of winter and placed them directly in water of about 20° C. Even under such a strong physiological shock, we found no evidence that the gametes obtained from the parents so treated were less viable than those from oysters that were brought to conditioning temperature more slowly.

Since the method for conditioning mollusks for out-of-season spawning has already been described (Loosanoff and Davis, 1950, 1963a), I shall not repeat the details here. It is sufficient to mention that the length of the conditioning period can be shortened or prolonged at will by keeping the mollusks at different temperatures (Loosanoff and Davis, 1952a, 1952b). We may conclude this section by stating that gametogenic behavior of many other bivalves conditioned at our laboratory at different temperatures resembles, in general, that of *C. virginica*.

Delaying Spawning by Low Temperature

Our next step was to prolong the spawning period by extending it into late summer and early fall, from the end of the normal spawning season in August until approximately the end of November. During this part of the year many mollusks of Long Island Sound, including oysters, cannot be artificially ripened because they are still recovering from natural spawning (Loosanoff, 1942). The problem of providing ripe mollusks during that period was solved by artificially delaying the final stages of gonadal development, and thus preventing spawning (Loosanoff and Davis, 1951). The basic idea was again extremely simple, consisting of transplanting, early in the season, usually during the second half of May, oysters and clams from Long Island Sound to the waters of Boothbay Harbor, Maine, where the temperature is considerably lower than in Long Island Sound. The water temperature in Maine, nevertheless, is sufficiently high to permit the full development of gonads but not high enough to permit, or induce, normal spawning. Thus oysters and clams of Long Island Sound suspended in the waters of Maine retain their spawn during late summer and early fall, while the Long Island Sound oysters are normally already spent. Ripe oysters from Maine were brought back to Milford Laboratory, as they were needed, and were easily induced to spawn. On occasion, instead of shipping oysters to Maine we used various cooling devices to keep the mollusks from spawning during summer and early fall. One such device is now installed at Milford Laboratory.

By the combination of our two methods—first, of ripening mollusks outside of the normal propagation period and, second, of delaying their gonadal development and preventing normal spawning during summer and early fall (in addition to utilizing the normal reproductive period)—we can now have ripe bivalves throughout the entire year. The larvae can thus be always available for any type of hatchery work, for studying various problems of larval development, for study of their physio-

logical and ecological requirements, for genetic studies, and for study of larval diseases and parasites (Loosanoff, 1954; Loosanoff and Davis, 1963a).

While developing methods for providing ripe gametes outside of the normal reproductive season, we wondered whether an individual oyster could be induced to spawn at intervals of several months or at least twice a year. This matter was of considerable practical importance because it often may be desirable, particularly in the studies of genetics, to spawn the same bivalve several times during the year. Our experiments showed that, given the proper conditions, nothing in the physiological pattern of the oyster prohibits normal gametogenesis more than once a year (Loosanoff and Davis, 1952a). Approximately 200 oysters were made to spawn at six-month intervals producing normal gametes; some were conditioned to spawn even three times a year.

Applicability of Methods to Oysters of Different Geographical Areas

In experiments to determine whether the above-mentioned methods of conditioning were applicable to groups of oysters and other mollusks from different geographical areas along the Atlantic and Gulf coasts, we soon discovered that our methods were not equally successful when applied to some of the southern populations. This inconsistency strengthened the hypothesis that the entire population of *C. virginica* was not genetically homogeneous but consisted of several physiologically different races. This point of view was based partly on our earlier field studies in Long Island Sound (Loosanoff and Engle, 1942a), on the suggestion offered by Stauber (1950), and on extensive observations of several groups of southern oysters kept in Milford Harbor for a prolonged period (Loosanoff and Nomejko, 1951). Recent studies have definitely settled this question by proving that southern oysters require a considerably higher temperature for their gonad development and spawning (Loosanoff, 1969). Nevertheless, the recent work of Hidu et al. (1969) showed that southern oysters can also be conditioned to spawn by certain modifications of the basic methods developed at Milford. Moreover, Maurer and Price (1968) clearly demonstrated that it may be possible to retard seasonal spawning of Delaware Bay oysters up to six months by using virtually the same methods as were developed for northern oysters.

Methods of Obtaining Fertilizable Eggs of Different Bivalves

Methods for inducing ripe oysters to spawn have varied somewhat, but the basic and most common one has already been described by Galtsoff (1930, 1932), Loosanoff (1937), and Loosanoff and Davis (1963a). Our method consists of placing ripe oysters, or other bivalves, in glass dishes of about one-liter capacity containing seawater of the same temperature as that at which the mollusks have been conditioned. These dishes are then immersed in a large tray or water table filled with hot tap water, and the temperature of the water in the dishes containing the oysters is quickly brought to the desired level. Simultaneously with the rapid increase in temperature, a suspension of eggs or spermatozoa may be added to the dishes.

As we discovered in our earlier work, some bivalves cannot be induced to spawn by the above-described method (Loosanoff and Davis, 1963a). With such bivalves stripping of ripe gonads can provide some uninjured ripe eggs that can be fertilized and that will develop normally. This method is useful, however, only for those species in which the germinal vesicle is dissolved automatically after stripping, for example, as with oysters of the genus *Crassostrea*. In some other species—for example, *Pitar morrhuana* and *M. mercenaria*—the stripped eggs fail to become fertilized because the germinal vesicle remains unbroken and prevents fertilization. This situation can be overcome in some species by placing the eggs in a weak solution of ammonium hydroxide for a short time, then washing them in seawater. This contact with ammonium hydroxide causes the germinal vesicle to break and the eggs become physiologically prepared for fertilization (Loosanoff and Davis, 1963a).

Japanese workers were successful in inducing spawning of some bivalves by injection of certain chemicals. For example, Sagara (1958a) induced spawning in *Meretrix* by injection of NH_4OH 1/2ON into their gonads. Sagara (1958b) also reported that he was successful in inducing spawning in *Mactra veneriformis, Mactra sulcataria, Crassostrea gigas, Corbicula japonica,* and *Trapezium japonicum,* during their spawning season by placing these mollusks in ammoniated seawater. In such cases the spawnings occurred without thermal stimulation. *Meretrix lusoria* and *Tapes japonica,* on the other hand, could not be induced to spawn by placing them in ammoniated seawater but they responded when two cc. of NH_4OH were injected directly into the gonadal mass. Sagara (1958b) also thought that a solution of KCl was effective in inducing discharge of gametes from the mantle of *M. edulis*. W. P. Breese (personal communication) found that elevated temperatures alone failed to induce spawning in some bivalves, including *Sacidomus giganteus,* the butter clam of our Pacific Coast, but this mollusk usually spawned after the addition of one or two grams of KCl per liter of water in which ripe clams were held. We have induced spawning of *M. edulis* by gently pricking the adductor muscle with a thin needle (Loosanoff and Davis, 1963a).

Davis and Chanley (1956) showed that properly conditioned northern oysters and clams (*M. mercenaria*) can be induced to spawn on many occasions during the same spawning season. For example, one female oyster spawned 16 times and one clam 11. No significant difference was found in the average number of eggs released during the entire spawning season by oysters that were induced to discharge spawn at 3-, 5-, or 7-day intervals. Obviously, the fact that a single oyster or clam can be made to spawn many times within a single season, or after a single conditioning, is of considerable practical importance to students of such branches of biology and genetics or physiology. It is also of practical significance to people who are involved in commercial hatchery operations because their best parental stock may be depended upon not for only a single spawning but for a long series of spawnings extending over a period of several weeks.

Another series of observations, which may be of equal interest to theoretical scientists and practical oystermen, demonstrated that no significant difference exists in the quality of spawn developed and discharged by individual oysters and clams of different ages and sizes. Some of the oysters employed in these studies were

approximately 40 years old and yet their eggs were as viable as those of oysters that were only 2 years old (Loosanoff et al., 1953).

Using our method for inducing gonad development of bivalves out of season, we were able to obtain larvae of *O. edulis* from the end of January to the end of the normal spawning period of this species, which in Milford Harbor extended through September. We introduced this species into the United States in 1949 (Loosanoff, 1955) and have reared numerous broods of its larvae to metamorphosis. We also furnished Milford-grown juvenile *O. edulis* to biologists of several states, including those of Washington and California.

Adult *O. edulis* were placed in conditioning aquaria during the winter, when they were far from being ripe, and were kept for several weeks in running warm seawater to which a culture of mixed plankton was added automatically. When these oysters approached ripeness, the continuous flow of water was stopped; instead, the water in the tank was changed once or twice a day. Food was added to the tank in the morning and evening.

Occasionally, we could induce spawning of conditioned *O. edulis* by using the standard method, which consists of increasing the temperature of the water and the addition of suspension of sex products, but such attempts were often unsuccessful. As a rule, however, we depended on the natural spawning of these oysters. The spawnings were easily ascertained by finding recently discharged eggs on the black, asphaltum-painted bottom of the aquaria near the female oysters (Loosanoff and Davis, 1963a). These females, now holding the eggs in their mantle cavity, were gently removed and placed into so-called maternity tanks of 15-gallon capacity where only two to four oysters were kept at the same time. The water in the maternity tank was changed daily and was always vigorously aerated. Phytoplankton, known to be a good larval food, was added twice daily.

Normally, release of larvae by the oysters which had spawned in our conditioning tanks took place from six to nine days after spawning was observed, provided the temperature in the tank was near 20° C or above. The release was easily noticed because the larvae tended to congregate in the surface layer of the water. Sometimes a single mother oyster continued to release larvae for almost two days. To collect the larvae the water in the tank was gently siphoned out and the larvae retained on the screen were then placed in rearing containers. The tank was then refilled with filtered seawater. Collection of the released larvae was made as often as three or four times a day, depending on the numbers of larvae discharged. During the release of larvae all other oysters, except the ones engaged in the release, were gently removed and placed into another tank so that they would not ingest the larvae.

In Wales, Walne (1956b), working with *O. edulis*, used methods similar to those of Milford, with the exception that he did not transfer gravid females into maternity tanks.

Handling of Eggs and Rearing of Larvae

The procedure of handling eggs and larvae in our cultures is relatively simple (Loosanoff and Davis, 1963a). Fertilized eggs found in the dishes at the end

of spawning are first passed through a comparatively coarse metal screen, which allows the eggs to pass through but retains masses of mucus, particles of pseudofeces, and other undesirable materials, such as fragments of shells. Then the eggs and water containing them are passed through a finer screen, which retains the eggs but lets the fluid containing excessive spermatozoa, blood cells, etc., pass and be discarded. In species that have such small eggs that sieving or screening is quite difficult, the eggs can be partially freed of sperm, etc., by letting them settle on the bottom of the beaker and then siphoning or decanting most of the fluid. If this operation is repeated several times, virtually all of the undesirable materials that are suspended or dissolved in the water will be discarded. The eggs can then be washed once more in filtered, ultraviolet-light-treated seawater and placed in the vessel where the developing embryos and resulting larvae remain undisturbed from one to two days.

The water in the vessels is changed, as a rule, every 24 or 48 hours, depending upon density of the cultures. During this change the water is again strained through a fine screen, which retains the larvae but lets the water pass through. The larvae are then returned to the jars, which are filled again with new seawater that has been filtered and treated with ultraviolet light. Normally, cultures are not aerated. Antibiotics are often used to control larval diseases.

Because this article is, to a certain extent, a historical review of the development of methods for cultivation of bivalve larvae, I would like to mention some of the difficulties we experienced before we finally arrived at the present procedures. These difficulties arose in part because Prytherch (1924) left the impression that it is almost imperative to grow larvae in flowing water. Accordingly, we wanted to continue in the same way and were not especially interested in standing-water, unfed cultures as grown by Wells. True, I had a set of fine screens with which I could retain larvae during the change of water but, nevertheless, during 1945 and early 1946 the only rearing was in slowly running water. The emphasis placed on this approach was justified by the belief that this method eliminated the need for supplementary food for larvae. Unfortunately, because of purely technical difficulties, consisting in rapid clogging of screening devices, nearly all our efforts were unsuccessful.

During the winter of 1946, Harry C. Davis joined my "staff." Before his arrival the "staff" consisted of myself, a high school girl, and a young man without any previous training in biology or any other natural science. Upon his arrival in Milford, Davis was presented with approximately 50 conditioned oysters, ripe enough to be induced to spawn, a set of fine screens, a few beakers, and a long lecture with the request to help develop a method of growing larvae in slowly flowing water.

We tried virtually all methods that were described in the literature and those we invented ourselves. All these devices were basically intended to let the water pass through some filtering material that would, nevertheless, retain the larvae. None of these devices worked well and, as a rule, they were responsible for an extremely high bacterial population in larval cultures. Finally, it became clear that regardless of the type of filtering devices used, the difficulty of keeping them clean would always present a problem. After we came to that conclusion we decided to try the milk

separator used earlier by Wells (1926, 1927). This separator, loaned to us by Mr. J. Glancy, was used for several months. We found it to be unsatisfactory and strongly suspected that the lack of success was due to the fact that parts of the separator, which came in contact with larvae, were made of metal containing a large proportion of copper, which is toxic to larvae. When coating these parts with a thin layer of paraffin did not solve the problem we returned the separator with our thanks.

Meanwhile, our numerous but disappointing attempts were making it evident that our lack of success was due not only to mechanical difficulties but also to our inability to provide the larvae with good food. This weakness was clearly demonstrated by some of our observations that even though in some of our experiments larvae lived for as long as 4–5 weeks, they still did not pass beyond straight-hinge stage. After long deliberation and study, therefore, we finally decided to give up the idea of raising larvae in continuously flowing water and, instead, concentrate on methods of rearing in standing water and simultaneously searching for microorganisms that would be good food for larval mollusks.

Development of Methods of Producing Larval Food

As mentioned above, we realized for a long time that a dependable method of rearing bivalve larvae on a laboratory or hatchery scale would require reliable production of sufficient quantities of good quality larval food. Spärck (1927) was probably the first to demonstrate that by addition of fertilizer (which in his work was liquid manure), the plankton flora of small natural ponds containing larval oysters could be significantly increased. In water fertilized in this manner Spärck grew larvae of European oysters to the size of 300 μ. Gaarder and Spärck (1933) made further studies of the organisms present in the water of Norwegian oyster ponds and found a large number of unicellular algae resembling *Chlorella*. Numerous small flagellates measuring only 2 or 3 microns were also present. Kändler (1933) also tried to grow larvae of European oysters on a diet of small green algae but was not successful. Cole (1937) demonstrated that not all forms of phytoplankton were equally good as food for larvae of *O. edulis*. He found that these larvae were not able to utilize green algae, such as *Chlorella*, but grew well on yellow-brown chrysomonads. Bruce et al. (1940), whose work has already been mentioned, continued research in food requirements of larvae of *O. edulis*. Even though these studies were not entirely successful and were later strongly criticized (Gross, 1947), they have added to our knowledge of food requirements of bivalve larvae in general.

As early as 1938 we were attempting to develop methods for providing food organisms not only for adult but also for juvenile oysters. At this time our mass culture grown in the "greenhouse" came into existence (Loosanoff and Engle, 1942b). Using some of the cultures grown under these conditions, we were able to establish experimentally that the amount of water pumped by adult oysters varied according to the species of microorganisms and also with their numbers (Loosanoff and Engle, 1947). We also found later that our mixed mass cultures were very often poor food for bivalve larvae.

Soon after H. C. Davis joined our staff, systematic efforts were begun to isolate various small phytoplanktonic forms from our mass cultures and also from

the waters of Milford Harbor and Long Island Sound, to rear them in pure cultures, and finally to ascertain their value as food for oyster and clam larvae. I doubt that anyone, except the people who were then at Milford Laboratory, realizes how much time and effort this work required. To summarize the situation, I may say that many forms were tried but almost none gave really satisfactory results. Finally, after many unsuccessful, or only partially successful, efforts at raising larvae on cultures of microorganisms isolated from local waters or received from other laboratories in the United States, I asked Dr. F. S. Russell of the Plymouth Laboratory, England, to send us several cultures from Dr. Mary Parke's collection. The cultures received from Plymouth were used to prepare bacteria-free samples; this work was accomplished with the help of Drs. Provasoli and McLaughlin of Haskins Laboratories. Soon an effective method for mass culture of phytoplankton under virtually sterile conditions developed at Milford assured laboratory workers a steady and sufficient supply of high quality food for larval and juvenile mollusks (Davis and Ukeles, 1961).

Among Dr. Parke's samples were cultures of *Isochrysis galbana* and *Monochrysis lutheri,* which were found by Davis to be good foods for oyster larvae. This discovery radically changed the situation, as far as successful rearing of oyster larvae was concerned. Soon an article describing the relative value of several groups of microorganisms as foods for oyster and clam larvae was published (Davis and Guillard, 1958). Finding which phytoplanktonic forms were excellent larval foods, another important breakthrough at Milford, led to the development of a not only reliable but highly effective method of cultivation of bivalve larvae.

Even before we received the cultures from Plymouth, and while we still depended principally upon our own mixed mass cultures grown under only partially controlled conditions, we had already accumulated a great deal of useful information concerning the food requirements of larvae (Loosanoff et al., 1955). For example, it was clearly demonstrated by our earlier experiments that larvae of different species of bivalves needed different planktonic forms for food. This observation led us later to the development of an analytical method of determining the qualitative and quantitative food requirements of different species (Davis, 1958).

We also found that, contrary to the generally accepted opinion prevailing at that time, organic detritus cannot be used by larvae of the American oyster or hard shell clam (Loosanoff et al., 1951). Soon it was found also that oyster larvae cannot consume either sulphur bacteria or any other of the several species of marine bacteria that were isolated in relatively pure culture and fed to larvae at our laboratory. This work also showed that even though young oyster larvae cannot utilize cells of green algae, such as *Chlorella,* older larvae of the same species are able to do so (Davis, 1953).

In the early 1940's we made several attempts to feed pulverized dry algae, such as *Ulva* and *Laminaria* to juvenile and adult oysters kept in our experimental tanks. About 20 years later this old material was given to one of our colleagues at Milford Laboratory to be tried as food for larvae of *M. mercenaria*. Strangely enough, the larvae were able to use it and grew to metamorphosis. Since it was difficult, however, to grind the algae into particles small enough for larvae to ingest, I obtained, through the courtesy of Dr. Hiroshi Tamiya of Japan, samples of dry uni-

cellular algae, *Scenedesmus* sp. and *Chlorella* sp., and suggested that our associate, H. Hidu, use this material as a food in rearing larvae. The results of this and similar studies were summarized by Hidu and Ukeles (1964). They concluded that dry cells of these algae can be used and, under suitable conditions, possess the physical properties desirable in artificial larval food.

Production of sufficiently large quantities of plankton of quality good enough for survival and vigorous growth of bivalve larvae still remains a major problem. Most commercial hatcheries are now using cultured phytoplankton as the primary source of food; others use cultured algae only as a supplement to the method used by J. Glancy, which is discussed later in this article. We know now, on the basis of practical experience, that production of algal food can be solved by experienced hatchery operators as is being done now at the hatchery of Pacific Mariculture, Inc., at Pigeon Point, California. This group has succeeded in rearing about 10 different species of bivalves and several species of abalone, and is growing such forms as *Isochrysis* and *Monochrysis* by using basically our formula to produce sufficient quantities of plankton to meet their hatchery requirements. *Phaeodactylum* is also grown at that hatchery, as a matter of routine, and is used in feeding larvae of advanced stages.

In England the work of Cole (1939) and Bruce et al. (1940) was continued by Walne. His studies were largely confined to observations of the food value of several species of phytoplankton in relation to larvae of *O. edulis*. His early studies (Walne, 1956b) agreed with those of Davis at Milford (Davis, 1953). His later work (Walne, 1963) confirmed his previous conclusion that *Chlorella* sp. has little value as food for oyster larvae. Although we found that *I. Galbana* and *M. Lutheri* were the best foods for larvae of *C. virginica* and *M. mercenaria*, Walne determined that *Dunaliella* and *Phaeodactylum* induced better growth of larvae of European oysters than did *Isochrysis*. He also found that larvae receiving *Isochrysis* and *Phaeodactylum* with and without bacteria showed no consistent differences in growth. Walne agreed with Milford workers that *Monochrysis* and *Isochrysis* are of about equal value for larvae of *O. edulis* (Walne, 1966). In the 1966 article Walne offered a description of the construction and maintenance of the large-scale algal culture apparatus, closely similar to that described by Davis and Ukeles (1961), and gave a formula for enriching the medium for cultivation of *I. galbana* and other similar forms.

Comparison of the "Loosanoff-Davis" and "Glancy" Methods of Larval Culture

Some recent publications contain comparisons of the "Loosanoff-Davis" or "Milford" method with the "Glancy" method. These comparisons usually suggest that the only difference between the two methods is that for rearing bivalve larvae we, at Milford, used cultured unialgal foods for the larvae, whereas Glancy, who worked at Great South Bay, following the technique of Wells, depended entirely upon the microorganisms normally present in seawater as food for the larvae. Unfortunately, some of these comparisons have not only failed to recognize the importance of this difference but also have failed to recognize other important contributions originating at Milford.

The "Glancy" method, following that of Wells (1920, 1926, 1927), consisted of centrifuging the seawater to eliminate zooplankton, the larger phytoplankton and the large particles of detritus, and then holding this water for about 24 hours in shallow tanks indoors, under a translucent roof, before using it in the larval culture vessels. This system permitted some reproduction of the smaller phytoplankters that pass through the centrifuge and, particularly on sunny days, brought the temperature of the water up to 80–85° F. If the small phytoplankters that pass through the centrifuge are good larval foods and are sufficiently abundant to supply the quantity of food required by the larvae, this method works very well, is relatively inexpensive, and requires little technical skill; the volume is limited only by the capacity of the centrifuges and holding tanks. The disadvantage is the lack of control of the quantity or quality of phytoplankton in the water. If the centrifugal water contains few or no good food organisms, holding it in the tanks for 24 hours brings small benefit. On other occasions the microorganisms developing in the holding tanks may be very poor foods for the larvae or actually toxic enough to kill them. Because of these difficulties inherent in the "Glancy" method, most commercial hatcheries and most investigators are now growing some of the algae that studies at Milford have shown to be good larval foods (Loosanoff and Davis, 1963a). Some hatcheries now use the cultured algae as the sole source of food for their larvae, as is the practice at Milford, and other hatcheries use the cultured algae to supplement the natural food in centrifuged seawater.

Although Glancy obtained a patent (# 3,196,833, July 27, 1965), there seems to be little that is original in his method since Wells (1920, 1926, 1927) used the same techniques and at Milford we were using a greenhouse-type structure since 1938 to culture algae (Loosanoff and Engle, 1942b).

Other techniques originating at Milford, which have contributed greatly to the development of shellfish hatcheries, include methods for conditioning mollusks for winter spawning (Loosanoff, 1945) and for delaying spawning until late summer and fall by keeping the shellfish at low temperatures (Loosanoff and Davis, 1951). These two components of the "Loosanoff-Davis" method have made it possible to possess spawnable mollusks throughout the year. Now all commerical hatcheries, on both the East and West coasts, systematically employ these parts of our method.

The use of sulfa drugs, antibiotics, and other substances to control diseases of larvae also, to the best of our knowledge, originated at Milford and constitutes an integral part of the "Loosanoff-Davis" method. We began to use these materials as early as 1952 when a compound, known as Bursoline, was used in an attempt to control fungus. The sulfa drugs and antibiotics have been used almost routinely since 1953 (Loosanoff, 1954).

The use of ultraviolet irradiation for treatment of seawater to aid in the control of bacteria, fungi, small protozoans, and other undesirable microorganisms was also pioneered at Milford, where ultraviolet treatment has been used routinely since 1954 (Loosanoff and Davis, 1963a).

Finally, the studies at Milford of the comparative value of different foods for larvae (Davis, 1953; Loosanoff et al., 1955; Davis and Guillard, 1958), and the effect of temperature and salinity on growth of larvae (Loosanoff et al., 1951; Davis,

1958; Davis and Ansell, 1962; Davis and Calabrese, 1964, 1969) give information vital to the successful operation of commercial hatcheries.

We may add that in the development of our methods we demonstrated that the food requirements of the larvae of different species may vary a great deal. For example, in the late 1940's we already were disseminating the information that larvae of *M. mercenaria* are not highly selective in their food, being, in this respect, different from larvae of *C. virginica* (Loosanoff and Davis, 1950). We also observed (Loosanoff, 1954) that the proper concentrations of food organisms in larval cultures are of extreme importance and that overfeeding may cause mortality of the larvae that may be due either to the large number of cells themselves or to the heavy concentration of their toxic metabolites (Loosanoff, 1954; Davis and Guillard, 1958).

Possibly, we may also consider as part of our method the techniques by which we prevent infestation of our large open outdoor cultures of plankton; these cultures often become contaminated with various forms of zooplankton, such as rotifers, tunicates, or crustaceans, which prey upon the algae, multiply rapidly, and quickly consume all the phytoplankton. We solved this problem by finding certain chemicals that kill undesirable crustaceans and then rapidly hydrolyze to yield nontoxic substances. These insecticides do not seriously affect bivalve larvae to which the algae are subsequently fed (Loosanoff et al., 1957). Ways have also been devised to eliminate ciliates and other undesirable forms (Loosanoff and Davis, 1963a). The studies by Ukeles (1962) of the growth of pure cultures of marine phytoplankton in the presence of different toxicants give important information on the effect of pesticides on growth of algae.

OTHER RECENT STUDIES IN CULTURE OF LARVAL MOLLUSKS

Determining Optimum Ranges of Environmental Factors

Because of the development of a dependable method for obtaining ripe spawn of many mollusks, and for rearing their larvae under controlled conditions, it became possible to initiate studies of the effect of various factors of the environment on the embryonic development of commercial bivalves and on survival and growth of larvae and recently metamorphosed individuals. Many of these studies were conducted at the Bureau of Commercial Fisheries Biological Laboratory at Milford, and were confined principally to *C. virginica, M. mercenaria, M. edulis*, and *O. edulis*.

The early experiments of this nature were on the effect of temperature on development and growth of *M. mercenaria* (Loosanoff et al., 1951). They were designed to ascertain the range of temperature within which development is possible and also to determine what may be considered the optimum temperature range. These studies, possibly the first of this nature to be made on eggs and larvae of commercial mollusks, provided much information of theoretical and practical value. They showed that fertilized eggs of these clams can develop into straight-hinge larvae at temperatures ranging from 18 to 31° ± 1° C. Early straight-hinge larvae can survive and grow, however, at temperatures as low as 15° and as high as 33° C. Larvae grew most rapidly at 30° C. In general, clam eggs required a narrower temperature range than was suitable for the survival and growth of shelled larvae. A sharp decrease in temperature, such as from 25 to 10° C within a few minutes, did

not kill or seriously injure clam larvae. Moreover, clam larvae could be kept for several days at low temperature, which virtually arrested their growth, and then returned to more favorable temperature, where they resumed their development and eventually metamorphosed. The larvae grown at 18° C began to metamorphose the sixteenth day after fertilization, but at 30° C setting of larvae began as early as the seventh day. These basic facts, of which I have cited a few, obviously are urgently needed by hatchery men to be able to rear larvae under the most promising conditions.

Davis (1958), working on eggs and larvae of *C. virginica* and *M. mercenaria*, found that the most favorable salinity range for development of straight-hinge larvae from eggs of American oysters appeared to be governed by the salinity at which the parent oysters developed gonads. He also found that the optimum salinity for development of eggs of *C. virginica* from Long Island Sound was about 22.5 parts per thousand (ppt). The optimum salinity for development of eggs of *M. mercenaria* of the same area was near 27.5 ppt.

Walne (1956b) experimented with larvae of *O. edulis* grown at salinities of 21.1, 25.9, and 31.3 ppt. Since the water in his cultures was not changed during the experiment, the evaporation that occurred caused salinity to increase several parts per thousand. For example, in cultures at 21.1 ppt the salinity increased to 25.9 ppt. Under conditions of his experiments the larvae survived and grew at all salinities but no setting was recorded in cultures initiated at 21.1 ppt. Davis and Ansell (1962) conducted a series of somewhat more critical experiments on salinity requirements of *O. edulis*. Using Milford methods these two investigators grew the larvae to metamorphosis at salinities of 20 and 22.5 ppt, both of which were lower figures than those reported by Walne. These studies have shown that the lowest salinity for good growth and metamorphosis of larvae of *O. edulis* was near 22.5 ppt, even though some of the larvae had metamorphosed at only 20 ppt. These investigators could not obtain normal larvae released by adult oysters conditioned at a salinity of 20 ppt or lower.

In most of the earlier studies principal attention was given to the effects of a single environmental factor, but it was soon realized that the effects of any one factor can be considerably altered by variation in other factors; studies to clarify these important relationships were accordingly undertaken (Davis and Calabrese, 1964). As expected, the temperature tolerance of clam and oyster larvae proved to be significantly affected by salinity. At near-optimum salinity, larvae of both species, *C. virginica* and *M. mercenaria*, survive and grow over a much wider range of temperature than at salinities near the lower limit of their tolerance.

In the same series of experiments it was also learned that the rate of growth of larvae at different temperatures was critically affected by the type of food organisms available. These authors believed that enzyme systems required to digest naked flagellates were active at lower temperatures than were the enzyme systems required to digest forms with thick cell walls. Because of this difference, when the larvae were reared at relatively low temperature, such forms as *M. lutheri* and *I. galbana* were preferable to such forms as *Chlorella*.

Soon after the above-mentioned series of experiments of Davis and Calabrese, observations of similar nature were reported on the larvae of the European oyster, *O. edulis* (Walne, 1965). These studies, however, were principally con-

cerned with the influence of variations in quantity of food and temperature on the growth of larvae. When *I. galbana* was used as a food, assimilation of it by larvae and growth of larvae were clearly affected by small variations in food-cell densities. Additional observations performed with six other species of algae showed that food requirements of the larvae of the European oyster were satisfied at much lower cell densities when the cells were large. This finding corroborated that of Loosanoff, Davis, and Chanley (1955), who reported that it took approximately 400,000 cells per ml of *Chlorella* 3 μ in diameter to give the same rate of growth of clam larvae as given by 50,000 cells per ml of *Chlorella* measuring 8 μ in diameter. It was for this reason that in our later studies of the relative value of different microorganisms as foods for larvae, equal packed cell volumes were used.

Interesting studies of the effects of salinity, temperature, and food requirements of larvae of the soft shell clam, *M. arenaria*, one of the most important commercial species of our northeastern coast, were conducted at Boothbay Harbor, Maine, by Stickney (1964). He found that the optimum temperature range for the larvae of this clam was between 17.2 and 23.2°C, although poor development was possible at a temperature as low as 10°C. The acceptable range for salinity extended from 16.2 to 32.2 ppt, the latter figure being the highest value tested. Some differences in response to temperature and salinity, between larvae which originated from parents of different geographical areas, were recorded. These observations possibly indicate the presence of geographically different races of *M. arenaria*, as has been ascertained for *C. virginica* (Loosanoff, 1969). Stickney also reported that *Cyclotella nana, Dicrateria inornata*, and *Phaeodactylum tricornutum* were the algal foods on which larvae of *M. arenaria* grew very well.

The recent article of Calabrese and Davis (1966), on effect of pH on embryos and larvae of some of our commercial mollusks, is possibly the first contribution of this nature to give the precise pH ranges for normal embryonic development of *M. mercenaria* and *C. virginica*. These workers determined that the pH for normal growth of clam larvae ranges from 6.75 to 8.5, and for oyster larvae from 6.75 to 8.75. In both species the rate of growth rapidly decreased when the pH fell below 6.75. The optimum pH range for growth of clam larvae was from 7.50 to 8.0, and for oyster larvae from 8.25 to 8.50. This type of information is, obviously, important not only for men engaged in hatchery practices but also for information of federal and state biologists working on establishment of standards for water quality.

Chemical Pollutants and Their Effects on Eggs and Larvae of Bivalves

Extensive experiments, conducted principally at Milford Laboratory, on the tolerance of eggs and larvae of bivalves subjected to different concentrations of various chemicals—including pesticides, weedicides, antibiotics, bacteriostatic compounds, and detergents—have demonstrated beyond all doubt that any of these substances can have a profound effect on the development of embryos and survival and growth of larvae, and at sufficient concentrations can cause abnormalities and death of these organisms. Some of these substances affect eggs and larvae in extremely low concentrations (Davis, 1961; Hidu, 1965; Calabrese and Davis, 1967; Davis and Hidu, 1969). Obviously, studies of this nature should be continued on a

broad basis, including studies of the effects of biological, chemical, and physical pollutants.

Our early observations showed that normal water may sometimes contain substances, still unidentifiable, which strongly affect development of eggs and larvae (Loosanoff et al., 1951; Davis, 1953; Loosanoff and Davis, 1963a). Walne (1956b) reported similar observations in his studies on larvae of the European oyster, and Millar and Scott (1968) recently discussed the effect of water quality on the growth of larvae of *O. edulis*. These authors came to the same conclusion as we did at Milford—that some substances, probably of natural organic origin, may be responsible for slow growth of larvae. This is the reason why it is advisable, before choosing a location for a future hatchery, to ascertain the acceptability of the water of that region by bioassay of its quality. The last time I rendered such a service to the industry was in 1963 when I bioassayed the water at Pigeon Point, California, where the present Pacific Mariculture, Inc. Hatchery is located and is successfully rearing the larvae of a number of pelecypods and abalone.

Diseases of Larvae

Ever since we have been able to rear bivalve larvae under controlled laboratory conditions regularly, we have experienced occasional sudden heavy mortalities among them. It was soon found that such mortalities may be due to a definite pathogen (Davis et al., 1954). The organism responsible for this epizootic was the fungus, *Sirolpidium zoophthorum*. Originally, this fungus was noticed in cultures of *M. mercenaria;* soon it was also found in cultures of *Teredo navalis, P. irradians, Tapes semidecussata,* and *C. virginica*. In *M. mercenaria* all stages of larvae can be attacked by fungus and the same probably holds for many other genera and species of larval mollusks.

Precautionary measures, consisting principally in maintenance of general cleanliness and the ultraviolet treatment of water in which the larvae were to be reared, gave promising results. As mentioned above, the use of ultraviolet light to prepare the water for growing larvae has been a part of our standard method since 1954. In England, Walne (1958) also used ultraviolet light in preparing water for culturing larvae of *O. edulis*. At present the use of the ultraviolet unit is probably standard in most laboratories and in some commerical hatcheries where bivalve larvae are reared (Loosanoff and Davis, 1963b).

Just as the pathogenic role of fungi was recognized by Davis and his associates, various bacteria became suspected of causing larval epidemics (Guillard, 1959; Tubiash et al., 1965). We began testing numerous fungicides and antibiotics to develop a method for prevention of larval diseases, determining at the same time the effects of these substances on survival and growth of the larvae themselves (Davis and Chanley, 1956). A large number of chemicals have been tried and at present the use of streptomycin at about 100 parts per million or Sulmet at about 33 parts per million is more or less standard practice among the larvalogists (Loosanoff and Davis, 1963a). The importance of bacteria in laboratory experiments on rearing larvae of *O. edulis* was also demonstrated by Walne (1958), who found that some of the antibiotics brought about an increase in setting of larvae of the European oyster.

Studies of diseases of larval and juvenile mollusks and development of their control are now continued at Milford Laboratory and probably at some other places. I have every reason to believe that because of these studies we will eventually be able to control most of the mortalities of larvae and recently metamorphosed mollusks that are caused by microorganisms.

Selective Breeding

The development of reliable methods for rearing bivalve larvae offers a broad field for studies of selective breeding of these mollusks. We may now begin to apply the principles of genetics in developing strains of commercially important bivalves with desirable qualities, such as rapid growth, resistance to certain diseases, and ability to propagate under suboptimum conditions. Studies in this field are now being conducted at several laboratories, including those chiefly interested in development of strains of oysters that are resistant to MSX.

Davis (1950) and Imai et al. (1950) were probably the first biologists to start crossbreeding of commercial mollusks. Both of these investigators came to the conclusion, by crossing *C. virginica* with *C. gigas*, that virtually all larvae resulting from this cross die about five days after fertilization, normally without progressing farther than the straight-hinge stage. More extensive studies of this nature, conducted by Imai and Sakai (1961), included crossing of different strains of the Japanese oyster, *C. gigas*. These authors reported that hybrids can be grown from crosses of *C. gigas* and *Crassostrea angulata*, but that in crosses of *C. gigas* with *C. virginica* or with *Crassostrea rivularis* fertilization may occur but the larvae produced will not survive.

In other bivalves it has been shown that two species of hard shell clams, *M. mercenaria* of Long Island Sound and *Mercenaria campechiensis* of the Gulf of Mexico, can be cross fertilized successfully and the hybrids grown to maturity (Loosanoff, 1954). Thousands of small hybrid clams resulting from these crosses were grown at Milford until they were approximately 1 cm long and then shipped to several marine laboratories along the Atlantic and Gulf coasts for observations on their behavior and growth under the different ecological conditions prevailing in widely spaced geographical areas. Several papers, including those of Haven and Andrews (1957), Chestnut et al. (1957), Woodburn (1963), and Menzel (1963), appeared as as result of this cooperative effort originating at Milford.

Interesting experiments were also carried on by Chanley (1961), who demonstrated that shell markings of *M. mercenaria notata* were inherited as a simple Mendelian character with incomplete dominance. Chanley also crossed two unselected clams and produced some fast-growing offspring. He later compared the growth of the progeny of these faster growing clams with that of the progeny of two randomly selected individuals and found that after 15 months the progeny of the faster growing individuals were 60 percent larger than those of individuals selected at random.

It was only a few years ago, however, that an independent project for studies of selective breeding and hybridization of commercially important mollusks was firmly established. These studies are now carried on at Milford Laboratory by Dr. Arlene Longwell and her assistants, and even though they have been underway for

only a short time, they have already produced much information which, no doubt, will eventually be of practical importance (Longwell et al., 1967; Longwell and Stiles, 1968). Extremely interesting work of the same nature has been reported by Menzel and Menzel (1965) and Menzel (1968a, 1968b) on other bivalves and also on *C. virginica*. We are looking forward to the results of these studies, which should greatly add to our knowledge and to our ability to develop a better quality of commerical mollusks.

Development of Chemically Treated Spat Collectors

Since it is well documented in scientific literature that larval marine invertebrates may be attracted by chemicals released by young and adult individuals of their own species, we suggested, many years ago, to one of our associates a study of the possibility of attracting ready-to-set larvae to collectors by incorporating in them excrements of adult oysters (Loosanoff and Davis, 1963b). To make these collectors, oyster feces were collected from the adult oysters kept in laboratory conditioning trays; the feces were air-dried and then mixed in a 3:1 ratio by volume with Portland cement to make a concrete panel approximately 4 × 2 × 1/4 inches. Other panels of similar size were made with dried silt collected in pans in the laboratory or with washed and dried sand. All panels were seasoned for some time to minimize the influence of toxic products from the fresh cement.

In several tests, panels made with oyster feces consistently collected more spat than the panels serving as controls (W. Landers, personal communication). A few years later Walne (1966) conducted similar studies on larvae of *O. edulis*. His experiments, however, consisted in preparing an active extract of oyster meats, clarifying it by filtering and centrifuging, and finally painting it on a glass plate. Walne found that the average ratio of setting was considerably higher on treated plates than on the controls.

Extensive studies were also made at Milford to find a method of preventing fouling of oyster shells that are used as spat collectors, by dipping them in various chemicals (MacKenzie et al., 1961). This experiment demonstrated that highly chlorinated benzenes, such as Polystream, could be used for this purpose because treated shells collected almost twice as many oyster spat as untreated ones. After these promising preliminary studies a massive experiment on a commercial scale was conducted by the staff of Milford Laboratory in New Haven Harbor in cooperation with F. Mansfield & Sons Company. In addition to letting us use their oyster bed, the company also supplied approximately 5,000 bushels of oyster shells, labor, and a boat needed for carrying on this extensive undertaking. To obtain reliable controls several acres of oyster bottoms were planted with untreated oyster shells (Loosanoff, 1961b).

The shells were planted in New Haven Harbor on August 11 and examined on September 26, 1961. Almost three times as many living oyster spat were found on chemically treated as on untreated shells. Moreover, the number of drilled young oysters on treated shells was about nine times lower than on the controls. Finally, as expected, the treated shells were much less fouled than the untreated ones. This condition was especially well illustrated by the great reduction in the numbers of *Crepidula*, which virtually covered untreated shells.

Recently, Castagna et al. (1969) conducted commercial-scale field experiments on the eastern shore of Virginia to evaluate our method of treating shell cultch with Polystream. These workers came to the conclusion that treatment of cultch by commercial growers may be economically feasible and may significantly increase their net production.

In connection with improvement of the cultch for collection of spat it may be appropriate to mention here the recent successes in producing "cultchless" spat of oysters. The method of production on a commercial scale was developed at the Pacific Mariculture, Inc., hatchery at Pigeon Point, California. I do not know the details of this approach, but I am aware that the method is efficient and that similar methods are now also being employed by some hatcheries on the Atlantic Coast. "Cultchless" spat is nothing new to the members of Milford Laboratory, however, because as long ago as 1955–56 we were already getting a large number of "cultchless" spat which, in reality, were recently set oysters that were dropping off the polyethylene film we had used as an experimental spat collector (Loosanoff, 1958). At that time "cultchless" spat was not considered especially desirable because of the difficulty of taking care of these virtually microscopic organisms. Because of this consideration I stated (Loosanoff, 1958), "At present, we find that the surfaces of most polyethylene films are too smooth and this condition causes the oyster set to peel off the collectors as soon as it reaches the size of 1/8-inch or somewhat larger." By making the surface of the film coarser by various means, we managed, nevertheless, to retain the oyster set on these collectors for a considerably longer period. When the oyster set was about 1/8" or larger, it could then easily be peeled off, giving a real "cultchless" spat which was already large enough to be shipped to oyster farmers or, perhaps, even suspended in open waters.

Rearing of Abalone

The scope of my review should include only the development of methods for cultivation of bivalve mollusks. I don't intend to deviate from this plan but, nevertheless, I cannot finish my discussion without mentioning the interesting and important work on rearing of larval and juvenile abalone by Japanese scientists and also some Americans. In Japan several men, led by Messrs. Ino, Imai, Sakai, and Oba, are engaged in this work. The initiator of these studies is, in my opinion, Dr. Ino of the Ministry of Agriculture and Forestry, who widely published on biological studies of the Japanese abalone.

In the United States several species of abalone, and even their crosses, have been successfully reared at the Pigeon Point hatchery. Several hundred of these hatchery-grown gastropods were shipped to the State of Oregon to be planted in selected localities by the state biologists. Abalone are also reared at the California Marine Associates hatchery in the Morro Bay area. Unfortunately, these efforts seem to be commercially unprofitable. No customers are willing to pay for the abalone set because abalone cultivators are not protected. In other words, as the laws are now formulated, if anyone plants hatchery-raised abalone set in open waters, he will not be able to restrain scuba divers from harvesting these mollusks after they reach legal size. Obviously, rewriting of our obsolete, often unfunctional

laws is necessary to give abalone farmers and other mariculturists badly needed protection.

CONCLUSION

From my review it should be clear that the Milford Laboratory of the Bureau of Commercial Fisheries has played an important role in the development of the present methods of cultivation of commercial mollusks. As the founder of this laboratory, as its Director for over 30 years, and as the man who was among those initiating the revival of interest in the methods for rearing of molluscan larvae, I am, naturally, proud of the achievements of the Milford group, just as the Japanese are proud of Dr. Imai and his associates, and our British colleagues of Dr. Cole and his followers. It should be understandable, therefore, that I would like to end this recitation in a somewhat personal manner. In short, I would like to share with you some of the experiences that we went through before the value of larval work was generally recognized and officially approved.

I began the preliminary experiments with larvae in 1944, but attained very little success. As mentioned before, there were no scientists on my staff at that time. Early in 1946 I spent several hours with Mr. Elmer Higgins, the Chief of the Division of Scientific Inquiry of the Bureau of Fisheries, talking with him about my hopes and plans and asking him to support our larval work. Mr. Higgins was a most understanding and receptive man and, as a result of this meeting, a new position of Marine Biologist was established at Milford Laboratory. The man who filled this position happened to be Mr. Harry C. Davis, who is at present Acting Director of this laboratory. Harry, who arrived full of enthusiasm and desire, plunged into a relatively new-to-him sphere of activities. That day in the early winter of 1946, when Harry came into my office for the first time, remains a "red letter day" on my scientific calendar.

The situation was abruptly changed after Mr. Higgins left the position which he occupied. Our work on larvae was considered in some quarters an undesirable folly and between 1949 and 1953 we went through an extremely difficult period. Our chief support at that time came from J. Richards Nelson, David Wallace, and John Glude. Because of their support and because of our rapid success, John Glude, who at that time was in charge of the Clam Investigations of the Bureau of Commercial Fisheries, transferred some of his funds to the budget of Milford Laboratory, thus saving us from "withering on the vine." Because of the help of these men and the organizations they represented, and because of our success in rearing larvae, we knew by the end of 1953 that we were successfully overcoming the opposition to our studies, and when, in 1954, our article entitled, "New advances in the study of bivalve larvae," appeared in *The American Scientist* and received general acclaim, we knew that we had won. From then on our progress became more and more rapid, resulting in numerous contributions to the techniques of rearing molluscan larvae, and to our knowledge of the physiological and ecological requirements of these larvae.

Parallel with the progress of our laboratory studies we published numerous articles, many of which are included in the bibliography of this review. Encouraged by our success, other individuals began to employ our methods, and many came to

us to learn our techniques. While being proud of our contributions to scientific literature, we are equally proud of the contribution that Milford Laboratory has made in the field of training people, both scientists and practical men, in the methods of cultivation of molluscan larvae and in related fields. According to the incomplete list of trainees recently prepared at Milford Laboratory, almost 60 U.S. nationals received training under our direction. This list includes college professors, state biologists, university students, and men who wished to enter the field of shellfish rearing. It is gratifying to know that virtually all commercial hatcheries on the East Coast have Milford trainees, most of them in executive positions. Moreover, Messrs. W. W. Budge and C. Black of Pigeon Point, California, although they did not go through a prolonged training at Milford, nevertheless received considerable help from us to prepare them for hatchery work.

The list also includes numerous names of foreign nationals beginning with Alan Ansell of England, Neil Bourne of Canada, Michael Crowley of Ireland, Robin Millar of Scotland, W. Ockelmann of Denmark, Mirjana Brenko of Yugoslavia, Juan Ribas Gonzalez of Spain, Tomoron Langkulsen of Thailand, Albert Lucas of France, A. Sastry of India, Won Tack Yang of Korea, and many others. This is certainly an impressive array of names.

I consider Edwin Fordham of Stratford, Connecticut, as the first practical shellfish hatchery man in the United States. Approximately in 1954, 4 or 5 years before J. Glancy began his operation, Fordham was already rearing bivalve larvae on a large scale at his temporary hatchery on the Housatonic River. Fordham learned the techniques of raising larvae at Milford Laboratory, where he was employed for some time, and has always remained in touch with members of our staff.

Because so many capable men from so many different countries were schooled in our techniques, the art of rearing larvae should not be lost. We hope, therefore, that some day the foundations that we, at Milford, and our colleagues in other parts of the world, have laid during the last two decades will revolutionize many important aspects of shellfisheries and, consequently, lead to production of more food for generations to come.

We also want to believe that our contribution takes mariculture out of its infancy and places it in a position from which it may soon begin to compete with agriculture in food production by supplying humanity with a wide variety of highly nutritious, delicate-tasting mollusks.

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LITERATURE CITED

BAUGHMAN, J. L., 1948. *An Annotated Bibliography of Oysters, With Pertinent Material on Mussels and Other Shellfish and an Appendix on Pollution.* Texas A. & M. Res. Found., College Station, Texas, 794 p.

BROOKS, W. K., 1880. The development of the American oyster. *Stud. Biol. Lab., Johns Hopkins Univ. IV*, 1–104.

BRUCE, J. R., M. KNIGHT, AND M. W. PARKE, 1940. The rearing of oyster larvae on an algal diet. *J. Mar. Biol. Assn. U.K.*, 24: 337–374.

CALABRESE, A., AND H. C. DAVIS, 1966. The pH tolerance of embryos and larvae of *Mercenaria mercenaria* and *Crassostrea virginica*. *Biol. Bull.*, 131:427–436.

CALABRESE, A., AND H. C. DAVIS, 1967. Effects of "soft" detergents on embryos and larvae of the American oyster *(Crassostrea virginica)*. *Proc. Nat. Shellfish. Assn.*, 57: 11–16.

CASTAGNA, M., D. S. HAVEN, AND J. B. WHITCOMB, 1969. Treatment of shell cultch with Polystream to increase the yield of seed oysters, *Crassostrea virginica*. *Proc. Nat. Shellfish. Assn.*, 59: 84–90.

CHANLEY, P. E., 1961. Inheritance of shell markings and growth in the hard clam, *Venus mercenaria*. *Proc. Nat. Shellfish. Assn.*, 50: 163–168.

CHESTNUT, A. F., W. E. FAHY, AND H. J. PORTER, 1957. Growth of young *Venus mercenaria*, *Venus campechiensis*, and their hybrids. *Proc. Nat. Shellfish. Assn.*, 47: 50–56.

CHURCHILL, E. P. JR., 1920. The oyster and the oyster industry of the Atlantic and Gulf coasts. *U.S. Bur. Fish. App. 8 to Rep. U.S. Comm. Fish.*, 1–51.

COLE, H. A., 1937. Experiments in the breeding of oysters *(Ostrea edulis)* in tanks, with special reference to the food of the larva and spat. *Fish. Invest., Min. Agric. Fish., London*, Ser. 11, 15: 1–28.

COLE, H. A., 1939. Further experiments in the breeding of oysters *(Ostrea edulis)* in tanks. *Fish. Invest., Min. Agric. Fish.*, London, Ser. 11, 16: 1–47.

DANNEVIG, ALF, 1945. Osterbassenget ved Flødevigen. Fiskeridirektoratets Skrifter Ser. Havundersøkelser. *Rep. Norwegian Fish. Mar. Invest.*, 8: 5–11.

DAVIS, H. C., 1950. On interspecific hybridization in *Ostrea*. Science, 111: 522.

DAVIS, H. C., 1953. On food and feeding or larvae of the American oyster, *C. virginica*. *Biol. Bull.*, 104: 334–350.

DAVIS, H. C., 1958. Survival and growth of clam and oyster larvae at different salinities. *Biol. Bull.*, 114: 296–307.

DAVIS, H. C., 1961. Effects of some pesticides on eggs and larvae of oysters *(Crassostrea virginica)* and clams *(Venus mercenaria)*. *Commer. Fish. Rev.*, 23: 8–23.

DAVIS, H. C., AND A. D. ANSELL, 1962. Survival and growth of larvae of the European oyster, *O. edulis*, at lowered salinities. *Biol. Bull.*, 122: 33–39.

DAVIS, H. C., AND A. CALABRESE, 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. *U.S. Fish Wildlife Serv., Fish. Bull.*, 63: 643–655.

DAVIS, H. C., AND A. CALABRESE, 1969. Survival and growth of larvae of the European oyster *(Ostrea edulis* L.) at different temperatures. *Biol. Bull.*, 136: 193–199.

DAVIS, H. C., AND P. E. CHANLEY, 1956. Effects of some dissolved substances on bivalve larvae. *Proc. Nat. Shellfish. Assn.*, 46: 59–74.

DAVIS, H. C., AND R. R. GUILLARD, 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. *U.S. Fish Wildlife Serv., Fish. Bull.*, 58: 293–304.

DAVIS, H. C., AND H. HIDU, 1969. Effects of pesticides on embryonic development of clams and oysters and on survival and growth of the larvae. *U.S. Fish Wildlife Serv., Fish. Bull.*, 67: 393–404.

DAVIS, H. C., AND R. UKELES, 1961. Mass culture of phytoplankton as foods for metazoans. *Science*, 134: 562–564.

DAVIS, H. C., V. L. LOOSANOFF, W. H. WESTON, AND C. MARTIN, 1954. A fungus disease in clam and oyster larvae. *Science*, 120: 36–38.

GAARDER, T., AND R. SPÄRCK, 1933. Hydrographisch-Biochemische Untersuchungen in Norwegischen Austernpollen. *Bergens Mus. Årbok 1932 Naturv. Rekke No.* 1: 1–144.

GALTSOFF, P. S., 1930. The fecundity of the oyster. *Science*, 72: 97–98.

GALTSOFF, P. S., 1932. Spawning reactions of three species of oysters. *J. Wash. Acad. Sci.*, 22: 65–69.

GALTSOFF, P. S., 1964. The American oyster *Crassostrea virginica* Gmelin. *U.S. Fish Wildlife Serv., Fish Bull.*, 64: 480 p.

GROSS, F., 1947. Review of paper by Bruce, Knight and Parke, 1940. *J. Cons. Perm. Int. Explor. Mer.*, 15: 86.

GUILLARD, R. R., 1959. Further evidence of the destruction of bivalve larvae by bacteria. *Biol. Bull.*, 117: 258–266.

HAVEN, D., AND J. D. ANDREWS, 1957. Survival and growth of *Venus mercenaria*, *Venus campechiensis*, and their hybrids in suspended trays and on natural bottoms. *Proc. Nat. Shellfish. Assn.*, 47: 43–48.

HIBBERT, D. H., 1897. Hibbert patent oyster bed and method of propagating therein. pp. 1–14. *Media, Delaware County, Pa.*

HIDU, H., 1965. Effects of synthetic surfactants on the larvae of clams (*M. mercenaria*) and oysters (*C. virginica*). *J. Water Pollut. Contr. Fed.*, 37: 262–270.

HIDU, H., AND R. UKELES, 1964. Dried unicellular algae as food for larvae of the hard shell clam, *Mercenaria mercenaria*. *Proc. Nat. Shellfish. Assn.*, 53: 85–101.

HIDU, H., K. G., DROBECK, E. A. DUNNINGTON JR., W. H. ROOSENBURG, AND R. L. BECKET, 1969. Oyster hatcheries for the Chesapeake Bay region. *Contrib. No. 382, Natur. Resour. Inst., Univ. Maryland, Spec. Rep. No. 2*, 1–18.

HORI, J., AND D. KUSAKABE, 1927. On the artificial culture of oyster larvae (II). *J. Imp. Fish. Inst.*, 23: 79.

HUGHES, E., 1940. The breeding of oysters in tanks. *J. Mar. Biol. Ass. U. K.*, 24: 543–547.

IMAI, T., AND S. SAKAI, 1961. Study of breeding of Japanese oyster, *Crassostrea gigas*. *Tohoku J. Agric. Res.*, 12: 125–163.

IMAI, T., M. HATANAKA, R. SATO, S. SAKAI, AND R. YUKI, 1950. Artificial breeding of oysters in tanks. *Tohoku J. Agric. Res.*, 1: 69–86.

IMAI, T., S. SAKAI, H. OKADA, AND T. YOSHIDA, 1954. Breeding of the Olympia oyster in tanks and culture experiments in Japanese waters. *Tohoku J. Agric. Res.*, 5: 13–25.

KÄNDLER, R., 1933. Die Kultur der Auster. Emil Abderhalden's Handbuch der Biol. Arbeitsmeth., Abt. 9, Meth. der Erforschung der Leistungen des Tierischen Organismus, Teil 5, Band 1. *Meth. der Meerwasserbiologie*, 599–716. Urban and Schwarzenberg, Berlin.

KORRINGA, P., 1952. Recent advances in oyster biology. *Quart. Rev. Biol.*, 27: 266–308, 339–365.

LONGWELL, A. C., S. S. STILES, AND D. G. SMITH, 1967. Chromosome complement of the American oyster *Crassostrea virginica*, as seen in meiotic and cleaving eggs. *Can. J. Genet. Cytol.*, 9: 845–856.

LONGWELL, A. C., AND S. S. STILES, 1968. Fertilization and completion of meiosis in spawned eggs of the American oyster, *Crassostrea virginica* Gmelin. *Caryologia*, 21: 65–73.

LOOSANOFF, V. L., 1937. Spawning of *Venus mercenaria* (L.). *Ecology*, 18: 506–515.

LOOSANOFF, V. L., 1942. Seasonal gonadal changes in the adult oysters, *Ostrea virginica*, of Long Island Sound. *Biol. Bull.*, 82: 195–206.

LOOSANOFF, V. L., 1945. Precocious gonad development in oysters induced in midwinter by high temperature. *Science*, 102: 124–125.

LOOSANOFF, V. L., 1949. Method for supplying a laboratory with warm seawater in winter. *Science*, 110: 192–193.

LOOSANOFF, V. L., 1954. New advances in the study of bivalve larvae. *Amer. Scient.*, 42: 607–624.

LOOSANOFF, V. L., 1955. The European oyster in American waters. *Science*, 121: 119–121.

LOOSANOFF, V. L., 1958. Use of plastics for collecting oyster set. *Commer. Fish. Rev.*, 20: 52–54.

LOOSANOFF, V. L., 1961a. Recent advances in the control of shellfish predators and competitors. *Gulf Carib. Fish. Inst., Proc. Thirteenth Annu. Session November 1960*, 113–127.

LOOSANOFF, V. L., 1961b. Treatment of cultch to collect heavier sets of oysters. *Bur. Commer. Fish., Biol. Lab., Milford, Conn., Bull. No. 8*, 25: 1–5.

LOOSANOFF, V. L., 1965. Gonad development and discharge of spawn in oysters of Long Island Sound. *Biol. Bull.*, 129: 546–561.

LOOSANOFF, V. L., 1966. Time and intensity of setting of the oyster, *Crassostrea virginica*, in Long Island Sound. *Biol. Bull.*, 130: 211–227.

LOOSANOFF, V. L., 1969. Maturation of gonads of oysters, *Crassostrea virginica*, of different geographical areas subjected to relatively low temperatures. *The Veliger*, 11: 153–163.

LOOSANOFF, V. L., AND H. C. DAVIS, 1950. Conditioning *V. mercenaria* for spawning in winter and breeding its larvae in the laboratory. *Biol. Bull.*, 98: 60–65.

LOOSANOFF, V. L., AND H. C. DAVIS, 1951. Delaying spawning of lamellibranchs by low temperature. *J. Mar. Res.*, 10: 197–202.

LOOSANOFF, V. L., AND H. C. DAVIS, 1952a. Repeated semiannual spawning of northern oysters. *Science*, 115: 675–676.

LOOSANOFF, V. L., AND H. C. DAVIS, 1952b. Temperature requirements for maturation of gonads of northern oysters. *Biol. Bull.*, 103: 80–96.

LOOSANOFF, V. L., AND H. C. DAVIS, 1963a. Rearing of bivalve mollusks, pp. 1–136. *In:* F. S. Russell, Ed., *Advances in Marine Biology, Vol. 1. Academic Press Inc., London and New York.*

LOOSANOFF, V. L., AND H. C. DAVIS, 1963b. Shellfish hatcheries and their future. *Commer. Fish. Rev.*, 25: 1–11.

LOOSANOFF, V. L., AND J. B. Engle, 1940. Spawning and setting of oysters in Long Island Sound in 1937, and discussion of the method for predicting the intensity and time of oyster setting. *U.S. Bur. Fish., Bull. No. 33,* 49: 217–255.

LOOSANOFF, V. L., AND J. B. ENGLE, 1942a. Accumulation and discharge of spawn by oysters living at different depths. *Biol. Bull.*, 82: 413–422.

LOOSANOFF, V. L., AND J. B. ENGLE, 1942b. Use of complete fertilizers in cultivation of microorganisms. *Science,* 95: 487–488.

LOOSANOFF, V. L., AND J. B. ENGLE, 1947. Effect of different concentrations of microorganisms on the feeding of oysters *(O virginica). U.S. Fish Wildlife Serv., Fish. Bull.*, 51: 31–57.

LOOSANOFF, V. L., AND C. A. NOMEJKO, 1951. Existence of physiologically-different races of oysters, *Crassostrea virginica. Biol. Bull.*, 101: 151–156.

LOOSANOFF, V. L., H. C. DAVIS, AND P. E. CHANLEY, 1953. Lack of relation between age of oysters or clams and quality of their spawn. *Oyster Inst. North America, Trade Rep. No. 133,* 1–2.

LOOSANOFF, V. L., H. C. DAVIS, AND P. E. CHANLEY, 1955. Food requirements of some bivalve larvae. *Proc. Nat. Shellfish. Assn.*, 45: 66–83.

LOOSANOFF, V. L., J. E. HANKS, AND A. E. GANAROS, 1957. Control of certain forms of zooplankton in mass algal cultures. *Science,* 125: 1092–1093.

LOOSANOFF, V. L., W. S. MILLER, AND P. B. SMITH, 1951. Growth and setting of larvae of *Venus mercenaria* in relation to temperature. *J. Mar. Res.*, 10: 59–81.

MACKENZIE, C. L. JR., V. L. LOOSANOFF, AND W. T. GNEWUCH, 1961. Use of chemically-treated cultch for increased production of seed oysters. *Bur. Commer. Fish., Biol. Lab., Milford, Conn., Bull. No. 5,* 25: 1–9.

MAURER, D., AND K. S. PRICE JR., 1968. Holding and spawning Delaware Bay oysters *(Crassostrea virginica)* out of season. I. Laboratory facilities for retarding spawning. *Proc. Nat. Shellfish. Assn.*, 58: 71–77.

MENZEL, R. W., 1963. Seasonal growth of the northern quahog, *Mercenaria mercenaria,* and the southern quahog, *M. campechiensis,* in Alligator Harbor, Florida. *Proc. Nat. Shellfish. Assn.*, 52: 37–46.

MENZEL, R. W., 1968a. Chromosome number in nine families of marine pelecypod mollusks. *Nautilus,* 82: 45–58.

MENZEL, R. W., 1968b. Cytotaxonomy of species of clams *(Mercenaria)* and oysters *(Crassostrea). Proc. Symp. Mollusca, Mar. Biol. Assn., India,* Part 1, 75–84.

MENZEL, R. W., AND M. Y. MENZEL, 1965. Chromosomes of two species of quahog clams and their hybrids. *Biol. Bull.*, 129: 181–188.

MILLAR, R. H., AND J. M. SCOTT, 1968. An effect of water quality on the growth of cultured larvae of the oyster *Ostrea edulis* L. *J. Cons. Perm. Int. Explor. Mer.*, 32: 123–130.

MOORE, H. F., 1898. Oysters and methods of oyster-culture. *Rep. U.S. Comm. Fish. for 1897,* 23: 263–338.

NELSON, T. C., 1921. Aids to successful oyster culture. I. Procuring the seed. *N.J. Agric. Exp. Sta., Bull. No. 351,* 1–59.

PRYTHERCH, H. F., 1924. Experiments in the artificial propagation of oysters. *U.S. Bur. Fish. App. 11 to Rep. U.S. Comm. Fish., Fiscal Year 1923,* 1–14.

RYDER, J. A., 1883. Rearing oysters from artificially fertilized eggs, together with notes on pond culture. *Bull. U.S. Fish. Comm.*, 3: 281–294.

SAGARA, J., 1958a. Artificial discharge of reproductive elements of certain bivalves caused by treatment of seawater and by injection with NH_4OH. *Bull. Jap. Soc. Sci. Fish.*, 23: 505–510.

SAGARA, J., 1958b. On the optimum temperature and salinity for the development of hard clam, *Meretrix meretrix lusoria* (Röding). *Bull. Tokai Reg. Fish. Res. Lab.*, No. 22, 27–32.

SPÄRCH, R., 1927. Studies on the biology of the oyster *(Ostrea edulis). Rep. Danish Biol. Sta.*, 33: 43–65.

STAUBER, L. A., 1950. The problem of physiological species with special reference to oysters and oyster drills. *Ecology,* 31: 109–118.

STICKNEY, A. P., 1964. Salinity, temperature, and food requirements of soft-shell clam larvae in laboratory culture. *Ecology*, 45: 283–291.

TUBIASH, H. S., P. E. CHANLEY, AND E. LEIFSON, 1965. Bacillary necrosis, a disease of larval and juvenile bivalve molluscs. *J. Bacteriol.*, 90: 1036–1044.

UKELES, R., 1962. Growth of pure cultures of marine phytoplankton in the presence of toxicants. *Appl. Microbiol.*, 10: 532–537.

WALNE, P. R., 1956a. Bacteria in experiments on rearing oyster larvae. *Nature*, 178: 91.

WALNE, P. R., 1956b. Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory. *Fish. Invest., Min. Agric. Fish. Food, London*, Ser. 11, 20: 1–23.

WALNE, P. R., 1958. The importance of bacteria in laboratory experiments on rearing the larvae of *Ostrea edulis* (L.). *J. Mar. Biol. Assn. U.K.*, 37: 415–425.

WALNE, P. R., 1963. Observations on the food value of seven species of algae to the larvae of *Ostrea edulis*. 1. Feeding experiments. *J. Mar. Biol. Assn. U.K.*, 43, 767–784.

WALNE, P. R., 1965. Observations on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis* L. *Fish. Invest., Min. Agric. Fish. Food*, London, Ser. II, 24: 1–45.

WALNE, P. R., 1966. Experiments in the large-scale culture of the larvae of *Ostrea edulis* L. *Fish. Invest., Min. Agric. Fish Food, London*, Ser. II, 25: 1–53.

WELLS, W. F., 1920. Growing oysters artificially. *Conservationist*, 3: 151, New York Conserv. Com.

WELLS, W. F., 1926. A new chapter in shellfish culture. *N.Y. St. Conserv. Comm., Fifteenth Annu. Rep.*, 1–36.

WELLS, W. F., 1927. Report of the experimental shellfish station. *N.Y. St. Conserv. Dep., Rep. No. 16*, 1–22.

WILSON, D. P., 1941. Oyster rearing on the River Yealm. *J. Mar. Biol. Ass. U. K.*, 25: 125–127.

WINSLOW, F., 1884. Report of experiments in the artificial propagation of oysters, conducted at Beaufort, N.C., and Fair Haven, Conn., in 1882. *Rep. U.S. Comm. Fish. for 1882*, 741–761.

WOODBURN, K. D., 1963. Survival and growth of laboratory-reared northern clams (*Mercenaria mercenaria*) and hybrids (*M. mercenaria x M. campechiensis*) in Florida waters. *Proc. Nat. Shellfish Assn.*, 52: 31–36.

DISCUSSION

MAURER: Dr. Loosanoff, one of the main efforts in our Sea Grant Project is to develop a completely controlled system. What is the present probability of realizing this controlled oyster farm or shellfish production factory? With the degradation of natural environments could one of these factories be established in an inland area like Nebraska or Illinois? Do you think the biological know how and technology are available?

LOOSANOFF: I do think a ranch like this is possible. However, since I am a practical man, I would not think in terms of inland states, but would prefer to concentrate these efforts for the present along the shore. Theoretically, you can develop this controlled system, but regardless of the chemical approaches, it is going to be an effort to maintain the oysters in their own metabolites. So I think it is better to depend upon normal seawater. We have some immense opportunities in many respects, principally because we can combine technology of other industries with our knowledge of biology, ecology and parasitology. For example, an associate of mine, Dr. Joiner, is extremely interested in developing various methods to utilize thermal pollution for the production of clams, oysters, and mussels. This is not a new idea of utilization of thermal effluent. As early as 1958, Harry Davis was working on this problem at the request of Mr. Waugh of England. Mr. Davis found that European oyster larvae can stand increased temperature and that they grew remarkably well. Nevertheless, I prefer to use natural approaches first. For example, there are hundreds of bays and harbors in Alaska absolutely unutilizable now. By constructing dams across the bays to utilize the effects of thermal effluents in the water, we may create great areas for oyster growing. This is why I fully agree with Dr. Daiber who said that we would have to consult ocean engineers to help us develop these methods.

Introduction to NUTRITIONAL REQUIREMENTS IN SHELLFISH CULTURE

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The requirements for the commercial operation of a "closed" system of shellfish production are: 1) rapid growth rates of larvae, spat, juveniles, and adults that are predictable and controllable; 2) maximization of market quality in adults; and 3) control of reproductive condition of brood stock. At the present time, all of these requirements can be met but only for small numbers of organisms and only for short periods of time. In theory, the ability to control the required parameters of oyster production will also be the means by which the system is freed from the spectre of "bad water."

Since the shellfish under consideration are primarily filter-feeders, the spectrum of possible physical configurations of their energy sources is limited. In addition, depending on the stage in the life history of the organism, the required food characteristics will change. Thus, there is an interaction between the life history stage of the organism and the appropriate food source.

Although there is still some controversy on the question, it is fairly clear that wild oysters are deriving their energy input from some component of the phytoplankton. Thus, a commercial operation might depend simply on the phytoplankton introduced with the culture water. The difficulty with this technique lies in the fact that natural phytoplankton systems are extremely variable in quality and quantity of organisms in both time and space. This is translated as uncontrolled and undesirable variability in shellfish production. Modifications to reduce this variability have employed concentration and/or enrichment of wild phytoplankton held in greenhouse pools. Often, however, these procedures result in the rapid growth of a laboratory "weed" of low nutritional value with the exclusion of the desired plankton species.

In an effort to rigorously examine the nutritional requirements of various pelecypods, selected species of algae are often grown either as unialgal or axenic cultures and then used as food in controlled quantities. Growth of oysters fed these special foods is seldom as good as oysters grown on wild food, but absolute control of food quantity can be realized only by the axenic technique.

Nonliving sources of energy have also been tried as oyster food. Some of the materials that have been studied are corn starch and freeze-dried phytoplankton.

Results, however, have not been conclusive. It is felt by some workers that the bacteria associated with the organic particles are, in fact, the only material utilized by the oyster. Reports have sporadically appeared claiming that invertebrates, including various shellfish, are able to assimilate organic compounds directly from the water. This suggests an additional pathway for the input of energy and materials into oyster biomass.

The crux of the problem of oyster production from the standpoint of nutrition is the management of an adequate food supply. This requires the development of techniques for the mass culture of microalgae of proven food value.

NUTRITIONAL REQUIREMENTS IN SHELLFISH CULTURE

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Increasing of human food resources through aquaculture has been widely discussed in recent years, and the culture of shellfish is often cited as one of the most promising possibilities (Bardach, 1968; Ryther and Bardach, 1968). Another proposal in aquaculture is the utilization of microalgae as a food supplement (Witsch, 1960; Spoehr, 1953). An economical method of harvesting algal cultures is to utilize grazing animals for this purpose (Gibor, 1957), and since fish and shellfish are already an acceptable dietary staple in most cultures, microalgae could be used to increase the yields of these high protein foods. Consideration of shellfish aquaculture brings to mind immediately the questions of what shellfish eat and whether our current thought that nutrition, particularly of oysters, is dependent on phytoplankton is indeed correct. The following discussion on shellfish nutrition will be concerned more specifically with nutrition in the oyster. For many years interest in large-scale shellfish culture has centered about the oyster, although larval rearing presents a challenge since oysters appear to be more restricted in their food utilization than other species (Loosanoff and Davis, 1963).

To put this discussion into proper perspective, I wish to recall some of the statements of past workers, as these are just about as true today as when they were made some years ago. In 1942 Galtsoff stated the following: "At present we must confess our ignorance of the principles of mollusk nutrition and consequently our inability to suggest a practical solution of the problem of forced feeding and production of fat oysters at will." Nelson (1947) commented: "May I admit with complete candor and humility that after half a century of research on the oyster we are still not in a position to say with certainty just what this mollusk can or cannot use as food." Korringa (1952) was only slightly more optimistic: "The nutrition of the oyster, though one of the basic problems of oyster culture, is only partially understood."

In the past, investigators in shellfish biology believed that the solution to the problem could be found in a study of feeding mechanisms, digestive processes, and ecology. While this approach is still valid, contemporary biology can make special contributions in terms of new techniques, but even more significantly, in an analytical approach aimed at elucidating basic mechanisms in bivalve nutrition. We know

that substances needed for specific cellular reactions may be obtained through synthesis or, if the synthetic apparatus is not available, must be taken in with the diet. Dietary needs are dependent on physical, physiological, and biochemical functions in the animal.

The structure and function of food collecting and digestive organs in different stages of development impose certain requirements on foods for them to be suitable for oyster nutrition. The most obvious of these is the size of the mouth and esophagus. Development of feeding organs and details of anatomy in larvae of *O. edulis* (Horst, 1883; Yonge, 1926) and *C. virginica* (Brooks, 1880; Stafford, 1913) were described and show development to the straight-hinge stage of both species to be similar (Galtsoff, 1964). This stage is especially important in the American oyster (about 60 hours in 18–21°C) when they reach 67–78 μ and begin to feed actively (Amemiya, 1926). In the European oyster, larvae are released as larger, well-developed, straight-hinge oysters, able to feed, having been sustained by a large amount of yolk in the eggs to carry them through to this stage (Yonge, 1960). The esophagus of straight-hinge 165 μ × 200 μ *O. edulis* is about 20 μ, the stomach 46 μ, and the midgut 12 μ (Yonge, 1926). On this basis it can be estimated that 78 μ × 67 μ *C. virginica* larvae have a mouth opening of less than 10 μ. Carriker (1951) observed a wide range of size measurements of *C. virginica* in straight-hinge, early umbo, late umbo, mature, and eyed larvae. This observation implies enough of a variation in size of the oral opening to be significant in terms of the kind of food that can be utilized.

Veliger larvae feed on suspended particles that cilia of the velum collect and direct to ciliary tracts leading to the mouth. A funnel-shaped esophagus is followed by a constricted stomach from which appears a blind sac (the liver rudiment) and a convoluted intestine; the entire internal surface of the alimentary tract is covered with cilia. There appears to be no food sorting mechanism in the larval gut other than the exclusion of large particles by the small diameter of the mouth and esophagus (Yonge, 1926; Millar, 1955). On metamorphosis the upper mouth parts develop from the apical region and take over the function of food collecting. In the early stages, before the gills have fully developed, the mantle cilia may contribute to the creation of food currents.

The time of development of the crystalline style appears to be ambiguous and yet could be significant in the utilization of certain foods by free-swimming larvae. Brooks' (1880) figures of six-day *C. virginica* larvae show no evidence of a style. In figures of straight-hinge *O. edulis* larvae a style and style sac appear (Yonge, 1926; Millar, 1955). The first stage at which Nelson (1918) found a style in *C. virginica* was in spat of 4 mm. Shaw and Battle (1957) also reported a style sac in 4 mm spat as a posteriorly directed diverticulum of the stomach. Chestnut (1949) observed spat of *C. virginica* on glass slides and did not observe a style or style sac in the earliest stages of spat at 0.25–4.5 mm. At this stage food was brought forward by pulsations in the foregut and stomach which ceased in larger spat as the crystalline style developed when the function of mixing and movements of nutrients through the stomach was carried out by ciliary activity and rotation of the style. The digestive tract of adult *C. virginica* was described (Shaw and Battle, 1957) as follows: the mouth dorsoventrally compressed is bounded by two pair of labial palps

leading to a crescentric esophagus, then to the anterior portion of the stomach from which a complex diverticulum extends. The posterior chamber of the stomach leads to the gastric shield, style sac, midgut, and descending intestine to the rectum. The style, composed of H_2O, salt, and globular protein, is stored as a flexible solid in a caecum, but the end projecting into the stomach is slowly dissolved as it mixes with food particles (Mitra, 1901). The complete digestive tract is lined with ciliated columnar epithelia with the exception of palps, gastric shield, and some cells in the digestive diverticula. Mucous secreting and eosinophilic cells occur along the tract; phagocytes are present between the lining epithelial cells, as well as in the lumen of the tract (Millar, 1955; Shaw and Battle, 1957).

The feeding mechanism in adults is dependent on the ciliary action of gills driving a current of water through the ostia. During passage, particulate matter is filtered off, wrapped in mucus, and transported to the labial palps, where it is ingested, or large spiny objects rejected. *C. virginica* effectively retains diatoms and 2–3 μ graphite particles but allows 70–80 percent *Escherichia coli* and 80 percent of 1–2 μ graphite particles to pass (Owen, 1966). Yeast cells as a food for adult *C. virginica* were rejected, most of them appearing in the pseudofeces. *Chromatium perty* fed together with a mixed phytoplankton culture was also vigorously rejected in the first few days (Loosanoff, 1949). The suggestion was made that palps of the oyster possess certain specialized cells that act as chemo-receptors. This work confirmed the earlier studies of Lotsy (1895) and Grave (1916) where oysters showed a definite selection of particles with food value in opposition to other investigators who thought that selection was mainly quantitative (Kellogg, 1915; Yonge, 1926). It was also postulated that selection of food particles may occur by a change in filtering efficiency as the result of the presence or absence of a mucous sheath during feeding (MacGinitie, 1941). According to this theory the sheath can retain fine particles as bacteria and colloids but in its absence only particles too large to pass the ostia are retained. Owen (1966) stated that a more efficient way to remove particles from currents of water would be the combined effects of musculature activity, mucous secretion (but not as a sheath), and a straining of particles by lateral frontal cilia. In heavy concentrations of microorganisms the rate of pumping of water was reduced and the tonus of the adductor muscle became impaired (Loosanoff and Engle, 1947). According to one investigator, "Feeding in the oyster is accomplished, therefore, through the delicate coordinations of nervous, muscular, ciliary, and mucous-secreting elements in which mechanical sorting of materials plays the most important part." (Nelson, 1923b).

As a physiological process that affects the nutrition of oysters, digestion is no doubt more complex than some of the earlier works imply. The importance of phagocytes in the physiology of digestion and distribution of food was emphasized by Vonk (1924) and Yonge (1926). In starved oysters fed iron saccharate, blood cells, olive oil, and diatoms, particles were engulfed by phagocytes. Takatsuki (1934) found that starch, carmine, and India ink were also accepted. Chestnut (1949) observed that a starch suspension introduced to the stomach of the oyster was phagocytized in three hours, and diatoms in one hour, with complete plasmolysis occurring in two hours. Phagocytes in a hanging drop suspension engulfed *Platymonas* in 37 minutes and complete dissolution took place in 2 hours, 45 minutes.

With a large species of *Nitzschia* two or three phagocytes could be seen trying to engulf the same diatom. Although phagocytes ingested all particles without selection as to nutritive value, nonnutritive particles were ejected after ingestion. Feng (1965) studied the response of oysters to the introduction of soluble and particulate material and noted intracellular degradation of certain bacteria and spinach chloroplasts. There is evidence that migration of host leucocytes through epithelial surfaces is the normal physiological process, but whether pinocytotic activity of the leucocytes is primarily defensive or is also a way to obtain nutrients is still not clear.

Yonge's (1926) view was that protein and fat digestion occurred only intracellularly within wandering phagocytes, and starch digested only extracellularly by the action of style amylase. However, Takatsuki (1934) reported protease, lipase, as well as amylase in phagocytes, and in *C. virginica* tryptic, lipolytic, and amylolytic activities were found (Chestnut, 1949). Sawano (1929) observed protease, amylase, as well as butyrase, in style extracts, and protease, poly, and dipeptidases were also detected by Rosen (1950). Although lamellibranchs exist mainly on plants of vegetable origin with cellulose a common polysaccharide, some workers concluded that there was no evidence of cellulase in the oyster and that, except for *Helix*, such enzymes were exceptional in the mollusks (Yonge, 1938). Occurrence of cellulase and related polysaccharide enzymes in the mollusks was discussed by Stone and Morton (1958) with the conclusion that in addition to bacterial activity there is an innate mechanism for splitting higher polysaccharides. Mansour-Bek (1948) described amylase, maltase, saccharase, cellulase, and chitinase in amoebocyte-free stomach juice in lamellibranchs. There is some doubt as to whether these enzymes were produced by animals themselves or by contaminants. Evidence of a cellulolytic factor in the crystalline style was also reported by Newell (1953). However, in view of the ability of spirochetes to split cellulose, there may be a relationship between spirochetes found in vicinity of the style and cellulose splitting activity. George (1952) showed that hydrolysis of neutral fat does occur extracellularly in the cavity of the stomach, and positive tests for lipases in the style extract showed this organ to be the primary source of the enzyme. A current survey of digestive enzymes that would more clearly reflect the types of substrates that can be utilized for food at different stages of development would be extremely valuable.

In numerous references to the food of oysters each of the following has been selected by different investigators as being the most important in providing nutrition: (1) dissolved substances of organic origin, (2) organic detritus, (3) living organisms: plant, animal, or both. Some of these investigations are based on careful studies and others on insufficient data and assumptions, but some information and insight to the problem can be gained from a brief review of these three categories of potential foods.

One of the earliest theories on the food of oysters was proposed by Pütter (1909), and supported by the work of Churchill and Lewis (1924) on mussels. Their view, that dissolved organic matter was utilized directly, was at one time considered extreme. The hypothesis was based on the argument that there is a small amount of plankton in the ocean coupled with a large need of animals for nutrients, and also, that there was actually a large amount of dissolved organic matter in the sea

and only small numbers of plankton forms to be found in the stomachs of marine animals. Many subsequent workers showed that Pütter's estimates were incorrect and his arguments inconclusive. Lohman (1909) and Lipschütz (1913) anticipated contemporary concepts by stating that preserved samples represent only a small amount of the phytoplankton food supply, that stomach content examination must be made immediately on removing animals from the water, and that there exists a vast number of important but unknown nannoplankton. Krogh (1931) concluded that phytoplankton do not release large amounts of soluble metabolites into the water and that there was little evidence to support Pütter's thesis.

Pütter's hypothesis was upheld by the work of Mitchell (1916), who observed that dextrose was absorbed, converted, and rapidly stored as glycogen; as much as 4.9 gm in 24 hours from 0.25 percent glucose. Glycogen was not formed from dextrin, and excess sodium phosphate in the medium checked glycogen formation from glucose. In addition, Yonge (1928) showed that there was an average uptake of 9 mg glucose/oyster/hour, but if the mouth was plugged by paraffin, uptake was reduced to zero. Collier et al. (1950, 1953) reported that carbohydrates may be made available by being absorbed on mucous strands and carried to the mouth. It was later concluded that oysters utilize dissolved carbohydrates for energy in a series of tests in which the caloric intake of oysters was compared with output of energy (Collier, 1959). By concentrating glucose from seawater, oyster life was prolonged 68.2 days and a significant increase in oyster meat was demonstrated in glucose-containing media (Gillespie et al., 1964). Nelson (1934) tested a series of substances as artificial foods for oysters, including cornstarch, ground alfalfa, soya bean meal, and ground crab meat, in which only cornstarch was useful. This finding was confirmed in experiments where oysters receiving cornstarch and wheat flour as a dietary supplement increased in dry meat weight over the controls not receiving this supplement (Haven, 1965). Supplements of the vitamins riboflavin, calcium pantothenate, thiamine, and pyridoxine had no effect on *V. mercenaria* larvae but significantly increased the rate of growth of *C. virginica* and *O. lurida* larvae, both when given alone and in combination with plankton foods (Davis and Chanley, 1956). Pomeroy (1952) and Pomeroy and Haskin (1954) concluded that although the major source of phosphorus is in food materials, significant amounts of phosphate and calcium ions are derived from what is available in the water, thus partially filling the requirements of oysters for these ions, both for carbohydrate metabolism and shell deposition (Bevelander, 1952). This observation suggests that other dissolved substances may also serve to supply metabolic needs of the oysters. The conclusion of Stephens and Schinske (1961), that the capacity to remove amino acids from solution in seawater is broadly distributed among marine animals, was based upon the examination of 35 genera in 11 phyla (*Mytilus* being the representative of the phylum Mollusca). Species that were examined in an antibiotic medium removed significant quantities of glycine in 24 hours whether the acid functioned as an anion or a cation. These investigators proposed that this uptake makes a substantial contribution to the food supply. Wood (1965) also detected amino acids in marine and estuarine waters and suggested that these compounds play a fundamental role in the economy of the marine community. According to Jorgensen's (1955) calculations, 0.05 mg/liter of organic matter must be derived from each liter of sea-

water processed by a filter-feeding organism in order to provide maintenance metabolism and three to four times this amount is needed for growth. Thus, if 3–6 mg/liter of dissolved organic matter are present in seawater, a 5 percent efficiency in removal would yield 0.15–0.30 mg/liter of organic material. Potentially useful classes of lipids were found in seawater in the range of 0.5–0.6 mg/liter (Jeffrey, 1966) and reports of carbohydrates in coastal waters ranged from 0.1–0.4 mg/liter and to 8 mg/liter in coastal lagoons (Lewis and Rakestraw, 1955). In view of these findings the possibility that shellfish can assimilate soluble substances should again be considered, especially in relationship to the carbohydrates.

A second theory on the nature of oyster food was upheld by many European workers who classified oysters as pure detritus eaters. Peterson and Jensen (1911) expressed doubt that plankton and bottom diatoms actually played a role in nutrition and were convinced that detritus comprised the most important foods. Blegvad (1914) also agreed that living phytoplankton were of no importance for bottom fauna, a view based largely on the observation that dinoflagellates passed through the oyster gut undigested. Savage (1925) concluded that growth was due to detritus since the greater part of the food found in oysters was inanimate. He also noted that feeding oysters appear to ingest anything suitable that is captured with no evidence of selection, and that in beds that resulted in rapid fattening of animals the food was highly variable. Gavard (1927) fed oysters artificial detritus prepared from plant and animal material and obtained a significant increase in weight. Davis (1950), however, found that marine detritus from several different sources added to larval cultures did not result in an increase in growth.

Fox (1950) made the important observation that dissolved and particulate matter are not well-defined terms with respect to organic matter of the sea. Dissolved organic matter may be adsorbed on particles of colloidal dimensions and so become available. The utilization of such matter as food is an interesting possibility since in some cases it can be shown that a collection of phytoplankton by filter feeders does not provide sufficient material to support growth and metabolism (Fox and Coe, 1943). Sutcliffe et al. (1963) showed that soluble organic matter can be removed from seawater by aeration or bubble formation, thus converting soluble organic matter to particulate form. The nutritional value of such aggregates, produced by bubbling air through filtered seawater, was demonstrated for the brine shrimp, *Artemia salina* (Baylor and Sutcliffe, 1963). This phenomenon may be the basis of recent reports (personal communication) of good larval growth on centrifuged seawater with very low phytoplankton densities. Profiles of organic carbon in particulate matter from various depths showed that carbohydrates decayed more rapidly than proteins. D-glucose and its polymers being preferentially removed during descent (Handa and Tominaga, 1969). Since there has been some evidence for carbohydrate utilization, the change in organic carbon profiles suggests that suspension filter feeders may utilize carbohydrates in the aggregates. Thus, the theory of aggregate formation may have significant implications in shellfish nutrition.

Utilization of living food is the third possibility for oyster nutrition. Since a strong amylase was found, some investigators reached the conclusion that the food of oysters must consist only of organisms rich in carbohydrates and that lamelli-

branchs are specialized herbivores (Yonge, 1926). However, this concept was not completely in accord with others, such as Nelson (1933), who claimed that 80 percent of oyster food is of animal origin and believed that Yonge was not correct in limiting the style enzymes to the digestion of starch and glycogen. Nelson observed the following in various stages of digestion: *Skeletonema*, *Coscinodiscus*, *Melosira*, as well as various protozoans, rotifers, nematodes, cestodes, snails, clams, oysters, tunicates, and fish eggs. The possibility that disintegration could be a result of bacterial action was ruled out but that some secretion of the style could penetrate the chitin of crustaceans and the cuticle of nematodes, reducing particles to a size small enough to be phagocytized, was postulated. Mitchell (1916) brought evidence to show that protozoa and seaweed fragments (*Ulva lactuca*) may serve as food for oysters. Mansour-Bek (1948) also challenged Yonge's view and asserted that proteolytic and lipolytic activity could occur extracellularly in the stomach and that bivalves are indeed able to utilize animal forms.

Bacteria also fall into the category of a living food source. The role of bacteria as useful or harmful agents in the nutrition of oysters at different stages of development is still quite unclear. Spärck (1927) found that oysters may thrive in small limited volumes of water without frequent renewal and that "development of the bacteria does not seem in any way to hurt the oysters." Galtsoff (1928) and Galtsoff and Arcisz (1954) concluded that the greater part of a bacterial population passes through the gills and only a small fraction of the total number remains. Imai et al. (1949, 1950) used a colorless flagellate that was cultured on bacterial diet to feed oysters, leaving the strong suspicion that bacteria, as well as flagellates, were supporting growth. Carriker (1956) reared clam larvae to metamorphosis on cereal and concluded that good growth of larvae was a result of an increased microbial population stimulated by the cereal. Davis (1950, 1953) examined 13 species of bacteria for effect on oyster larval growth but observed no increase in low bacterial concentrations over the control which reached 94.05 μ, while parallel cultures fed mixed phytoplankton increased to 146.75 μ. Walne (1963) found no consistently improved growth in a comparison of cultures of *Isochrysis* and *Phaeodactylum* with and without bacteria. However, Hidu and Tubiash (1963) observed a 25–100 percent increase in rate of growth of larvae dependent on unknown bacteria in a nonsterile "Combistrep"* solution. Also suggesting bacterial utilization was the report that impure cultures of the unicellular green alga, *Coccomyxa littoralis*, gave satisfactory growth of spat, but pure cultures lacked a factor essential for growth (Cole, 1936). Zobell and Feltham (1938) studied bacterial utilization by mussels and concluded that in nature bacteria are probably an important part of the diet only below the photic zone but may be indirectly important in nutrition by synthesizing phytoplankton nutrients, or by converting dissolved organic matter to particulate form. Adult mussels survived and grew when fed 10^8 to 10^9 washed bacteria/ml, but, if peptone was added to the water, the animal died in 10^5 bacteria/ml. Guillard (1959) reported that two clones of bacteria were toxic to clam larvae but a third was without effect. Tubiash et al. (1965) also reported massive mortalities of clam and oyster larvae with certain clones of bacteria. The type of bacterial flora selected for

*Reg. U.S. Pat. Off., Chas. Pfizer & Co., Inc.

by different metabolites in seawater appears to be responsible for populations that may be nutritious, toxic, or without effect.

The view that has persisted longest and is most widely accepted is that phytoplankton and nannoplankton constitute the principle source of shellfish food. One of the earliest studies concluded that 88 percent of oysters fed on diatoms, 1 percent on desmids, and 3 percent on spores and particulates of seaweeds (Dean, 1887). Lotsy (1895) also regarded the diatoms as practically the only source of food and stated that the reproductive cells of large algae and animal or ground material were of no importance. Moore (1908) and Kellogg (1915) studied filtering rates and stomach contents and concluded that diatoms constituted 95 percent of the food of oysters. Grave (1916) listed 13 organisms that he considered the bulk food of Chesapeake Bay oysters; 10 diatoms, 2 peridines, and 1 small green flagellate. Stafford (1913) reported that *O. virginica* thrives best in shallow bays and estuaries where there is an abundance of small nannoplankton. Other early supporters of this view were Hunt (1925), Yonge (1926), Hinard (1923), Dodgeson (1926), and Martin (1923, 1928). It was the laboratory work of Martin that gave considerable impetus to the theory that diatoms were of less importance than previously indicated and that nannoplankton constituted the bulk food. In these experiments oysters were fed pure cultures that resulted in the following growth increments: *Amphora*, 11.17 percent, *Gleocystis*, 9.75 percent, detritus, 6.4 percent, and the control, 2.7 percent. Other experiments included *Nitzschia*, *Chlorella*, yeast, detritus, as well as a naked flagellate. The greatest increase was observed in pure cultures of a brown naked flagellate. Other evidence gathered from nature also pointed to the importance of the phytoplankton. Gaarder and Spärck (1932) observed that the dominant organism in good oyster plots was a small flagellate and a nonmotile green organism.

Although British attempts at artificial propagation of oysters date back to 1867 (Philpots, 1890), the importance of Cole's work (1937, 1938) was in the use of large clean tanks, sound mature breeding stocks, clean offshore water, and controlled organic enrichment to supplement the natural foods. The conclusion that emerged was that the essential factor for tank culture was the character of the food organisms rather than the condition of the water; also, that oyster larvae during the free-swimming stage utilized as foods only minute naked flagellates of the Chlamydomonaceae, Cryptomonadaceae, and Chrysomonadaceae but were unable to utilize nonmotile species with cellulose. He reached the tentative conclusion that spat can utilize green unicells with cellulose because enzymes slowly penetrate the cell wall, and that compared to spat the passage of ingested material through the gut of larvae is very rapid; therefore, green cells appear undigested.

Although Cole was successful in obtaining spat in tank culture on a commercial scale, Bruce et al. (1939) were virtually the first to develop good laboratory methods for raising larvae. Experiments were carried out where suitable larval foods were cultured and added to the seawater from which the natural phytoplankton had been removed by filtration. In this work attention was directed to flagellates rather than nonmotile forms and to the interesting fact that the six organisms differed in their usefulness as foods although each was of an ingestible size. The difference in color, hence storage material, was the most obvious variation, and these authors suggested that the varying usefulness of the flagellates depended directly on

the degree to which the algal food reserves served the immediate needs of developing larvae. The most successful flagellates in feeding experiments that maintained growth from liberation to settlement stage were greenish-yellow or golden-brown and measured 3 μ in diameter; these were later identified as *Isochrysis galbana* (flagellate 1) and *Pyraminomonas grossi* (flagellate H).

Experiments with marine phytoplankton were generally continued in the direction of evaluating utilization of photosynthetic species as foods. The colorless flagellate, *Monas*, with which Imai et al. (1949, 1950) reared spat, or the colorless species, *Astasis klebsii*, was not utilized by *C. virginica* (Davis, 1950), nor was the colorless *Bodo* useful to the European oyster (Walne, 1956). Walne (1963) reported that *C. stigmatophora* and *C. marina* were not good larval foods and were even inferior for spat although some growth did occur. Davis (1950) found that *Chlorella* sp. was not utilized by very young *C. virginica* larvae but was of some use to larvae over 125 μ. Clam larvae can exist on a diet made up chiefly of *Chlorella* (Loosanoff and Marak, 1951) and also utilize unialgal cultures of *Chlorella* (Davis and Guillard, 1958). However, if concentrations became too heavy, most of the larvae were killed and those swimming were abnormal (Loosanoff et al., 1955). The best single foods for clam larvae were *Chlorococcum*, *Isochrysis*, and *Monochrysis* but a combination provided better growth than did quantities of any of the single foods tested. A mixture of *I. galbana*, *M. lutheri*, *Dunaliella euchlora* and *Platymonas* sp. gave very good results. Reasonably good growth was also obtained on other species with cell walls, such as *Chlamydomonas* and *Phaeodactylum* (Davis and Guillard, 1958).

The following flagellates supported growth of oyster larvae: *Dicrateria inornata*, *Chromulina pleiades*, *Isochrysis galbana*, *Hemiselmis rufescens*, and *Pyraminomonas grossi*, but an unidentified cryptomonad and the flagellate, *Chlamydomonas*, were of no value (Davis, 1950; Davis, 1953). Equal numbers of different species fed to larvae resulted in different rates of growth in *C. virginica* larvae and in these experiments it became clear that *I. galbana* was a very good food. It was later reported that pure cultures of *I. galbana* and *M. lutheri* were the best larval foods and that addition of the chlorophytes, *Platymonas* sp. and *Dunaliella euchlora*, improved the food value of the chrysophytes (Davis and Guillard, 1958). The following species generally were found to be of mediocre value: *D. euchlora*, *Platymonas* sp. *Cyclotella* sp., *Chlorococcum*, *Phaeodactylum*, and *Cryptomonas* sp. Certain species were toxic or without food value to both larval and juvenile clams and oysters, e.g., *Prymnesium parvum*, *Stichococcus* sp., *Chlamydomonas* sp., *Amphidinium carteri*, and *Gymnodinium* sp. (Guillard, 1958; Davis and Guillard, 1958). The suggestion was made that the value of good chrysomonad foods was due to their small size, production of little or no toxic metabolites, and absence of a thick cell wall.

Whereas only small naked flagellates benefited oyster larvae in the earliest stages, they are capable at about the sixth day of using other forms, such as *Platymonas*, *Phaeodactylum*, and *Chlamydomonas*. Clam larvae are able to use a greater variety of foods than oyster larvae of the same age. Juveniles of both species utilize a still wider range. The naked flagellates good for larvae were also relatively good for juveniles but those foods best for juveniles, as cryptomonads, *Skeletonema* or *Actinocyclus*, were useless to larvae. Walne (1963) observed that if *D. tertiolecta* were utilized, it had a higher value than *I. galbana;* the difference in results between *C.*

virginica and *O. edulis* was attributed to the larger size of the latter larvae. *Monochrysis* appeared to be a similar or slightly better food than *Isochrysis* for *O. edulis*. *Phaeodactylum tricornutum*, like *Dunaliella*, was utilized by different broods of larvae to a different degree. A complicating factor in food testing is that some results with the European oyster larvae suggest that a given species may not be acceptable to all broods of larvae, which emphasizes the need for many replications of experiments before conclusions may be reached on the food value of a given species.

Although it is now established that certain small naked flagellates support growth and development of larvae, more information is needed on factors that affect feeding, particularly in the earliest stages on which successful artificial propagation is dependent. Some of these factors were recently investigated with ^{14}C labeled *Monochrysis lutheri* (Ukeles and Sweeney, 1969). It was observed that the ingestion of food cells started immediately upon addition of algae to the larval culture and although the number of food cells ingested increased rapidly at first, a plateau was reached in 24 hours after which there appeared to be little additional ingestion. Ingestion of food cells was stimulated by an increase in temperature. The number of food cells ingested was proportionate to the number available. However, retention or utilization of food cells did not similarly increase but reached a plateau at a relatively low number of food cells. The data suggest that feeding is a continuous process and at a certain cell concentration an equilibrium is reached between cells entering the mouth and those leaving the gut. Walne (1965) observed that *O. edulis* larvae did not increase in growth if food concentrations were increased beyond a satiation level and this was reached at a lower density when cells were larger than when they were small. At critical concentrations nonuseful foods, such as trichocysts, liberated from dinoflagellates, innocuous bacteria, and small nonnutritive phytoplankton, may block ingestion of such useful food organisms as *M. lutheri*. The conclusion was reached that even innocuous bacteria, if present in large numbers, can interfere with normal feeding and digestive processes by being preferentially ingested by virtue of size, and packing the gut with material that may be of no nutritional value (Ukeles and Sweeney, 1969).

The nutritional inadequacy of micro-algae may have one or a combination of sources, such as wrong size, indigestibility, deficiency in some essential nutrient, or toxicity. The size of an organism obviously limits its usefulness at particular stages of development and is especially important in young larvae. Digestibility is a function of the oyster's enzymatic capabilities, as well as algal chemical constituents. Dean (1958) emphasized that the difference between good and bad foods may be due to resistance to digestion. He observed that a cryptomonad disintegrated when swimming near an undissolved style, but cells swam freely when the style was completely dissolved. Whereas *M. lutheri* behaved similarly, *I. galbana* could swim near or touch the style for more than 72 hours without any discernible effect.

Differences between good and bad foods are often attributed to the chemical composition of the plankton. However, this concept is probably not as significant as was once believed since Parsons et al. (1961) reported that marine phytoplankton have similar overall organic compositions when grown under similar physical and chemical conditions, regardless of the size of the organism or the class to which it belongs. Wherever algal foods appear to be deficient in meeting the nutritional re-

quirements of a filter feeder, toxic or inhibiting factors may be indicated. It should then be demonstrated that increasing concentrations of algae parallel an increasing detrimental effect. Since the movement of food in larvae takes place by means of ciliary action in the digestive tract, compounds or ions affecting ciliary beat can influence the extent of digestion and movement of the food mass. Toxicity of some chlorophytes, *Chlorella*, *Chlamydomonas*, and *Stichococcus*, may be due to liberation of unsaturated fatty acids (Proctor, 1957; Spoehr et al., 1949) and in *Chlorella* has been associated with senescent cells (Ryther, 1954). Even good foods occasionally display toxicity (Guillard, 1958) presumably due to bacteria. Interactions within a culture are complex and factors supplied by other organisms in the water may affect utilization or toxicity of a given species, e.g., the normal toxicity of *Prymnesium parvum* may be decreased by a bacterial population (Shilo and Aschner, 1953). However, there is evidence that the presence of certain species of bacteria in food cultures may cause oyster and clam larvae mortality (Guillard, 1959; Tubiash et al., 1965). There is also evidence that large numbers of nontoxin-producing bacteria may cause a normally good food to become poor (Ukeles and Sweeney, 1969).

Criteria for utilization of foods have been varied and in most experiments are not as rigorous as is desirable. Although ingestion is not digestion or utilization, stomach content examinations were often used as a means of studying feeding in the adult oyster. Presence of a given species was considered evidence of utilization and its absence nonutilization. This procedure led to the often quoted conclusion that diatoms constituted 95 percent of the food of oysters. Obviously, among the contents of the stomach, diatoms would be easily recognized but other species would disintegrate rapidly and escape detection. The presence or absence of a style has also been used as an index of a feeding oyster (Chestnut, 1946). This procedure may also be criticized since under certain conditions, e.g., winter, a style has been observed in a nonfeeding oyster (Galtsoff, 1964); and Yonge (1926) observed that a style may be present in healthy animals even when starved. Nelson's (1923a) method of studying feeding under different conditions was to judge an open oyster as actively feeding, although Hopkins (1936) showed that an oyster may be open without feeding.

The excretion of living cells and the study of algal pigments in feces have also been used as an indication of the utilization or nonutilization of food. However, Currie (1962) showed that a rapid degradation of ingested chlorophyll takes place resulting in false chlorophyll values. The passage of living phytoplankton cells through the gut of planktonic herbivores often appears to originate from animals feeding at an excessive rate on dense cultures (McMahon and Rigler, 1965). Floyd (1952) utilized radioactive phosphorus in plankton to demonstrate digestion, absorption, and assimilation into organic phosphorus containing compounds in tissues. A positive correlation between the number of cells passing through the intestinal tract and the amount of nutrient material assimilated by the cells was evidence that digestion and utilization occurred. Walne (1965) and Ukeles and Sweeney (1969) also utilized radioactive food cells as a means of studying food ingestion in larvae. This is an extremely useful method of studying nutritional problems through all stages of development, but care must be taken to provide adequate controls before valid conclusions may be drawn. An interesting nonradioactive method of

following food uptake was suggested by Ackman and Hingley (1968) based on the occurrence and prolonged retention of dimethyl-B-propiothetin (DMPT) by oysters. Since a substantial proportion (7 out of 10 classes) of phytoplankton available to filter feeders in temperate latitudes contains DMPT, evidence that DMPT levels correspond roughly with the intake of phytoplankton could provide useful data on phytoplankton availability and provide a supplement to the conventional techniques used in feeding studies.

Growth changes from the planktonic stage over a period of time until metamorphosis are also criteria of food utilization (Davis, 1953; Guillard, 1958; Davis and Guillard, 1958). A more rapid technique was used by Walne (1963), who considered the mean growth in a 24- to 48-hour assay on larvae as a reliable index of the comparative value of foods. In all these studies phytoplankton was fed in equal packed cell volumes to allow for differences in size of organism. It is important to test species in a range of concentrations since each food organism may result in better growth at one concentration than at another, and packed cell volumes have only a rough relationship to cell counts even in the same organism. It would perhaps be useful to employ other standards of comparison, such as carbohydrate reserves, pigment, or protein concentrations, in addition to packed cell volume and cell count determination. Another error inherent in the technique of adding algal suspensions to oyster cultures as a method of comparing food value is that the addition of a particular suspension includes components of the growth medium in several stages of utilization and metabolites produced by the cells. In addition to these variables, cells from different stages of the growth curve may have a varied size and structure and thus could influence utilization by larvae. A more ideal method of food comparisons would be to use algal cells from the same phase of the growth curve that were washed free of additives and metabolites. Although increases in size and time of metamorphosis are very good criteria on which to compare the relative value of different foods, the most rigorous method of assaying the usefulness of a particular food source is to determine the ability of an animal to survive normally and reproduce for many generations on an experimental diet under axenic conditions.

Korringa (1949) stated that the solution to the problem of what an oyster eats is really not difficult and can be understood by three types of investigations: (1) ascertain what an oyster eats, (2) study the process of digestion, and (3) put an oyster on an artificial diet. Numerous investigators have attempted to find the answers by using the first two procedures, with only minimum success. The third method is the only one that can yield the answer to the question, what does an oyster eat?

Animals have been defined as "organisms that are essentially phagotrophs, ingesting food in chunks" (Hutner and Provasoli, 1965). The essential questions in relation to the nutrition of filter feeders, such as oysters, are whether they are obligate or facultative phagotrophs; if they can utilize solutes, does this support growth? These questions were examined for *Artemia* in a series of papers that provide an excellent procedure for investigating similar nutritional questions in other filter feeders while suggesting some answers to nutritional problems in oysters (Provasoli and Shiraishi, 1959; Shiraishi and Provasoli, 1959; Provasoli and

D'Agostino, 1969). *Artemia* could be grown without living food but was an obligate phagotroph. The central problem in designing media was how to supply nutrients effectively. Whereas vitamins and amino acids were utilized as solutes, albumin and starch could not be replaced by water-soluble ingredients.

Progress in axenic culture was established as an important goal for critical studies in the invertebrates (Dougherty, 1959) but at the time this symposium was held there were no contributions from oyster biologists. In a consideration of the future direction of work in oyster nutrition it is apparent that significant observations can only be made with axenic cultures of oysters on an artificial diet.

Although this type of research is the one that can result in answers to basic problems of oyster nutrition, it does not hold any immediate promise for the rearing of large numbers of animals with the presently available techniques. Current information on oyster nutrition appears to indicate that the oyster is probably an obligate phagotroph but can fill some of its needs by solutes. Young larvae have more limited digestive capabilities and are more sensitive to toxic and adverse conditions in the food supply than older animals. According to this picture it is possible that at different stages of development some nutritional needs are filled by solutions, aggregates, detritus, or a variety of living things, including phytoplankton, bacteria, and zooplankton. However, the food supply that yields the most consistent results in feeding experiments, that is most reliable and amenable to control, duplication, and adaptation for large-scale development, is living phytoplankton.

For the present, the culture of phytoplankton foods must be carried out on a scale suitable to the artificial propagation of commercially valuable shellfish. Our methods of culturing micro-algae were developed so that they could be adapted for this purpose with the goal of providing a variety of foods, such as unialgal cultures of high densities, relatively free of bacterial or other contaminants. The following four types of culture systems are in operation to fulfill the dietary requirements of shellfish, both for research and hatchery programs: (1) small volumes of axenic cultures for maintenance of stocks, for starting larger cultures, and for use in critical larval physiology experiments; (2) cultures in 18-liter Pyrex carboys for larval feeding and growth experiments; (3) closed transparent polycarbonate (160-liter) tanks for large-scale larval rearing; (4) open fiberglass (1,000-liter) tanks for feeding of juveniles and adults. This variable culture scale allows for flexibility and continuity of cultures while ensuring adequate food production to meet different types of feeding requirements.

The success of food production in hatcheries is often dependent on an adequate supply of good stock cultures, to ensure continuance of the strain and consistent results in food production. Cultures are best maintained in small volumes of an enriched seawater medium and should be bacteria free if at all possible. Convenient culture vessels are 120 or 150 mm screw-capped test tubes filled with 10 ml of media or 125 ml screw-capped flasks filled with 60 ml of media. Cultures may also be maintained on solid media, such as seawater agar slants, and are particularly useful for the long-term storage of stocks. Some type of pasteurization or sterilization procedures should be used for all glassware and media. Where standard autoclaves are not available other processes should be instituted to give some measure of bacteriological control, e.g., pressure cookers, boiling, filtration, ultra-

violet or chemical treatment (the latter used with extreme caution). Proper areas for incubation, away from dust, under fluorescent lights, and relatively cool (20° ± 2°C), are needed. Subcultures are made about every six weeks. Fernbach flasks inoculated with cultures from 125-ml flasks, filled with about 1,200 ml of media, and fitted with cotton plugs holding siphons and aseptic filling devices need not be agitated or aerated. Fernbach flask cultures are useful for culturing foods needed in critical larval growth experiments and for inoculating five-gallon carboys.

Pyrex carboys are used for culturing foods either in batch or semicontinuous culture. The advantages of this size vessel are that moderately large volumes of several species can be made simultaneously available and that cultures may be discarded if they are not satisfactory foods while still maintaining adequate food supplies. For most purposes batch cultures in which algae are harvested at some useful density are adequate and simple to prepare and maintain. We have been experimenting for some time with semicontinuous cultures in which cultures are harvested as needed and the volume of culture removed is replaced with sterile media. For long-term maintenance these carboys are outfitted with four-hole "steril-cap"* rubber stoppers containing the following: a siphon (if there is no aperture at the bottom of the carboy for withdrawing liquids), a cotton-plugged air outlet, an inoculating port, and a glass tube reaching to the bottom of the carboy attached to a stone aerator. Carboys are autoclaved empty and, after cooling, four liters of autoclaved media and 1,200 ml of inoculum are added. Cultures are mixed by bubbling an air CO_2 mixture, and incubated in a cold water bath or cold room at 15–20°C, near a bank of fluorescent lights. Additional media are added over a period of several days as the culture increases in density until the capacity of the carboy is reached. Such carboys have been maintained in semicontinuous culture by harvesting two to four liters daily on an average of four months and as much as 18 months.

Where oyster rearing facilities are extensive, a much larger volume of food may be needed than could be provided by the multiplication of carboy cultures, unless facilities and maintenance help are extensive. To fill such requirements tank cultures that hold considerably larger volumes than carboys may be used. We are currently employing two types of tank culture—closed and open. The closed tanks are composed of a polycarbonate plastic "Lexan"† that is crystal clear and stable to autoclave sterilization. The tank is rectangular (35″ × 18″ × 16″) with a maximum capacity of 160 liters but filled with about 80 liters of culture. The cover has three openings (2-1/4″ × 2-1/2″); one is plugged with a cotton stopper, one is outfitted with a "steril-cap" as on the glass carboys and one covered with a media-filling bell.‡ Harvesting takes place through an opening in the bottom of the tank. The vessels are placed on a shelf in a cold room in front of a vertical bank of fluorescent lights. The tanks are inoculated with four–eight liters of a dense culture from the five-gallon carboy and filled with 20 liters of Millipore-filtered seawater and sterilized nutrient supplements.

Since very large amounts of food cultures are needed for maintaining adult and juvenile animals, the large-scale culture of phytoplankton in open tanks appears

*Product of Baltimore Biological Supply
†Product of Commercial Plastics and Supply Co.
‡Product of Belleo Glass, Inc.

to be the only practical method of filling this need. At one time a shallow wooden tank of only 3,000-gallon capacity in a greenhouse-type enclosure was used for this purpose. The tank was fertilized with a commercial garden fertilizer and filled with seawater. Once started, and harvesting 1/20th of the volume each day, cultures could be maintained for long periods of time without emptying and washing the tank (Loosanoff and Engle, 1942; Loosanoff, 1951). Although an open fertilized seawater tank supports a variety of phytoplankton species, this type of culture method is no longer compatible with the current status of shellfish-rearing techniques, except for certain uses, e.g., feeding of adult animals. The advantage of such a culture system is that in the absence of complex equipment large volumes of food are always available at a minimum expenditure of effort and funds. The disadvantage is that an increase in phytoplankton is often followed by unwanted protozoan and metazoan species, and under the best of conditions the dominant population cannot be controlled and may not be a useful food. For hatchery purposes a greater degree of control and a measure of predictability are needed in food production. In another type of open tank culture used more recently at Milford, an effort was made to gain some of the advantages of the tank method while minimizing the disadvantages. This was done by putting into operation an open tank culture that was under some control (Ukeles, 1965). The salient points of this culture system are as follows: (1) cultures be started with the dominant species desired, rather than depending on the natural phytoplankton bloom; (2) several tanks of moderate size, rather than a single very large tank, be used for culture containers and each with different species; (3) cultures be harvested after short periods of time as dense populations are reached, rather than be kept for long periods; (4) efforts be made to set up enrichment conditions for the particular species desired. Cultures were maintained outdoors during the summer months in 280-liter fiberglass tanks with clear plastic covers. To provide for some temperature control, each culture container was nested inside a larger fiberglass tank and the outside tank used as a water bath. Seawater pumped from the nearby harbor was continuously circulated in the outer tank, thereby serving to cool the cultures. Bubbles from stone aerators connected to a small air pump provided aeration and stirring. To avoid the immediate introduction of an algal population with the seawater, a medium was devised consisting of an enriched artificial sea salt and tap water. Each tank was inoculated with 10 liters of a dense carboy culture. A similar type of fiberglass tank of 1,000-liter capacity is now being used indoors with an artificial light source. Tanks are located in a 20° C culture room, and media prepared as in the outdoor tanks. Cultures of 80 liters from the closed polycarbonate tanks constitute inocula for open fiberglass tanks. The growth medium is added as the culture increases in density so that maximum culture capacity is reached in a few days.

Densities of cultures are quickly determined by cell counts or by packed cell volumes in a centrifuged sample. Specially modified Hopkins tubes are used in which readings of .001 ml packed cells per 10 ml of media can be made. Observations on the appearance of the supernatant of a centrifuged sample can often give information on the condition of a culture. A cloudy supernatant is an indication of a heavily bacterized culture and such cultures should be discarded. Preservation of harvested algal cultures until such times as particular foods are needed would be a valuable adjunct to the food production process. Such a procedure would allow food

production to become independent of larval rearing and ensure a uniformity of food supply. We have on occasion centrifuged large volumes of culture in a Sharples centrifuge, resuspended the sedimented cells in sterile seawater, and stored the suspension in the refrigerator for several weeks without difficulties being encountered. Lyophilized preparations of chlorophytes and chrysomonads were resuspended and used to rear *M. mercenaria* to metamorphosis. The growth of clam larvae was comparable to those receiving living foods, but dried preparations resulted in little or no growth of oyster larvae (Hidu and Ukeles, 1962).

The most acute problem in fulfilling the goals of mass culture was in devising methods of securing large volumes of seawater from which the microbial population was removed. Although elaborate devices are now in use in research and industrial plants for raising axenic microorganisms and even axenic metazoan species, their use does not appear to be indicated at the present time. Heat sterilization, ultraviolet, chemical, or ultrasonic treatment were possibilities that were explored but were not fruitful. Filtration appeared to be the most practical approach and after numerous trials on different types of filters (Davis and Ukeles, 1961) we are currently using a series of filters for cold sterilization of seawater, 15 μ, 1 μ, 0.45 μ, and 0.22 μ, the last of which is sterilized and replaced frequently. Growth of a cell population will depend to a large extent on the physical and chemical environment. Dilution rates become extremely critical in starting new cultures. A rapid dilution rate will usually result in lysis and death of the culture. The optimum temperature for growth will vary with species and to some extent is a complex factor that depends on other environmental conditions. Cultures should be maintained at the lowest temperature that is consistent with a good yield to avoid encouraging bacterial growth. A satisfactory temperature for most algal species is 15–20° C. Cultures may be incubated at this temperature in cold rooms, air-conditioned areas, and on water tables cooled by circulating cold water pipes. Where cooling devices are not available, it may be necessary to depend on high temperature strains for foods. Chlorophytes, foods that are useful to clam larvae and to juvenile clams and oysters, are generally more tolerant to higher temperatures than are the chrysomonad oyster larval foods. Agitation, as such, does not necessarily have a beneficial effect and in some experiments has been observed to retard growth. However, in cultures of large volumes, mixing serves as a mechanism to provide light intermittency, reduce sedimentation, and to transfer heat, gases, and dissolved materials. A satisfactory method to produce mixing is by passing a mixture of CO_2 in air through stone aerators, the gas bubbles providing the necessary mixing. Aeration is provided by an "oil-less" compressor and filtered through absorbent cotton. Excessive evaporation in cultures may be avoided by hydration of gas with distilled water before delivery to the culture vessels.

The CO_2 concentration required is a complex function of the pH, light intensity, culture density, and growth rate. In large volume dense cultures a CO_2 concentration of less than 2 percent is maintained. Discontinuing addition of CO_2 becomes necessary if the pH falls below pH 7.0–7.5. An increase in density will ensue if the culture is in good condition, but a low pH is often indicative of heavy bacterial contamination and cultures should then be discarded. The pH range of growth for most marine species is between pH 7.0 and pH 9.0 with the most opti-

mum range at pH 8.2–8.7. The light intensity received by cells in a culture is dependent on the degree of agitation, the density of a culture, and the position of a cell in the suspension. A newly inoculated culture may be light saturated initially and then become light limited. About 500 footcandles of light is adequate for various suspension densities. Numerous formulations for growth of marine algal species have been prepared to gain maximum growth of different species. Our formulations are prepared so that they will be adequate for all food species. Currently we are using an enriched seawater medium for cultures receiving autoclaved and filter-sterilized media and an enriched artificial seawater medium for all open tank cultures (Table 1).

Table 1.
Media for Mass Cultures

	Non sterilized	Filter sterilized	Heat sterilized
*Rila Marine Mix	15 gm.	—	—
KH_2PO_4	40 mg.	20 mg.	20 mg.
$NaNO_3$	232 mg.	310 mg.	310 mg.
Vitamin B_{12}	4 µg.	3 µg.	3 µg.
Thiamine.HCl	0.4 mg.	0.3 mg.	0.3 mg.
†NaFe EDTA	—	10 mg.	5 mg.
"TRIS"	500 mg.	250 mg.	1 gm.
$FeCl_3.6H_2O$	1.5 mg.	—	—
Na_2 EDTA	1 mg.	—	—
Trace metals	—	‡	‡
Sea H_2O	—	1000 ml.	500 ml.
Demineralized H_2O	1000 ml.	—	500 ml.

*Rila Products, Teaneck, New Jersey
†Geigy Industrial Chemicals, Ardsley, New York
‡Guillard and Ryther, 1962

There may be several sources for culture failure in carboys. Damp filters permit moisture to enter and so contaminate filters and cultures. Plastic tubing and worn rubber tubing often form unreliable seals. Many materials used in construction of culture vessels have factors that are potentially toxic, e.g., natural and synthetic rubbers, some flexible plastic formulations, and metal alloys (Dyer and Richardson, 1962). High quality control in food cultures may be maintained by frequent observations of cultures, both macroscopically and microscopically, as well as density and pH measurements. To ensure food reliability, 24- to 48-hour assays for toxicity should be run on developing eggs and on free-swimming larvae. Abnormality in development or a high percent mortality are indications of toxicity in food cultures.

The solution to the problem of mass culture of algal foods for the artificial propagation of commercially valuable species lies in good quality control through adequate maintenance, engineering, and sanitation.

LITERATURE CITED

ACKMAN, R. G. AND H. J. HINGLEY, 1968. The occurrence and retention of Dimethyl-β-Propiothetin in some filter-feeding organisms. *J. Fish. Res. Bd. Canada* 25, 267–284.

AMEMIYA, I. 1926. Notes on experiments on the early developmental stages of the Portuguese, American and English native oysters, with special reference to the effect of varying salinity. *J. Mar. Biol. Assn. U.K.* 14, 161–175.

BARDACH, J. E., 1968. Aquaculture. *Science* 161, 1098–1106.

BAYLOR, E. R. AND W. H. SUTCLIFFE JR., 1963. Dissolved organic matter in seawater as a source of particulate food. *Limnol. Oceanogr.* 8, 369–371.

BEVELANDER, G., 1952. Calcification in molluscs. III. Intake and deposition of Ca^{45} and P^{32} in relation to shell formation. *Biol. Bull.* 102: 9–15.

BLEGVAD, H., 1914. Food and conditions of nourishment among the communities of invertebrate animals found on or in the sea bottom in Danish waters. *Rept. Dan. Biol. Sta. to the Board of Agric.* 22, 43–78.

BROOKS, W. K., 1880. The development of the oyster. *Studies from the Biol. Lab. Johns Hopkins Univ.* 4, 1–106.

BRUCE, J. R., M. KNIGHT, AND M. W. PARKE, 1939. The rearing of oyster larvae on an algal diet. *J. Mar. Biol. Assn. U.K.* 24, 337–374.

CARRIKER, M. R., 1951. Ecological observations on the distribution of oyster larvae in New Jersey estuaries. *Ecol. Monogr.* 21, 19–38.

CARRIKER, M. R., 1956. Biology and propagation of young hard clams, *Mercenaria mercenaria*. *J. Elisha Mitchell Sci. Soc.* 72: 57–60.

CHESTNUT, A. F., 1946. Some observations on the feeding of oysters with especial reference to the tide. Address Nat. Shellfish. Assn., New York City Meeting, 22–26.

CHESTNUT, A. F., 1949. Studies on the digestive processes in *Ostrea virginica*. Doctoral thesis. Rutgers Univ.

CHURCHILL, E. P. AND S. I. LEWIS, 1924. Food and feeding in fresh-water mussels. *Bull. U.S. Bur. Fish.* 39, 437–471.

COLE, H. A., 1937. Experiments in the breeding of oysters (*Ostrea edulis*) in tanks, with special reference to the food of the larva and spat. *Fish. Invest., Min. Agric. Fish., London*, Ser. II, 15, 1–24.

COLE, H. A., 1938. A system of oyster culture. *J. Cons. Perma. Int. Explor. Mer.* 13, 221–235.

COLLIER, A., 1959. Some observations on the respiration of American oyster *Crassostrea virginica* (Gmelin). *Inst. Mar. Sci.* 6, 92–108.

COLLIER, A., S. RAY, AND A. W. MAGNITZKY, 1950. A preliminary note on naturally occurring organic substances in sea water affecting the feeding of oysters. *Science* 111, 151–152.

COLLIER, A., S. M. RAY, A. W. MAGNITZKY, AND J. O. BELL, 1953. Effect of dissolved organic substances on oysters. *U.S. Fish Wildlife Serv. Fish. Bull. 84*, 54, 167–185.

CURRIE, R. I., 1962. Pigments in zooplankton faeces. *Nature* 193, 956–957.

DAVIS, H. C., 1950. On food requirements of larvae of *Ostrea virginica*. *Anat. Rec.* 108, 132–133.

DAVIS, H. C., 1953. On food and feeding of larvae of the American oyster, *C. virginica*. *Biol. Bull.* 104, 334–350.

DAVIS, H. C. AND P. E. CHANLEY, 1956. Effects of some dissolved substances on bivalve larvae. *Proc. Nat. Shellfish. Assn.*, 46, 59–68.

DAVIS, H. C. AND R. R. GUILLARD, 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. *U.S. Fish Wildlife Serv. Fish. Bull. 136*, 58, 293–304.

DAVIS, H. C. AND R. UKELES, 1961. Mass culture of phytoplankton as foods for metazoans. *Science* 134, 562–564.

DEAN, B., 1887. The food of the oyster: its conditions and variations. *Second Rep. Oyster Invest.*, State of New York, Albany.

DEAN, D., 1958. New property of the crystalline style of *Crassostrea virginica*. *Science* 128, 837.

DODGESON, R. W., 1926. Multigraphed reports from fisheries experiment station, Castle Bank, Conway.

DOUGHERTY, E. C., 1959. Introduction to Axenic Culture of Invertebrate Metazoa: A Goal. *Ann. N.Y. Acad. Sci.* 77, 27–54.

DYER, D. AND D. RICHARDSON, 1962. Materials of construction in algal culture. *Appl. Microbiol.* 10, 129–131.

FENG, S. Y., 1965. Pinocytosis of proteins by oyster leucocytes. *Biol. Bull.* 129, 95–105.

FLOYD, D. J., 1952. Foods and feeding of oysters as observed with the use of radioactive plankton. Address Nat. Shellfish. Assn. 171–180.

FOX, D. L., 1950. Comparative metabolism of organic detritus by inshore animals. *Ecology* 31, 100–108.

FOX, D. L. AND W. R. COE, 1943. Biology of the California sea mussel *(Mytilus californianus)*. II. Nutrition, metabolism growth and calcium deposition. *J. Exp. Zool.* 93, 205–249.

GAARDER, T. AND R. SPÄRCK, 1932. Hydrographisch-biochemische untersuchungen in Norwegischen austern-pollen. Bergen Mus. Arbok. I. Naturv. Rekke No. 1: 1–144.

GALTSOFF, P. S., 1928. Experimental study of the function of the oyster gills and its bearing on the problems of oyster culture and sanitary control of the oyster industry. *Bull. U.S. Bur. Fish.* 44, 1–39.

GALTSOFF, P. S., 1942. Problems of productivity of oyster bottoms. Address Nat. Shellfish. Assn., Philadelphia, 7 p.

GALTSOFF, P. S., 1964. The American oyster *Crassostrea virginica* Gmelin. *U.S. Fish Wildlife Serv. Fish Bull.* 64, 480 p.

GALTSOFF, P. S. AND W. ARCISZ, 1954. Observations on the rate of propulsion of water and retention of coliform bacteria by the oyster. *Proc. Nat. Shellfish. Assn.* 44, 1–8.

GAVARD, D., 1927. De quoi se nourrissent les huîtres? Leur nourriture envisagée au point de vue "Ostréiculture". *Bull. Trav. Stat. Aquic. Pêche Castiglione Alger.* 2, 237–254.

GEORGE, W. C., 1952. The digestion and absorption of fat in lamellibranchs. *Biol. Bull.* 102, 118–127.

GIBOR, A., 1957. Conversion of phytoplankton to zooplankton. *Nature* 179, 1304–1305.

GILLESPIE, L., R. M. INGLE, AND W. K. HAVENS, 1964. Glucose nutrition and longevity in oysters. *Quart. J. Florida Acad. Sci.* 27, 279–288.

GRAVE, C., 1916. The process of feeding in the oyster. *Science* 44, 178–181.

GUILLARD, R. R., 1958. Some factors in the use of nannoplankton cultures as food for larval and juvenile bivalves. *Proc. Nat. Shellfish. Assn.* 48, 134–142.

GUILLARD, R. R., 1959. Further evidence of the destruction of bivalve larvae by bacteria. *Biol. Bull.* 117, 258–266.

GUILLARD, R. R. AND J. H. RYTHER, 1962. Studies on marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 8, 229–239.

HANDA, N. AND H. TOMINAGA, 1969. A detailed analysis of carbohydrates in marine particulate matter. *Mar. Biol.* 2, 228–235.

HAVEN, D. S., 1965. Supplemental feeding of oysters with starch. *Chesapeake Sci.* 6, 43–51.

HIDU, H. AND H. S. TUBIASH, 1963. A bacterial basis for the growth of antibiotic-treated bivalve larvae. *Proc. Nat. Shellfish. Assn.* 54, 25–39.

HIDU, H. AND R. UKELES, 1962. Dried unicellular algae as food for larvae of the hard shell clam, *Mercenaria mercenaria*. *Proc. Nat. Shellfish. Assn.* 53, 85–101.

HINARD, G., 1923. Les fonds ostréicoles de la Seudre et du Bélon. Notes et Mémoires No. 31. Office Scientifique et Technique des Pêches Maritimes, 1–27.

HOPKINS, A. E., 1936. Adaptation of the feeding mechanism of the oyster *(Ostrea gigas)* to changes in salinity. *Bull. U.S. Bur. Fish.* 48, 345–364.

HORST, R., 1883. A contribution to our knowledge of the development of the oyster. *(Ostrea edulis L.)* *Bull. U.S. Bur. Fish.* 2, 159–167.

HUNT, O., 1925. The food of the bottom fauna of the Plymouth fishing grounds. *J. Mar. Biol. Assn. U.K.* 13, 560–599.

HUTNER, S. H. AND L. PROVASOLI, 1965. Comparative physiology: Nutrition. *Ann. Rev. Physiol.* 27, 19–50.

IMAI, T. AND M. HATANAKA, 1949. On the artificial propagation of the Japanese common oyster, *Ostrea gigas* Thun., by non-colored naked flagellates. *Bull. Inst. of Agric. Res., Tohoku Univ.* 1, 33–46.

IMAI, T., M. HATANAKA, R. SATO, S. SAKAI, AND R. YUKI, 1950. Artificial breeding of oysters in tanks. *Tohoku J. of Agric. Res.* 1, 69–86.

JEFFREY, L. M., 1966. Lipids in sea water. *J. American Oil Chem. Soc.* 43, 211–214.

JØRGENSEN, C. B., 1955. Quantitative aspects of filter feeding in invertebrates. *Biol. Rev.* 30, 391–454.

KELLOGG, J. L., 1915. Ciliary mechanisms of lamellibranchs with descriptions of anatomy. *J. Morph.* 26, 625–701.

KORRINGA, P., 1949. More light upon the problem of the oyster's nutrition? *Bijdr. Dierk.* 28, 237–248.

KORRINGA, P., 1952. Recent advances in oyster biology. *Quart. Rev. Biol.* 27, 266–308.

KROGH, A., 1931. Dissolved substances as food of aquatic organisms. *Biol. Rev.* 6, 412–441.

LEWIS, G. J., JR. AND N. W. RAKESTRAW, 1955. Carbohydrate in sea water. *J. Mar. Res.* 14, 253–258.

LIPSCHÜTZ, A., 1913 Die Ernährung der Wassertiere durch die gelösten organischen Verbingunden der Gewässer (Eine Kritik) Ergebn. *Physiol.* 13, 1–46.

LOHMAN, H., 1909. Über die Quellen der Nahrung der Meerestiere und Pütter's Untersuchungen hierüber. *Int. Rev. Hydrob. und Hydrog.* 2, 10–30.

LOOSANOFF, V. L. AND J. B. ENGLE, 1942. Use of complete fertilizers in cultivation of microorganisms. *Science* 95, 487–488.

LOOSANOFF, V. L. AND J. B. ENGLE, 1947. Effect of different concentrations of micro-organisms on the feeding of oysters (*O. virginica*). *U.S. Fish Wildlife Serv. Fish. Bull.* 51, 31–57.

LOOSANOFF, V. L., 1949. On the food selectivity of oysters. *Science* 110, 122.

LOOSANOFF, V. L., 1951. Culturing phytoplankton on a large scale. *Ecology* 32, 748–750.

LOOSANOFF, V. L. AND R. R. MARAK, 1951. Culturing lamellibranch larvae. *Anat. Rec.* 111: 129–130.

LOOSANOFF, V. L., H. C. DAVIS, AND P. E. CHANLEY, 1955. Food requirements of some bivalve larvae. *Proc. Nat. Shellfish. Assn.* 45, 66–83.

LOOSANOFF, V. L. AND H. C. DAVIS, 1963. Rearing of bivalve mollusks. *In Advances in Marine Biology* 1, 1–136.

LOTSY, J. P., 1895. The food of the oyster, clam, and ribbed mussel. *Rept. U.S. Comm. Fish and Fisheries for 1893,* 19, 375–386.

MCMAHON, J. W. AND F. H. RIGLER, 1965. The feeding rate of *Daphnia magna* Straus in different foods labeled with radioactive phosphorous. *Limnol. Oceanogr.* 10, 105–113.

MACGINITIE, G. E., 1941. On the method of feeding of four pelecypods. *Biol. Bull.* 80, 18–25.

MANSOUR-BEK, J. J., 1948. On the proteolytic and lipolytic enzymes in the stomach juice of some Lamellibranchia. *Enzymologia* 12, 221–231.

MARTIN, G. W., 1923. Food of the oyster. *Bot. Gaz.* 75, 143–169.

MARTIN, G. W., 1928. Experimental feeding of oysters. *Ecology* 9, 49–55.

MILLAR, R. H., 1955. Notes on the mechanism of food movement in the gut of the larval oyster, *Ostrea edulis*. *Quart. J. Mic. Sci.* 96, 539–544.

MITCHELL, P. H., 1916. Nutrition of oysters: Glycogen formation and storage. *Bull. U.S. Bur. Fish.* 35, 151–161.

MITRA, S. B., 1901. The crystalline style of Lamellibranchia. *Quart. J. Micro. Sup. Serv.* 44, 591–602.

MOORE, H. F., 1908. Volumetric studies of the food and feeding of oysters. *Bull. U.S. Bur. Fish.* 28, 1297–1308.

NELSON, T. C., 1918. On the origin, nature and function of the crystalline style of lamellibranchs. *J. Morph.* 31, 53–111.

NELSON, T. C., 1923a. On the feeding habits of oysters. *Proc. Soc. Exptl. Biol. Med.* 21, 90–91.

NELSON, T. C., 1923b. The mechanism of feeding in the oyster. *Proc. Soc. Exptl. Biol. Med.* 21, 166–168.

NELSON, T. C., 1933. On the digestion of animal forms by the oyster. *Proc. Soc. Exptl. Biol. Med.* 30, 1287–1290.

NELSON, T. C., 1934. Studies on the food and feeding of oysters. 53rd, 54th Annual Reports of the New Jersey State Agricultural Experiment Station and the 45th, 46th Annual Reports of the New Jersey Agricultural College Experiment Station for the two year period ending June 30, 1933, p. 19–21.

NELSON, T. C., 1947. Some contributions from the land in determining conditions of life in the sea. *Ecol. Monogr.* 17, 337–346.

NEWELL, B. S., 1953. Cellulolytic activity in the lamellibranch crystalline style. *J. Mar. Biol. Assn. U.K.* 32, 491–495.

OWEN, G., 1966. Feeding. *In* Wilbur, K. and Yonge, C. M., eds. Physiology of Mollusca. Vol. II. Academic Press, Inc., New York, 1–47.

PARSONS, T. R., K. STEPHENS, AND J. D. H. STRICKLAND, 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Can.* 18, 1001–1016.

PETERSEN, C. G. J. AND P. B. JENSEN, 1911. Valuation of the sea. Animal life of the sea-bottom, its food and quantity. *Rep. Danish Biol. Sta.* 20, 1–81.

PHILPOTS, J. R., 1890. Oysters, and all about them. John Richardson and Co., London.

POMEROY, L., 1952. Radioisotope studies of the utilization of dissolved phosphorus and calcium by the American oyster. *Proc. Nat. Shellfish. Ass.* 167–170.

POMEROY, L. R. AND H. H. HASKIN, 1954. The uptake and utilization of phosphate ions from sea water by the American oyster, *Crassostrea virginica* (Gmel.). *Biol. Bull.* 107, 123–239.

PROCTOR, V. W., 1957. Studies of algal antibiosis using *Haematococcus* and *Chlamydomonas*. *Limnol. Oceanogr.* 2, 125–139.

PROVASOLI, L. AND K. SHIRAISHI, 1959. Axenic cultivation of the brine shrimp, *Artemia salina*. *Biol. Bull.* 117, 347–355.

PROVASOLI, L. AND A. D'AGOSTINO, 1969. Development of artificial media for *Artemia salina*. *Biol. Bull.* 136, 434–453.

PÜTTER, A., 1909. Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer. Jena, Fisher. 168 p.

ROSÉN, B., 1950. Proteases in the digestive gland of lamellibranchs. Arkiv fur Kemi. 1, 205–211.

RYTHER, J. H., 1954. Inhibiting effects of phytoplankton upon the feeding of *Daphnia magna* with reference to growth, reproduction, and survival. *Ecology* 35, 522–523.

RYTHER, J. H. AND J. E. BARDACH, 1968. The status and potential of aquaculture, particularly invertebrate and algae culture. Prepared for National Council on Marine Resources and Engineering Development, P. B. 177767 (Clearinghouse Fed. Sci. Tech. Info., Springfield, Va.).

SAVAGE, R. E., 1925. The food of the oyster. *Fish. Invest., Min. Agric. Fish., Lond., Ser. II*, 3, 1–50.

SAWANO, E., 1929. Studies on the digestive enzymes of *Ostrea circumpicta*. *Sci. Reports of the Tohoku University, Biology* 4, 327–334.

SHAW, B. L. AND H. I. BATTLE, 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). *Can J. Zool.* 35, 325–347.

SHILO, M. AND M. ASCHNER, 1953. Factors governing the toxicity of cultures containing the phytoflagellate, *Prymnesium parvum* Carter. *J. Gen. Microbiol.* 8, 333–343.

SHIRAISHI, K. AND L. PROVASOLI, 1959. Growth factors as supplements to inadequate algal foods for *Tigriopus japonicus*. *Tohoku J. Agric. Res.* 10, 89–96.

SPÄRCK, R., 1927. Studies on the biology of the oyster., II. The feeding and growth of the pelagic larvae. III. Some observations on the condition of the food supply. *Rep. Danish Biol. Sta.* 33, 46–59.

SPOEHR, H. A., 1953. The need for a new source of food. *In* J. S. Burlew (ed), *Algal culture from laboratory to pilot plant. Carnegie Inst. Wash. Publ. 600*, 24–28.

SPOEHR, H. A., J. SMITH, H. STRAIN, H. MILNER, AND G. HARDIN, 1949. Fatty acid antibacterials from plants. *Carnegie Inst. Wash. Publ. 586*, 67.

STAFFORD, J., 1913. The Canadian oyster. The Mortimer Co. Ltd. Ottawa, 159 p.

STEPHENS, G. C. AND R. A. SCHINSKE, 1961. Uptake of amino acids by marine invertebrates. *Limnol. Oceanogr.* 6, 175–181.

STONE, B. A. AND J. E. MORTON, 1958. The distribution of cellulases and related enzymes in Mollusca. *Proc. Malac. Soc.* 33, 127–141.

SUTCLIFFE, W. H. JR., E. R. BAYLOR, AND D. W. MENZEL, 1963. Sea surface chemistry and Langmuir circulation. *Deep-Sea Research* 10, 233–243.

TAKATSUKI, S., 1934. On the nature and functions of the amoebocytes of *Ostrea edulis*. *Quart. J. Micro. Sci.* 76, 379–431.

TUBIASH, H., P. CHANLEY, AND E. LEIFSON, 1965. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. *J. Bact.* 90, 1036–1043.

UKELES, R., 1965. A simple method for the mass culture of marine algae. *Limnol. Oceanogr.* 10, 492–495.

UKELES, R. AND B. M. SWEENEY, 1969. Influence of dinoflagellate trichocysts and other factors on the feeding of *Crassostrea virginica* larvae on *Monochrysis lutheri*. *Limnol. Oceanogr.* 14, 403–410.

VONK, H. J., 1924. Verdauungsphagocytose bei den Austern. *Zeit Vergl. Physiol.* 1, 607–623.

WALNE, P. R., 1956. Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory. *Fish. Invest., Min. Agric. Fish. Food, Lond.*, Ser. II. 20, 1–23.

WALNE, P. R., 1963. Observations on the food value of seven species of algae to the larvae of *Ostrea edulis*. I. Feeding experiments. *J. Mar. Biol. Assn. U.K.* 43, 767–784.

WALNE, P. R., 1965. Observations on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis* L. *Fish. Invest., Min. Agric. Fish. Food, Lond.*, Ser. II. 24, 1–45.

WITSCH, H. V., 1960. Biological possibilities of mass culture of algae for food production. *In* P. Kachroo (ed), *Proc. Symp.* Algology. Indian Council Agric. Res. and UNESCO South Asia Coop. Office, New Delhi, 147–155.

WOOD, L., 1965. Determination of free amino acids in seawater. Contribution No. 201 from the Virginia Institute of Marine Science, 652–655.

YONGE, C. M., 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. *J. Mar. Biol. Assn. U.K.* 14, 295–386.

YONGE, C. M., 1928. The absorption of glucose by *Ostrea edulis*. *J. Mar. Biol. Assn. U.K.* 15, 643–653.

YONGE, C. M., 1938. Recent work on the digestion of cellulose and chitin by invertebrates. *Sci. Progr.* 32, 638–647.

YONGE, C. M., 1960. Oysters. Collins, London, 209 p.

ZOBELL, C. E. AND C. G. FELTHAM, 1938. Bacteria as food for certain marine invertebrates. *J. Mar. Res.* 312–327.

Introduction to SHELLFISH DISEASES IN HATCHING OPERATIONS

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Intensive cultivation of plants or animals often leads to increased disease and mortality. Increased population densities, often maintained under marginal conditions, magnify effects of stresses that might be unnoticed or minimized in natural populations. As shellfish cultivation progresses consideration of causes and effects of disease will become increasingly important. What now seem to be abstract considerations and minor or theoretical problems may assume major proportions and demand practical solutions before shellfish cultivation can be carried on routinely.

An obvious and painful example of this problem is near at hand. Massive oyster mortalities in Delaware Bay due to disease associated with MSX (*Minchinia nelsoni*) are still fresh memories for many of us. In this case high-density transplanted oyster populations were hosts for a virulent infectious agent and resulted in extreme levels of infectivity and mortality. The events that triggered this epidemic are still not known precisely, but some general lessons were learned:

1. Infection of natural populations is common, but disease is uncommon. Under natural conditions an infectious agent may be present in many members of a population but only occasionally are hosts killed.
2. In artificially dense populations potential host organisms probably are exposed more frequently to infection. They may also be stressed by crowding, and it is possible that environmental stresses (e.g., temperature, salinity, pollution, etc.), acting singly or in concert, may initiate or magnify the disease problem. The relative importance of these notions has not been evaluated in any detail thus far but answers are badly needed if we are to initiate rational shellfish breeding programs.
3. Epidemics are self-limiting, but the reasons for this are obscure—except, of course, when all the potential hosts have been killed. Hopefully there is a genetic component that can be manipulated so that truly resistant oysters can be bred artificially. This has not been demonstrated unequivocally as yet, however, and the whole genetics problem needs intensive study.

The MSX disaster may have been a well-disguised blessing. It dramatized a problem that may become very common in the future and it caused many people to

examine basic biological problems. It also made abundantly clear the fact that we know very little about mechanisms of disease in shellfish. It is ironic, and perhaps prophetic, that the first massive MSX mortalities were detected in Delaware Bay where Dr. Stauber had done the earliest studies on oyster defense mechanisms and where his students were continuing those studies. Dr. Haskin had his attention forcefully drawn to the problem and quickly interest spread from the Rutgers group to many other East Coast laboratories. It was soon evident that this was a general problem and so, to enhance information exchange, the first annual Oyster Mortality Conference was called. Initially these conferences dealt with the MSX problem exclusively, but soon the programs included more peripheral research reports of basic biologic phenomena. In my laboratory, for example, since 1961 we have attempted to culture oyster cells *in vitro* and we have examined the composition of oyster and other molluscan bloods in considerable detail. We have tried to elucidate factors affecting phagocytosis by oyster cells and intracellular events that follow phagocytosis. In short we have tried to analyze the defense mechanisms of mollusks at the cellular level. We and many others have added to a growing, but still woefully incomplete, body of fundamental knowledge that will be useful in the future. We are now turning our attention to aspects of noninfectious disease in mollusks, namely the effects of pesticides on oysters, particularly the effects of chronic exposure to pesticides and their possible effects on oyster reproduction.

OYSTER DISEASES IN NORTH AMERICA AND SOME METHODS FOR THEIR CONTROL*

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INTRODUCTION

In much of the U.S. today, the abundance and exploitation of wild stocks or populations of oysters as well as the harvesting practices imposed by tradition apparently preclude extensive use of refined aquaculture techniques. In those areas where wild stocks are not abundantly available and where the use and cost of aquaculture operations could be competitive with harvesting and marketing of wild stocks, the application of aquaculture methods holds great promise.

With increasing use of the nation's coastal zones for industry, residential development, and recreation, less area remains available for shellfish aquaculture. More efficient means to increase U.S. production must be found, and currently there is vigorous activity to find new or modified methods to increase reproduction, "seed" capture, and survival of larvae, juveniles and adults. Hatchery and closed system methods of aquaculture are becoming increasingly popular and more efficient. However, the use of natural or open environments for the capture, growth and development of oysters and other shellfish species accounts by far for the greatest oyster production today and probably will continue to do so for years to come.

COMMON SPECIES OF OYSTERS IN NORTH AMERICA

Because much of the oyster industry in the U.S. today still concerns itself with traditional practices of oyster propagation and harvesting, some background information on the species and management practices involved should be provided.

*This report is not intended to represent a highly technical or comprehensive review of oyster diseases or methods for their control. Its purpose is to familiarize university, state and federal management officers, scientists and the general public with disease problems associated with shellfish production and management.

The fact that citations are omitted is not intended as a slight to the many, many competent and outstanding individuals who have contributed so greatly to the study of oyster diseases and various aspects of shellfish management. A selected bibliography is provided for those interested in further pursuit of pertinent literature.

Only four of the many species of North American oysters are of real or potential commercial value:

Crassostrea virginica (American oyster, Eastern oyster, Virginia oyster) is indigenous to the east and Gulf coasts of the U.S. and the east coast of Canada. These oysters are moved extensively from location to location during various stages of their lives and during various seasons of the year. In some instances, transfers of young oysters (spat, yearlings, juveniles) are made to private or public beds where they grow to market-size adults and are harvested. In other instances, transfers of adults are made to certain locations for brief periods prior to harvest until they acquire particular qualities (for example, saltiness, fatness) that increase their value. This species is generally harvested from September to April. Attempts have been made to introduce the Eastern oysters onto the west coast of the U.S. without much success, but some success has been achieved in Hawaii where they readily reproduce and grow.

The Japanese or Pacific oyster *(Crassostrea gigas)* is usually imported from Japan as young "seed" and planted on vast beds on the U.S. and Canadian Pacific coasts. There they grow to market size and are harvested. Off-bottom culture of this species is widely practiced in Japan and to only a slight extent on the west coast of North America. Since these oysters are not native to the area in which they are grown, spawning and setting can best be described as erratic or unpredictable. As a consequence the West Coast industry is still largely dependent on foreign sources of seed. However, greater effort is being made to develop and improve methods for efficient seed capture in the few areas of British Columbia and Washington where spawning has been observed.

Ostrea lurida (Olympia oyster, native oyster) is a diminutive animal that rarely grows larger than 2½ inches. It is native to the west coast of the U.S. and Canada but the growing areas formerly devoted to the production of this species are largely being replaced by the faster-growing, higher-yielding Japanese oyster. Local but significant markets for this species still exist. As far as we are aware, no extensive efforts have been made to introduce this species into areas where they are not already indigenous. Its life history is very similar to the European oyster.

Ostrea edulis (Dutch oyster, European oyster or the flat oyster) is the oyster of commerce in most of northern Europe. It requires relatively cold, salty, clear water for growth and reproduction. Like *O. lurida*, the females retain their eggs and early larval stages within the mantle cavity until the motile, shell-bearing larvae are released to planktonic life and eventual setting. Female oysters bearing gonads packed with whitish or cream-colored larvae are sometimes erroneously said to be suffering from the "white sickness." After further development of the eye spot, the larvae take on a grayish coloration and the oyster is then said to have the "gray sickness." With increased shell formation and further development the larvae take on a more blackish appearance and the oysters are now said to have the "black sickness." The conditions described, of course, are not sicknesses in the true sense; however, aesthetically, the oysters are not appealing. Since these spawning conditions are found during the months lacking "r," we in the U.S. have been burdened with a tradition that has been transferred to the American oyster *(C. virginica)*. Fertilization and larval development of the Eastern oyster (and the Japanese oyster)

is completely external; yet, we are still required to follow the dictates of the expression, "Oysters 'R' in Season." It is a fact that American oysters are seldom harvested and are more difficult to sell in months that have no "r." The U.S. industry could probably produce a better product if regulations permitted the harvesting of oysters into May–June, with the fall harvest delayed until October–November when oysters are in better condition after spawning.

In the early 1950's, largely through the efforts of Dr. Victor Loosanoff, the European oyster was introduced into Boothbay Harbor, Maine, where small, isolated, but self-sustaining populations exist. No concerted efforts have been made to increase the production or productivity of this species in Maine, although in Canada more serious efforts are currently being made. The species has great potential as a food resource in these cold waters.

A BRIEF REVIEW OF DISEASES AND PARASITES OF OYSTERS

Metabolic Diseases

Oyster mortalities have occurred from time to time for which no rational explanation could be given. Even after exhaustive examination of appropriately collected and processed tissue materials and comparisons of environmental parameters in mortality and nonmortality areas, investigators could find no organisms that could be attributed as the causative agent of death. Such mortalities have occurred in Matsushima Bay, Japan, and more recently in the State of Washington. Both sexes were affected, usually in their second year of growth, during the period when spawning would normally take place. These oysters appeared to be in excellent condition at the time of death and gonads were ripe. Since these mortalities seem to be associated with the spawning cycle, it is speculated that abnormal metabolism or perhaps hormonal effects may play an important role. Toxins or toxic metabolic by-products resulting from digestion of food organisms cannot be ruled out.

Virus Diseases

No direct evidence exists to implicate viruses in mortalities or diseases of oysters. However, in two epizootics viruses are suspected as the disease agents.

Malpeque Bay Disease—an epizootic that virtually destroyed the oyster industry, occurred in Malpeque Bay, Prince Edward Island, about 1915 and over a period of years spread to other areas within the Canadian Maritime Provinces. The cause of this disease remains unknown although many organisms have been suggested as possibilities. Histopathological studies suggest the infectious etiological agent may be a virus. The oyster industry in the affected areas reached its former level of production after several years, and it is hypothesized that the current populations are resistant strains that have been developed from survivors. Evidence that the infectious agent is still present is suggested by the fact that nonindigenous oysters are susceptible to the agent and die within the first or second year after introduction.

Neoplasm-like conditions from both coasts have recently been observed in shellfish—oysters, mussels, and possibly clams. These abnormalities have been quite rare in the Eastern oyster and can be of various types. Although it is possible that these conditions may be caused by unrecognized microparasites or bacteria, it is not inconceivable that a virus or chemical agent is responsible. We know absolutely nothing about the etiology or possible transmission of these disease conditions within species, across species lines, or to higher taxa.

Bacterial Diseases

Second to the nutritional requirements of the oyster larvae, the largest problem for successful closed hatchery systems is the control of bacterial growth, while permitting larval development. Experimental evidence is available to indicate that heavy burdens of bacteria in larval cultures can cause mortalities which reduce survival, setting, and development. More recent evidence shows distinct differences between bacterial species found in the environment from an enzootic area (Pocomoke Sound, Maryland) and a disease-free area (Eastern Bay, Maryland). It is hypothesized that, since setting is poor in Pocomoke Sound and rather substantial in Eastern Bay, bacteria may play a role in survival of larval shellfish in the natural environment.

A bacterial disease has been found in oysters from a mortality area in Japan (Matsushima Bay). The same disease has also been observed in Japanese oysters in the Naselle River, a tributary of Willipa Bay, Washington. Oyster beds in this river receive shipments from the infected area in Japan and oyster mortalities have been reported from the Naselle River. The infections, which occur as pockets of bacteria-filled abscesses, have been seen by both Japanese and American investigators. In the U.S. the infection is called "focal necrosis." Several attempts to isolate the organisms have been unsuccessful. Although a number of suspicious bacteria have been isolated, their pathogenicity has not been confirmed.

Fungal Diseases

Dermocystidium marinum. A large body of literature has resulted from studies on this organism. Its name was recently changed to *Labyrynthomyxa marina* but the colloquial name "Dermo" still persists. The organism has been implicated experimentally and under natural conditions as a pathogen causing heavy mortalities, and its presence can be diagnosed by a culture technique. It is probably not a single species but a complex of related species. First described from oysters from the Gulf of Mexico, it has also been found in oysters and many other species of bivalve mollusks along the Atlantic coast, including Chesapeake and Delaware Bays. A species of *Dermocystidium* has been isolated from oysters from Long Island Sound, and a few years ago a positive culture test for a similar organism was recorded by our laboratory in oysters from the Far East.

Mortalities caused by *Dermocystidium* occur in some areas of the Gulf throughout the year. In more northerly latitudes outbreaks occur primarily in the summer if oysters are crowded and when water temperature and salinity are relatively high.

Mycelial Disease. A disease associated with oyster mortalities in the Gulf of Mexico and in aquaria of Gulf-held oysters has been referred to as mycelial disease. The disease has also been observed in oysters from Chesapeake Bay. More recently mycelial growths have been observed in Portuguese oysters (*C. angulata*) sent to the BCF Oxford Laboratory from France. These oysters came from beds where mortalities were occurring and they were presumably suffering from the so-called "gill" disease. It was believed that the disease was reintroduced into France with shipments of *C. gigas* from Japan. However, close examinations of oyster samples from the presumed disease points of origin in Japan failed to reveal the presence of any disease entities resembling mycelial disease or the gill disease. Interestingly, French scientists were dispatched to Japan to examine future shipments of oysters for disease. Apparently the French oyster industry is willing to risk the introduction of exotic species into France, and it is reported that they imported several hundred tons of Japanese seed oysters during 1969.

Other Possible Fungal Diseases

Microcell Disease: Other oyster diseases possibly of fungal etiology have been reported. A disease of Japanese oysters in the Denman Island area of British Columbia has been observed. Although fungi have not been definitively implicated, an organism having stages resembling slime molds has been isolated from oysters from the disease area. Some investigators have speculated that the disease may be caused by a virus. The disease has also been called "microcell disease" and has been observed in hatchery-grown oysters (*O. edulis*) in Connecticut and in European oysters planted in California.

Shell Disease: Mass mortalities of oysters occurred in Europe beginning in 1930. The disease was characterized by formation of pustules on the inner shell surfaces. Thin parts of the oyster shells were perforated by a fungus which proliferated in the tissues after reaching the inner surfaces of the shell. Infections were common on beds where old shells were abundant. Activity of the fungus was correlated with temperature and the outbreak was said to be intensified by widespread use of cockle shells as spat collectors.

Other diseases attributed to fungi are "foot disease" ("maladie du pied"), noted by the French in European oysters, and a disease of hatchery-reared clam and oyster larvae attributed to a species of *Sirolpidium*.

Protozoan Diseases

The most devastating oyster epizootic reported in the U.S. was caused by a haplosporidan, *Minchinia nelsoni*, formerly called MSX (Multinucleate Sphere Unknown). Although oyster mortalities observed from time to time along the northeast coast of the U.S. may have been caused by haplosporidan infections, none were as severe as those that occurred in Delaware Bay beginning in 1957, and in lower Chesapeake Bay beginning in 1960. Much has now been written about the epizootiology, pathology, life history, and distribution of this parasite in oysters from the East Coast. Despite numerous attempts by many competent investigators, true

laboratory transmission or transplantation of the disease from one oyster to another or even to other animals has not been demonstrated. Attempts to grow the organism *in vitro* have also failed. It is, of course, possible that some stages in the life history of the parasite require development in an intermediate host before the organism can become infective.

Apparently infectious stages are water-borne, since infections occur initially in the gills, then spread to adjacent tissues. Ultimately, all tissues except muscle are affected. Sporulation occurs infrequently and appears to take place only in the lobes of the digestive diverticulae. Salinity apparently plays some role in the ecology of *M. nelsoni*. High prevalences of the disease in oysters and concomitant mortalities are more often observed in waters consistently above 15 ‰. This again suggests that an ecologically restricted alternate host or carrier may be involved. Mortalities caused by the parasite can occur through the year, but peaks of mortality occur during the summer.

Another haplosporidan parasite, *Minchinia costalis* (formerly *Haplosporidium costale*) is held responsible for extensive oyster mortalities in seaside bays of the Delmarva Peninsula with waters of close-to-oceanic salinities. Vegetative or plasmodial stages of this organism have also been observed in oysters from Long Island Sound. It has a similar life history to *M. nelsoni* with which it was at first confused. However, sporulation is more regular. Spores are more often observed in infected oysters and sporulation apparently takes place in most all of the tissues. The disease is colloquially called SSO (Sea Side Organism) in the older literature.

Other Protozoan Diseases and Parasites

Several other protozoan organisms have been observed in oysters. Most of these organisms induce a host response, but normally cause relatively minor damage to the host. However, it is entirely possible that under conditions of stress these parasites may act as facultative or adventitious disease agents.

Species of the flagellate *Hexamita* are ubiquitous and have been observed in several species of oysters from Europe, North America and Asia. Trophozoite stages are observed particularly in oyster samples collected and examined during the winter months.

Ancistrocoma sp. and several other ciliates have also been observed in oysters from several locations and a great deal of confusion still exists regarding their taxonomy and pathogenicity for oysters and other species of bivalve mollusks.

Amoebae isolated from Eastern oysters have been described. Recent *in vitro* culture experiments have resulted in the isolation of several other protistan organisms having amoeba-like stages. Much confusion also remains about the taxonomy and pathogenicity of these organisms, particularly the question, are they true amoebae or are the amoeboid organisms merely life cycle stages of another taxon?

While searching for alternate seed sources of Japanese oysters for the West Coast industry, oysters from several areas in the Far East were examined for the presence of pathological conditions and disease entities. A presumed coccidian and a myxosporidan were observed in tissue samples of oysters from Taiwan but the

taxonomy and pathogenicity of these organisms is presently unclear. Spores of the gregarine *Nematopsis* were also observed in these oysters. However, it is not certain if the species involved is the same as those commonly found in oysters from the Atlantic coast of the U.S. Apparently, *Nematopsis* is not responsible for oyster mortalities.

Metazoan Diseases and Parasites

Several metazoan parasites have been observed in many species of oysters and shellfish from many locations. Most of these organisms are larval stages of helminths merely occupying oysters and other bivalve mollusks as intermediate hosts. Larval trematodes of the genus *Bucephalus* have been found in American, European and Japanese oysters. They apparently do not cause extensive mortalities. However, since they are primarily parasites of gonadal tissues they are responsible for functional castration of the oysters. In heavily infested populations the reproductive potential may be reduced. Larval cestodes of the genus *Tylocephalum* have been reported in oysters from the warmer waters of the U.S. east coast, Gulf coast, Hawaii, and more recently in Pacific oysters from Japan and Taiwan.

Common crustacean parasites of oysters are the copepod, *Mytilicola*, and the pea crab, *Pinnotheres*.

DISEASE PREVENTION AND CONTROL MEASURES

The application of fishery management techniques to shellfish, particularly to shellfish in unfavorable environments, could serve to minimize unfavorable ecological factors, thereby reducing mortality and strengthening the economy of industries and communities dependent upon the shellfish resources. Animal husbandry and wildlife management both control population as a means of improving food supplies and protecting certain wildlife resources. However, disease often is the single most important factor contributing to declines in oyster resources. With increasing application of shellfish aquaculture methods and hatchery techniques where shellfish populations reach a maximum density, and the transfer of susceptible stocks from one location to another becomes a routine procedure, consideration of the effects of disease on survival and ultimate production is essential.

Although disease is ever-present in open aquatic environments, new factors have been introduced by man to stress the animals that comprise shellfish resources and their habitats. Oysters are often densely crowded together; profound physical and chemical changes have been imposed on the environment and predators and competitive species of plants and animals have been introduced. These stress conditions could enhance the importance of disease as the cause of mortality. Disease must be controlled if economically successful production of cultivated shellfish is to be achieved.

Before any meaningful and reliable disease control measures can be taken, it is essential that basic biological and ecological information on the disease agent and the host be accumulated. Knowledge of vulnerable life cycle stages, types of restrictive environmental conditions, and conditions of stress would be useful in designing

disease control measures. Multidisciplinary approaches and a wide array of research effort would, of course, be necessary. Such effort would include epizootiology and pathology studies; cytochemical, biochemical and immunological studies of the host and pathogen; culture studies of disease agents, associated organisms and host tissues; selective breeding studies and genetic studies of the host and infectious agents.

The transfer of susceptible animals to or from epizootic or enzootic areas should be strictly forbidden. The promiscuous, often indiscriminate transfer of oysters from one location into another has caused serious oyster mortalities in indigenous and introduced stocks. The possible consequences of any transfer should be carefully considered before introductions are made. The introduction of susceptible animals into enzootic areas will almost guarantee renewed outbreaks in the introduced stocks. From a genetic standpoint it is always possible that the introduced animals will "dilute the gene pool" of indigenous resistant animals and increase susceptibility of new generations.

Resistant animals from mortality areas, when introduced into new environments, may carry exotic parasites, diseases and predators that could affect resident populations of the same or different species.

The repeated introduction of animals carrying disease agents is probably the best way to establish a disease entity in a new environment. A nonvirulent microparasite might undergo several life cycles or generations, then mutate into a more virulent form in its new environment.

Restrictive environments and modified planting and harvesting schedules can be used to good advantage. In some cases an intimate knowledge of the biology and environmental requirements of a pathogen has successfully permitted continued oyster production. In *M. nelsoni* epizootic areas of Chesapeake Bay, plantings have been restricted to low salinity areas, since the parasite is apparently noninfective in salinities consistently below 15 ‰. In the future, it may be possible to avoid or cure infections through knowledge of the infective periods and favorable environmental conditions. For example, if a disease occurs only during spring through fall, infections could be prevented by moving the hosts to restrictive areas, and returning them when the infective period has passed. Similarly, infected animals could be moved upriver to effect a possible "cure."

The damaging effects of *Dermocystidium* disease have been mitigated by planting the beds thinly, harvesting within two years, and altering planting and harvesting schedules to take advantage of decreased pathogen activity during the colder months.

Production of shellfish in artificial and natural environments where diseases can be controlled should be encouraged. Bacterial and fungal epizootics among larvae can be reduced under hatchery conditions by ultraviolet treatment of uncontaminated filtered seawater, antibiotics, and general sanitation of utensils, vessels and tanks. Production of shellfish in artificial ponds holds great promise. The use of disease-free and disease-resistant brood stocks, filtration and ultraviolet treatment of recirculated, possibly even natural waters, would reduce or eliminate predators and alternate or intermediate hosts of disease agents. Practical methods for producing mass quantities of pure phytoplankton foods are still lacking (see R. Ukeles, this volume).

Natural ponds, embayments and lagoons may also be used for shellfish production particularly if multiple use is not a consideration. In suitable locations bottoms can be cleaned or treated with agents that would inhibit entry or survival of predators and alternate, intermediate and reservoir hosts.

Disease resistant stocks should be developed through selective breeding of survivors. It is possible empirically to develop resistant strains of oysters by breeding survivors of mortalities, either by design or by natural selection, as occurred in Prince Edward Island (Malpeque Bay disease) and, to some extent, in parts of Chesapeake Bay (*M. nelsoni* infection). Concentrations of survivors on well-managed natural beds could do much to improve recruitment and return to full production.

The development of disease-resistant shellfish populations is probably the most difficult and time-consuming of the control methods suggested. In Prince Edward Island where disease resistance through natural selection has been achieved, an interval of approximately 10–20 years was required to rehabilitate the population. Meanwhile, industry suffered dramatically. However, resistant stocks were drawn upon to repopulate relatively quickly other oyster growing areas of the Gulf of St. Lawrence that had been decimated by the same disease. Apparently, the same pattern of resistance is beginning to emerge in parts of Chesapeake and Delaware bays, where oysters have taken several years to show initial resistance.

Experimental laboratory and field attempts to develop resistance are also a slow and difficult process. Resistant strains against *Dermocystidium* disease have not been developed. Efforts to develop resistance against the haplosporidans, *Minchinia nelsoni* and *M. costalis* are being attempted. However, efforts are handicapped by: 1) lack of knowledge of oyster genetics and the mechanisms involved in sex changes, 2) inability to effect consistently successful natural or experimental transmission of disease, 3) inability to recognize resistant animals and progeny early enough for use as parental breeding stocks, 4) the long generation time of the oyster, and 5) expensive facilities and equipment necessary to maintain large numbers of oysters as parental stocks.

Chemical and mechanical control measures can be used with some success. In northern Europe shell disease in oysters is caused by a fungus that perforates the shell. The fungus thrives in old shells and is particularly abundant in cockle shells used as cultch. The disease declined when the cultch was spread in disease-free areas, old shells were cleared from the beds, and infected young oysters were dipped in mercuric chloride.

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LITERATURE CITED

ANDREWS, J. D., 1966. Oyster mortality studies in Virginia. V. Epizootiology of MSX, a protistan pathogen of oysters. *Ecology* 47: 19–31.

CHENG, T. C., 1967. Marine molluscs as hosts for symbioses, with a review of known parasites of commercially important species. *Advan. Mar. Biol.* 5: 1–424.

COUCH, J. A., C. A. FARLEY and A. ROSENFIELD, 1966. Sporulation of *Minchinia nelsoni* (Haplosporida, Haplosporidiidae) in *Crassostrea virginica* (Gmelin). *Science* 153: 1529–1531.

DAWE, C. J. and J. C. HARSHBARGER, editors, 1969. Neoplasms and related disorders of invertebrate and lower vertebrate animals. *Nat. Cancer Inst. Monogr.* 31. GPO, Washington, D.C. 772 pp.

ENGLE, J. B. and A. ROSENFIELD, 1963. Progress in oyster mortality studies. *Proc. Gulf Carib. Fish. Inst. 15th Annu. Sess.*: 116–124.

FARLEY, C. A., 1968. *Minchinia nelsoni* (Haplosporida) disease syndrome in the American oyster *Crassostrea virginica*. *J. Protozool.* 15: 585–599.

GALTSOFF, P. S., 1964. The American oyster *Crassostrea virginica* Gmelin. *U.S. Fish Wildl. Serv., Fish. Bull.* 64: 1–480.

HASKIN, H. H., L. A. STAUBER and J. G. MACKIN, 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): causative agent of the Delaware Bay oyster epizootic. *Science* 153: 1414–1416.

IMAI, T., K. NUMACHI, J. OIZUMI and S. SATO, 1965. Studies on the mass mortality of the oyster in Matsushima Bay. II. Search for the cause of mass mortality and the possibility to prevent it by transplantation experiment. *Bull. Tohoku Reg. Fish. Res. Lab.* 25: 27–38. (In Japanese with English summary)

KATKANSKY, S. C., W. A. DAHLSTROM and R. W. WARNER, 1969. Observations on survival and growth of the European flat oyster, *Ostrea edulis*, in California. *Calif. Fish Game* 55: 60–74.

KORRINGA, P., 1952. Recent advances in oyster biology. *Quart Rev. Biol.* 27: 266–308; 339–365.

LAIRD, M., 1961. Microecological factors in oyster epizootics. *Can. J. Zool.* 39: 449–485.

LOGIE, R. R., 1956. Oyster mortalities, old and new, in the Maritimes. *Fish. Res. Bd. Can., Progr. Rep. Atl. Coast Sta.* 65: 3–11.

LOOSANOFF, V. L. and H. C. DAVIS, 1963. Rearing of bivalve mollusks. *Advan. Mar. Biol.* 1: 1–136.

MACKIN, J. G., 1961. Status of researches on oyster diseases in North America. *Proc. Gulf Carib. Fish. Inst. 13th Annu. Sess.*: 98–109.

MACKIN, J. G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publ. Inst. Mar. Sci. Univ. Tex.* 7: 132–229.

MORI, K., H. TAMATE., T. IMAI and O. ITIKAWA, 1965. Studies on the mass mortality of the oyster in Matsushima Bay. V. Changes in the metabolism of lipids and glycogen of the oyster during the stages of sexual maturation and spawning. *Bull. Tohoku Reg. Fish. Res. Lab.* 25: 65–88. (In Japanese with English summary)

NEEDLER, A. W. H. and R. R. LOGIE, 1947. Serious mortalities in Prince Edward Island oysters caused by a contagious disease. *Trans. Roy. Soc. Can., Ser. 3, Sect. 5*, 41: 73–89.

SINDERMANN, C. J. and A. ROSENFIELD, 1967. Principal diseases of commercially important marine bivalve Mollusca and Crustacea. *U.S. Fish Wildl. Serv., Fish. Bull.* 66: 335–385.

TUBIASH, H. S., P. E. CHANLEY and E. LEIFSON, 1965. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. *J. Bacteriol.* 90: 1036–1044.

YONGE, C. M., 1960. Oysters. Collins, London. 209 pp.

DISCUSSION

KINCHELOE: Are you familiar with Senate Bill # 1151 that is soon to have hearings?

ROSENFIELD: Yes, I think it was brought up because of the spread of certain diseases of fresh water fishes in the United States. It came about after the introduction of an amendment to consolidate Federal Regulations Title 50 for international importation of certain fish diseases. Shellfish are included in the bill. Pertinent to this I believe there have been two introductions of the European oyster to the United States.

LOOSANOFF: I have several comments to offer. The first is that there was only one introduction of European oysters to Maine waters. This was done in 1949 in Boothbay Harbor and sampling was subsequently taken over by Mr. Davis. The new population of the oyster now extends in a line about 520 miles along the Coast. There was no second introduction.

My next comment refers to the MSX-related mortality of oysters discovered in 1957 by Rutgers University. I think the discovery was made in 1952 in Long Island by Dick Nelson, brother of the late Professor Nelson. Dick Nelson asked us to accompany him and dredge for oysters. We spent a day and found dead almost a half a million bushels of large oysters. I called Washington and requested technical help from them. Washington contacted Professor Nelson who expressed the opinion that the oysters were killed by crabs. Yet during the entire day we did not find a dozen crabs. When I wrote Washington, I informed them that the mortalities were not due to crabs because small oysters would be preferentially killed by the crabs, and all the large oysters were dead. If we had received support at that time, we might have been able to make plans to dampen the effect of the ensuing epizootic.

ROSENFIELD: Did you see organisms in the tissues that might have caused the mortalities?

LOOSANOFF: No, I did not. We had no facilities or trained staff for this specialty. But I believe the mortality occurred in 1952 and not in 1957. According to Dick Nelson, those diseased oysters came from Virginia. Another point frequently missed in these discussions is mortality of the spat. We had witness of this mortality in Long Island in 1944 and 1945. Spat would grow to a certain stage and then begin to die. For example, of 100 spat on a shell only one or two would remain alive. In this case, we could have used a pathologist. Moreover, the men and women working in genetics have an opportunity to develop genetically resistant races. Finally, for the last five or six years I have been free to pursue a variety of things. In one case, I aided a man in developing a hatchery on the Pacific Coast. Presently, this man can grow millions of spat but according to you, he should not ship them. Thus, the hatchery becomes a liability instead of an asset. What can we do to facilitate interstate commerce involving shipping this stock from one state to another? Even if you take set from the Pacific Coast and ship it here, the state of California will not permit you to ship oysters into California from here. At the same time, as Dr. Menzel pointed out, the Japanese send about 46 shiploads of oysters into California waters every year.

ROSENFIELD: I have no objections to the introduction of species provided that one carefully considers the consequences in a particular instance. The introduction of *Ostrea edulis* into Maine does not meet with my objections as much as the introduction of oysters from Japan into Chesapeake Bay. My feeling is that the European oyster does not compete with any other species in the United States. So, I do not object to something like this. On the other hand, the Japanese oyster might grow at such a fantastic rate in Chesapeake Bay as to overwhelm the native oyster. Under controlled conditions this might not occur. Still, this is what we are up against in addition to the fact that Japanese oysters may also introduce parasites that might affect other commercially important species.

LOOSANOFF: This is exactly why I think this is a proper time to form a committee of scientists and industry people to develop criteria by means of which we can solve this problem. This brings to mind an incident where an oysterman purchased 150 bushels of French oysters that nobody knew about. These oysters began dying by the millions and some were brought to us for examination. I said, "For God's sake, what are these French oysters doing here?" The point is that the man who intro-

duced the oysters did not realize the seriousness of his action. Let us educate oystermen, and provide them with the laws to go into the hatchery business, produce spat, and sell it for profit.

ROSENFIELD: I am not advocating prohibition of importing oysters from current Japanese sources into the West Coast of the United States. It is too late to do anything about this anyway. What I do advocate is that any species from foreign seas be carefully screened before introduction into the U.S.—for example, Pacific oysters from Korea or Taiwan.

LOOSANOFF: Many years ago at a meeting in Philadelphia, recommendations were made against introducing foreign species without previous consultation with scientific authorities. I fully agree with this course of action. However, I do not believe we can suffocate the industry by localizing it and restricting it to specific geographic areas, and that is what a comprehensive import law would do.

MAURER: In this discussion several points emerged that should be summarized. One view urges greater control over interstate shipping of exotic species. Assuming sufficient controls, the other view advocates the feasibility of interstate shipping. In another vein, people in shellfish pathology are just now beginning to determine what particular pathogens are involved. Further, perhaps less is known about disease and disease prevention under controlled hatchery conditions than any of the other areas covered in this conference.

Introduction to SELECTIVE OYSTER BREEDING

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One of the subprojects in Delaware's sea grant program is directed toward the selective breeding of oysters. The primary objective of this subproject is to improve the growth and survival rate of our local oyster species *C. virginica* by selective breeding in the laboratory. The secondary subproject objective is to investigate the feasibility of developing hybrid oysters by cross breeding *C. virginica* with other oyster species in the genus *Crassostrea*.

The University Marine Laboratories have been actively engaged in selectively breeding MSX-resistant oysters since 1962. Under the sea grant program, the selective breeding program has been expanded to include experiments in hybridization of oysters, in which have been produced viable hybrids of *C. virginica* and *C. gigas*.

In the genus *Crassostrea*, there are approximately six commercially important species of oysters and many of these species have been cross bred in the laboratory. In experimental work involving nonindigenous species of oysters, precautions must be taken to insure that less desirable species are not accidentally introduced into local oyster growing waters. Seawater that is used to maintain, condition, spawn, or rear exotic species and hybrids must be prevented from directly entering local waters. Continuous land disposal of large volumes of seawater is often a problem. Many problems are encountered in obtaining and conditioning exotic species of oysters for spawning on the same day and hour with *C. virginica*. And in handling and rearing hybrid oyster larvae, additional precautions must be taken to prevent the mixing of wild and hybrid oyster larvae.

Our next speaker, Dr. R. W. Menzel, has surmounted the aforementioned obstacles and has produced viable hybrids of a number of *Crassostrea* species.

SELECTIVE BREEDING IN OYSTERS*

R. W. MENZEL
Professor, Department of Oceanography
Florida State University

At the present time we do not have enough information on selective breeding of oysters to even know what is possible. In the opening address to the National Shellfisheries Association 20 years ago, the late Dr. Thurlow Nelson's topic was "What Can Science Offer the Oyster Grower." At that time Dr. Victor Loosanoff and his associates at the Bureau of Commerical Fisheries Biological Laboratory at Milford, Connecticut had begun to have consistent success with the laboratory spawning and rearing of clams and oysters. Dr. Nelson said, "Armed with such techniques, there is every reason to hope that, through selective breeding, we can obtain oysters and quahogs capable of attaining market size in half the time now required."

After 20 years, even in this age of greatly accelerated scientific advancement, Dr. Nelson's "hopes" are still just that, hopes. We are making progress, however, and there is every reason to hope again, especially with the increasing interest in controlled farming of the sea. The investment in maricultural ventures is often considerable and modern oyster farmers will seek every means possible to obtain greater returns on their investments.

Commercial shellfish hatcheries are now a reality, based on the techniques of the Milford Laboratory and those of the late Mr. Joe Glancy, as well as research and development in foreign countries. I am aware of one hatchery practicing selection by retaining only the larger larvae at each water change. I have heard (but not verified) that one hatchery rears hybrid oysters. The Milford Laboratory has an active program in the genetics of shellfish.

There are precedents, in that certain aquatic animals have been selected for more desirable traits. The aquarium hobbyists have many bizzare types of fish, e.g., the many forms of goldfish. The trout farming industry has commercially superior strains adapted to pond culture. The catfish farming industry in this country is relatively young, but already hatcheries are experimenting with hybrids between species and practicing selection.

Cultivation of oysters, at least in some form, has been practiced for a long time, but in some respects is not even comparable to terrestrial farming two or

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three centuries ago. Advances have been made, besides the starting of hatcheries, including better mechanization in processing, planting and harvesting, although in the interest of conservation, the old inefficient hand tongs are used in many areas. In addition there are concentrated efforts to eliminate mortality caused by diseases and predators. New areas where oysters do not normally occur are continuing to be exploited, e.g., leased planted bottom land along our coasts and the "Japanese raft culture."

Despite the advancements, the bounties of nature, along with the vagaries, are the main limiting factors. Despite the steadily decreasing overall harvesting of oysters in this country there is every reason to believe that there could be a manifold increase through further controls and domestication. (I shall not discuss the inroads made by pollution on oyster production.) To truly domesticate the oyster we need to control every facet. Such complete controls are ideals and seldom met in application, although poultry growers are coming close.

Oysters are the most thoroughly studied marine invertebrate animal. They have a sessile life, except for the larval stages, and these can be controlled through hatcheries. We understand the basic requirements for growing oysters. We know that variability occurs among individuals and populations. Capitalizing on the variables through selective breeding should be feasible. Environmental conditions cause many of the variables but it is inconceivable that the majority are not under some genetic control, even though modified by environmental factors.

What is needed is an intensification of programs in selective breeding, similar to the many programs for terrestrial organisms that are being conducted by the states and the U.S. Department of Agriculture. We are all aware of what can be done. I can remember the barnyard turkey in comparison to what I have for Thanksgiving dinner now. Better understanding of the nutritional requirements and the supplying of these have been responsible for part of the increase in agricultural production, but I have heard that at least 30 percent is estimated to be from the growing of selected and hybridized strains.

I shall give some examples of variables of commercial importance, that might be selected for, realizing that each advance, or even failure, will open up new vistas. The many researchers whose data I am using will not be mentioned by name, but I thank them and will include a representative bibliography. I shall discuss my attempts at hybridization of oysters in the genus *Crassostrea* and thank Mr. Theodore Ritchie and Mr. Harold Sims, who did most of the crossing and rearing of the oyster larvae.

SELECTION

Introduction

All natural populations exhibit many variable traits that are genetically controlled. Through selection, either natural or by controlled expériments, some of the variation can be lessened. An example of natural selection would be the passing on to future generations of a resistance to a disease, because of the mortality of the more susceptible individuals. Man has been able to exploit the variability, and even

introduce new variables, in the many organisms he has domesticated, through selective breeding.

Variation in growth, resistance to diseases and resistance to certain physiological stresses will be discussed. I shall confine my remarks to our native species on the Atlantic Coast, *Crassostrea virginica*.

Growth

Better growth is one of the first things that comes to mind when selective breeding for desirable traits is mentioned. Almost as many methods for determining growth in oysters exist as studies on the subject. The oyster farmer is interested in the greatest yield in the minimum amount of time. This yield can be in bushels per acre, or better, pounds of oyster meat, because the size of the shell does not always indicate what the yield will be in meat. There are marked seasonal differences in meat yield, governed primarily by the reproductive condition of the animals. Some other factors that influence the yield of meat are food abundance, temperature, salinity and turbidity.

Selective breeding could establish strains that have maximum growth under the existing conditions. It is conceivable that strains could be selected for that would grow better under certain conditions than others. Thus it might be possible to develop oyster seed that grow better at low salinity and high turbidity and another strain better suited to high salinity and low turbidity. Seed oysters from natural areas are variable in their growth when planted to other areas. I have heard experienced oystermen say that they preferred seed from certain areas for their planted ground. How much of the variation is due to environmentally influenced factors and how much is genetically controlled is not known.

Another desirable trait that could be selected for is growth at a uniform rate. This has been done for terrestrial organisms, especially plants that are machine harvested. Inbreeding will reduce the variables to some extent. I have found the growth to be more uniform in laboratory-spawned quahog clams in the third generation than those in the first generation, whose parents were from a wild population. Ecological conditions of the microhabitat that influence the growth rates would negate the full realization of this ideal.

The thickness of the shell might be selected. Shell thickness is directly correlated with the age and growth rate of the individuals and influenced by ecological conditions, but undoubtedly there are variables that are inherited. Thin-shelled oysters would be desirable when grown under the controlled conditions of ponds or tanks. Thick-shelled oysters would have a better survival in certain areas that have abundant shell-boring pests, outweighing the disadvantages of decreased ratio of meat yield to shell size.

Disease Resistance

In populations of organisms there is better resistance of some individuals to adverse conditions than others and this resistance is under genetic control. The survival of the species is dependent on this because most populations undergo

stresses of varying severity. Populations acquire immunity of varying degrees to diseases. If a new disease is introduced into a population there is often no genetical resistance and the mortality may be catastrophic. In horizontal time I know of no species that have been entirely eliminated because of a disease. The physiological condition of the organisms is of importance in the ability to resist a disease and often the combination of several adverse conditions causes the demise of individuals. In addition the disease organism may mutate and become more virulent causing high mortalities.

The reestablishment of the eastern Canadian oyster industry after the devastating disease-caused mortalities during 1915–20 is an example of natural selection, with a portion of the population having resistance. The techniques of hatchery culture allow for the controlled breeding of genetically resistant oysters. In addition new traits can be introduced into the oysters that may be important in the establishment of disease resistance. There are many examples of the establishment of resistance in terrestrial organisms through controlled breeding and selection.

I do not know the present status of the MSX disease (*Minchinia nelsoni*) in the Chesapeake and Delaware Bays, or if it has been demonstrated whether any of the oyster populations have any resistance. Efforts in both areas have been made to select for disease resistance. I understand in the Chesapeake Bay (Virginia) that resistance has been demonstrated in that oysters that attach and grow in an endemic disease area have better resistance than those that set in disease-free areas. It would seem that the former oysters are survivors of the disease and have some immunity, whether acquired or based on genetic factors.

There is a pathogenic fungus (*Dermocystidium marinum*) along the Atlantic and Gulf of Mexico coasts. This disease is primarily a warm water organism, causing mortality only when the temperature is above 20° C, and only to larger oysters after their first summer of life. These two characteristics of the disease may be partially influenced by the physiology of the host animal. Young oysters are physiologically more vigorous and it is during the warmer period when there is the additional stress caused by spawning. Up to half the adult oysters are killed by the fungus each summer in certain areas. Efforts to control the disease have been made but the main control has been to change the harvesting time before the disease has caused extensive mortalities. In addition the oyster farmer relies on the natural abundance of oysters, which still allows for successful commercial operations, even after the mortality caused by the disease.

One might expect that with such a virulent organism, natural selection would cause the development of disease resistance. The fungus was first described in 1950 and undoubtedly had been killing oysters for many years before. However, the rapid growth and early sexual maturity, in the Gulf area at least, would prevent natural selection from occurring. Oysters that attach in the spring spawn as early as the following fall and certainly by the following spring, a year later, before the disease has had a chance to eliminate the susceptible individuals.

Although there have been no dramatic breakthroughs in producing disease resistance through selective breeding, efforts should be continued. Not only should survivors of a disease be used for brood stock but other populations as well, where the disease does not occur, for these may have genetic traits for resistance. Based on

the experiences of the agricultural experiment stations it will be a continuing battle. There have been instances where strains or varieties have been selected that had resistance and then the pathogenic organism mutated so that the variety was no longer resistant.

Resistance to Physiological Stresses

Numerous physiological stresses are encountered in the natural habitat throughout the lifetime of oysters. These stresses often cause considerable mortality or may be harmful in other ways. Growth ceases or the "fatness" or the glycogen content is reduced. Like disease resistance, the manifestation of harmful effect is due partly to the physiological conditions of the individuals. The ability to survive physiological stresses is partly genetic and some oysters and populations of oysters are better adapted than others to adverse conditions.

Temperature is one of the environmental stress conditions over which the oyster farmer has no control when growing oysters under natural conditions. Extremes of temperature cause adverse reactions. Different populations react differently to temperature; e.g., there is a difference of 6–8° C between oysters from the northern and southern regions for the minimum threshold of temperature for the initiation of spawning.

Under experimental conditions the ciliary activity on the gills of oysters from more northern areas continues down to 0° C, whereas all activity ceases at lows of 5–6° C in the Gulf of Mexico area. Northern oysters cease feeding at temperatures of 30° C, which are rarely encountered in the colder areas. In the Gulf areas oysters feed at 30° C and above, as indicated by the presence of the crystalline style. These physiological traits might prove desirable if through selection a variety of oysters could be produced that feed in a wider range of temperatures.

Oysters are euryhaline but do not normally live in salinities below about 10 ‰. They survive in lower salinities for short periods but prolonged exposure causes mortality. Oysters are grown in the low salinity range of their tolerance in many areas. In fact, these areas are often the most successful, e.g., Maryland's Chesapeake Bay waters, because many of the pests are less tolerant of low salinities than oysters. Mortalities have occurred in such areas, however, because of freshets. A thriving oyster population has been reported from an area in Louisiana that were living in salinities down to 6 ‰. The ability to tolerate such low salinities may be correlated with the generalization that marine animals are better able to regulate their osmotic balance in low salinities at higher temperatures. The low salinity tolerance may be genetically controlled and should be investigated.

Oysters in the genus *Crassostrea* are predominantly estuarine animals and are adapted physiologically and morphologically to the conditions that occur in these areas. Oysters are filter feeders and species in the genus *Crassostrea* have the ability to select food items from the water and reject the nonnutritious particles or those otherwise unacceptable. Estuaries are more turbid than the open sea and the ability to select food is a definite advantage for the animals.

Investigators in the more northern areas, where the turbidity is usually less than in some of the oyster producing areas of the Gulf of Mexico, have found that

high turbidity is detrimental to oysters. In our local area of Florida the turbidity is so high during the summer months that a Secchi disk will disappear within several centimeters, yet the oysters live and thrive. The ability of oysters to function in such high turbidity might be "bred" into oysters from more northern areas, which have their own adaptations to their locality.

HYBRIDIZATION

Introduction

We are all aware of what has been done with domestic terrestrial organisms through hybridization between species or between varieties of the same species. Hybrid vigor often results as does the incorporation of desirable characteristics from both parent species. In my work with hybrids of northern and southern quahogs, I found that the hybrids have the desired commercial traits of the good growth of the southern parent and the good keeping qualities, when taken from the water, of the northern parent.

If the species are far enough apart genetically they may not hybridize and if they do the hybrids may be sterile. The mule is a good example of a sterile hybrid. If the hybrids are fertile, segregation will occur in the F_2 generation. In quahogs the F_1 hybrids are fertile and there is segregation in the F_2's.

If seed oysters are produced under the controlled conditions of a hatchery it is not necessary for the hybrids to be fertile; in fact it might be better if they were not. Fertility in the hybrids might result in the establishment of races or species into the natural habitat with resulting bad consequences. The exotic parent may become "weedy" and supplant the native species. If continual hybridization occurs the resulting progeny may be commercially inferior to the native species. No controls can be exercised in the natural habitat and the use of hybrids should be considered with extreme care.

My oyster research for the past several years has been on the cytotaxonomy of species of *Crassostrea*. Oysters are termed ecomorphic and it is often difficult to determine the species from the shell morphology unless one knows where they came from. For instance, I doubt very seriously if even a competent conchologist could separate the Portuguese oyster (*C. angulata*) from the Japanese (*C. gigas*), if I were allowed to select the shells of each and gave no information as to their origin.

It is suspected that many described species from certain areas are ecological variants of a species from another area. To further confound the species identification man has introduced oysters to different areas, because of their economic importance. We have good records of some of the introductions, but knowledge of other introductions have undoubtedly been lost in antiquity. To determine the systematic affinities of various species of *Crassostrea,* exotic species were obtained. These were reared from the fertilized egg under similar laboratory conditions. The species were crossed and if hybrids were obtained, these were reared under the same conditions.

This work was supported through a grant from the National Science Foundation during the period from May 1966 through April 1968. Unfortunately no

funds have been available since that time from outside sources and the press of other duties has prevented the continuation. A total of 11 species of *Crassostrea* were secured from various areas of the oyster-growing regions of the world, although attempts at crossing and rearing hybrid larvae involved only six species.

Methods and Results

The techniques followed closely those developed at the Bureau of Commercial Fisheries Laboratory at Milford, Connecticut, with modifications pursuant to the facilities available. Larval culture containers of five-gallon capacity were used, which permitted numerous simultaneous cultures in the space available. We found it more efficient to "strip spawn," than to induce spawning by the temperature shock and sperm suspension method. This method allowed for better controls in the hybridization attempts. With hybridization attempts the procedure was as follows: (1) reciprocal crosses between species, (2) each species selfed, (3) a portion of eggs of each species not selfed, as a further control to be certain that no sperm contamination occurred, either from the "female" or inadvertently.

If fertilization and cell cleavage occurred, the embryos in the two-four cell stage were sieved to remove extraneous material and placed in the larval culture tanks, filled with filtered (4 μ) seawater. The rearing of the larvae followed the established procedure, with changes of filtered water and the addition of cultured algae (*Monochrysis* and *Isochrysis*) plus Sulmet three times a week.

Shell strings were placed in the larval culture tanks at time of setting. After attachment the spat were marked and suspended in large aquaria of filtered seawater and fed with cultured algae. Upon reaching a size of 2–5 mm the spat on the shell strings were suspended in an aquarium with flowing seawater and given supplemental feeding of algae. Despite the naturally occurring food in the seawater and the supplemental feeding the growth rate was curtailed. *C. virginica* of the same set were planted in the adjacent bay and had almost twice the growth as those kept in the aquarium.

The species of *Crassostrea* were in two morphological groups. One had denticles along the anterior margins of the shell valve, similar to species of *Ostrea* that I am familiar with. The other group had no denticles like our native *C. virginica*. The group with the denticles were:

C. amasa (Iredale)—Australia
C. commercialis (Iredale and Roughley)—Australia
C. cucullata (Born)—India, Mauritius, Philippines and Singapore
C. echinata (Quoy and Gaimard)—Australia
C. margaritacea (Lamarck)—South Africa

The group without shell denticles were:

C. angulata (Lamarck)—cultured in England
C. brasiliana (Lamarck)—Brazil
C. gigas (Thunberg)—cultured in Washington
C. iredalei (Faustoin)—Philippines
C. rhizophorae (Guilding)—Canal Zone and Puerto Rico
C. virginica (Gmelin)—native

The six species available in the season of 1967, in which hybridization attempts were made were: *C. commercialis* in the first group with shell denticles; *C. angulata, C. gigas, C. iredalei, C. rhizophorae* and *C. virginica,* in the second group without denticles. All possible cross fertilization combinations were made with these species.

Attempts at crossing, using *C. commercialis* (with denticles), were unsuccessful. The gametes were completely incompatible with the five species without shell denticles. Reciprocal crosses were made, using *C. iredalei* with the other four species without shell denticles. Although fertilization and cell cleavage occurred, with larval development, repeated attempts to rear the larvae met with failure. Cytological examinations of the hybrid embryos showed mitotic anomalies of haploid, triploid and hexaploid chromosome numbers.

Table 1 shows the hybrids that were reared through metamorphosis, and their growth under laboratory conditions. The success of rearing these hybrids showed that *C. angulata, C. gigas, C. rhizophorae* and *C. virginica* are closely related, and will hybridize under experimental conditions. However, hybridization under laboratory conditions does not mean that the species would necessarily hybridize under natural conditions. The hybrids became sexually mature during the summer of 1968. These were self-fertilized and cell cleavage occurred in some. These embryos were preserved for cytological examination but the analysis has not yet been completed.

Discussion

The results of the hybridization experiments show that hybrids can be obtained, at least in the four species, *C. angulata, C. gigas, C. rhizophorae* and *C. virginica,* under laboratory conditions. I understand the Japanese have crossed *C. angulata* with their native *C. gigas*. So far this work is too preliminary to pursue commercial applications.

Casual observations were made on the several exotic species themselves. All the oysters were kept in aquaria, with flowing seawater, whose effluent was led to a pit in the ground. The conditions were far from ideal and during the summer months the temperatures in the aquaria were often above 30°C. All the species except those from tropical areas, had a complete cessation of shell growth, the meats were thin and watery, and excessive mortality occurred.

One tropical species, *C. iredalei* from the Philippines, thrived under the conditions. In the period from March through October the oysters grew several inches and the meats remained in good condition, although mostly because of mature gonads, and there was negligible mortality. *C. iredalei* is a large oyster (grows to more than six inches in shell height) and the flavor is good (at least to me). Repeated laboratory attempts resulted in no hybridization with the other species. This species might be suitable for introduction in our more southern regions, especially for growing in pond culture. Introductions of exotic species, however, are fraught with many hazards as discussed with the planting of hybrids. Also one should be certain that no diseases or pests are introduced.

Table 1.
Growth of Laboratory Oysters

Date Set # Set Date Examined	angulata × gigas 5 Aug 67 109 Size mm # L × W	gigas × angulata 18 Aug 67 24 Size mm # L × W	angulata × virginica 9 July 67 31 Size mm # L × W	gigas × virginica 15 June 67 41 Size mm # L × W
9/67	99 3.5 × 3.0	23 4.5 × 4.0	30 9.0 × 8.0	39 36.5 × 28.0
10/67	95 11.0 × 9.5	18 12.0 × 9.0	28 12.5 × 11.0	37 37.0 × 29.0
11/67	87 14.0 × 12.5	17 12.5 × 11.0	28 13.0 × 11.0	35 37.5 × 30.5
12/67	87 14.5 × 12.5	16 12.0 × 11.5	24 13.5 × 11.5	35 37.5 × 30.5
1/68	87 14.5 × 12.0	16 12.5 × 11.5	24 14.0 × 11.5	35 37.5 × 30.5
2/68	84 14.0 × 12.0	16 12.5 × 11.5	24 18.0 × 14.5	32 38.0 × 31.0
3/68	83 14.5 × 12.0	16 12.5 × 11.5	23 19.5 × 16.0	29 37.5 × 31.0
4/68	81 21.0 × 17.5	16 15.5 × 13.5	22 31.0 × 25.5	29 38.5 × 33.0
5/68	79 22.0 × 17.5	16 19.0 × 16.0	22 33.0 × 27.0	23 41.0 × 33.5
6/68	78 40.5 × 31.5	13 34.5 × 27.5	21 48.5 × 38.0	17 52.0 × 41.5
7/68	73 40.0 × 31.0	11 46.5 × 34.5	21 52.0 × 40.0	17 53.5 × 41.5
8/68	60 46.0 × 35.0	11 46.5 × 34.0	20 52.0 × 38.5	15 52.5 × 41.0
9/68	58 46.0 × 34.0	11 46.0 × 33.5	20 52.0 × 38.5	13 53.5 × 42.5
Survival	53%	46%	65%	32%

Table 1. (continued)

Date Set # Set Date Examined	rhizophorae × angulata 28 Aug 67 159 Size mm # L × W	rhizophorae × virginica 28 Aug 67 151 Size mm # L × W	rhizophorae × rhizophora 3 Aug 67 40 Size mm # L × W	virginica × virginica 5 July 67 50 Size mm # L × W
9/67	111 1.5 × 1.0	145 2.0 × 1.5	34 11.5 × 11.0	44 22.0 × 18.5
10/67	98 8.0 × 6.0	143 6.0 × 5.0	32 16.0 × 15.0	44 27.5 × 19.0
11/67	95 9.0 × 7.0	142 6.5 × 6.0	32 17.0 × 16.0	43 27.5 × 19.0
12/67	95 9.0 × 7.0	130 6.5 × 6.0	32 17.0 × 16.0	43 27.5 × 22.0
1/68	88 8.5 × 7.5	121 7.0 × 6.5	28 17.0 × 15.0	41 27.5 × 22.0
2/68	40 8.5 × 7.0	111 7.0 × 6.5	20 17.0 × 14.5	40 27.5 × 23.0
3/68	40 8.5 × 7.0	78 7.0 × 7.0	18 17.0 × 14.5	40 27.5 × 23.0
4/68	36 10.5 × 9.0	68 7.5 × 7.0	13 17.0 × 14.5	40 28.0 × 23.5
5/68	36 11.0 × 10.0	58 13.0 × 11.0	12 18.0 × 15.0	40 30.0 × 24.0
6/68	21 27.0 × 22.5	54 27.5 × 22.0	10 28.5 × 23.0	39 40.5 × 30.5
7/68	21 32.0 × 27.0	52 39.5 × 32.0	10 33.0 × 28.0	36 46.0 × 33.0
8/68	19 34.5 × 26.0	52 40.0 × 32.5	10 35.5 × 28.0	36 46.0 × 34.0
9/68	18 36.0 × 27.5	52 41.0 × 32.0	10 36.0 × 28.0	36 47.5 × 34.5
Survival	11%	34%	25%	72%

CONCLUSIONS

We have made some progress in domesticating the oyster. We have many of the basic techniques and realize what we should do. The planting of seed oysters is already a widespread operation, and in many areas the entire industry is dependent on continual planting. Hatchery techniques are well established and offer the means for further domestication. There is a steadily increasing interest and investment in ventures in the controlled farming of the sea.

We still have a long way to go. One of the goals of domestication is the establishment of desirable strains. Selection in terrestrial organisms that have been domesticated has been going on for many years; in fact the majority of our domestic animals and plants were selected or developed long before man was aware of genetics, although it has been known for a long time that "by their fruit ye shall know them." With more awareness of genetical principles, man has been able to improve the domesticated organisms from a commercial standpoint with the development of new strains and varieties and hybrids. There is no reason why "certified seed" oysters cannot be developed. As of now we are trying to farm a bunch of wild animals.

LITERATURE CITED

ANDREWS, J. D., 1966. Problems in shellfish production. *Proc. Nat'l Shellfish. Assoc.*, 57: 9–10.

ANDREWS, J. D., 1967. Oyster mortality studies in Virginia. VII. Review of epizootiology and origin of *Minchinia nelsoni*. *Proc. Nat. Shellfish Assn.*, 58: 23–26.

BARDACH, J. E., 1968. Aquaculture. *Science*, 161: 1098–1106.

BUTLER, P. A., 1952. Growth and mortality rates in sibling and unrelated oyster populations. *Proc. Gulf Caribb. Fish. Inst.*, 71.

CROWDER, H. E., 1967. BCF role in farming the sea. *Proc. Nat. Shellfish Assn.*, 58: 16–18.

DRINNAN, R. E. and J. C. MEDCOF, 1961. Progress in rehabilitating disease-affected oyster stocks. *Fish. Rsch. Brd. Canada. Gen. Ser. Circ.*, 34: 3 pp.

GALTSOFF, P. S., 1928. The effect of temperature on the mechanical activity of the gills of oysters (*Ostrea virginica* Gm.) *Journ. Gen. Physiol.*, 11: 415–431.

GALTSOFF, P. S., 1964. The American oyster, *Crassostrea virginica* (Gmelin). *U.S. Bur. Comm. Fish.*, Bull. 64: 400 pp.

IMAI, T., M. HATANKA, S. SATO, and R. SUKI, 1950. Artificial breeding of oysters in tanks. *Tohoku Journ. Agric. Resch.*, 12: 69–86.

IMAI, T., and S. SAKAI, 1961. Study of breeding of Japanese oysters. *Tohoku Journ. Agric. Resch.*, 12: 125–163.

IVERSON, E. S., 1968. *Farming the edge of the sea*. Authur Heighway, London. 303 pp.

LOOSANOFF, V. L., 1949. On food selectivity of oysters. *Science*, 110: 122.

LOOSANOFF, V. L., 1954. New advances in the study of bivalve larvae, *Amer. Scient.*, 42: 607–624.

LOOSANOFF, V. L., 1958. Challenging problems in shellfish biology. *Perspectives in Mar. Biol.*, Pt. IV: 483–495.

LOOSANOFF, V. L., 1965. Mariculture—Its recent development and its future. *Agric. Engin.*, 46: 93–97.

LOOSANOFF, V. L., and H. C. DAVIS, 1963. Shellfish hatcheries and their future. *U.S. Comm. Fish. Rev.*, 25: 1–11.

LOOSANOFF, V. L., and H. C. DAVIS, 1963. Rearing bivalve larvae. *Advances in Marine Biology*, Academic Press, London, pp. 1–126.

LOOSANOFF, V. L., and C. A. NOMEJKO, 1951. Existence of physiologically different races of oysters, *Crassostrea virginica*, *Biol. Bull.*, 151–156.

MACKIN, J. G., 1961. Oyster diseases caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publ. Inst. Mar. Sci.*, 7: 132–229.

MATTOX, W. T., 1949. Studies on the biology of the edible oyster, *Ostrea rhizophorae* Guilding in Puerto Rico. *Ecol. Monogr.*, 19: 339–356.

MENZEL, R. W., 1955. Some phases of the biology of *Ostrea equestris* Say and a comparison with *Crassostrea virginica* (Gmelin). *Publ. Inst. Mar. Sci.* 4: 70–153.

MENZEL, R. W., 1956. The effect of temperature on the ciliary action and other activities of oysters. *F.S.U. Studies*, 22: 25–36.

MENZEL, R. W., 1961. Shellfish mariculture. *Proc. Gulf Car. Fish. Inst.*, 14: 195–198.

MENZEL, R. W., 1969. Cytotaxonomy of species of clams (*Mercenaria*) and oysters (*Crassostrea*). *Mar. Biol. Assoc. India, Symp. on Mollusca*, Pt. 1: 75–84.

NELSON, T. C., 1928. Relation of spawning of oysters to temperature. *Ecology*, 9: 145–154.

NELSON, T. C., 1949. What can science offer the oyster grower. *Proc. Nat. Shellfish Assn.* Conv. 1949: 1–9.

RAY, S. M., 1954. Biological studies of *Dermocystidium marinum*, a parasite of oysters. *Rice Inst. Pamph. Special Issue*, November, 1954, 114 p.

STAUBER, L. A., 1947. Possible physiological species in the oyster. *Ostrea virginica*. *Anat. Rec.* 99: 614.

THOMSON, J. M., 1954. The genera of oysters and the Australian species. *Australian Journ. Mar. and Freshwater Resch.* 5: 132–168.

DISCUSSION

H. C. DAVIS: Dr. Menzel, what was the percentage of successful crosses of *C. gigas–C. virginica* in your work?

MENZEL: We crossed them several times and achieved about 12–18 percent fertilized eggs. We found we could cross *C. angulata* with *C. gigas* readily.

H. C. DAVIS: The reason I asked is that we reported we were unable to make these crosses. Also, some of Dr. Longwell's recent work indicated that under certain conditions there is evidence that instead of the sperm nucleus taking part in the fertilization process, it initiates a doubling of the maternal chromosomes. We think Dr. Longwell is undertaking this genetic study in a logical manner. She is attempting to find what the possibilities of natural selection are: This can be determined by her heritability studies.

MENZEL.: Does she have some markers on oysters? Some characteristics that she marks from inheritance?

H. C. DAVIS: No, at the present time, she is using primarily the success of the larval cultures as a criterion for growth. She has been trying to establish purer lines by full sibling crossing. In one group of oysters she obtains absolutely no progeny from a full sib cross. This is a group of oysters from around Norwich, Connecticut. In another group of oysters from New Haven, Connecticut, about 30 miles away, she does get some normal progeny. Thus, there is evidence here of a difference between two oysters from localities within about 30 miles. The fact that she gets absolute mortality in this one set of full sib crosses and a high mortality in sib crosses from the other area indicates that the oysters do contain a fair number of deleterious genes; i.e., when you combine them in full sib crosses, you do obtain abnormal progeny.

MENZEL: That has been illustrated in larger animals.

H. C. DAVIS: Unless close crosses are made you would expect to produce larvae some of which do not survive while others do quite well. In this respect we think commercial hatcheries are doing the proper thing in discarding their slower-growing larvae, which eliminates some of these deleterious genes. Dr. Longwell also finds evidence that the oyster egg is able to select the sperm that it permits to enter. This is one way that the oyster, in the wild, can enforce hybridization rather than self-fertilization or close inbreeding.

MENZEL: Yes, I would agree but you certainly can force inbreeding in the laboratory

H. C. DAVIS: This absolute mortality is only from this one area. Dr. Longwell has not obtained full sib lines from more than these two areas at present. But it does indicate a very high content of deleterious genes. She finds that if the spat are irradiated, full sib crosses are more successful, which indicates that the irradiation caused mutation in some of the deleterious genes. Therefore the alleles are no longer matched.

In one of her irradiated females she obtained some extremely large eggs which when fertilized by normal sperm produced extremely large straight-hinge larvae. Upon analysis of the eggs she found their chromosome count to be triploid. Thus, we think that in this cross the large larvae were triploids.

MENZEL: In fertilization by stripping, I often get a few eggs that are polyploid, but most of the polyploid eggs are abnormal. Of course, I am unsure if these eggs would have produced viable larvae, because in examining the larvae you sacrifice them.

LOOSANOFF: Gentlemen, I have two practical proposals. The first concerns a recent observation I made in Tomales Bay, California, where I found that the Japanese oyster, *C. gigas* is absolutely immune to sponges. You can place American oysters and Japanese oysters side by side and two years later, the former are absolutely disintegrated whereas the latter remain untouched. Now is it possible genetically to transfer a gene in the Japanese oyster into the American oyster to prevent sponge infestation?

MENZEL: It would be possible to hybridize the back crosses and incorporate all the attributes and retain that gene, but the chances are that it would require several years.

LOOSANOFF: This is why I am discussing it, and I strongly suggest this problem to someone to perform because this could be an extremely important contribution to American culture of oysters.

The second suggestion concerns using thermal effluents to our advantage. It is not very often that the temperature of the water will exceed the desired level. That is, it will not rise above 96°F and kill the American oyster. Now, if we consider the Portuguese oyster which has even a greater tolerance to higher temperatures than the American oyster and introduce these genes into *C. virginica*, people using thermal additives will have a safety zone.

Introduction to
INFLUENCE OF THE CLIMATIC WATER BALANCE ON THE ESTUARINE ENVIRONMENT

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Most shellfish develop and live in an estuarine or coastal environment that is, to a considerable degree, influenced by the conditions of water flow from the surrounding land masses. In a coastal estuary such as the Delaware, a simple balance of water inflows and outflows may be written as follows:

$$RO + P - E \pm U = I/O$$

Where RO is the runoff from the surrounding land area
P is the precipitation on the estuary surface
E is the evaporation from the estuary surface
U is the inflow to or outflow from the estuary through the channel bottom.
I/O is the net inflow or outflow of water from or to the ocean needed to maintain the water level.

In practice it is extremely difficult to evaluate U. It is usually assumed that this term is negligible although the validity of this assumption may be questioned in certain areas. The remaining terms on the left-hand side of the relation can be evaluated with some precision so that the net inflow or outflow to or from the ocean can be determined quantitatively. This type of water balance approach provides the only practical method of determining net inflow or outflow of water to an estuary since direct measurement at a wide estuary mouth with alternating water currents is next to impossible.

The quality of the water in the estuary, especially its salinity, should be closely related to the net water exchange with the ocean. Other factors such as the level of stream pollution, turbidity and sediment load of the stream, and its nutrient condition must also be related to the factors of the hydrologic balance since they are responsive to the quantity of runoff from the land and the amount of mixing with the ocean.

The three left-hand terms of our water balance expression (RO, P, E) all involve climatic variables. Evaluation of the climatic water balance, thus, will provide

us with information on the quantity of water exchanged with the ocean and on its quality. Since climatic information is available for many years of record while actual measures in the estuary may be fragmentary and of short duration, the climatic approach can provide basic and needed information.

Quantitative evaluation of the terms on the left-hand side of the equation can be accomplished with knowledge of precipitation and temperature at a fairly dense network of stations well distributed over the watershed. Runoff of water from the land could, of course, be determined if all of the tributary streams were gaged. Lacking this, however, it is possible to compute runoff from land areas from the relation

$$P - E \pm \Delta S = RO$$

where ΔS is the change in water storage in the ground. All terms are expressed as depths of water.

The American climatologist, C. W. Thornthwaite, has provided a simple and usable expression by which evapotranspiration from a land area can be determined from information on temperature and day-length (1948). Thornthwaite and Mather (1955) have developed a simple climatic water balance bookkeeping procedure that permits direct computation of the change in storage in the soil and the runoff of water from a place knowing just the precipitation and evapotranspiration. Plotting the point data and analyzing the geographical patterns of runoff permits direct estimation of runoff from a basin with a fair degree of accuracy. Many previous comparisons between measured and climatically computed runoff justify present faith in the climatic bookkeeping approach.

Precipitation (P) and evaporation (E) over the estuary surface are seldom measured. In a few instances, lightships or islands in the water body may provide approximate values of precipitation and a measure of temperature from which evaporation can be determined. However, it is known that such observations are biased on the high side, generally because of exposure problems, so that corrections must be applied to approximate over-water values. Micrometeorologic theory is, however, far enough advanced to permit reasonable estimation of over-water evaporation and precipitation from the available land- or ship-measured values.

Carter (1958) has used the climatic approach to provide average monthly and annual values of net exchange between the Delaware estuary and the ocean. On the average, he found that there is a net discharge from the estuary to the ocean every month although there is a 17 to 1 variation in monthly outflow figures (from 137.5 to 8.0 billion cubic feet/month) through the year. Carter's average monthly figures do not consider diversions of water within the basin or the release of stored water from the reservoirs—both factors of increasing importance in recent years.

The present study of the influence of the climatic water balance on conditions in the estuarine environment seeks to elaborate on the earlier study by Carter by evaluating the changing pattern of monthly and annual volumes of water exchange between the Delaware estuary and the ocean with time over the past 20 years.

These values will then be related to salinity and other water quality measures in the estuary in an effort to predict estuarine conditions of importance to shellfish growth from climatic information. Two substudies are also to be undertaken—the first is concerned with the actual change in volume of runoff from subbasins of the Delaware with increasing modification of the environment (changing land use, urbanization, farm abandonment, tree cutting or reforestation) and the second with the change in water quality with increased industrialization, urbanization, and changing farming and conservation practices.

The present study only began in July of this year (1969) and so it is too early to report on significant achievements. The approach however, offers promise of providing estimates of important conditions within the estuary from parameters measured routinely at nearby shore stations.

LITERATURE CITED

CARTER, D. B., 1958. The average water balance of the Delaware basin. *Pub. in Climatology*, Lab. of Climatology, XI (3), 249-302.

THORNTHWAITE, C. W., 1948. An approach toward a rational classification of climate. *Geogr. Rev.*, XXXVIII (1), 55-94.

THORNTHWAITE, C. W., AND J. R. MATHER, 1955. The water balance. *Pub. in Climatology*, Lab. of Climatology, VIII (1), 1-104.

CLIMATIC AND ECOLOGICAL SETTINGS FOR GROWING SHELLFISH*

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Climatic Conditions and Biotic Adaptations

Most populations of commercial shellfish live in estuaries. Estuaries are areas where fresh and salt waters mix. Salinities and temperatures are usually the greatest variables and tend to determine the severity of estuaries as habitats for organisms. Relatively low diversities of species but large populations of a few species indicate relatively stringent environments. In view of present limitations of areas in the mid-Atlantic region where shellfish are grown (disease, predator, pollution, seed-supply problems, e.g.), the few successful species could be called fugitives, that is, capable of meeting environmental fluctuations more successfully than biotic competition. Yet in Chesapeake Bay oyster reefs up to 30 feet thick and continuous shell accumulations through 10,000 to 20,000 years attest the hardiness and tenacity of estuarine species. Before man's intervention they were very successful fugitives!

On the mid-Atlantic coast, the rigorous physical parameters include temperatures ranging from 0° C to 30° C and salinities varying 10 to 15 ‰ annually and up to 5 ‰ in one tidal cycle. These regular changes are augmented by the rampages of nature called droughts, flash floods and hurricanes. Virginia has just experienced (August and September 1969) extensive oyster kills from hurricane Camille. In summer, huge additions of freshwater are followed by complicated anaerobic conditions. These ensue from excessive inputs of organic matter and nutrients, and from stratification induced by heat and freshwater. The wide fluctuations in quality and quantity of planktonic food caused by these cyclic and catastrophic physical changes are recognized but poorly understood. The results are adjusted to painfully by oystermen when oysters are in poor condition in Virginia rivers but plump in the Potomac River and some of Maryland's waters as occurred in the 1968–69 season.

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Hydroclimographs representing estuaries on east and west coasts of North America are compared in Figure 1. The Willapa Bay polygon is from Hedgepeth (1951). Notable in the polygon for Gloucester Point on the York River are the predominances of low and high temperatures outside the range considered favorable for shellfish growth and conditioning for market. Five months exhibit means above 20° C and four months are below 10° C. Only three months of the year are truly favorable for shellfish activity. The Gloucester Point data are ten-year monthly means from 1953 through 1962. Annual and daily fluctuations are much greater than the ten-year means and these extremes must be endured by shellfish. Fluctuations of seasonal salinities typically exceed twice the range shown in Figure 1 and daily variations are much greater than monthly means. The lowest monthly means during the ten-year period were 14 ‰ (April and May) whereas the highest were 24 ‰ (October and November).

The coastal regions of the continents, where estuarine shellfish are grown, exhibit significant climatic differences. The western shores of the continents have prevailing on-shore winds from the Atlantic and Pacific oceans, hence, have oceanic climates; these greatly moderate temperatures of the coastal waters, both in warm and cold seasons. The eastern shores with weather fronts crossing large land masses have much more drastic seasonal changes and they are controlled by continental

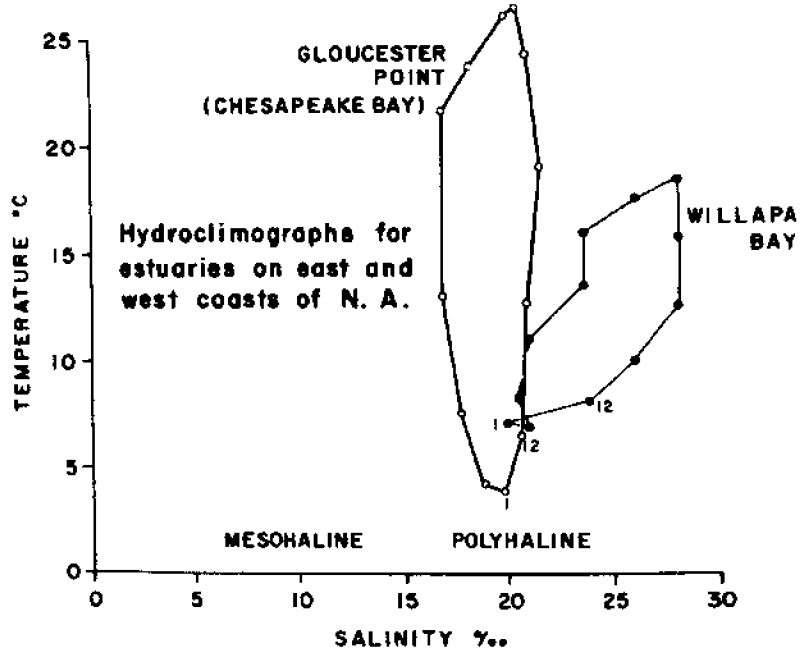


FIGURE 1. A comparison of East and West coast estuarine environments where shellfish are grown. The data for Gloucester Point are ten-year monthly means from 1953 through 1962. The Willapa Bay polygon is from Hedgepeth, 1951.

climates. The warm oceanic currents that impinge on European and Western North American shores limit annual ranges of monthly mean temperatures to about 10°C whereas the range along Eastern Asian and North American shores is about 20°C or more. Furthermore, these oceanic currents turn southward along the western continental shores, causing upwelling of nutrient-rich ocean waters. The eastern shores must depend largely upon runoff, mixing and recycling for nutrient supply, hence the vagaries of food supply become a problem.

The distributions of introduced commercial species of shellfish reveal the climatic adaptations of some estuarine bivalves. Many important commercial species (and predators) from rigorous eastern shores of continents have become well established on milder western coasts. The Pacific oyster, *Crassostrea gigas*, has long been imported as seed from Japan to our West Coast and in some areas reproduces naturally. The Manila clam from Japan is very abundant intertidally. Three of the most important commercial bivalves of eastern North America have been introduced to the West Coast; the mannose or soft-shell clam, *Mya arenaria;* the quahog or hard clam, *Mercenaria mercenaria;* and the eastern oyster, *Crassostrea virginica*. The first two are more adapted to cold waters, and hence have been more successful in breeding and spreading in the cool summer waters of the West Coast.

Introductions of shellfish and associated pests from eastern North America to Europe are numerous and an extensive literature exists. All of the commercial bivalves just listed have been imported to Europe—the latest being Pacific oysters to France. In contrast, few species from coasts with maritime climates have been successful in becoming established on shores with continental climates. The European flat oyster, *Ostrea edulis*, is precariously established in Maine waters with very limited breeding; it does not withstand hot summers in the mid-Atlantic region. On the ocean-cooled western coasts, the failure of species from continental climates to spawn in summer in many seasons and places is an advantage in marketing well-conditioned shellfish throughout the year. On these coasts, the shellfish culturist is provided with ready spawning stock for hatchery use and laboratory tests such as bioassays with larvae.

The ranges and distributions of several native commercial species on the rigorous eastern North American coast indicate the hardiness of these shellfish. The eastern oyster belongs to a family usually considered to be subtropical in preferences but it survives considerable freezing intertidally in the mid-Atlantic region and, where submerged, tolerates long cold winters as far north as the Gulf of St. Lawrence. The soft-shell or long-neck clam tolerates very cold waters, and is somewhat protected in burrows. The oyster is commercially important from Canada to Mexico and *Mya* ranges south to Chesapeake Bay in great abundance. Both these species withstand very low salinities, in part by a period of dormancy during cold winters and spring freshets. The hard clam ranges all along the western Atlantic coast with the center of abundance in the mid-Atlantic estuaries. *Mercenaria* has evolved into distinct species in the northern and the southern parts of its range, and there is firm evidence of geographic clines and races of oysters; probably localized races exist in other shellfish.

Where wide geographic ranges are accompanied by high tolerances for low salinities, shellfish may find refuge from predators, diseases and competitors. Low-

salinity sanctuaries have permitted populations of oysters and soft-shell clams in Chesapeake Bay to escape decimation by MSX *(Minchinia nelsoni)* and blue crabs respectively. Hard clams require salinities about half those of seawater, hence are confined to the lower half of Chesapeake Bay. Small hard clams less than an inch in length are scarce because the species is confined to areas of heavy predation. Shelly oyster beds provide the best habitats for survival of young clams, and most are harvested in such places. The commercial catch consists largely of old clams, probably mostly exceeding ten years, hence sampling gives a distorted picture of growth potential for clams. In the Long Island area, regular recruitment provides small clams, and favorable growth rates of young clams may be observed. The Bay scallop, *Aequipecten irradians,* has a range from Massachusetts to the Carolinas but requires polyhaline waters. Hence, this desirable estuarine species did not withstand the multifold pressures of man's harvesting, predators, and reduction of its favorite habitat—eel grass beds. Two species of mussels offer potential fisheries but are not used much in North America. *Mytilus edulis* is a northern species with its greatest potential in New England. It does not usually withstand summer temperatures in Chesapeake Bay and also is confined to high salinities in warm regions. The ribbed mussel, *Modiolus demissus,* is an intertidal species with wide salinity and temperature tolerances; it was once used as a source of vitamin D.

Two basic types of coastal waters are widely utilized to rear shellfish—lagoons and true estuaries or drowned river valleys. The characteristics of western Atlantic coast examples are most familiar to us. Shallow lagoons formed by barrier beaches and sea islands tend to exhibit low freshwater runoff, high salinities, hence relative stability and relatively high diversity of biotic communities. Lagoons often favor high spatfalls but low survival, and are not easily managed for shellfish culture. Biotic problems often prevail over physical ones. Drowned river valleys, on the other hand, such as Chesapeake and Delaware Bays, provide strong physical gradients, particularly of salinities, modified by seasonal freshwater runoff from their extensive drainage basins. Mixing, by tides as well as winds, recycles nutrients brought in by freshwater from the land. Fauna and flora tend to become impoverished in species as salinities decline, and fluctuating physical parameters and nutrient supplies cause rapid successions of plankton organisms. In this type of estuary, adaptations to survive and grow in extreme physical conditions permit a few tolerant species to thrive without strong biotic competition. Topography makes generalizations about tidal activity in the two types of estuaries impossible, and fertility may depend upon factors other than runoff and mixing, such as extensiveness of marshes around coastal lagoons. Although clearly rich in nutrients and highly productive compared to the ocean, the food webs for transferring energy are complex, wasteful, and extremely variable in estuaries. Hence, it is difficult to manage nature's estuarine "gardens" to produce crops of usable sizes and kinds of organisms.

Estuaries can be quite inhospitable to shellfish because of bottom conditions. Instability in terms of shifting sand, soft muds and inadequate cultch often limit the areas that can be inhabited. Silting and sanding from seasonal storms have important effects, particularly in winter and especially on small young shellfish. Physical removal of *Mya* and oysters from their beds to the shore by heavy winter winds

is not uncommon, and hurricane storms move whole beds. The bottom is the natural home of most shellfish but currents are slow and silt and detritus are excessive in abundance for these filter feeders to thrive there. It is widely recognized that better growth and yields are obtained in suspended or off-bottom stocks. The use of three-dimensional culture has tremendous advantages. However, our estuaries are wide and shallow and exposed, hence unless submerged structures can be engineered, suspended culture will likely be restricted to creeks, ponds and sheltered areas. As Ryther (1969) points out, concentrated raft culture depends upon natural food from much larger areas of primary production. Hence, the problem narrows to one of finding suitably protected natural areas where currents and physical parameters permit culture of dense populations.

Prospects of Manipulating Environment for Artificial Culture

The descriptions of estuarine environments indicate what coastal shellfish must endure, but what do they prefer? Their hardiness and prolific traits enable them to persist in repopulating environments inhabited by predators, diseases, competitors and subject to drastic and rapid physical changes. They are both plastic and tenacious.

Intensive culture implies control of biotic and physical factors of a shellfish-dominated ecosystem. Optimal conditions would vary for each stage of culture and they are poorly known as yet. Fluctuations of nutrients and food supply must be minimized. As primary consumers, shellfish are in a favorable trophic position to encourage food-enhancing practices such as fertilization, inoculation of impounded waters, and addition of food. These are already common practices in rearing shrimp and fish in artificial enclosures. Ryther (1969) has reviewed the potential for production of shellfish in estuaries and warned against excessive optimism based on high yields of raft cultures in Japan and Spain.

The life cycle of bivalve mollusks may be divided conveniently into four stages for management purposes. The pelagic larval stage, lasting some 10 to 15 days in nature, is the most wasteful period and the least controllable in estuarine waters. Rapid strides in hatchery techniques now permit almost unlimited numbers of larvae to be grown rapidly to setting (oysters in 8 days). By controlling environments, dense cultures are reared by a variety of cultured and natural food sources. The list of places over the earth where hatcheries are in operation is growing rapidly and indicates that this phase of artificial shellfish culture has a firm empirical base.

Food and disease problems are accentuated in hatcheries but it has been shown that oyster larvae can be bred in a wide range of salinities (10 to 12 ‰ and up) and temperatures (18 to 30° C) comparable to those found where natural reproduction occurs.

The second phase of shellfish culture may be described as the nursery period. Small shellfish have many enemies, for their size permits crabs, snails, starfish, flatworms and fish, to name a few, to devour them in quantities. Often young-of-the-year predators are ready to take their toll as soon as shellfish spat are settled. Furthermore, storms moving silt and sand frequently smother large numbers. Observations of commercial plantings indicate that the nursery stage is virtually ig-

nored and the waste of seed (oysters, particularly) is prodigious. Although shellfish growers prefer large seed, and in crowded seed areas this usually means prolonged slow growth, they readily accept losses up to 90 percent with no effort to avoid predators and smothering. For example, seed oysters from the James River contain several year classes of which the spat and yearlings often comprise a high percentage of the bushel count. In practice most of these younger oysters are lost by planting on soft bottoms or predator-infested beds. *Mya* regularly produces heavy spatfalls annually in lower Chesapeake Bay, yet mature clams are mostly limited to intertidal zones. No culture is involved and no survival is the usual result. The regularity and intensity of spatfall of *Mercenaria* is much less clear. The scarcity of small clams is attributed to blue crab predation. Plantings of one-inch clams in sandy bottoms revealed rapid and heavy losses. In mesh-lined trays, seed clams survived exceptionally well. Recently, planting hard clams on shelled beds has been advocated to increase survival, and the Maryland hydraulic escalator clam harvester is just the rig to bring buried shell to the surface.

Suspension culture has long been practiced in Japan and other countries to avoid losses in the nursery period. Considerable experimentation has been done on the east North American coast to explore the problems and costs of rafting and suspension of strings, bags and trays. A major problem has always been serious fouling by quick growing pests such as tunicates, sponges, tube worms and barnacles. The best control appears to be a system of regular exposure to air and sun for drying. This is not easy to accomplish without losses on a coast with freezing winters and very hot summers. Intertidal exposure in the maritime climate of the West Coast is much more feasible.

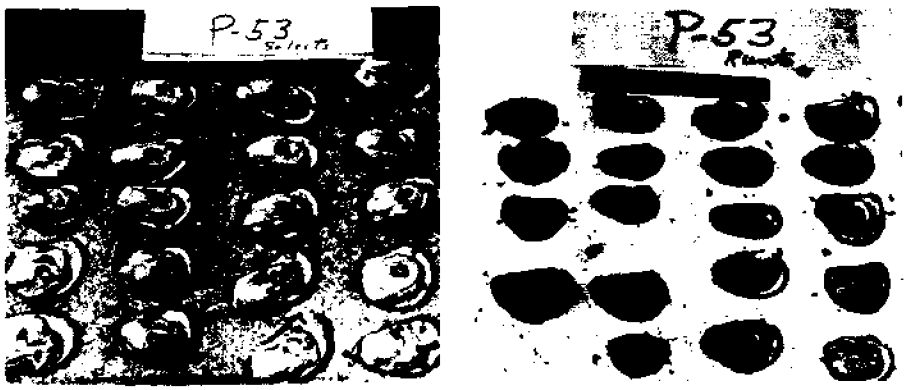
The recent innovation of free or cultchless oyster spat, thus relieving hatcheries of the cultch-cleaning job, creates new and formidable problems in nursery practices. The free spat are denied the protection of cultch in their early life and must be grown to sizes of one inch or larger before excessive losses from predation, silting and tidal movements can be prevented. Little information is available on present practices of those hatcheries using free spat. It appears that traying, with its attendant costs and fouling problems, may be necessary. Heavy concentrations of young oysters may increase disease and metabolic waste problems. Ponds and protected creeks, also sheds and tanks to which water is pumped have been tried as nurseries. Most nursery operations appear to obtain good survival (hard clams, oysters, and scallops) but growth that is inferior to that experienced in nature. Usually the culturist has little basis for judging growth except by comparing with shellfish grown in natural waters, and the variability in nature has already been emphasized. Probably those oyster fisheries already adjusted to some type of suspension culture will find no use for free spat. They already have most of the advantages of off-the-bottom culture including fast growth, three-dimensional culture, freedom from nonswimming predators and rapid turnover. However, most other shellfish in artificial culture are confronted with the same initial problems of free spat that must be protected, hence nursery practices need much more exploration.

The third and fourth stages of culture are primarily concerned with growth and conditioning of shellfish for market. These phases of culture are perhaps least amenable to artificial manipulation, and tend to overlap in their requirements.

Shellfish planted as seed have reached a size that requires much space and quantities of food. Suspension culture alleviates both problems by utilization of tidal waters relatively free of silt and suspended detritus and by avoidance of the slow currents associated with bottom friction. Temperature changes and seasonal successions of plankton organisms result in sporadic growth in temperate zones; hence the period of growth to marketable size varies from one to many years in most species. Along the western Atlantic coast, growth may be interrupted in winter for about three months in Chesapeake Bay and as much as six months at the northern end of ranges (e.g., oysters). Furthermore, growth is often slowed by too-warm waters in summer and by long periods when energy is dissipated in reproduction. Stabilizing temperatures at suitable levels for each species (about 20° C for oysters) would moderate most growth problems.

Predator problems tend to decline as shellfish become larger and disease problems become more important. Off-bottom culture accentuates these trends particularly where shellfish are grown densely on rafts or racks. Any delay in marketing caused by slow growth or failure to store glycogen and yield plump meats is costly. Competitors for space and food quickly become intolerable in suspended stocks if provisions for air exposure or reduced salinities are not available. The fungus *Dermocystidium* must be avoided in warm waters by isolation or removal to low-salinity waters. It is highly contagious in clumped populations. The protozoan disease caused by MSX, *Minchinia nelsoni*, can be minimized by choosing genetic and exposed seed stocks with resistance capacities. The effects of MSX on growth and glycogen storage in susceptible oysters is shown dramatically in Fig. 2. This population from the 1968 yearclass grown from free spat had a mortality of 61 percent from June through September 1969. Yet, samples of 25 oysters selected by size from survivors as stunted (sick) and healthy animals revealed large differences in size, condition of meats, and prevalences of MSX on 7 October 1969.

FIGURE 2. Susceptible progeny from Long Island stock, set in California hatchery. At left, large oysters, selected before opening by size, are creamy white with glycogen whereas the "runts" are dark and thin, and obviously many were sick. Note that the runts appear larger than they really are because of a 25% photographic enlargement.



The Sea-Rac operation at Queens Creek in the York River in the late 1930's illustrated the importance of rapid growth and marketing of shellfish (Evans, 1943). Half-grown Long Island oysters were placed in trays in the spring and marketed the following fall and winter. These Sea-Rac trays were placed at a level to obtain air exposure during most low tides for handling and for control of fouling. Unfortunately, hard freezing killed oysters exposed by persistent northwest winds in 1942, following their removal to Week's Creek in the Rappahannock River to escape military pollution in the York River (Fig. 3). This Sea-Rac oyster farm was no small trial, for 11,000 trays holding about a bushel each were suspended on three miles of creosoted sills. In Australia similar low intertidal racks of oysters on sticks must be sprayed with water during certain periods of low tides and hot weather to avoid summer kills.

The shellfish mariculturist must obtain fast growth, good survival and rapid turnover to compete economically with cheaper wild stocks grown on the bottom with little attention. Often potential growth rates are not appreciated because in most areas over-age wild stock is harvested. Furthermore, seasonal variations are so great that prime growth is not attained or appreciated. Wide variations in growth of wild stocks with many runts suggest that genetic selection of breeding stocks for hatcheries offers much promise. Often shellfish are held an extra year or more to attain some traditional size for the consumer. Fortunately, hard and soft clams are

FIGURE 3. A view of the Sea-Rac operation of the Chesapeake Corporation of Virginia in Week's Creek, Rappahannock River, Virginia. (Photo by William Booth)

considered prime at sizes small enough to permit harvesting before growth has become excessively slow. Geometric growth rate declines very rapidly with size. Simple linear measurements are not precise enough to show this decline clearly. Volume and weight may reflect ecotype variations; hence culturists have no easy measures or standards of growth. The usual size measurements require periods of one to several months to indicate growth rates during which the suitability of the environment is unknown. The Havinga method of weighing oysters under water for shell deposition is sensitive for short periods (Andrews, 1961), and other metabolic measures should be developed for other species.

Rapid tidal currents usually favor shellfish growth according to observations on planted oysters, but if food is abundant, equally good growth can be attained in ponds with little current and low tidal exchange. An artificial one-acre pond near VIMS, used for a nursery area, regularly produces tray oysters superior in condition to those in adjacent open waters. Yet this pond, with only a one-foot culvert for exchange of water, in about four acre feet of pond volume, has only about one foot of tidal range (mean in adjoining river is 30 inches), and occasionally it stratifies enough to induce anaerobic conditions in the deeper parts. Obviously, limited mixing and exchange of water still permit localized phytoplankton production to grow and "fatten" oysters. The shallow claires of France are well known for their conditioning of oysters. Programs for artificial propagation will probably augment nutrients and food supply rather than try to duplicate nature's tidal flows.

It is perhaps significant that the few common bivalves of commercial importance are essentially suspension filter feeders although they undoubtedly use detritus and organic materials ingested incidentally. Possibly, only by feeding on small primary producers with rapid turnover rates can the dense populations often found in nature be sustained. Competition is intensive and survival rates low but tidal movement of food permits high production from small acreages. This is dramatically demonstrated in Japan and western Europe where three-dimensional culture is utilized. It is interesting that in Chesapeake Bay one example each of the three evolutionary types of bivalve mollusks occurs as a commercial species. The hard clam with a hatchet-shaped foot for ploughing through the bottom is nearest the basic type, whereas oysters are sessile, and *Mya* represents the deep fixed-burrow group with long siphons. Yet all shellfish have a common preference for small phytoplankton that has led to much culturing of microscopic algae. Successful use of carbohydrate supplements and comminuted dried algae experimentally suggests that growing phytoplankton may not be the only way of providing adequate nutrition. Programs to use supplements grown on land or sea probably have as their inspiration poultry and livestock feeding operations. It would be unfortunate to turn away from the enormous potential and high efficiency of phytoplankton production freely distributed by tides that Ryther describes.

Artificial Propagation in Modified Environments

One must not underestimate the effect of man's technology on estuarine environments. Population growth resulting in demand for water and power promises rapid alterations in our shellfish growing areas. Even the oceans no longer seem

safe from harmful and irrevocable changes. Diversion and storage of water on scales that make present Delaware River problems seem trivial are being proposed frequently. Each catastrophe such as Camille's deluge in Virginia brings demands that the rivers be "controlled" with dams. A whole series is now being planned for Chesapeake Bay. It is improbable that any set of impoundments would have prevented Camille's floods in Virginia because a very rainy season would have already filled all reservoirs.

It is possible that we may learn how to utilize man's "tamed" rivers in the sequences of artificial propagation of shellfish. Regulated flows could conceivably be used to manipulate nutrients and phytoplankton populations more advantageously. Concurrent control of predators, diseases and competitors would be essential, for our hardy shellfish species depend heavily upon seasonal physical extremes to limit these biotic factors. It would be relatively easy to mix and oxygenate ponds and shallow impounded bays wherein fertilization tends to produce organic matter exceeding the carrying capacity.

An example of planning to utilize stored water for manipulation of physical and biotic characteristics of an estuarine tributary is the Salem Church reservoir proposed for the Rappahannock River. This river has a salinity regime in the oyster-growing sector that favors oysters but is marginal for several important pests. In wet years oyster drills and oyster diseases are inhibited in activity or eliminated. Planned water releases in wet years only could conceivably control or eliminate drills without harming oysters. Moderate spat-falls now lost to predation could greatly increase production of oysters in the river. An important factor is the location of this river in that intermediate mixing zone between ocean and freshwater river where the greatest seasonal fluctuations of salinities occur normally (15 to 18 ‰ late summer values).

The estuaries and lagoons of the western Atlantic coast exhibit extremely wide seasonal variations of temperatures and salinities, hence it seems unlikely that large natural areas will be moderated for efficient mariculture by man. Hatcheries, ponds, plastic-covered areas utilizing the greenhouse effect, and power-plant heated impoundments may contribute to production of larval and early seed stages. Beyond these stages, all shellfish are now grown in natural waters with climatic limitations applicable. However, shellfish culture in this country seems much too localized in all stages of growing and marketing. Each culturist seems content to live with the advantages and problems of his confined area. Cultural practices in Europe and western North America appear much more flexible with certain regions used as seed areas and others for growth and fattening. Transplanting frequently and for long distances is commonplace.

If the environment cannot be modified feasibly, it seems appropriate that shellfish should be manipulated into the best habitats according to season and objective. Estuarine shellfish are seasonally stressed by physical extremes of freshwater and low oxygens in wet years, and by predators, diseases and competitors that accompany high-salinities in periods of drought. Prolonged spawning and waste of excessive seed supplies are common south of Chesapeake Bay; large bodies of northerly waters are excellent "finishing" areas but are lacking adequate recruitment of shellfish. Upper Chesapeake Bay contains thousands of acres of excel-

lent shellfish beds that are barren, particularly in the Potomac River and in Maryland. Delaware Bay and the sounds of Long Island north to Massachusetts are not fully used. If shellfish producers can haul oysters in the shell from Louisiana to Chesapeake Bay to shuck, would it not also be feasible to move oysters north for conditioning before harvesting? The fear of introduction of predators and diseases seems to concern biologists more than industry members, judging by their respective activities. The lack of efficient harvesting gear to minimize losses from transplanting surely is a poor excuse in this technological society. The problem is real in Chesapeake Bay but can be surmounted. Should each shellfish producer be concerned with the product from setting to delivery to the consumer? Can each producer master the ecology of culture and the economics of marketing? By specialization each stage of production could be conducted intensively and on a large scale in the areas of most suitable environments, and a superior product sold to consumers. The problem of southern seed not surviving in northern climates can now be resolved by growing northern strains in hatcheries in Florida, for example. Furthermore, most shellfish reach excellent marketing condition in periods as short as a few weeks, given favorable habitats.

Modification of environments for rearing early stages of shellfish seems well within reach but it must be followed by reorganization of later cultural procedures to reap the full benefits of artificial propagation. Natural conditions are seldom "right" for long in any shellfish growing area along our coast.

LITERATURE CITED

ANDREWS, J. D., 1961. Measurement of shell growth in oysters by weighing in water. *Proc. Natl. Shellfish Assoc.* 52: 1–11.

EVANS, G. L., 1943. Story of the Sea-Rac. *The Commonwealth, the magazine of Virginia*. 10(8): 10–12.

RYTHER, J. H., 1969. The potential of the estuary for shellfish production. *Proc. Natl. Shellfish Assoc.* 59(1968): 18–22.

Introduction to
FEASIBILITY OF OYSTER HATCHERIES IN THE DELAWARE BAY REGION

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Our interest in hatchery work was stimulated by a collapse of the oyster industry in Delaware caused by MSX. Oyster landings fell from a high in 1954 of 4,340,000 lbs. worth $2.75 million to about 34,000 lbs. worth $28,000 by 1965. Thus, today the dockside landings and, indeed, the oyster industry of Delaware are worth approximately one percent of their value just 15 years ago.

The University of Delaware's approach to the rehabilitation of our state's industry has been: 1) to select brood stock from survivors of the MSX epidemic which may be disease resistant, 2) to produce progeny from these stock by means of artificial culture methods, and 3) to practice selective breeding by testing the progeny for disease resistance, fast growth and good market qualities. We are still in the early stages of this effort.

We developed our initial hatchery design with the help of Mr. Philip Campbell and Mr. George Vanderborgh, presently of Long Island Oyster Farms. We employ the natural algae feeding method, begun by Wells in 1920. Most of our hatchery environmental control consists of water temperature regulation and seiving or straining the natural water to reduce competition by wild zooplankton for food and setting space. We have been reasonably successful in producing spat with this method.

However, it is quite obvious to us, as it is to most workers in this field, that the natural feeding and growing method is at times highly undependable due to the vagaries of water quality and plankton populations. Therefore, it is our ultimate goal to develop rearing techniques for oysters utilizing a completely controlled culture environment including regulation of salinity, temperature, oxygen levels, oyster foods, contaminating biota including human and oyster pathogens, and waste products of the oyster, to name a few of the major considerations.

We have unquestionably set our sights high and the achievement of the ultimate goal will depend on how well we do our job of creating and refining oyster culture and processing techniques that are economically feasible.

The pursuit of our goal of factory-produced oysters will undoubtedly give rise to considerable "spin-off" that can be applied to the advantage of conventional

oyster farming, e.g., new domesticated strains of oysters possessing especially desirable characteristics, more efficient types of cultch, better ways of utilizing the natural water column for growing oysters, new methods of disease and predator control, more efficient handling, shucking and processing methods, and the development of stronger lines of communication between scientists, industry, and the consumer through marine extension programs.

THE FEASIBILITY OF OYSTER HATCHERIES IN THE DELAWARE-CHESAPEAKE BAY REGION*

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The question to be examined: "Are oyster hatcheries now, or will they ever be, commercially feasible in the Delaware-Chesapeake Bay region?"

Before looking at this we should examine the history of shellfish hatcheries in this region. Loosanoff yesterday gave an excellent review of worldwide hatchery activity including some remarks on persons who have developed methods in the Delaware-Chesapeake region. Notable in my literature search was first, Professor W. K. Brooks of Johns Hopkins University who in the late 1800's demonstrated that eggs could be taken from the female oyster and developed to free-swimming veliger larvae in the laboratory (Brooks, 1879). Brooks' student, Dr. Julius Nelson, recognized the potential of hatcheries for commercial seed production and tried to develop larval rearing methods in southern New Jersey from about 1889 to 1910 without success (Nelson, 1889; 1909). The failure was due to lack of knowledge of the complete larval life history. After 1910, there apparently was little hatchery effort here until the first MSX (*Minchinia nelsoni*) oyster kills in 1957–58. Of course, you are familiar with the other significant advances in the Long Island area, that of Wells and Glancy in the 1920's with larval feeding by natural algae (Wells, 1920) and that of Loosanoff and Davis in the 1940's using a cultured algal system (Loosanoff and Davis, 1963).

With the MSX oyster epizootic in this region, there followed a request for federal funding for research by the states of New Jersey, Delaware, Maryland and Virginia (Fig. 1) to attempt to rehabilitate the industry. These efforts have been funded throughout the 1960's by Federal PL 87-580 and PL 88-309. The research has involved the development of oyster hatchery techniques to investigate mechanisms of oyster resistance to the disease and as a possible rehabilitation measure. This effort has been an ideal proving ground for the various northern oyster rearing techniques in the Chesapeake area.

*CBL Contribution No. 396

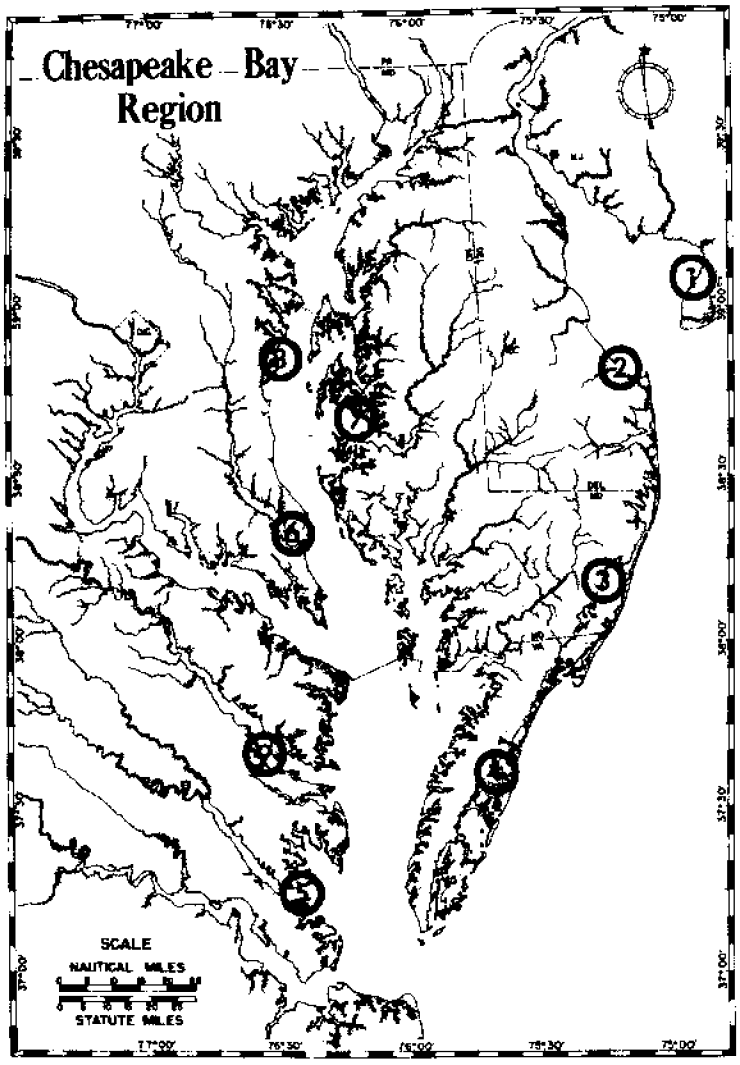

FIGURE 1. Laboratories and shellfish hatcheries in the Delaware-Chesapeake Bay region that have participated in "MSX" oyster research and/or hatchery development. No. 1—Rutgers, The State University, New Jersey Oyster Research Laboratory on Delaware Bay (NJORL); No. 2—University of Delaware Marine Laboratory at Lewes (UD); No. 3—Snow Hill Field Station of the Natural Resources Institute, University of Maryland on Chincoteague Bay (activities of this Station transferred to Solomons, Maryland); No. 4—Wachapreague Field Station of the Virginia Institute of Marine Science; No. 5—Virginia Institute of Marine Science at Gloucester Point, Virginia (VIMS); No. 6—Chesapeake Biological Laboratory of the University of Maryland at Solomons, Maryland (CBL); No. 7—U.S. Bureau of Commercial Fisheries Laboratory at Oxford, Maryland; No. 8—Frank Wilde private hatchery site on the West River at Shadyside, Maryland; No. 9—The Windmill Point Oyster Company (EDA affiliated) at Urbanna, Virginia.

A recent trend has been toward oyster hatcheries for commercial seed production in this area. This has been pursued by:

1. The University of Delaware (UDML) on Delaware Bay at Lewes in conjunction with Sea Grant funding (Fig. 1, No. 2).
2. The Chesapeake Biological Laboratory (CBL) at Solomons on the Chesapeake (Fig. 1, No. 6) and at Public Landing on Chincoteague Bay (Fig. 1, No. 3) has been determining the biological feasibility of hatcheries on both high (30–34 ‰) and low (10–20 ‰) salinity areas of the region. In addition, in the past two years CBL has cooperated with a private hatcheryman, Mr. Frank Wilde of the West River (Fig. 1, No. 8), attempting to use all available information to evolve the most workable commercial hatchery system for the area.
3. The Virginia Institute of Marine Sciences (VIMS) at Gloucester Point (Fig. 1, No. 5) has recognized the possible future role of hatcheries and has done research particularly on cultchless setting techniques.
4. The Windmill Point Oyster Company at Urbana, Virginia (Fig. 1, No. 9) represents several commercial oyster companies of the region who have cooperated with the U.S. Department of Commerce in establishing a pilot hatchery facility that has operated since 1965.

In speaking of the feasibility of commercial hatcheries in this region, I will borrow a theoretical framework developed by an anonymous author in the December 1968 *Potomac Newsletter** with the tital: "The Oyster Producing Potential of the Potomac Estuary." In discussing the potential of the river, the author stated that there were three types of factors—biological, economic, and finally political-sociological—that set successively lower limits on potential oyster production. For example, speaking of the biological limit, in Japan (Hiroshima Bay) using rafting techniques, a production of 20 metric tons per acre has been reported. At this level, the historic high of Potomac production could be matched in less than 1/2 square mile of area. The 1967–68 production in Maryland waters, where there are over 500 square miles of oyster bottom, could be matched in less than a square mile. But limits on Potomac production are governed at successively lower levels first by economic and finally by political-sociological factors. In speaking of the feasibility of shellfish hatcheries in the Delaware-Chesapeake region, I would like to draw a rough parallel and speak first of biological, then economic, and finally political-sociological feasibility.

BIOLOGICAL FEASIBILITY

The first consideration in hatcheries is, of course, biological feasibility. Will biological systems work? Just because one can spawn oysters and raise larvae in one area (Long Island Sound) doesn't necessarily mean that it will be possible in another area such as Chesapeake Bay. The experience, however, of the laboratories in the MSX program over the past 10 years has given us insight into biological feasibility of systems. I will speak of all the systems necessary to a hatchery: conditioning and spawning, larval rearing, setting, and spat rearing. Experience of the

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different laboratories in the MSX program will be summarized (From PL 88-309 Progress Reports) and some conclusions drawn.

Conditioning and Spawning

Earlier work has indicated that Chesapeake oysters may be a different physiological race than northern oysters, and thus, probably more difficult to condition and spawn. The theory was proposed by Stauber (1950), who noted that oysters throughout their range from Canada to the Gulf of Mexico spawned at similar times during the year, despite the fact that widely different temperatures prevailed. Loosanoff and Nomejko (1951) then brought spat to Milford from five sites on the coast from Wareham, Massachusetts to the James River, Virginia. After holding the groups two years, Loosanoff tried to spawn the oysters. He met with failure in the southern groups despite the fact that they carried a thickness of as much as 2.0 mm of unspawned gonad. Thus, it appeared that different temperature races were present geographically and that there might be difficulty in applying northern conditioning and spawning techniques to, say, the Chesapeake area oysters.

The 88-309 MSX projects have given us much insight into the conditioning and spawnability of Chesapeake-Delaware Bay stocks. Summaries of results from 88-309 progress reports are as follows: The New Jersey Oyster Research Laboratory (NJORL) at Cape May has conditioned and spawned Delaware and Chesapeake oysters since 1962. They have relied on an in-season program from May to August, with little attempt at winter and early spring conditioning. Oysters have been brought to, and held in, spawning condition in running-water laboratory tanks held below 24° C. There has been little difficulty in spawning properly conditioned Delaware-Chesapeake stocks. However, spawning baths of heated, running seawater have been more successful than the heated, standing spawning baths used by the Bureau of Commercial Fisheries at Milford (Haskin, 1964, 1965, 1966, 1967).

The University of Delaware (UDML) at Lewes has been successful with in-season spawning of Delaware Bay stocks (Ritchie, 1964, 1966). They have recently been investigating off-season conditioning regimes to lengthen the spawning season. Maurer and Price (1968) held potential spawners throughout the summer months into the fall and winter by taking Delaware Bay oysters from the Bay in the spring, when it was below 15° C, and holding at 15° C throughout the summer. Secondary conditioning regimes of 20° C then allowed spawning that fall and winter.

The Virginia Institute of Marine Sciences (VIMS) at Gloucester Point was originally successful in spawning Chesapeake oysters in May and June of 1964. However, since that time they have obtained gametes by stripping rather than by a stimulation of natural spawning. Also, there has been difficulty in winter and early spring conditioning. Andrews stated that it takes up to six weeks to condition a winter Chesapeake oyster, regardless of treatment (Andrews, 1964a, 1964b, 1965, 1966, 1967, 1968a, 1968b).

The Chesapeake Biological Laboratory (CBL) at Solomons, Maryland has investigated conditioning and spawning regimes for Chesapeake oysters (1968,

1969) in moderate to low salinity (10–20 ‰) Chesapeake areas (Hidu et al., 1969). The trials, of rather broad spectrum, have identified potential problem areas for later research. Conditioning was begun in February of each year by holding several Chesapeake stocks at 24° C. When ambient temperatures rose over 22° C, running seawater was refrigerated to below 20° C. Periodically, oysters under these conditions are subjected to spawning stimuli and spawning success noted.

From these trials and the experience of other laboratories in the Delaware-Chesapeake region, we have these tentative conclusions on conditioning and spawning of regional oyster stocks:

1. An early spring to early summer regime of 24° C for six weeks should produce a spawnable oyster. This is in contrast to U.S. Bureau of Commercial Fisheries (BCF) results on Long Island Sound (Loosanoff and Davis, 1963) where a two to four week regime at 24° C will produce a spawnable oyster. This substantiates differences in temperature requirements for oyster gonad production in these two regions.
2. Preseason spawning before March may be difficult or impossible without supplemental feeding. In February and March in each of two seasons at CBL for example, oysters placed at 24° C in running water have merely lost condition instead of increasing thickness in gonad layers. These results are similar to those experienced at VIMS. Additional biological work is needed to develop early season conditioning requirements.
3. Properly conditioned Chesapeake oysters are easily stimulated to spawn in the hatchery. Running water, 30° C baths, and stripped gamete addition are more satisfactory than the standing-water baths of Loosanoff and Davis (1963) at Milford, Connecticut.
4. Oysters should be held below 20° C to prevent spontaneous spawning during the summer months. It is not absolutely necessary to stop spontaneous spawning, however, since Chesapeake oysters appear to repeatedly build up additional gonad during the summer months. We have found spawnable oysters in the Bay on the October and November bar surveys.*

Larval Rearing

The MSX projects since 1958 have provided a real test of the applicability of northern rearing techniques in the Delaware-Chesapeake region. I speak especially of the cultured algal system of USBCF at Milford and the Wells-Glancy, Long Island natural algal feeding system. Summarizing the experiences of various laboratories and hatcheries in this area:

NJORL at Cape May began its efforts in 1962 with cultured algae, *Monochrysis lutheri, Isochrysis galbana,* and *Dunaliella euchlora.* In subsequent years they have utilized a natural algal system in the rearing of experimental stocks. The system has been quite simple; water is drawn freshly from the tidal flats at Cape May and merely passed through a 25-micron plankton mesh to remove most zooplankton. Water is changed daily, with the larvae held at 10 per ml, and temperatures at

*In cooperation with the Maryland Department of Chesapeake Bay Affairs fall bar survey of Mr. Harold Davis.

24° C. Pfizer "Combistrep" is added at 200 ppm. In five years of operation since 1964, more than 50 broods of larvae have been reared with inconsequential loss. (Haskin, 1964–67).

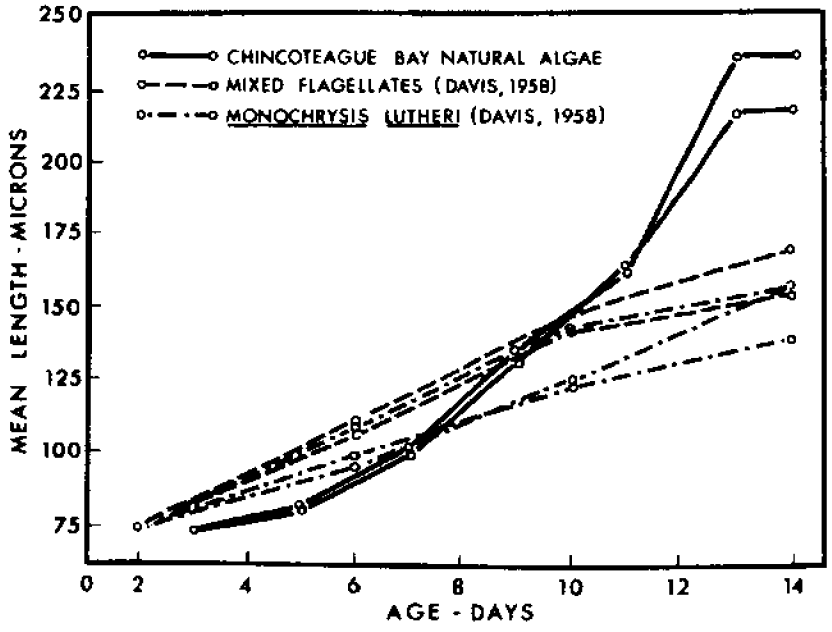
The University of Delaware at Lewes has utilized both cultured and natural algal systems. In recent years, they have had especially good luck with a relatively simple system of filtering natural water with an AFCO Filter Bag (5-10 μ) and greenhouse aging for a day (Ritchie, 1968).

VIMS at Gloucester Point and Wachapreague have reared their experimental MSX oysters by using cultured algae exclusively, (*Monochrysis lutheri, Dunaliella euchlora* and *Isochrysis galbana*). Although they have tried, significantly, they have been unable to utilize natural algal feeding at their Gloucester Point Station (Andrews, 1964-68).

The Chesapeake Biological Laboratory (Public Landing on Chincoteague Bay and Solomons on mid-Chesapeake Bay), in conjunction with potential Chesapeake hatchery operators, has extensively tested larval rearing methods by natural feeding. We began at Public Landing in the mid-1960's (Sprague et al., 1967). Then in 1968 and 1969 at our Solomons hatchery, we tested the applicability of natural feeding techniques throughout the season from February to September. The methods have been similar to those used by NJORL.

The Chincoteague larval rearing results of 1967 are compared with results received by Davis and Guillard (1958) using artificially cultured algae at Milford, Connecticut (Fig. 2). Davis' larval growth rates were quite constant through 12 days

FIGURE 2. Comparison of growth of oyster larvae receiving Chincoteague Bay natural food with growth rates obtained (Davis and Guillard, 1958) by feeding unialgal cultures of *Monochrysis lutheri* and a "mixed flagellate" diet. Culture temperature in each case was 24° C.



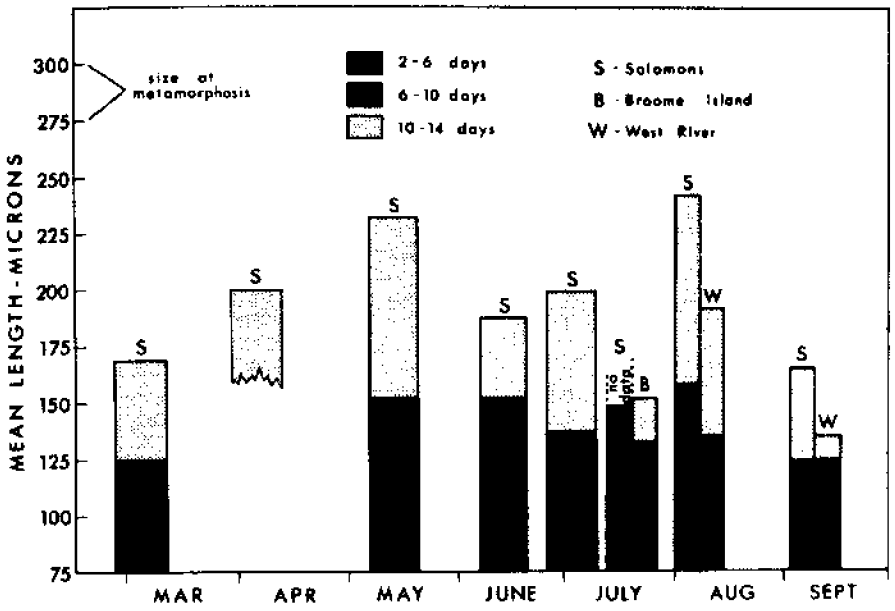


FIGURE 3. Growth to 14 days of several broods of oyster larvae reared by natural algal feeding in three low salinity Chesapeake locations from March to September 1968.

of feeding, probably in response to a single feeding rate (.01 ml packed cell volume/liter/day). Growth rates with natural algal feeding appear to be quite different. An initial lag period is followed by very rapid growth after larvae reach 100 μ in size. At Chincoteague, mean lengths of 225 to 250 μ were attained in 14 days at 24° C. The reasons for the very rapid growth in later larval stages are not clear, but perhaps the older larvae are able to utilize a greater variety of algal species present.

The 1968–69 low-salinity Chesapeake natural feeding trials produced similar results. Figure 3 shows our 1968 results at Solomons and at two other low salinity hatchery locations. Overall growth rates were excellent from the earliest trials in March to latest trials in September. Despite considerable qualitative and quantitative differences in plankton content of natural waters throughout the season, all trials produced very acceptable larval growth rates and survival. Extensive second year trials, at Solomons and at the Wilde hatchery in 1969, gave good results also.

Thus, in summary the last six to eight years have seen extensive trials of natural and cultured algal systems of larval feeding in the Delaware-Chesapeake area. The most noteworthy fact is the widespread success of various natural feeding methods. Their applicability in a commercial system will be discussed in an economic context.

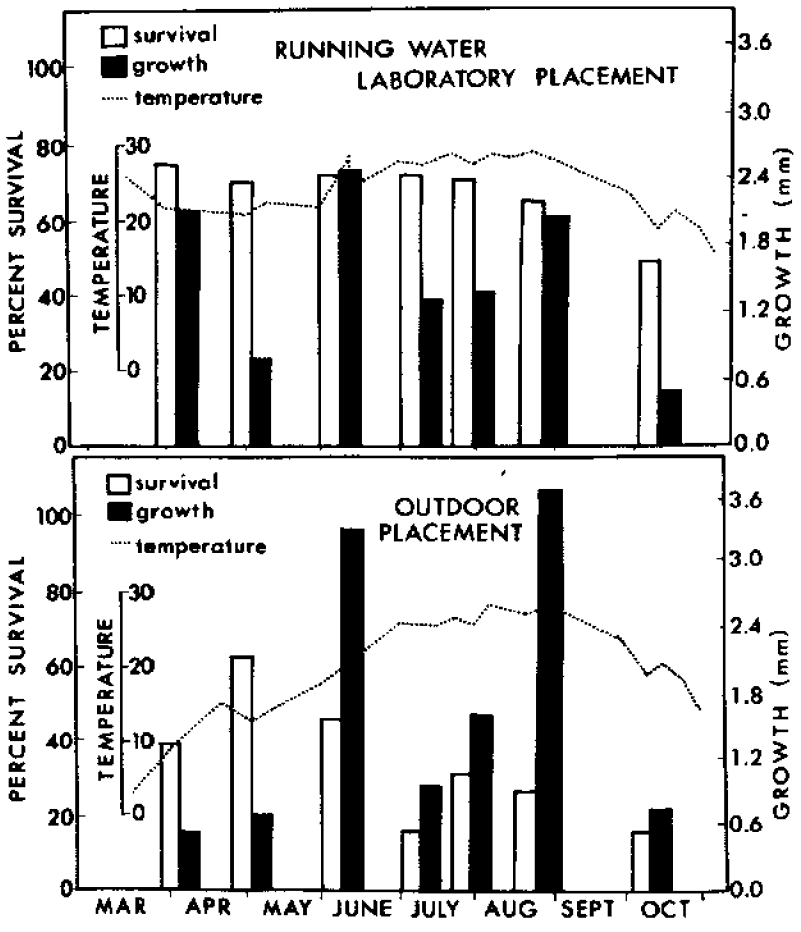
Setting and Survival of Spat

Problems of setting in the hatchery are similar in all areas, so this will not be discussed. However, the new cultchless techniques have changed the picture radically and this will be discussed in an economic context.

First-year growth and survival of postmetamorphic oysters (the nursery stage of Dr. Andrews) is a critical area for a hatchery and should be examined in the Chesapeake area. Although little information is available, it is reasonable to suspect that first-year juvenile mortality will be much higher in high-salinity (> 15‰) areas than in low salinity areas. This, of course, is due to the salinity barrier on the range of several oyster predators and disease. Notably absent in low-salinity areas are the oyster drills, *Eurosalpinx* and *Eupleura*, and MSX (*Minchinia nelsoni*) disease. The presence or absence of predators and disease governed by the salinity factor may be of great importance in choosing hatchery locations.

CBL, in the low salinity Solomons area, has run spat survival trials throughout the 1968 season. Tests were of two types: first were determinations of growth

FIGURE 4. Growth and survival of several broods of oyster spat from setting to two weeks' post-setting placed in laboratory vs. outdoor conditions in low salinity Chesapeake Bay at Solomons, Maryland.



and mortality in new spat from 0–2 weeks old; second were similar trials from 2 weeks to 1 year of age. Spat from several broods were placed in different low-salinity environments at several times throughout the 1968 season.

Oyster spat in low-salinity waters of Chesapeake Bay suffered their greatest mortality during the first two weeks of their lives. A comparison of new spat placed in running-water laboratory conditions with those placed directly outdoors is particularly interesting (Fig. 4). In the direct outdoor placement, 0 to 2 week losses were heavy, in most cases greater than 50 percent, regardless of season. The periods of June and late August provided excellent growth. However, mid-summer growth and survival was hindered by setting of competitive organisms and the predaceous flatworm, *Stylochus ellipticus*. Losses of spat from 2 weeks of age to 1 year of age were extremely light and were estimated at 10 to 30 percent. Growth of the trayed oysters placed at several locations on the Patuxent River was excellent with average length ranging between 26 and 57 mm.

With regard to growth and survival of cultchless spat we have a void of information in the Delaware-Chesapeake area. The Windmill Point Hatchery at Urbana has a cultchless process (Edwin Powell—Personal communication). They fear that blue crab predation may be a significant mortality factor if the cultchless oysters are placed directly on the bar. Mr. Wilde (Fig. 5) of the West River, presently has about 500,000 hatchery-reared cultchless oysters that he has reared to half-

FIGURE 5. First-year pilot hatchery of Mr. Frank Wilde of the West River, Maryland. With a very minimal $1,000 investment, Mr. Wilde tested the workability of hatchery systems at this site. With about a half year of his time, he has produced about 500,000 one-inch cultchless spat and intends to expand into a more permanent operation in future years.

FIGURE 6. First-year handling of cultchless juvenile oysters at the Wilde pilot hatchery. Low salinity backwaters have given excellent growth and survival of trayed oysters in their first year. Additional methods must be developed for the handling of later cultchless stages.

inch size, trayed in a saltwater creek (Fig. 6). He is now entering an unknown area of survival as he attempts to place the cultchless oysters on his leased oyster bar.

Summing up, it is obvious that oyster hatcheries are biologically feasible in the mid-Atlantic region. All systems are workable; however, the greatest unknowns remain in the later nursery stages.

ECONOMIC FEASIBILITY

The more pressing and important question is now: "Are oyster hatcheries now, or can they ever be, economically feasible in the mid-Atlantic region, especially the Delaware-Chesapeake Bay area?" Here in the Chesapeake area we are at a crossroad. On the one hand, we observe the Long Island Oyster Farms and other commercial hatcheries going full speed ahead in the Long Island area with the impetus of industry capital. On the other, we see probably much better biological habitat here going unused with respect to hatcheries and this is in the face of per-

sistent seed shortages. Why, for example, is there such limited use put to certain natural seed sources as at the Delaware Bay shore of Cape May, where in a usual year more oysters set than will set in all the hatcheries for the next thousand years?

How real are seed shortages on the public and private grounds of Delaware and Chesapeake Bays? It is, I believe, generally known that these areas form one of the finest oyster-growing habitats anywhere, but the region as a whole, especially Maryland's Chesapeake Bay, suffers from lack of good setting areas. Mr. Joseph Manning, Director of the Maryland Department of Chesapeake Bay Affairs (DCBA), summed up his department's feelings in 1968 at the Maryland Governor's Conference on the Chesapeake: "The critical limiting factor on oyster production in most of Maryland's waters is recruitment, and in this respect we are much less fortunate than most of the other oyster-producing states. In only a few areas of very limited size is spatfall consistently heavy enough to produce seed oysters of commercial quality." Manning continued, "The private planter of oysters has long been the stepchild of the Maryland industry. Currently, 10,045 acres of Maryland bottoms" (of 400,000+ acres of oyster bottom plotted in the Yates survey of 1901)" are leased to 766 persons, of whom relatively few are actively engaged in growing oysters. Records of the Department covering more than 15 years indicate that private planters account for something less than 10 percent of the annual harvest. Inability to purchase seed oysters, or to lease bottom on which seed oysters can be produced, is the major deterrent to growth of the private segment of the industry. Liberalizing legislation enacted in the past three years will, it is hoped, permit the investment of additional private capital in Maryland's efforts to maintain its recently regained position as the leading oyster-producing state. It has been demonstrated that an increase of more than 150 percent in production has had no significant effect on the unit price received by the public oystermen for their catch. Furthermore, it appears unlikely that production in any of the leading oyster-producing states will increase materially in the foreseeable future; in most, a continuing decline is predicted. We find no reason to believe that Maryland's private oyster fishery cannot undergo orderly development without harm to the public fishery, and without abandonment of the time-honored concept that the natural oyster bars of the state are the common property of its citizens.

In summary, it may be said with confidence that Maryland's oyster fishery, with intelligent management and reasonably good fortune, can continue to grow in both volume and value without expense to the taxpayers of the State, adding many millions of dollars to the economy of Maryland and providing employment to a very significant number of persons in areas where other employment opportunities are severely limited." (Manning, 1968).

There are similar problems in Delaware Bay and Virginia's Chesapeake Bay, although I have had no first-hand contact with these areas. The situations are somewhat different in the different states with Maryland's Chesapeake Bay largely in public management and Delaware Bay and Virginia's Chesapeake Bay largely under private control.

Recently, I questioned several Maryland private growers, plus the Potomac River Fisheries Commission (PRFC) (Mr. Robert L. Norris—Personal communication), who manage over 50 miles of prime oyster bottom on the Potomac River,

asking these questions: "Have seed oysters been available in the Chesapeake area over the past 5 to 10 years and what price have you paid or would you have been willing to pay?"

Their answers provide a general confirmation of the statements of Manning that there has been a general lack of availability of seed oysters in recent years. Specifically, the recent declines in traditional Virginia sources, such as the Great Wicomico and James River, have curtailed operation. The PRFC, for example, began purchasing seed in 1966 and in all subsequent years have not been able to use their allotted budget because of the unavailability of seed. Maryland's public seed program, managed by DCBA, has been authorized to sell surpluses over a million bushels. The surpluses have been so sporadic that it has not been worthwhile for the PRFC to consider them in recent years. The experiences of Maryland private planters have been similar. Some say that from the 1950's to early 1960's, it hasn't been worth the effort to work grounds because of seed scarcity. In the late 1960's, private planters have also suffered with the loss of traditional seed sources. The DCBA surplus has been only intermittently available.

With the perennial seed oyster shortages in the Delaware-Chesapeake area, is there any hope then that the hatchery method can fill the gap? This is entirely a matter of cost comparison now and the comparisons might change in the future. Overboard costs for natural seed in this area in the past five years, from my information, have ranged from $1.35 per bushel to $2.85 per bushel. *Local private Chesapeake oystermen and the PRFC presently appear to be extremely reluctant to pay over $2.00 per bushel overboard costs for seed oysters.*

There have been at least two estimates of cost of hatchery seed in the Long Island area. Mercer (1963) estimated that his costs at the Bluepoints Hatchery ranged between $5.50 and $7.00 per bushel. The Bluepoints Hatchery sets its seed on oyster shell cultch and produces between 2,000 and 5,000 bushels of seed oysters per year. Matthiessen and Toner (1965) established a pilot hatchery on Martha's Vineyard using largely USBCF, Milford techniques. Their cost estimates approximated $5.00 to $15.00 per bushel of seed depending on how costs were figured.

Thus it appears that present hatchery costs (using the cultch method) range between three and seven times that of naturally produced seed in the Chesapeake area. These cost differences appear to be too great to overcome in a commercial hatchery operation.

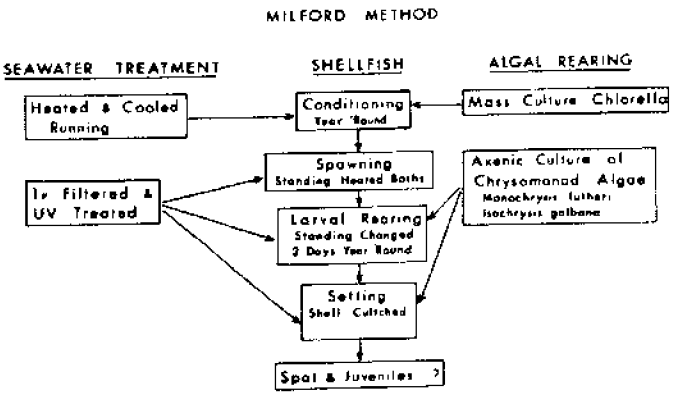
Is there any hope then that an oyster hatchery will ever be commercially successful in this area? Yes, with three developments:

1. A streamlining of existing techniques to produce maximum efficiency. This alone will not make hatcheries successful here since cost differences appear to be too great.
2. A full development of the new cultchless setting techniques. The cost advantages of this method are obvious with elimination of cultch-handling problems and probable increased efficiency of conversion of mature larvae to spat. However, cultchless spat require new methods of handling in the later nursery stages that must be solved before the method can be of practical value.
3. The development of desirable genetic strains of oysters. This development will obviously completely revolutionize hatchery economics. The subject was reviewed by Dr. Menzel in his presentation yesterday.

CBL has been working on several tacks to eventually bring about a commercially feasible hatchery system to Maryland's Chesapeake Bay. Originally, in 1968–69, we worked on the biological feasibility of hatchery systems (Hidu et al., 1969). At the same time, we have been attempting to find and select a fast growing race of Chesapeake oyster for use as a hatchery broodstock (Klaus G. Drobeck, in progress). Most recently, in 1969, we have been working with a private oysterman, Mr. Frank Wilde of the West River on Chesapeake Bay to attempt to evolve a commercially workable system. This operation is interesting because it demonstrates a way to advance hatcheries to an economic reality. Mr. Wilde, who has been interested in hatcheries for many years, has added his innovations and limited private financing. We at CBL have advised in techniques, borrowing information from several sources: the Loosanoff and Davis techniques from Milford, the Long Island Industry methods and the Chesapeake area 88–309 hatchery experience. The result has been a considerable advance to a commercially workable hatchery for this region.

In outlining our progress and techniques, I will contrast them with the basic parts of the Milford method and Long Island Sound Industry methods. The Milford method (Fig. 7) has as its important features the year-round conditioning and spawning of oyster stocks and the rearing of larvae using cultured algae as food. Various cultch types have been used in setting with little development of technique of handling later juvenile stages. Costly parts of the operation appear to be the maintenance of algal cultures and the heating and cooling of large quantities of seawater for conditioning spawning stock. The Long Island Industry techniques (Fig. 8) have evolved from the original Wells-Glancy natural algal feeding method. Methods now include the rearing of chrysomonad algae as a backup feeding system and the incorporation of cultchless setting techniques. Costly items in the operations are the handling of oyster shell cultch at Mercer Bluepoints, the maintenance of the backup algal systems, and the holding and heating of seawater for larval rearing.

FIGURE 7. Simplified outline of essential systems in the USBCF—Milford hatchery system.



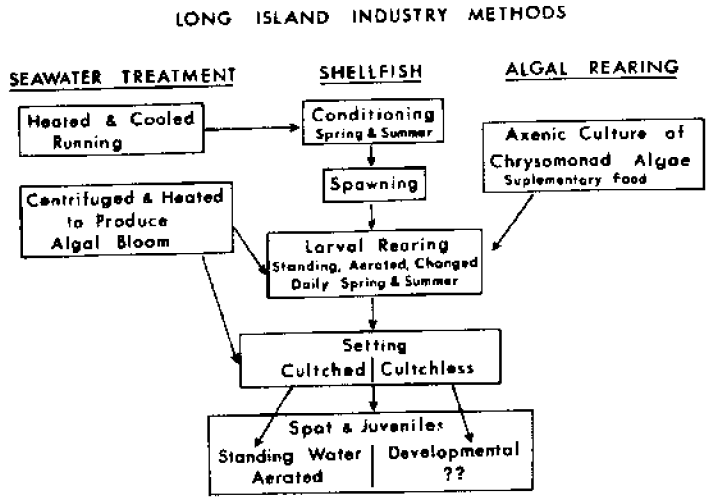
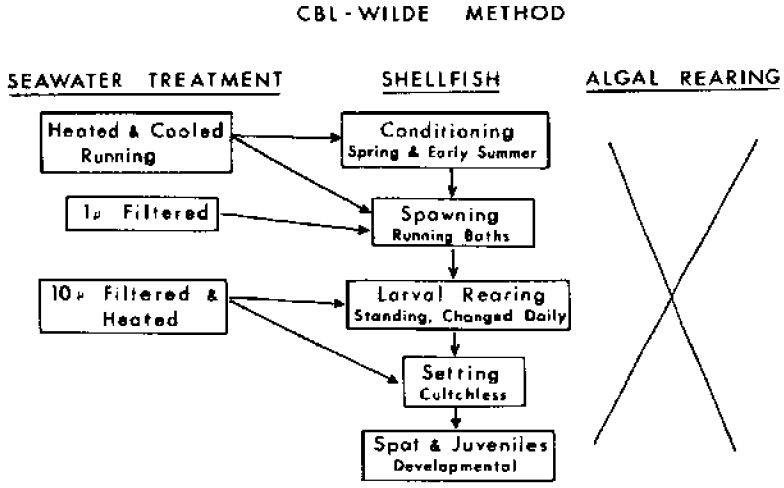


FIGURE 8. Simplified outline of essential systems in Long Island commercial oyster hatcheries. In most Long Island hatcheries, backup algal rearing systems have been added to the basic Wells—Glancy natural algal methods.

The CBL-Wilde method (Fig. 9) borrows from the Milford and Long Island methods and, in addition, draws from the Chesapeake area experience of the past 10 years. Mr. Wilde in his first year of operation, 1969, spent about $1,000 on a very modest pilot hatchery facility (Fig. 5) plus a half-year's time to see if biological systems were workable here before considering a larger investment. In the first year, he produced about 500,000 cultchless oysters of half-inch size and intends with additional investment to expand to a 10,000,000 spat/year operation.

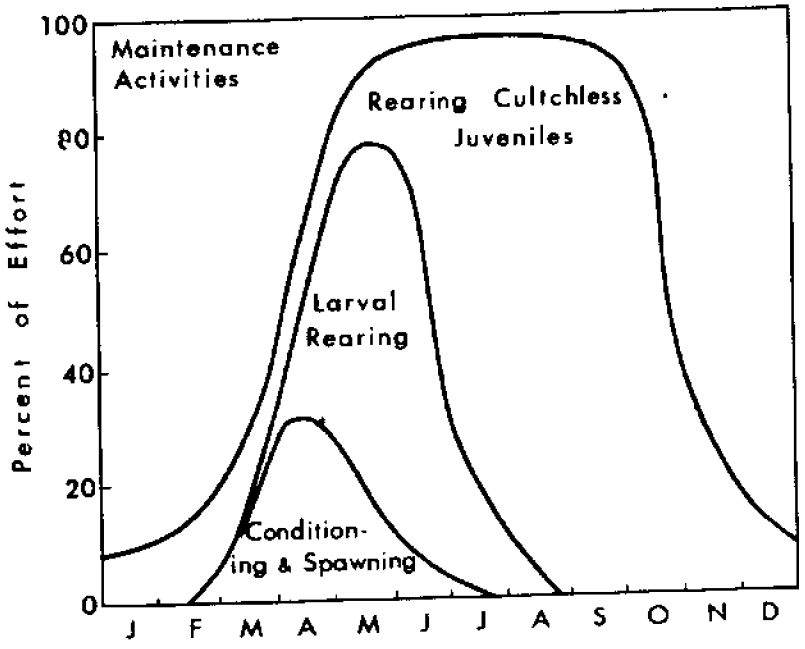
FIGURE 9. Outline of essential systems in the CBL—Wilde pilot oyster hatchery. The system incorporates 88–309 hatchery experience in simplifying existing Milford and Long Island techniques.



Every effort has been made to eliminate costly items in the operation (Fig. 9). At this point, the Wilde hatchery is not rearing algae but utilizing the simple natural algal systems that have been successful on much of Delaware and Chesapeake Bays. Setting, of course, is cultchless. Proper seasonal timing of operations promises to greatly aid in cost reduction (Fig. 10). For example, conditioning and spawning are not attempted before March because of the poor luck of CBL and VIMS in early season conditioning. Also, the expense of heating water for conditioning and larval and spat rearing is eliminated. Larval rearing is carried on exclusively in the spring months, April to July, when natural algal foods are plentiful and, more importantly, sets of the natural predator, *Stylochus ellipticus*, appear to be absent. By rearing larvae in the spring the spat may take advantage of optimal spring growth conditions and are later of large enough size to be greatly immune to mid-summer sets of *Stylochus* and shell competitors. Larval rearing is curtailed after June because of our experience with mid-summer losses of early spat. The period after June is reserved for handling the cultchless juveniles in the nursery stage. Great increase in volume with growth and problems of fouling organisms and predators will require much attention, no doubt. As stated, handling problems remain after juveniles leave the early nursery stage.

The state of development of commercial hatcheries in this area is shown in a different way by a flow diagram of expected mortalities with various life stages (Fig.

FIGURE 10. Expected seasonal work effort at the Wilde pilot hatchery. Each hatchery system is strongly restricted seasonally to minimize costs and maximize biological efficiency.

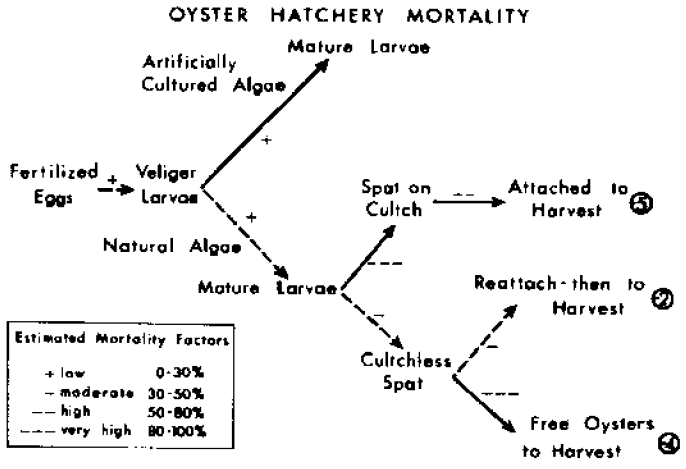


11). For example, there is little trouble in rearing fertilized eggs to veliger larvae with low mortality and low expense by the method of Loosanoff and Davis (1963). Larvae can be reared to a setting stage here with low mortality by artificial algae or by natural algae. The widespread success and low cost of natural algae make this the attractive alternative. At setting there again is a choice, i.e., to go with cultched or cultchless oysters. With the cultch method there are problems of high mortality in conversion of larvae to spat plus large expense in handling cultch. However the final product is seed oysters which lend themselves to established American field techniques. Cultchless oysters have obvious advantages in the hatchery with ease and economy of handling plus probable efficiency in conversion from larvae to spat. However, the cultchless spat pose new culture problems in later stages that have not been solved.

It is essential that much thought be given now to culture of the cultchless juvenile to harvest if the hatchery is to be successful economically. As stated previously, little or no information is presently available on later culture of cultchless juveniles. There is thought in the Chesapeake (Edwin Powell—personal communication) that cultchless oysters may suffer extreme loss from blue crab predation if placed directly on the bottom. Apparently the blue crab is able to pick up and manipulate a free spat much more readily than cultched spat (William Shaw—personal communication).

All alternatives should be tested and evaluated to bring the cultchless spat to an economic harvest. Possibly free oysters may be placed on the bar late in the season, October to November, after blue crab activity lessens, but this remains to be tested. Some type of three-dimensional culture might be workable to take advantage

FIGURE 11. Estimated mortality factors at several oyster life stages using the available hatchery techniques. Estimates are based only on opinion of shellfish workers in the field. The dashed line indicates the sequence of techniques that probably will produce the least overall mortality from fertilized egg to harvest in the hatchery.



of very rapid growth rates commonly experienced by oysters located off the bottom. The traying of cultchless oysters appears to be very feasible for the first half year; however, crowding and handling problems probably will be apparent after that time. An attractive, although untried, alternative with larger cultchless oysters would be to reattach the oysters to a substrate by a rapid-drying adhesive. This would permit a spacing of oysters plus three-dimensional placement on rafts for maximal growth to harvest. The cultchless oyster has obvious advantage in the hatchery. The only barrier to its economic use is a bit of thought on new handling techniques after the first year.

SOCIOLOGICAL-POLITICAL FEASIBILITY

A hatchery system can be biologically and economically feasible and yet not be workable, or never get off the ground, if it is in conflict with sociological or political forces. We have observed the New Jersey watermen and have attempted to interest several Maryland watermen in a hatchery operation. These folks are by nature conservative and extremely resistant to new ways of operation. The Maryland watermen (3,000+), for example, are a culturally closely knit group, who have through the years made a somewhat marginal living on the public oyster bars by very traditional methods of sail dredging and hand and patent tonging.

A change in production method could be looked upon as a threat to their livelihood. They might fear perhaps that outside interests would eventually compete with their trade. The watermen, especially in Maryland, thus form a particularly vocal political force, one the state management agencies must listen to. And in so doing, the management agencies, too, become conservative and resistant to change. This kind of an interaction can just as effectively block a new development as any of the other factors.

The type of waterman that has become interested in hatcheries is atypical—a person who is perhaps interested in oysters but who has some other alternate income and wishes to try something new. The field has a high risk with possible extreme financial benefit but also with something new to allow the person merely to lead a more interesting life. There are people like this around and occasionally we are lucky enough to find one.

There is the thought that hatchery operation would lend itself to a big business enterprise, and thus eventually replace traditional ways of oystering, and replace a traditional social culture with irretrievable loss. If this were true, we would also be reluctant to foster such a change.

But I say that the traditional waterman in this area is fighting a rear-guard action to other uses of the estuary that are potentially destructive to the oyster industry as it is presently practiced. For example, the formerly reliable James River Chesapeake seed sources have sharply declined in recent years, no doubt in response to the cumulative effects of increased industrial and domestic pollution plus effects of channel dredging that may change flow patterns. The projected large-scale use of the mid-Atlantic estuaries for power plant cooling also threatens to degrade the shellfish environment (Mihursky, 1969).

Thus, it is time for the mid-Atlantic oyster industry to attempt to diversify production methods to provide the necessary industry stability in times of change in the estuary. The hatchery method of production should thus be thought of as a method that is supplementary to natural methods of seed production and oystering. Both should be worked on with similar vigor to allow the region to achieve and maintain its full shellfish harvest potential.

ACKNOWLEDGMENTS

Appreciation is expressed to Dr. Harold H. Haskin, Mr. Klaus G. Drobeck, Mr. Elgin A. Dunnington, Jr., Mr. Willem H. Roosenburg and the USBCF, Milford, Connecticut, for much counsel and aid in the development of Chesapeake aquacultural techniques. Thanks to Mr. Harold Davis of the Maryland Department of Chesapeake Bay Affairs for aid in initiating activities between CBL and the Wilde hatchery. Dr. L. Eugene Cronin added much direction to the CBL studies throughout.

LITERATURE CITED

ANDREWS, J. D., 1964a. Quarterly report to U.S. Bur. of Comm. Fish. for period ending June 20, 1964. PL 87-580, Grant # 14-17-0007-212. V.I.M.S., Gloucester Point, Va. (unpub. mimeo. rept.).

ANDREWS, J. D., 1964b. Quarterly report to U.S. Bur. of Comm. Fish. for period ending Sept. 30, 1964. PL 87-580, Grant # 14-17-0007-212. V.I.M.S., Gloucester Point, Va. (unpub. mimeo. rept.).

ANDREWS, J. D., 1965. Quarterly report to U.S. Bur. of Comm. Fish. for period ending Sept. 30, 1965. PL 88-309, Grant # 14-17-0007-330. V.I.M.S., Gloucester Point, Va. (unpub. mimeo. rept.).

ANDREWS, J. D., 1966. Annual report to U.S. Bur. of Comm. Fish. for period July 1, 1965 to June 30, 1966. PL 88-309, Grant # 14-17-0007-330. V.I.M.S., Gloucester Point, Va. (unpub. mimeo. rept.).

ANDREWS, J. D., 1967. Quarterly report to U.S. Bur. of Comm. Fish. for period ending March 31, 1967. PL 88-309, Grant # 14-17-0007-554. V.I.M.S., Gloucester Point, Va. (unpub. mimeo. rept.).

ANDREWS, J. D., 1968a. Annual report to U.S. Bur. of Comm. Fish. for period July 1, 1967 to June 30, 1968. PL 88-309, Grant # 14-17-0007-798. V.I.M.S., Gloucester Point, Va. (unpub. mimeo. rept.).

ANDREWS, J. D., 1968b. Oyster mortality studies in Virginia. VII. Review of epizootiology and origin of *Minchinia nelsoni*. *Proc. Nat. Shellfish Assn.*, 58: 23–36.

ANONYMOUS. 1968. The oyster-producing potential of the Potomac estuary. The Potomac Newsletter 2(12). Potomac Basin Center, 1250 Connecticut Ave., N.W., Washington, D.C. 20036

BROOKS, W. K. 1879. Abstract of observations upon artificial fertilization of oyster eggs and embryology of American oyster. *Amer. J. Sci.*, New Haven, XVIII: 425–527.

DAVIS, H. C., and R. L. GUILLARD. 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. *Fish. Bull.* U.S. No. 136, 58, 293–304.

HASKIN, H. H. 1964, 65, 66. Annual reports to U.S. Bur. Comm. Fish. Contract # 14-17-0007-226 of PL 87-580 and Sub-projects 3-3-R of PL 88-309, Rutgers, The State Univ., New Brunswick, N.J. (unpub. mimeo. repts.).

HASKIN, H. H., 1967. Quarterly progress report for period July 1 to Sept. 30, 1966 to U.S. Bur. of Comm. Fish., Sub-project 3-3-R of PL 88-309. Rutgers, The State Univ., New Brunswick, N.J. (unpub. mimeo. rept.).

HASKIN, H. H., L. A. STAUBER, and J. A. MACKIN. 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae: Causative agent of the Delaware Bay epizootic. *Science*, 153 (3742): 1414–1416.

HIDU, H., K. G. DROBECK, E. A. DUNNINGTON JR., W. H. ROOSENBURG, and R. L. BECKETT. 1969. Oyster hatcheries for the Chesapeake Bay region. N.R.I. Special Report No. 2., Contrib. No. 382, Natural Resources Institute, University of Maryland, Solomons, Maryland, 18 p.

LOOSANOFF, V. L., and H. C. DAVIS. 1963. Rearing of bivalve mollusks. In: F. S. Russel (ed.), Advances in Marine Biology, Academic Press, London 1: 1–136.

LOOSANOFF, V. L., and C. A. NOMEJKO. 1951. Existence of physiologically different races of oysters, *Crassostrea virginica*. *Biol. Bull.* Woods Hole, 101, 151–156.

MANNING, J. H. 1968. Bay fisheries resources. Proceedings of the Governor's Conference on Chesapeake Bay. Sept. 12–13, 1968. Annapolis, Maryland pp. 91–108.

MATTHIESSEN, G. C. and R. C. TONER. 1966. Possible methods of improving the shellfish industry of Martha's Vineyard, Dukes County, Massachusetts. The Marine Research Foundation, Inc. Edgartown, Massachusetts, 138 p.

MAURER, D., and K. S. PRICE, Jr. 1968. Holding and spawning Delaware Bay oysters (*Crassostrea virginica*) out of season. 1. Laboratory facilities for retarding spawning. *Proc. Nat. Shellfish Assn.* 58: 71–77.

MERCER, H. 1963. Comment on L. I. commercial shellfish hatcheries. Unpub. mimeo leaflet. The Bluepoints Company. West Sayville, Long Island, N.Y. 3p.

MIHURSKY, J. A. 1969. Patuxent thermal studies—summary and recommendations. N.R.I. Special Report No. 1. Natural Resources Institute, University of Maryland, Solomons, Maryland. 20 p.

NELSON, J. 1889. Report of the Department of Biology of the New Jersey Agricultural Experiment Station for the year 1889. 197–220.

NELSON, J., 1909. Report of the Department of Biology of the New Jersey Agricultural Experiment Station for the year 1908.

RITCHIE, T. P. 1964. Pilot studies of the spawning and rearing of MSX resistant oysters. Report to U.S. Bur. of Comm. Fish., Quart. Period of July 1 to Sept. 30, 1964. Contract # 14-17-0007-222 of PL 87-580. Univ. of Dela., Lewes, Dela. (unpub. mimeo. rept.).

RITCHIE, T. P., 1966. Pilot studies of the spawning and rearing of MSX resistant oysters. Report to U.S. Bur. of Comm. Fish., Quart. Period of March 12 to June 30, 1966. Contract # 14-17-0007-507 of PL 88-309. Univ. of Dela., Lewes, Dela. (unpub. mimeo rept.).

RITCHIE, T. P., 1968. Pilot studies of the spawning of MSX resistant oysters. Report to U.S. Bur. of Comm. Fish., Quart. Period of July 1 to Sept. 30, 1968. Contract # 14-17-0007-507 of PL 88-309. (Unpub. mimeo. rept.).

SPRAGUE, V., R. L. BECKETT, and H. HIDU. 1967. Development of a disease resistant oyster brood stock. Report to U.S. Bur. of Comm. Fish., Quart. Period of July 1 to Sept. 30, 1967. Sub-project No. 3-20-R-3 of PL 88-309. Univ. of Md., Solomons, Md. (unpub. mimeo. rept.).

STAUBER, L. A. 1950. The problem of physiological species with special reference to oysters and oyster drills. *Ecology*, 31, 109–118.

WELLS, W. F. 1920. Growing oysters artificially. Conservationist. 3: 151.

DISCUSSION

ZHARADNIK: Some of the future problems listed on the last chart on the slides were particularly interesting. You indicated that a great deal of work has been done up to the point where there are either cultchless spat or spat set and from there on the hatchery operation is possibly in jeopardy. It is interesting to me that a systems analysis of traditional methods in the New England area has yielded essentially the same conclusion. As far as we are concerned at the University of Massachusetts, the refinements that have taken place in the techniques developed to produce set need to be carried on into the juvenile and mature oyster area. At the last two National Shellfisheries meetings, we presented a progress report on a system to carry on with the animals past the setting stage. It becomes primarily a problem of economic feasibility of the pumping operation. We are very much interested in following this up to see how cultured spat fit into this picture. Recently, we put into operation a 50-bushel pilot plant and hope to obtain some larger-scale operational data available to the industry.

LOOSANOFF: Concerning spawning techniques I would like to say that even though Milford and Chesapeake Bay are quite separate geographic areas, and while certain details of conditioning oysters to spawn for larval rearing need to be refined from one area to the other, basically, the problem is still the same.

HIDU: Right, just some differences in technique is about all it amounts to—in some cases, taking advantage of different ecological situations, but essentially the same.

LOOSANOFF: In comparing Milford's activities with a commercial hatchery I believe there are a few distinctions to consider. Milford cannot commit themselves exclusively to a year-round production of spat because this would interfere with other studies like genetics, for example. Milford uses three or four precise methods involving conditioning, spawning, rearing and axenic cultures and is constantly experimenting to develop new techniques. Thus, direct comparison between Milford and commerical hatcheries is difficult to make because Milford is an institution for basic research. This should be emphasized when comparing different methods. Since workers at Milford are able to dedicate 12 months a year to basic research rather than just 6 because of a growth hatchery commitment, rapid progress has been made in the past.

The second point I have to offer involves physiological races. I do think we should remember that this concept is not anything new. You quoted it from 1951 papers. Actually, if you read Mitchell's work in 1920, you will find that he was speaking about different physiological races then. He stated very clearly that a bushel of oysters from Bridgeport responded very differently to spawning stimuli than those originating from more southern areas. At the time, we were performing experiments with five different groups of oysters and were surprised at how differently these oysters responded. Have you ever tried to condition and spawn a Florida oyster?

HIDU: No, we never have. Oysters from the Virginia Chesapeake is as far south as we have ever tried.

LOOSANOFF: We came to the conclusion that oysters from different geographic areas are rather different. We had an extremely difficult time in conditioning Florida oysters even when they were held at a variety of conditions.

HIDU: We had some hatchery progeny from Great Bay, New Hampshire and Patuxent River, Maryland, and we performed conditioning experiments early this spring at 23° C. But we began the experiment too early in the season and found the oysters losing condition. However, after six to eight weeks we saw no difference, and I am still unsure about our results. The experiment may have been complicated by the lack of food in the water this time of the year.

LOOSANOFF: Are you familiar with several cases in Long Island Sound? For example, Dick Nelson brought oysters from Virginia and planted them in New Haven Harbor. The oysters remained for about two years and never spawned. Another case was a load of oysters from the Hudson River planted further North, and they failed to spawn after two years.

SHAW: I have several questions concerning seed production. You know we are interested in this too. I believe there is no shortage of potential seed in Maryland. I emphasize potential because in my usage it means utilizing what is naturally available. The Maryland part of Chesapeake Bay is unique in that setting failures are rare in certain tributaries, i.e., St. Mary's River, Little Choptank, Harris Creek, Broad Creek, and Eastern Bay. The problem is utilizing the natural setting that is available. For example, this year alone during our monitoring service we observed as much as 6,000 spat on a 4" × 4" plate. This indicates the potential that exists there. Unfortunately, it is not used, and it is a shame that this waste occurs. It is true that natural planters want seed, and so we try to determine how to apply this natural seed.

For your purposes hatcheries make sense. Still, it might be advisable to include additional costs for spat and juvenile development because there is going to be a new cost from constructing trays and maintaining the trays daily. This is a cost that should be considered with some indication of the capital. But I hope we can develop for economical purposes the natural setting that is so great in Maryland. For example, by planting thinner beds of cultch we can spread out the seed bars and perhaps almost double the acreage. By doubling the natural seed reserves in Maryland, you would have enough seed to go around.

In terms of cultch it seems accurate to say that shells on the bottom catch spat with about 50 percent efficiency. In the hatchery you are only 50 percent efficient because one half of the surface area is lost. If one tries raft culture, at least the cultch is available on both sides, which tends to increase the efficiency of the cultch. The point here is that I am unsure whether there is a shortage of seed or just a failure to develop the potential in Maryland.

HIDU: I agree that we need several methods to enhance seed production. In passing, I can indicate several differences between hatchery and natural production. Obtaining natural set by rafting of-

fers problems of predation by the flatworm, *Stylochus*. An excellent set might occur and this could be obliterated in mid-summer by predation. Another point is that the hatchery technique offers the big advantage of genetically selected stock whose value we have already discussed in this conference.

DEAN: Herb, do you know whether there is any data on the effect of intensive raft culture of shellfish on bottom conditions resulting from tons of fecal material dropping to the bottom?

HIDU: No, I do not.

DEAN: This factor will have to be considered by commerical growers if they pursue intensive raft culture in a small confined area, without a great tidal exchange, with less than a three-foot tidal amplitude. Under these conditions a great mass of fecal material would accumulate on the bottom.

SHAW: This point is of interest because if raft culture and hatcheries are going to be successful, they will probably be limited to ponds and small creeks for purposes of control. Since we are biologists, we have not been able to engineer rafts that would be used in open waters. The Japanese are more realistic and are able to use open waters quite successfully. We thought that protected ponds would be the ideal location for off-bottom culture. But, if oysters are held in these ponds, the amount of silt and food wastes becomes tremendous. We had a raft in a pond much like you suggested with a limited tide of a foot and a half. Within two years there was an accumulation of 1.5 feet of waste products which actually buried and killed a third of the oysters in their own waste. Without good tidal exchange, the pond will fill up. Either we move into open waters with these rafts or call upon the engineers to design equipment to flush these wastes away. Otherwise this will remain a serious problem.

DAVIS: Herb, we found in Milford that we had to feed oysters from New Hampshire to get them to develop gonads. Apparently, they do not contain enough reserve to develop gonads readily.

In other experiments we have been trying to condition oysters at lower salinities and have encountered some difficulty. A group of oysters which will develop gonads quite readily at normal salinity may not progress at a lower salinity. This may be attributed to the fact that we are mixing freshwater with seawater. What happens is that we are reducing the food content, but since these experiments were in the winter I rather suspect that lowering the salinity on these oysters has affected their gonad development considerably. The same thing may be happening in your experiments when you expose New Hampshire oysters to a lower salinity.

Another comment concerning our feeding experiments and your natural feeding experiments is that we were feeding larvae at a constant rate. We have known for a long time that as the larvae grew larger, they should have an increased supply of food. Recently Mr. Rhodes, of our laboratory, has made quantitative studies of the optimum concentration of algae at each successive stage in larval development. But the fact that your natural food was poorer than our foods at the early stages of growth and then shot upwards and finally leveled off again would indicate to me that your natural foods may be somewhat toxic and/or in too high a quantity. The larvae are probably running out of food again as you level off at the top of your curve.

Introduction to DESIGN AND DEVELOPMENT OF AN ENVIRONMENTAL CONTROLS SYSTEM FOR CULTURING OYSTERS

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In production of oysters an important factor is the environment. Application of techniques used in agriculture environmental control to the rearing of aquatic animals will result in enormous increases in yields. Using these techniques, man has developed from the hunting of the animals of the forest to culturing these animals for his needs. He is now starting to culture a few of the many aquatic animals available.

There are few places in the world where the environment is continuously optimum for animals throughout the year. Wild animals survive because the majority are able to move about and select a comfortable environment. The oyster is not able to do this, and therefore is required to live in the extremes. Continuation of the oyster depends upon propagation in large numbers and adaptation to a particular environment.

The engineer in collaboration with the marine biologist must design structures and environmental control systems that are economically optimum. This may or may not be optimum in the biologists' sense, but optimum economically for profitable production.

The environment is of importance to the biologists in obtaining the fullest genetic expression from the aquatic animals or plants.

To answer the question of a profitable environment, an understanding is needed of the physiological and biological responses to the environment; also an understanding is needed of the physical aspects of the environment and their effect on the animal's energy loss or gain to the environment. The engineer with some understanding of the physiological principles developed by the biologist and a knowledge of the environmental factors, can then design a shelter with an environment controlled to the extent that it is economically and genetically justified for profitable production.

An animal's environment is the total of all external conditions that affect its development, response and growth. Literally, it could include the equipment, the type, shape, depth of tank, and in the case of the oyster, the kind of materials of

construction. These environmental factors can be separated into physical, social and thermal factors. The physical factors are such things as space, light, sound, pressure, water and equipment. Social factors include such parameters as oyster density, flow rates per oyster and oysters per tank. Finally, thermal factors include such things as water temperature and radiation.

DESIGN AND DEVELOPMENT OF AN ENVIRONMENTAL CONTROLS SYSTEM FOR CULTURING OYSTER LARVAE

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It should be obvious to all that we cannot have complete control of environmental factors that affect shellfish as long as we are using natural seawater. It is equally clear to those of us who have tried to use artificial seawater that it is not easy to devise a synthetic medium satisfactory for development of oyster eggs and larvae and that such waters are not practical for large-scale rearing experiments or for shellfish hatcheries. Moreover, to grow enough algal food to feed a large number of shellfish, particularly a commercial quantity, to market size in an artificial medium or in seawater devoid of natural food would be in itself an enormous task. I do not believe it will be economically feasible, with our present knowledge, to rear shellfish to market size on artificial food. It is much more practical, after an oyster gets to be about 1/4-inch in diameter, to let it glean its own food from natural seawater. What I shall discuss, therefore, will be certain aspects of the history of the design and development of facilities for the modest degree of control of environmental factors we have attained at Milford Laboratory.

Seawater Systems

The seawater system of our old laboratory consisted of a single lead intake line and check valve, a hard rubber-lined pump, a wooden storage tank, lead delivery lines, and hard rubber stopcocks as outlets. This system continues to be very satisfactory, except that in spite of the best maintenance we could give, after almost 30 years *Teredo* finally made their way completely through the walls of the wooden tank and we have had it fiber glass-lined. Almost as important as nontoxic and noncorrodible incoming lines are noncorroding waste seawater disposal lines. In the old laboratory these were of duriron and now, after about 30 years, they have rusted until our maintenance crew is afraid to clean them lest they break holes through the pipe, and we are faced with the problem of replacing them.

Such a variety of materials is now available that it is difficult to recommend what is best, for the choice of materials must depend upon the particular requirement and the relative cost. In our new laboratory all seawater and fresh well water intake, delivery, and waste discharge lines are of PVC (polyvinyl chloride) and our

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wooden storage tank is fiber glass-lined. PVDC (polyvinyl dichloride) or polypropylene lines would be better, particularly for the hot seawater lines, but PVDC was more expensive and not all the required fittings were available in this material when our laboratory was built. The availability and cost of PVDC or polypropylene should be investigated, however, by anyone installing new facilities because these materials have a higher temperature tolerance than PVC. Ideally, all seawater lines should be in duplicate to allow alternate treatment of the lines to kill fouling organisms. In our new laboratory we have duplicate lines from intake to storage tank but it was not feasible (both from cost and space considerations) to duplicate delivery and waste lines in our laboratory where each of the 13 wet laboratories is supplied with hot and cold seawater and fresh well water, in addition to hot and cold tap water, gas, and air. Here, we depend upon periodic 48-hour treatment of the delivery *and waste* lines with freshwater to kill fouling organisms.

All pumps are the centrifugal type, rubber lined, and all valves are of PVC or are rubber-lined metallic valves. At the time we built, satisfactory PVC valves were not available in sizes above two inches, for we believe all valves should be capable of being serviced without removal from the line. Piping and valves are arranged so that either of the two pumps supplying seawater to the laboratory can use either of the two intakes and either of the two lines to the storage tank. This permits alternating use of lines even though one pump should be inoperable. We use lead check valves of the top opening type placed in our intake lines, with the top of the valve one foot above mean low water, to permit opening and clearing the valve when occasional foreign objects get caught in the valve seat and prevents closing.

Controlling temperature

Although Loosanoff (1945) does not describe his first discovery that even in winter oysters would develop gonads if held in seawater at high temperatures, from personal conversation I know that the discovery was an example of what we would now call "serendipity." He was trying to develop a method for killing boring sponge in the shells of oysters by high temperature and, in the course of examining the oysters, discovered that they contained ripe gonads. This led to the experiments he described in the 1945 paper. At that time the oysters were conditioned by keeping them in aquaria of standing seawater where the temperature was maintained by ordinary electrical aquarium heaters and thermostats. Soon after I came to Milford we decided that flowing seawater would be better and I rigged up a copper coil, through which the seawater passed, immersed in a bucket of tap water on a tripod with a Bunsen burner under it. By regulating the flow of seawater and the intensity of the flame of the burner a moderate degree of control was attained. After the bucket sprung a leak and put out the burner and Dr. Loosanoff and Mr. Lucash found the laboratory filled with gas, they decided a *better* system must be devised before I blew up the laboratory. The net result was the type of apparatus shown in Figure 1, consisting of a coil of lead pipe, through which the seawater passed, immersed in an old hot water tank and using side arm gas hot water heaters with the flame controlled by an electric thermostat operating solenoid gas valves (Loosanoff, 1949). This is still a good workable system and is probably the cheapest and best device for heating relatively moderate flows of seawater. In our old lab-

oratory we finally had three such setups with up to three sidearm heaters per tank to provide the volume of warm seawater needed.

The sources of warm seawater for our new laboratory are two "shell and tube" Karbate* heat exchangers (Fig. 2). In these the seawater flows through a series of carbon tubes and hot water from the furnace is circulated inside the shell and surrounding the tubes. Circulation of the furnace water is by standard circulating pumps and the temperature is controlled by pneumatic thermostats operating pneumatic proportioning valves that permit just enough water from the furnace to circulate through the exchanger to maintain the set temperature.

For these controls to work properly a closed loop system with a circulating pump is needed for the hot seawater. This insures a constant flow of the hot seawater over the sensing elements regardless of the amount of hot seawater being used in the laboratory. Theoretically, for optimum operation the circulating pump should deliver about twice the maximum volume used to insure a return flow through the exchanger at all times. This circulating pump must be nontoxic since it is handling seawater. This appeared to be no problem since rubber-lined centrifugal pumps are made in all sizes. The difficulty encountered, however, was that these pumps are not designed for a head of pressure on the intake side. When such a pressure exists, as in this application, these pumps, or at least the ones we have tried, leak very badly around the shaft seal. We are now using a magnetic drive pump with a nylon head. These pumps are not yet available in large enough size to be entirely satisfactory but are the best we have found.

*Mention of commercial products in this article does not imply endorsement by the Bureau of Commercial Fisheries.

FIGURE 1. Heat exchangers in old laboratory, consisting of copper hot water tank with side arm gas heaters, with lead coil inserted through which seawater passes.

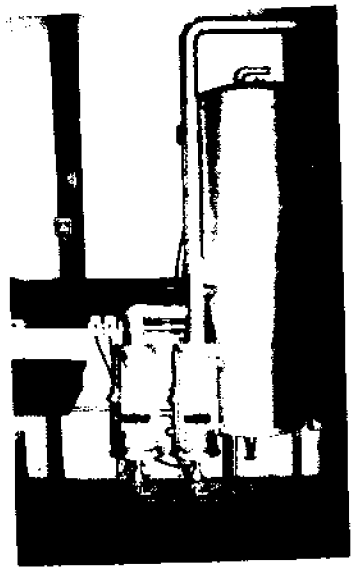


FIGURE 2. "Karbate" heat exchangers in new laboratory. Seawater passes through "Karbate" tubes surrounded by a steel jacket through which hot water from the furnace passes.

Our Chief of Maintenance also uses these heat exchangers to provide hot freshwater to kill fouling organisms in our seawater delivery and waste lines. He thinks that without the heat exchangers it would be virtually impossible to accomplish this because he needs a constant flow at a very uniform temperature high enough to kill the fouling organisms but not hot enough to ruin the PVC piping. Union Carbide makes a number of types and sizes of Karbate heat exchangers, so that it should be possible to design several types of systems to fit your individual needs. Ours seem to be quite efficient.

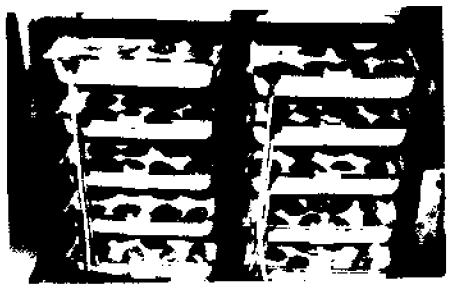
The upper exchanger (Fig. 2) is designed to deliver 20 gallons per minute of seawater bringing it from 0° C to 40° C. This is the source of warm seawater for inducing gonad development in our shellfish and for our larval cultures. The middle exchanger in Figure 2 is designed to give 75 gallons per minute of seawater bringing it from 0° C to 15° C; this supplies the five 36′ × 4′ tanks in the hatchery (Fig. 3) used for holding juvenile shellfish reared in the hatchery until they can be put in the outdoor tanks or natural waters.

The lower heat exchanger (Fig. 2) is designed to cool seawater. I will discuss this later.

To complete our arrangement for inducing gonad development we have trays for keeping the animals in flowing seawater supplied with water of the desired temperature. One system for maintaining the flow of seawater at the desired temperature is to regulate by stopcocks the amount of hot and cold seawater entering a mixing jar which supplies the trays. We have used this system extensively with good results (Loosanoff and Davis, 1963) (Fig. 4). It takes constant care, however, as stoppages in the stopcocks on the hot or cold seawater can cause drastic changes in temperature. A stoppage of cold water, for example, can allow the trays to fill with hot seawater, which may cause spawning of shellfish in an entire bank of trays. In our new laboratory we still use this method for some work since it allows control of individual banks of trays at different temperatures (Fig. 5). For general conditioning, however, we have another system in which the amount of hot seawater is controlled by a normally closed pneumatic proportioning valve (Fig. 6) operated by a pneumatic thermostat. This allows just enough hot seawater to enter the pipe to maintain the set temperature. This system has the advantage that if the cold sea-

FIGURE 3. Tanks in hatchery room. Each tank is 4′ wide by 27′ long by 18″ deep and can be supplied with 15 gallons of seawater per minute at not less than 15° C.

FIGURE 4. Banks of enameled trays in wooden racks in old laboratory.



water supply, or the air for the controls, should fail, the hot seawater is automatically shut off. These controls are expensive enough, however, that they are feasible only when a number of trays are to be held at the same temperature (Fig. 7). We have two such control systems: one controls the temperature on a series of banks of trays for shellfish being brought up to spawning condition and the other controls the temperature of a series of trays kept at a lower temperature used to hold animals already in spawning condition. Note also that we are using fiber glass trays instead of the old enameled trays which eventually chip and rust.

We have recently been trying to induce gonad development at different salinities. For this we use the constant level jars and regulate both temperature and salinity by juggling the stopcocks that regulate the flow of hot seawater, cold seawater, and cold fresh well water (Fig. 8). This, of course, requires constant checking to maintain both factors reasonably constant. Unfortunately, I know of no automatic devices for maintaining a given salinity.

We are now using our well freshwater for diluting seawater for all our salinity work. Oyster larvae appear to tolerate somewhat lower salinities using this source of freshwater instead of the demineralized tap water as used in our previous experiments.

FIGURE 5. Banks of fiber glass trays in fiber glass racks in new laboratory.

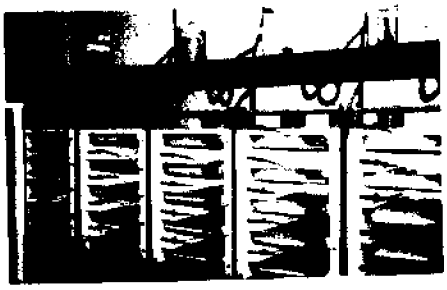


FIGURE 6. Pneumatic thermostats and water valves to control temperature of running seawater to banks of trays.

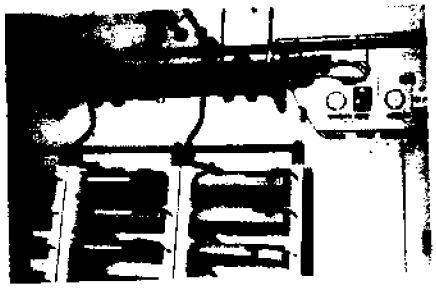


FIGURE 7. Series of banks of trays in new laboratory room for conditioning oysters for spawning in winter.

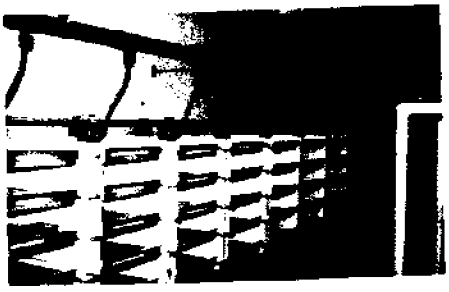
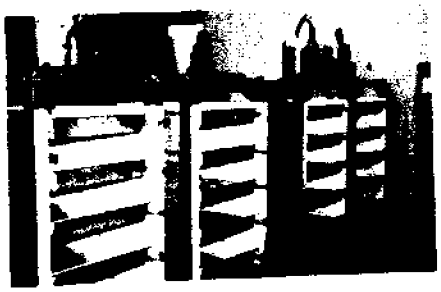


FIGURE 8. Banks of trays with both temperature and salinity controlled.



Equipment

Since gonad development and maturation of gametes in shellfish is speeded up by an increase in temperature, it was reasoned that lowering the temperature would retard gonad development and spawning (Loosanoff and Davis, 1951). Our first attempt along this line was with a field-rigged cooling device, concocted out of an old refrigerator unit, to cool the seawater supplied to the oysters. The oysters were held for some time in this rig before it broke down and the temperature of the seawater rapidly came up to its normal summer temperature, resulting in a mass spawning of our entire stock of oysters. This experiment did indicate, however, that the theory was sound. For several years, during the latter part of May, we would take a supply of oysters and clams to Boothbay Harbor, Maine, where summer temperatures of seawater are appreciably lower than at Milford and not high enough to permit spawning of our clams and oysters. Small groups of these animals were then brought back to Milford as needed for spawning. Oysters kept under these conditions could be spawned until early- to mid-October when those remaining in Maine waters would start resorbing gonads. It was found that oysters that had been induced to develop gonads in the spring and were spawned out just before taking them to Maine apparently required a longer time, at Maine temperatures, to develop gonads and did not resorb gonads as early in the fall, so that some of these oysters could be spawned as late as the following December or January. Clams do not resorb gonads so clams from the stocks kept in Boothbay Harbor could be spawned throughout the fall, winter, and even in the following spring and summer. When *Codium* was found in certain areas of Long Island Sound and diseases of oysters became better recognized, biologists began to question the wisdom of transplanting Long Island Sound oysters and clams to Boothbay Harbor and it became necessary to develop methods of holding oysters and clams at Milford for late-season spawning. Our first apparatus for doing this consisted of Frigid Units, Inc. insulated tanks in one end of which the cooling coil of a Frigid Units water chiller was inserted (Fig. 9). This did a satisfactory job except that, because there were a

FIGURE 9. Frigid Units water chiller used for cooling seawater to retard spawning.

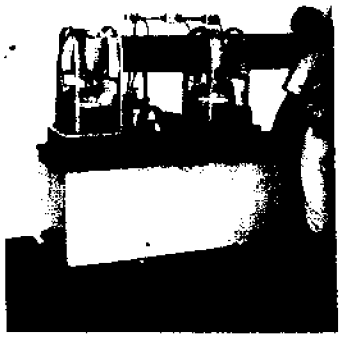


FIGURE 10. Insulated tanks for cold seawater in which clams and oysters are held.

number of dissimilar metals in the part that was immersed in the seawater, we had continuous trouble from electrolysis. The company was very good, however, in keeping us supplied with replacement units for the first summer so that we did not lose our spawners. By the next year we had developed the system so that the water chiller cooling coils were in a tank of fresh water and this chilled freshwater was circulated through coils of plastic coated copper tubing in the adjoining insulated tanks of running seawater where the clams and oysters were kept (Fig. 10). This eliminated the trouble with the water chillers but still left us with a relatively limited facility considering the needs of our pilot hatchery and our genetics program.

To augment our facilities for holding spawners for late season spawning we now have a 20.8-ton, water-cooled water chiller furnishing cold water for the bottom Karbate heat exchanger in Figure 2. This setup is designed to give 20 gallons per minute of cooled seawater bringing the temperature from 24° C down to 10°-15° C. This should be sufficient to supply about five of the insulated tanks.

Temperature Control in Larval Cultures

We have used a number of devices to control temperature in larval cultures. One of the simplest, yet quite effective devices was our constant temperature table (Loosanoff and Davis, 1963) (Fig. 11). It consisted of a water table that has a loop of lead pipe on the table connected to a stainless steel "can" under the table that had an electric heating unit inserted in it (Fig. 12). A sensing unit on the table, operating through a thermostat, controlled a relay which turned the current to the heating unit off and on. The hot water circulated through the loop by convection. A circulating pump picked up water at one end of the table and discharged at the other end to keep the water on the table in motion to prevent pockets of warm or cold water. As long as the temperature was kept above room temperature, convection currents in the larval containers prevented stratification and the entire culture was maintained at a temperature only slightly lower than the bath temperature, even though only a few inches of the bottom of the culture container were immersed in the water bath.

FIGURE 11. Constant temperature table in old laboratory.

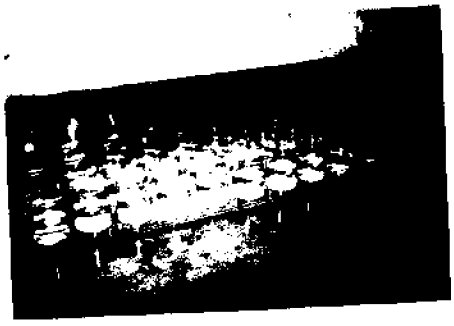
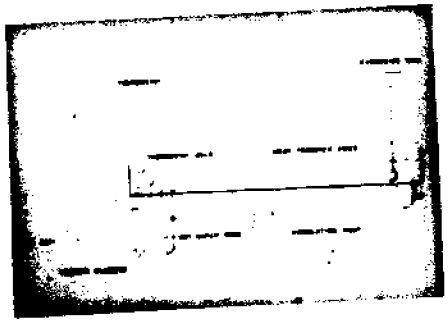


FIGURE 12. Schematic of constant temperature table in old laboratory.



A multiple temperature box of somewhat similar design built by Mr. Lucash of our staff was used in our studies of the effects of different temperatures on embryonic development and survival and growth of larvae (Fig. 13). This device has six separate water baths, each having both a hot water loop and a cold water loop with a double acting thermostat that opened and closed normally closed solenoid water valves to permit either hot or cold water to circulate through the loops to maintain the set temperature. The hot water was furnished by an apparatus like that used on the constant temperature tables and the cold water was furnished by a small Remcor water chiller. Both the constant temperature table and the multiple temperature box serve their purpose well and are reasonably inexpensive.

In our new laboratory, since the hot water furnace runs the year round, we have tapped the hot water from the furnace to heat the loops on our constant temperature tables (Fig. 14). The flow of furnace water through the loop is controlled by a pneumatic thermostat operating a normally closed pneumatic proportioning water valve. In this system the thermostat holds the valve open just enough to allow sufficient hot water from the furnace to circulate through the loop to maintain the set temperature. Simply by turning off the air to the thermostat the system is inactivated and the table can be used as an ordinary drain table. We have used a fiber glass lining for these constant temperature drain tables rather than PVC because it is easier to patch, seal on new pipes or otherwise modify.

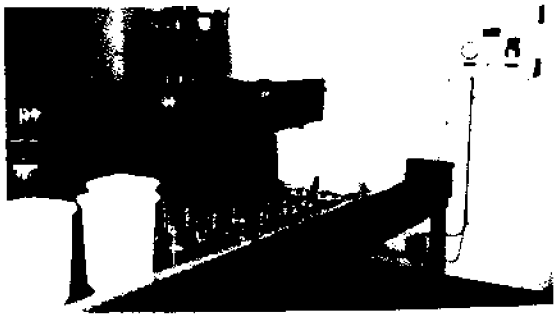
In our pilot hatchery we decided the most feasible system, as in most commercial hatcheries, was to maintain the room temperature at the temperature desired for the cultures (Fig. 15). One of the commercial hatcheries, however, uses electrical heating tapes wound around the culture vats to maintain the temperature, and another hatchery, I believe, still uses immersion-type electric heating units.

Pretreatment of Seawater

It is obvious that if you wish to keep your larval cultures free of debris and contaminating organisms, some pretreatment of the seawater is needed to remove

FIGURE 13. Multiple temperature box with six different water baths; each bath can be held at any temperature from 5° to 35° C within ±1° C.

FIGURE 14. Constant temperature table in new laboratory using the pneumatic controls.



them. Our first, most primitive method was to tie cotton batting held between layers of cheesecloth tightly around a hard rubber pipe that had been drilled full of 1/4-inch holes. This was moderately efficient but was difficult to make up and, because the cotton batting supports bacterial growth, the filters had to be changed frequently to eliminate buildup of bacteria and concomitant decomposition products. For our larval work we still use filters, made by several companies, consisting of a polyvinyl chloride body with a filter unit having a polyvinyl chloride or polypropylene core and wound with orlon, polypropylene, or some other nontoxic winding that will not support bacterial growth (Loosanoff and Davis, 1963) (Figs. 16, 17). Filter units in various sizes utilizing from one to as many as eight of the 10-inch cores are available (Fig. 18).

FIGURE 15. Hatchery-type larval rearing tanks in new laboratory pilot hatchery.

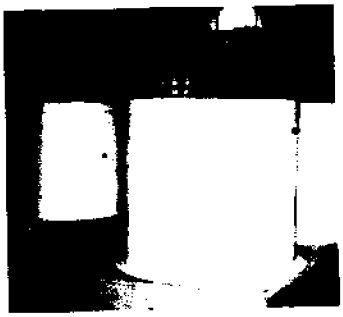


FIGURE 16. Filters, UV unit and fiber glass tank; a typical setup in each laboratory for preparing seawater for larval cultures.

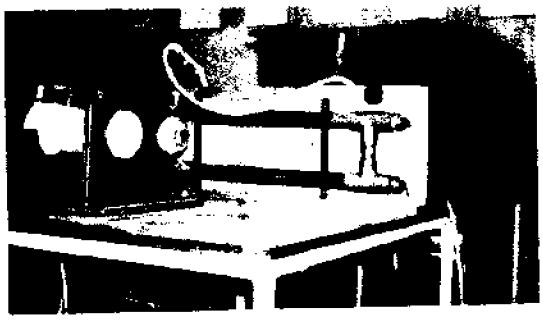


FIGURE 17. Schematic of filter unit.

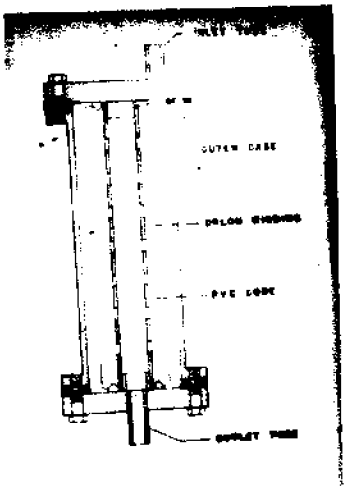
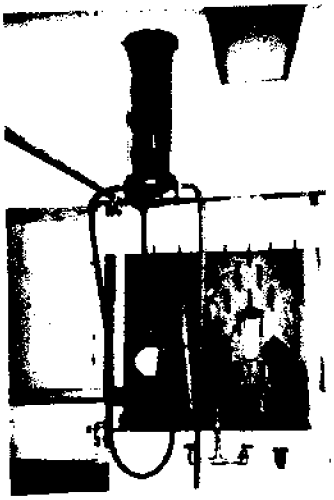


FIGURE 18. Filter holder using eight cores for filtering large volumes of seawater.



For our pilot hatchery work we have tried use of the centrifuge (Fig. 19) for clearing the water, as well as some of the multiple core type filters but have found that the most satisfactory device is a filter bag setup (Fig. 20). These nylon filter bags can be obtained in a number of pore sizes, and are made by Afco Filter Products, Division of American Felt Company, Glenville, Connecticut.

It is obvious, of course, that any type of filter unit restricts smaller and smaller particles as the filter surface becomes packed with debris, whereas a centrifuge unit of the Sharples type will take out only those particles of a given size or density and will continue to pass the smaller particulate matter that may be of food value to the larvae. Since, at least at Milford, it is necessary to add supplementary food throughout most of the year, this is of little advantage to us; therefore, we prefer the filter bags for clarifying the seawater. It is perhaps of significance that, with the centrifuged seawater, commercial hatcheries report that bubbling air through the cultures in their larval vats improves the rate of growth of the larvae, while with the cultured algal foods used at Milford we have not been able to demonstrate any beneficial effect of aeration. We believe that the centrifuges break up some of the larger phytoplankton cells and thus release appreciable quantities of dissolved organic matter and that bubbling air through such an enriched solution of dissolved organic matter forms organic particles (Baylor and Sutcliffe, 1963; Riley, 1963) that the larvae are able to utilize as foods. Since centrifuged Milford seawater, without supplemental algal foods, is capable of supporting larval growth only occasionally, we have not been able to carry out experiments to prove or disprove this assumption.

Ultraviolet Lights and Antibacterial Agents

We still lack really satisfactory methods for the control of bacteria in our larval and algal cultures. A good method for cold sterilization of seawater is urgently needed. We have routinely used ultraviolet treatment of the seawater used for larval culture since 1954 (Figs. 16, 21). We started using it in an attempt to control

FIGURE 19. Centrifuge of type used in many commercial hatcheries for clarifying seawater for larval rearing tanks.

FIGURE 20. Nylon filter bags for filtering seawater made by Afco Filter Products, Division of American Felt Company, Glenville, Conn.

the fungus disease that was creating havoc with our clam larvae (Loosanoff and Davis, 1963). This fungus disease occurred sporadically enough that we were never able to demonstrate that any method of control was really effective but we have not been bothered by fungus in our larval cultures since using the UV-treated seawater. We also know, from bacteriological analysis, that the bacterial count and the variety of bacteria are both reduced by ultraviolet treatment of the seawater. UV treatment does not completely eliminate bacteria, but we believe that the reduction in the number or possibly the types of bacteria is helpful.

We have also used Sulmet (American Cyanamid's veterinary grade of sodium sulfamethazine) and Combistrep (Pfizer's combination of dehydrostreptomycin and streptomycin sulfates) to control bacteria in our larval cultures. Neither of these substances is completely effective in preventing growth of bacteria known to be pathogenic to bivalve larvae, but in many instances they have greatly reduced mortality of larvae or increased the rate of growth or both. We suspect that either they selectively kill some of the toxin-producing bacteria or they tend to neutralize the toxins. It seems probable that the longer shellfish hatcheries operate, the more likely they are to develop endemic bacterial flora of toxin producers or actual pathogenic forms. Much more work needs to be done to identify these bacteria and develop methods for their control. We now use Sulmet with almost all of our cultures of oyster larvae and try to reserve Combistrep for use when all else fails.

Food Production Units

The amount of suitable foods in natural seawater varies from area to area and within any given area from time to time. As experimenting biologists and commercial hatchery operators have found, one must also control this environmental variable. In some areas and at some times the amount of phytoplankton in the water must be reduced to obtain satisfactory growth of larvae; in most areas at some times and in many areas at all times good larval foods need to be added to the seawater to augment that naturally present in the seawater.

Of the various types of food we at Milford have tested on bivalve larvae, the naked chrysomonad flagellates have proved to be the best (Davis and Guillard, 1958). We believe the foods must be particulate, they must be small enough to be ingested, they must contain all the essential elements, and they must stay in suspension. Many of the small unicellular algae seem to fulfill these requirements but

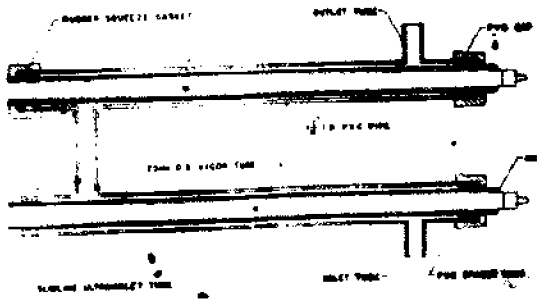


FIGURE 21. Schematic of UV light units as constructed at Milford.

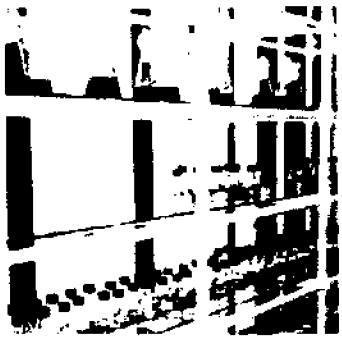
those lacking cell walls appear to be more easily digested, and among the naked ones those producing the least toxic metabolites are best. Those algae that produce little or no toxic metabolites, however, are the most difficult to maintain in pure culture probably because there are no toxic metabolites to repress growth of contaminating organisms. Consequently, we find that algae, such as *Monochrysis lutheri* and *Isochrysis galbana*, which in pure cultures are excellent larval foods, are easily contaminated by bacteria, some of which produce toxins that seriously interfere with growth of larvae or kill them. Apparently, some of these toxins that seriously interfere with larval growth have little or no effect on growth of the algae, i.e., cultures of algae that are growing beautifully may harbor enough toxin-producing bacteria to interfere seriously with larval growth. Other, even more heavily bacterized cultures of *M. lutheri* or *I. galbana* may remain reasonably good foods for larvae, i.e., not all bacteria are toxin producers and those that are not have little effect on larvae. It is significant that, in some instances, the majority of larvae that have received a toxic food at their first feeding will not grow even when transferred to new seawater and given nontoxic foods.

For an environmental controls system for culturing shellfish we urgently need a system for growth of relatively large volumes (several hundred gallons per day) of essentially bacteria-free algae. In the old laboratory temperature of the algal cultures (Fig. 22) was maintained at approximately 20° C by having the bottom three to four inches of the carboys submerged in a water bath maintained at about 18° C by a Remcor water cooler. Vigorous aeration of the cultures not only supplied the algae with the required air and carbon dioxide, but also prevented temperature stratification in the carboys. This system of temperature control worked very well on this scale. On the scale required for our new laboratory with our facilities for a hatchery program and an expanded genetics program this method for temperature control was considered inadequate.

In our new laboratory, therefore, in both the stock culture room and the mass culture room the entire rooms are maintained at about 20° C and the flasks in the stock culture room (Fig. 23) and the carboys in the mass culture room (Fig. 24) set on open shelves. The cool-white fluorescent lights and their ballasts are mounted behind glass in a duct supplied with a flow of cooled air, and temperature controls

FIGURE 22. Mass culture carboys in old laboratory.

FIGURE 23. Stock culture room, new laboratory, showing cultures on open shelves.



are arranged so that if the temperature in the room goes above a set temperature (about 24° C) due to failure of the air conditioning equipment or for any other reason, the lights are automatically turned off. This is an essential feature and was installed only after we had lost our cultures a time or two due to failure of the air conditioning equipment and the subsequent rise in temperature to nearly 30° C. The carboys are used to inoculate larger tanks (Fig. 25). We periodically check each carboy and tank for toxicity by feeding a sample of each culture to oyster larvae; when these tests show that the algal culture is toxic, contents of that carboy or tank are discarded, the apparatus is sterilized and a culture is started from a new inoculum. Once a culture has become toxic we have not been able to correct its condition.

Control of Quality of Seawater

During the past two or three years we have found some rather striking evidence that the "quality" of the seawater in which oyster embryos develop to the straight-hinge larval stage can affect the subsequent growth and survival of the larvae. This was first brought to our attention when I started raising larvae in the new laboratory. My associate, Mr. Rhodes, would spawn oysters in the old laboratory and give me some of the eggs for experiments in the new laboratory. First we noted that while his larvae grew quite normally, those I was culturing in the new laboratory did not grow well. To verify this we made sure we had sibling larvae that were fed the same food. As another check, I would give him some of the larvae I had raised to straight-hinge and take some of his. We would then try to grow larvae from both sources in each laboratory. Larvae brought to straight-hinge in the old laboratory would grow normally in either building, but of those reared to straight-hinge in the new laboratory only a few would grow at relatively normal rate, while the majority grew hardly at all and eventually died. We thought that this was due to some toxicity in the seawater system of the new laboratory which was subsequently leached out, but we have recently found that this apparently toxic water occurs from time to time and the apparent toxicity varies in intensity. Larvae brought to straight-hinge in some batches of seawater will all die with none showing appreciable growth, while almost 100 percent of sibling larvae brought to straight-hinge in

FIGURE 24. Mass culture carboys in mass culture room, new laboratory, on open shelves, with lights and ballasts back of plate glass in ventilated duct. Room maintained at about 68° F.

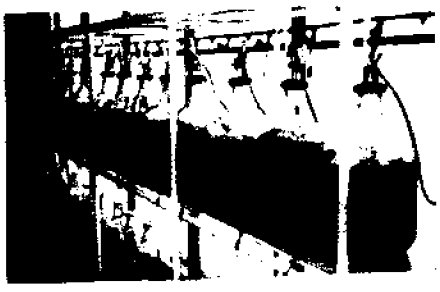
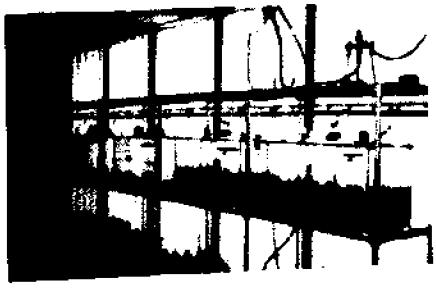


FIGURE 25. Larger tank in mass culture room on open shelves.



seawater from another source will grow normally. At other times, when the toxicity is apparently somewhat weaker, approximately 50 percent of the larvae brought to straight-hinge in the "bad" seawater will grow at almost the same rate as sibling larvae brought to straight-hinge in "good" water, while the remaining 50 percent never grow and eventually die. Since our geneticists have shown from full-sib crosses that our oysters must carry a very heavy load of deleterious genes (that result in virtually 100 percent mortality during fertilization, embryonic or early larval development in full-sib crosses), we believe the "bad" seawater is exerting a variable but comparatively stringent selective pressure that eliminates those genetic combinations least capable of surviving under these conditions.

These findings and our previous observations on the effect of toxic blooms indicate the desirability, particularly for experimental work on physiology of larvae and genetic studies, of a source of seawater free of such toxins or at least constant in its effect on embryos and larvae. In an attempt to obtain such a constant source at Milford, we have tried to develop a seawater well which we believed would give us a supply of seawater that would be at least more consistent in quality. Since we did not get seawater, we have no evidence on how effectively well seawater would overcome this difficulty.

Another development at Milford in which you might be interested is our tank farm (Fig. 26). Several years ago Mr. Landers found that juvenile clams kept in such tanks grew more rapidly than sibling clams suspended in Milford Harbor. The rate of growth was dependent upon the rate of flow of seawater and the size of the juvenile clams. The faster growth achieved in these tanks, we believe, is because in them there is a continuous exchange of the seawater in immediate contact with the clams so that the food supply is constantly being replenished. The rate of exchange of water over those suspended in the harbor, however, apparently was not rapid enough to replenish completely the water as fast as the clams pumped. Another evidence for this was that, particularly as the clams increased in size, the rate of growth of clams nearest the intake end of the tank was faster than for those at the overflow end unless the rate of flow was increased.

In our tank farm we would like to be able to control fouling and competing organisms. We found that we could control growth of filamentous algae by using black tanks and covers for the tanks. We have tried commercial UV units to prevent

FIGURE 26. New tank farm facility at Milford for rearing recently set hatchery-reared clams and oysters to appropriate size for planting in natural waters and for rearing various genetic lines to spawning size.

setting of barnacles, mussels, soft clams, coot clams, and sea squirts but have found that the UV light was only partly effective for this purpose. Treatment by UV does, however, do a reasonably effective job of killing bivalve larvae, so we do use it on tanks containing recently set oysters from our genetic studies to prevent setting of "wild" oysters that might interfere with the genetic studies. We believe also that a more effective UV system might reduce the number of other above-mentioned fouling organisms to an acceptable level.

In conclusion, I think that at Milford we have achieved fairly good control of the easily-controlled factors, such as salinity and temperature, but still are not adequately controlling some less well-known but important factors, such as the bacteria and their toxins in our algal and larval cultures, the quality of our seawater, and fouling organisms in our tank farm.

LITERATURE CITED

BAYLOR, E. R., AND W. H. SUTCLIFFE JR., 1963. Dissolved organic matter in seawater as a source of particulate food. *Limnol. Oceanogr.*, 8:369–371.

DAVIS, H. C., AND R. R. GUILLARD, 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. *Fish Bull., U.S. Fish and Wildlife Serv.*, 58:293–304.

LOOSANOFF, V. L. 1945. Precocious gonad development in oysters induced in midwinter by high temperature. *Science*, 102: 124–125.

LOOSANOFF, V. L. 1949. Method for supplying a laboratory with warm seawater in winter. *Science*, 100: 192–193.

LOOSANOFF, V. L., AND H. C. DAVIS, 1951. Delaying spawning of lamellibranchs by low temperature. *J. Mar. Res.*, 10: 197–202.

LOOSANOFF, V. L., AND H. C. DAVIS, 1963. Rearing of bivalve mollusks, pp. 1–136. *In:* F. S. Russell, Ed., *Advances in Marine Biology, Vol. 1*, Academic Press Inc., London.

RILEY, G. A., 1963. Organic aggregates in seawater and the dynamics of their formation and utilization. *Limnol. Oceanogr.*, 8: 372–381.

DISCUSSION

CASTAGNA: In regards to toxic water, have you ever treated for it—such as trying to detoxify with a chelating agent?

H. C. DAVIS: In regards to chelating agents we have tried a few things but found nothing that would chelate some toxins in our seawater system. One point about the use of chelators which may be of interest is that when I first started work on the effect of salinity, I used chelators on our tap water so that I could dilute seawater. I discovered very quickly that one has to know the proper amount of material to chelate because one can overchelate just as easily as underchelate. Also we have used chelators like Fuller's Earth and Kaolin in particular with our work on turbidity. At the proper concentration of these turbidity-producing agents you obtain a slight increase in the rate of larval growth over controls. In an experiment involving the use of Sulmet compared to no Sulmet with turbidity-producing agents there was almost no difference in growth between the two treatments.

ZAHRADNIK: Mr. Davis, concerning the cost estimates that you referred to in the American Cyanamid Report. It might be reassuring to biologists, since you all do not agree with one another, to know that engineers do not agree with one another either. Cost estimates made by us are much less than those made by the American Cyanamid people because we have taken into consideration that pumping costs are a function of the age of the animal that you are working with, the time of the year, the amount of food in the water. More important than this is the function of the kind of system that you are using to pump the seawater. We have designed a system that utilizes a siphon effect so that all the energy used raising water to some elevation is not wasted by allowing it to return to the sea via an open channel. If one applies a siphon principle, one can substantially reduce the pumping cost

The second point is that your approach has been to try to optimize each one of the subsystems. You have tried to optimize conditioning, spawning, rearing, and setting. This does not necessarily produce the optimal integrated system. It seems to me as a systems engineer that the great need is to integrate and optimize these various subsystems that biologists to a great extent, and with some amount of success, also developed. In integrating the subsystems we may find that we will have to accept less than optimal subsystem performance in order to achieve optimal systemal performance.

The third point I would like to offer is that there is great difference between the kind of total environmental control that researchers demand in their work compared to the total environmental control that is required for production. I can illustrate this by saying whenever you study some phenomenon you want to determine a range of variables like temperature, salinity, algal concentrations. However, once this range has been defined it is much more economically feasible to design a system to control at a given set point rather than a range of set points; this represents a tremendous difference in cost.

H. C. DAVIS: No, I thought I had made it abundantly clear that I do not think a completely controlled environment was either desirable or feasible at this time. Moreover, I think American Cyanamid's figures are based on the use of well water or water completely devoid of food so that they would have to produce all of their food requirements.

ZAHRADNIK: I know that you use supplementary feeding, but when you change your water temperature 10° or 15° C, does it destroy some of the value of the food? Simply, is the water all right for the oysters to feed on?

H. C. DAVIS: When you increase the water in the heat exchangers to 40° C, yes, you probably knock out almost everything. But we mix that water with cold seawater to achieve the temperatures we want; some food will still remain. It is decreased no doubt by some amount and this is evidenced by the sludge in the heated seawater.

ZAHRADNIK: The dead cells, for instance, if they are used right away, could the oysters utilize them?

H. C. DAVIS: I think so as long as the cells are not disintegrated.

Introduction to EFFECTS OF PHYSICAL STIMULI ON THE ENERGY REQUIREMENTS OF SEPARATING THE VALVES OF AN OYSTER: TECHNIQUES AND DEVELOPMENT OF AN OYSTER-SHUCKING MACHINE

THOMAS H. WILLIAMS
Instructor, Agricultural Engineering
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Any conference on shellfish should not overlook some of the aspects of processing. This session will deal with oyster shucking.

The Agricultural Engineering Department here at the University of Delaware has for the past year been working on an NSF Sea Grant subproject entitled "The Effects of Physical Stimuli on the Energy Requirements for Separating the Valves of Oysters."

The objective of this project is to determine which external physical stimuli will induce valve separation in the Eastern oyster without changing the raw food quality of the meat. We feel this is a preliminary step for developing an automated oyster-shucking device to replace the manual labor presently employed by the industry.

Experiments to date have utilized electrical stimulation of the adductor muscle, thermal shock, ultrasonics, and compressed air. None of these studies has shown results that suggest further development. However, we have many more energy sources to investigate.

TECHNIQUES AND DEVELOPMENT OF A RAW-OYSTER-SHUCKING MACHINE

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The problems involved in mechanically shucking raw oysters are perhaps the most significant facing the industry and have remained largely insoluble through the ages. In this day of ever-increasing technology we find that laborers skilled in hand shucking methods are becoming extremely scarce. In fact, the art of hand shucking on a commercial scale may dwindle to the point where increased production methods may actually create a glut of unshucked oysters. Therefore, it is imperative that a mechanical means of shucking be found and perfected soon.

This is not to say that a number of mechanical methods have not been developed, tested experimentally, and perhaps used in some commercial operations. However, all of them to date have had severe limitations and hand shucking still pervades the industry.

Because I grew up the son of an oysterman and have been involved in the oyster business several years of my life, the development of a mechanical shucking device has long been of keen interest to me. Actually, as many of you know, I've spent considerable time and effort in developing an automatic raw-oyster-shucking machine that was demonstrated to packers in Maryland and Virginia in the autumn of 1968. This prototype has been under constant study and experiment since and is rapidly approaching a stage of commercial production. A commercial model is now on the drafting board and I hope will soon be built and made ready for the market.

I would like to tell you something of how I have arrived at methods employed in this particular machine. To begin with, I studied the anatomy of the oyster to see if I could find an "Achille's heel" that could be exploited by a machine. I knew there were three attachment points holding the valves (shells) of the oyster together, the hinge ligament and the two adductor muscles. After investigation I learned that I could use a diamond-edged abrasive wheel to cut off the hinge end of the oyster thus eliminating one attachment point and also thus exposing the body of the oyster meat and the adductor muscles where they are attached to the shells. Next, by arranging two spring steel knives so they would slide through the hole made by cutting off the hinge and along the inside of the shell, the muscle attachments could be severed. Of course, an integral part of the machine is the spiked

clamp that holds the oyster tightly in position while the diamond wheel cuts off the hinge and later when the steel knives are in the process of shucking (severing the adductor muscles from the inside of the shell).

Briefly, the operation of my machine is as follows: The oysters are placed by hand, hinge side up, in a feeder wheel that carries them to the shell clamp. As the oyster proceeds through the machine the hinge portion is cut off by a diamond wheel. Afterward, two arms spread the attached shells apart at the aperture made by the diamond cutting wheel so that the two shucking knives may easily enter between the two shells and by sliding downward along the inner face of the shells will sever both muscle attachments from the shells. The shucking knives, after severing these adductor muscles, push the shells farther apart so the oyster meat drops freely below.

The smallest machine, operated by one feeder such as the prototype demonstrated, will shuck approximately 1200 oysters per hour, producing three or four gallons of oysters, depending on their sizes. Machines capable of larger volumes are considered desirable and feasible.

I have made a study of the quality of raw shucked oysters based on market acceptability and have found that oysters shucked by my present prototype machine are equivalent to hand-shucked oysters. It will also shuck single oysters ranging from legal size 3″ upward to 5″ without adjustments to the machine. Another machine grades the shucked oysters into four sizes or grades of meat.

The machine will shuck singles only. Clusters can be broken apart mechanically so that more than half of them can be recovered as singles and machine shucked.

Some oysters, because of extreme abnormalities and irregularities of shells, cannot be shucked by the machine. A study to determine what percentage of oysters harvested, including these clusters and abnormalities, must be shucked otherwise has been pursued for the past year with samples from many sections of the Chesapeake Bay and shows the figure to be about 20 percent. It also indicates that most of these are unprofitable to shuck by hand and often are discarded by the hand shucker and end up on the shell pile. One situation that will be given consideration will be to grade out mechanically such unshuckable oysters and provide nearby facilities for recovery of such meats by steaming and processing them as canned oysters or oyster stew.

In conclusion, the machine I have developed is not perfect but it is a major step toward automation of a very difficult job and the commercial model we shall be manufacturing in the months ahead should ease the shucking problem by increasing production and profits. At the same time others will be working on other methods to solve the same problem including irradiated heat, hot water dips, microwave energy, etc. So I am convinced that all of us working for the same objective, industry and academicians, will eventually (and I hope soon) solve the problems of mechanical raw oyster shucking.

Introduction to SYSTEMS ANALYSIS OF OYSTER PRODUCTION

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Historical Background

Growing concern over the marked decrease in oyster production on the East Coast prompted numerous studies for the revival of this industry. The most comprehensive report was one by Matthiessin and Toner published in 1966 entitled *Possible Methods of Improving the Shellfish Industry*. Using this as their major reference, the American Cyanamid Company published a problem analysis of controlled environment growth (*New Engineering Approaches for the Production of Connecticut Oysters*, 1968).

The systems engineering project at the University of Delaware has undertaken a comprehensive systems analysis that will combine not only the theoretical research as done by the American Cyanamid Company but also the practical research accomplished by the University's Shellfish Laboratory.

Goals of the Delaware Systems Program

Two specific goals have been established as being of significant value to the project and represent the direction of our present efforts:

1. A digital simulation of the entire oyster production process is being developed. This simulation is planned to facilitate:
   a) Studies of the economic feasibility of a prototype system
   b) Examination of the effects of experimental variables
   c) The design of a pilot facility
2. As a natural fallout from the planning of a digital simulation, new areas of necessary research are being identified. These new research areas pinpoint

significant inadequacies in our present understanding of oyster production process or control of the life cycle.

A digital simulation will be done for each subsystem and then each will be optimized. The optimization will include:

a) Changing experimental variables
b) Varying design control factors (i.e., oyster stacking, production rate, tank width)
c) Redesigning facilities

These subsystems will then be combined by a "main" program to unify the description of a controlled environment system for oyster production.

The Digital Simulation

A study of the life cycle of an oyster reveals numerous interrelated variables. A simulation of oyster growth in a factory situation involves the understanding and definition of these variables. The overall system has been broken into smaller subsystems. Listed below are these subsystems, their percentage of approximate plant costs, and the number of variables associated with each.

Subsystem	*Percentage of Physical Plant Cost	Identified Variables Associated with Each Subsystem
10. Algae System for Food Supply	5.7%	40
20. Hatchery Operations	4.2%	100
30. Oyster-Growing Tanks	60.0%	70
40. Packaging, Processing and Marketing	.5%	30
50. Water Supply System	19.5%	25
60. Waste Disposal System	5.1%	5
70. Instruments, Replacements	5.0%	—
	100.0%	270

*Based on American Cyanamid Report

Areas Requiring Further Research

The following sections are capsule descriptions of areas that may warrant additional research in the future. It would be desirable that the outcome of this research be parametric relationships that are needed to develop a reliable digital simulation.

1. *Breeding*
   a) *Spawning Control:* presently a large area of research but several approaches should be investigated.
      (1) Growth characteristics of oysters grown to market size at lower temperatures to prevent gameto-genesis.
      (2) The development of a sexless, or nonreproductive strain of oyster.
      (3) Infection of oysters with sporocysts which attack gonadal tissues and in effect castrate the animal.

b) *Selective and Cross Breeding:* a systematic study should be made of all known types of oysters to determine what varieties should be considered first in a selective breeding development program. Following these "paper" studies an optimum commercial oyster should be developed.

2. *Environmental Control*
   a) *Temperature Adaptation:* studies should be carried out to determine the ranges of temperature to which the most promising types of oysters will adapt.
   b) *Hydraulic Characteristics of Oysters:* understanding the natural and optimum flow patterns is important to optimize:
      (1) Water flow rate
      (2) Direction of flow
      (3) Type of flow (laminar, turbulent)
      (4) Orientation of oysters
      (5) Stacking depth
   c) *Shape Control of Shell:* methods should be developed to:
      (1) Maximize internal shell volume
      (2) Control production of shell material
   d) *Salinity:* studies should be carried out to determine:
      (1) Optimum salinity for maximum rate of growth
      (2) Effects of the rates of change of salinity by tidal cycles
   e) *Reactor Design:* oyster growing tanks may be regarded as chemical systems which might fruitfully be studied as chemical reactors.
   f) *Cultch Design:* investigation of setting techniques to find a cultch with some or all of these characteristics.
      (1) Limits the number of spat that adhere to cultch material.
      (2) May be separated or expanded as the oysters grow.
      (3) Is economically adaptable to a continuous or batch commerical process.
      (4) Is nontoxic or, better yet, is beneficial to oyster growth.
3. *Diet Control:* a carefully controlled study is needed that provides food to test oysters at a known rate to determine foods that:
   a) Maximize meat growth
   b) Affect flavor
   c) Minimize shell growth
   d) Provide the correct balance of trace elements
4. *Waste Control:* further investigation to determine:
   a) Mechanics of elimination
   b) Possible uses of waste
5. *Diseases:* studies should be initiated to identify:
   a) Potential diseases in a closed system
   b) Methods to minimize disease effects (isolation or early detection)
   c) Treatment of oyster diseases by either prevention or control
6. *Market Analysis:* an economic survey concerned with:
   a) Potential markets if a continuous oyster supply is available to the public

b) Market potential for:
   (1) Half-shell trade
   (2) Canned and processed oysters
   (3) Food concentrates
   (4) New food uses
c) Value of by-products such as oyster shell
d) Significance of a major advertising campaign on public consumption

LITERATURE CITED

MATTHIESSEN, G. C. and R. C. TONER, 1966. *Possible methods of improving the shellfish industry of Martha's Vineyard, Dukes County, Massachusetts*. The Marine Research Foundation, Inc., Edgartown, Massachusetts, 138 p.

New Engineering Approaches for the Production of Connecticut Oysters—A Problem Analysis, Vols. I and II, by American Cyanamid Company, Central Research Division, Stanford, Conn.

A SYSTEMS ENGINEERING APPROACH TO MOLLUSC PRODUCTION

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I wish to express my appreciation for being afforded the opportunity of addressing this conference on the Artificial Propagation of Commercially Valuable Shellfish, first, because the technology of aquaculture in general seems to represent a feasible candidate solution for increasing available protein for protein-poor nations of the world, and second, because system engineering, born and bred as a tool for the management of high technology developments in aerospace and electronics, when applied to aquaculture, represents a significant example of a tool or technique which can be economically transferred and adapted to this commercially oriented sector of our economy.

To properly set the stage for my comments on the application of system engineering to mollusc production, I must first insert a few additional qualifiers with regard to this paper. I am not reporting an application—I am anticipating one, so this will not be a case study. Rather, it is a description of problem characteristics that make system engineering appear suitable as a philosophy, process, or discipline for attacking the mollusc aquaculture problem. There are no results presented, rather a demonstration of parallelism between types of problems and a gross attempt at problem formulation.

The term "system" with its numerous appended supplementary descriptions (i.e., analysis, engineering, design, etc.) appears with increasing frequency in the jargon of the technical community, all too frequently with inadequate definition of terminology. Allow me, therefore, to define terminology, and to thus establish a common ground for understanding in this probe into aquaculture applications.

System Engineering

System engineering, which has its own eternal triangle, (Fig. 1), is variously defined as:

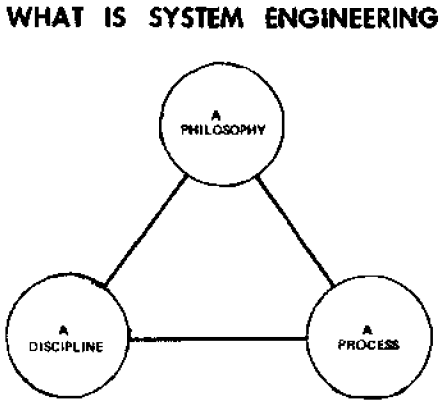
1. A philosophy that leads the engineer to consider complex systems 1) as an *integrated* problem rather than functional subproblems, and 2) in the light of total resources that must be brought to bear on the problem. The *system* engineer is thus concerned with equipment, performance, cost, spares availability, training of personnel, job safety, etc., etc.
2. A process—a multistage (frequently five), analytical and management control procedure covering a system's evolution from concept formulation to phase-out, and concerned with the tracking and evaluation of a system's performance through its many stages of evolution.
3. A discipline—which, in a professionally interdisciplinary environment, explores the feasibility and evaluates new technoeconomic systems using models, parametric analyses, tradeoff analyses, and other similar analytical tools and methodology which I will briefly explore later in this paper.

System Engineering (S.E.) is all of these things, but with a degree of applicability consistent with the complexity and economic constraints of the program to which applied. I am, however, discipline oriented, and from that viewpoint, also see S.E. as a management tool that affords the opportunity to assess engineering decisions in the light of quantifiable or qualitative constraints. *In a data-rich environment,* it permits the rapid and early evaluation of many more concept alternatives than normally made available to the program manager. *In a data-poor environment,* it provides a method for acquiring insight into program sensitivities in a well-ordered manner, quite frequently, pinpointing areas in which quantification efforts can *best* be devoted.

Stages of System Engineering

The stages of system engineering are characterized by stages of accomplishment the first of which is concept formulation. The disciplinary aspect of system

FIGURE 1. The three aspects of system engineering.



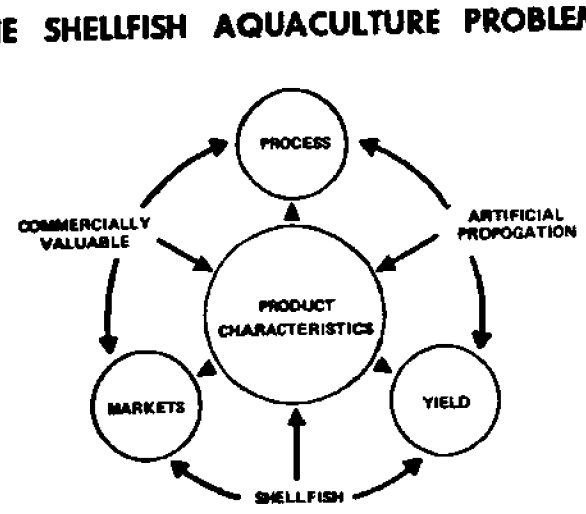
engineering manifests itself quite differently in each of the stages of system engineering as a process. In concept formulation, the system engineer is concerned primarily with problem definition, the establishment of requirements, and the mapping of alternative solutions in the broadest terms for the system as a whole (frequently more qualitative than quantitative).

Looking to the title of this conference as the problem definition (Fig. 2), for example, *Artificial Propagation of Shellfish* and *Commercially Valuable* would be interpreted in terms of qualitatively expressed objectives, constraints, and design concepts—thus, *Commercially Valuable* as an objective would suggest consideration of markets (and demand functions) as constraints. These in turn would suggest that *Product Characteristics* and *Yield* require consideration, the former as relates to *Species;* the latter as relates to *Process* specifications and design concepts.

In a similar manner, *Artificial Propagation* as an objective would be quite readily interpreted directly in terms of *Process* design concepts, and ultimately also in terms of *Product Characteristics* and *Yield*. Perhaps the single most significant factor that must be realized is that in the reconciliation of *Artificial Propagation* and *Commercially Valuable* as objectives there is a danger of suboptimization since the biological aquaculturists' objective function of *maximal yield* may not be consistent with optimization of the business aquaculturists' objective function of *commercial value* (profitability). Therefore, the general plan of attack that can be seen to be emerging with this early definition of interdependencies requires continuous consideration of the *system as a whole.*

In the second stage, that of System Definition, the system engineer builds a quantitative baseline as a yardstick for measuring the extent of system optimization resulting from considering alternative design concepts. The third, fourth, and fifth stages comprise the implementation stages terminating in either phase-out or growth.

FIGURE 2. Definition of the shellfish aquaculture problem.

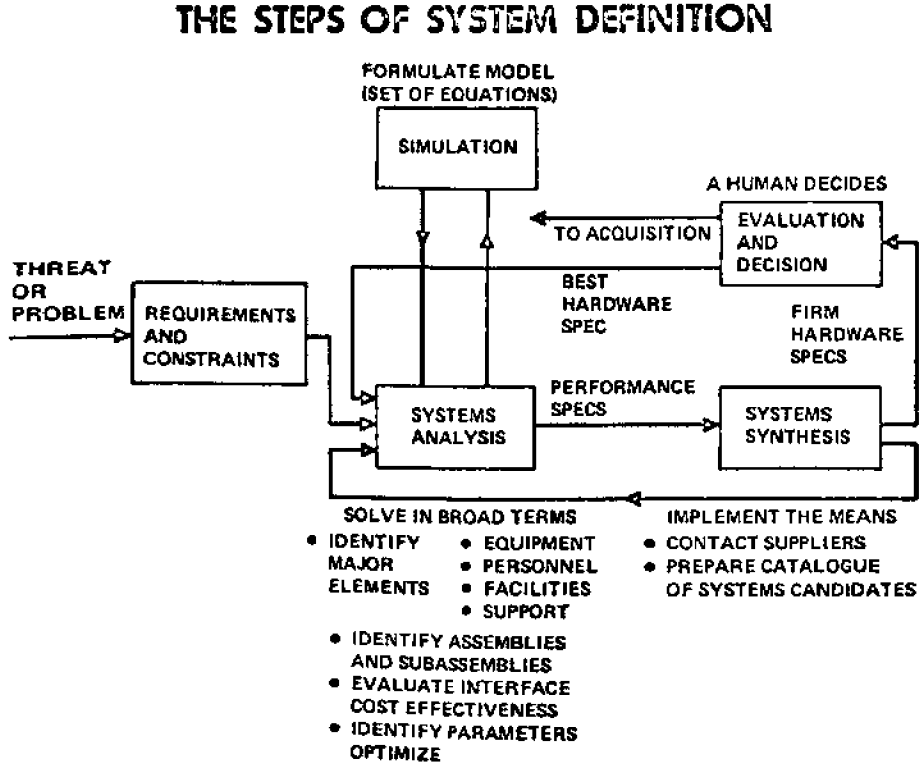


Systems Definition

Returning now to the Systems Definition stage, the activities performed in the four steps indicated in Figure 3 comprise the methodology that takes the process from analysis to decision making. Information accumulated at each step, directly and through feedback, determines the final choice among the candidate systems. The process starts with a rigorous analysis of requirements and constraints—an expansion of the information that had been more generally and qualitatively expressed in concept formulation. This is followed by a systems analysis step in which the system engineer delineates the functions and activities that comprise the operational system. It is this functional description that is the key to the entire analytical procedure and provides the means for identifying many of the system characteristics. Under some circumstances, it is valuable to create a simulation model to facilitate understanding functional interrelationships particularly when the system being analyzed contains:

1. Many processes and thus,
2. Many interfaces between process elements, where
3. Many constraints exist as do
4. Ill- or under-defined functional relationships, and
5. Ill-defined objectives.

FIGURE 3. The principle steps of the system definition process.



The model can be digital, analog, or hybrid and serves primarily as a means for exploring design trade-offs in an environment in which, because of the many alternatives previously mentioned, intuitive evaluation and decision making would be dangerous. The alternatives are subjected to sensitivity analyses with particular emphasis paid to exploring boundary conditions. The system is rarely simulated as a whole, but instead the subsystems are simulated and their models tied together.

The last two steps, synthesis and evaluation, are then performed with the help of the simulation model and within bounds that have been established by the sensitivity analyses. Thus, more effort can be expended on *relevant* alternatives.

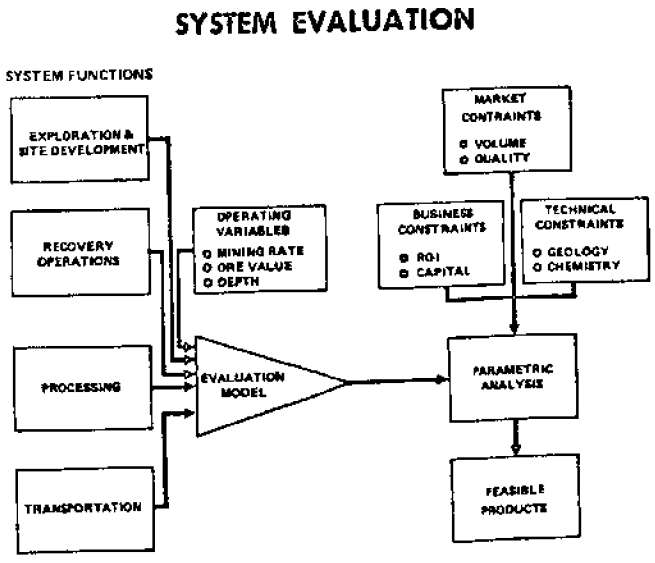
Some Illustrations

Having established the system engineering framework as a process and discipline, I would now like to explore a few applications to commercial systems that will serve to illustrate different stages, degrees of sophistication, and comprehensiveness with which the techniques can be applied, while simultaneously demonstrating how we can approach aquaculture in a similar manner.

The illustrations I have selected also derive from oceanic systems, namely Ocean Mining and Ocean Transportation. Functional similarities between aquaculture, agriculture, and other forms of animal husbandry appear reasonable in principle, but hardly similar in technology, and the quality and sufficiency of data for analytical purposes is an unknown. It would appear desirable under these circumstances to establish an analytical framework around which to organize a study of aquaculture as a system and to identify information gaps.

As a guide to structuring such a framework, Figure 4 portrays one developed for examining and evaluating ocean mining, a field similarly faced with extrapola-

FIGURE 4. The elements considered in the evaluation of ocean mining systems.



tive problems. As this indicates, four system functions were identified: exploration and site development, recovery operations, processing, and transportation, with many subfunctions defined for each major function. Preliminary engineering evaluations in concept formulation led to the conclusion that the impact of the new environment would more likely affect operational factors than require significant changes in the technical state-of-the-art. Operating requirements and technical constraints were specified and detailed design studies undertaken for a multiplicity of different concepts that could satisfy the specifications. Additionally, market and business constraints were specified in terms of boundary conditions such as minimum acceptable return on investment (ROI), maximum allowable capital investment, maximum penetration, and minimum quality of delivered ore.

Figure 5 depicts a possible organization for aquaculture not too dissimilar, you will note, from the one described for ocean mining. As a matter of fact, it will get to look a lot more like it as the framework is expanded. The paucity of data suggests the need for parametric analysis and thus *system models* for exploring *process interfaces* and other *sensitivity analyses*.

Functional Analysis

Having devised an analytical framework for examining aquaculture, the next activity to be undertaken is that of functional analysis.

FIGURE 5. An organizational framework for evaluating aquaculture activities.

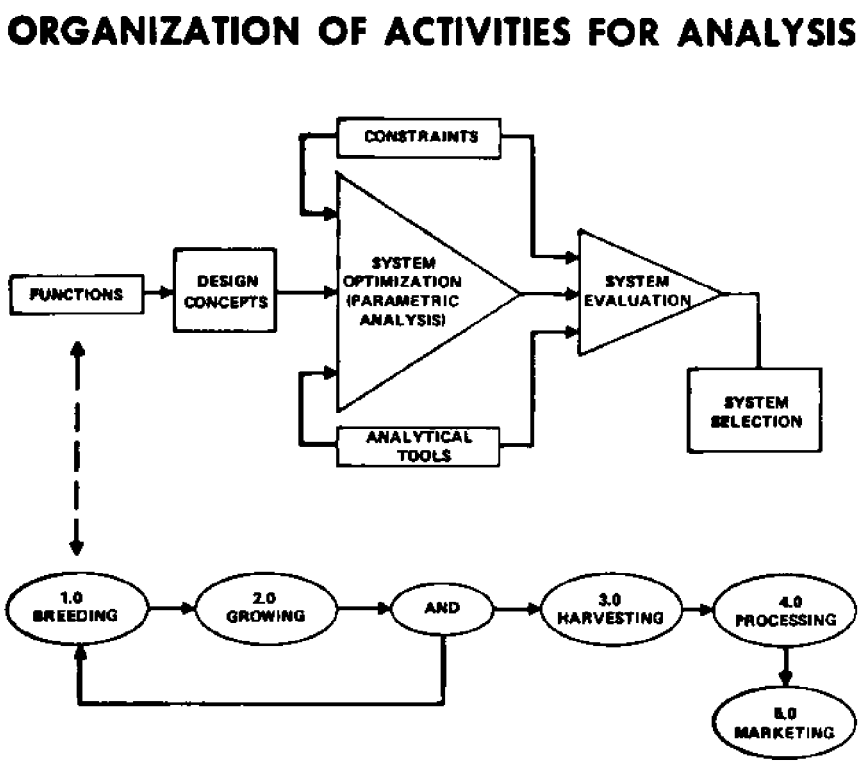


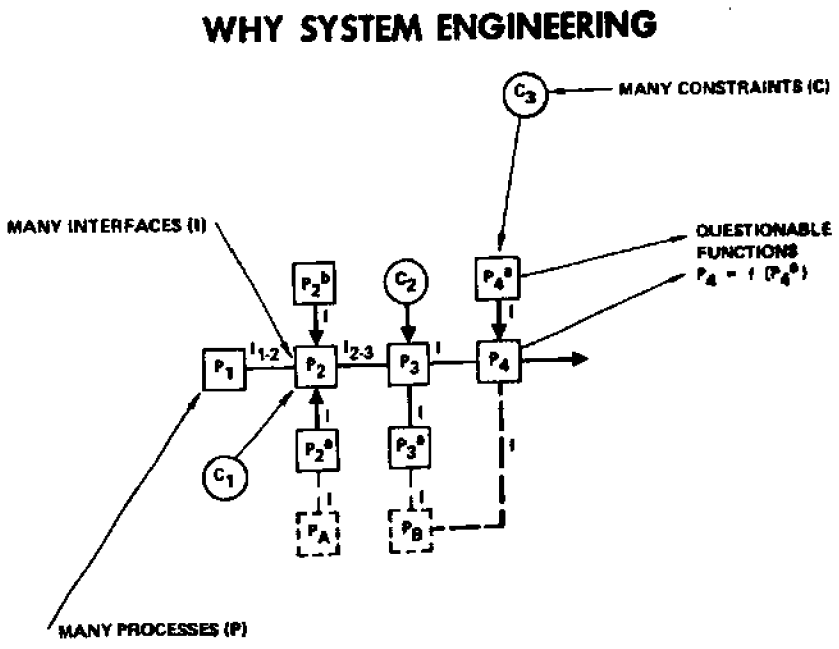
Figure 6 identifies the information developed in this step, including process elements and operations, intra- and inter-system interfaces, operational or process constraints, and functional relationships that relate inputs and outputs for each identified element.

Looking first at the functions of aquaculture, with little research we can intuitively describe the most obvious functions as shown on the lower half of Figure 5 namely: breeding, growing, harvesting, processing, and marketing. The primary functional loop between growing and breeding has been indicated. Information loops not shown might include market feedback to breeding, growing, and/or processing.

Since the purpose of analyzing the functions is to assure accountability for all systems and components necessary to implement the system, as well as to identify parameters that describe the system's operation, and the technologies upon which the system is dependent, a more detailed functional analysis is required. The previous intuitively defined functions might be expanded in any of several ways described by processes found in a number of literature sources including the work of John H. Ryther et al., in *The Status and Potential of Aquaculture*. The description of G. Vanderborgh and Son, Long Island oyster producers, is particularly enlightening in this regard, and has been used for the purpose of illustrating the development of more detailed functional descriptions.

Figure 7 depicts an expansion of the aquaculture functions in general, and in particular illustrates the second level of detail in the description of breeding—what was formerly a five-step process is now a nine-step one.

FIGURE 6. The purpose of functional analysis in the system engineering process.



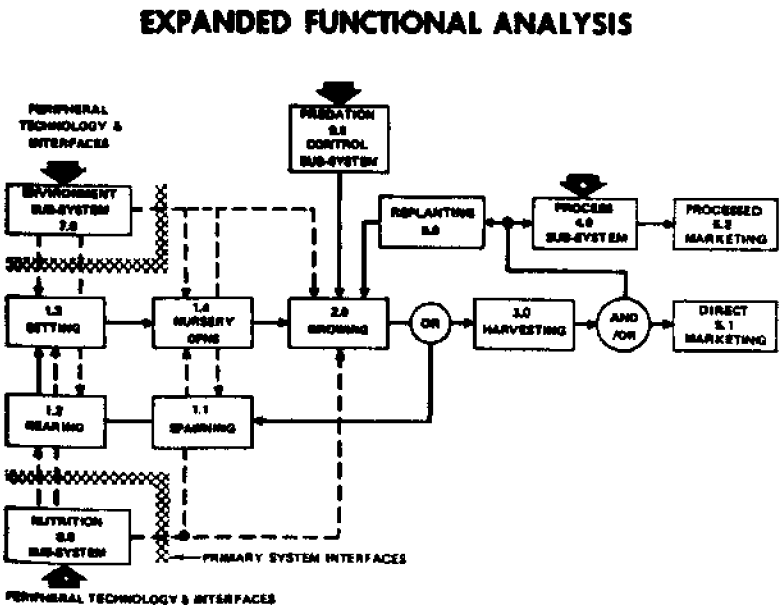
Although far too detailed for easy viewing, Figure 8 illustrates the level of functional analysis required to facilitate identification of the process parameters, process interfaces, and peripheral considerations necessary to suitably model the system. Environmental control interfaces have been coded to permit rapid recognition of their all-pervading influence on the process. Again, it should be noted that only the breeding function has been examined, and that for only one interpretation of the process.* Concurrent with the detailed functional analysis, external constraints upon the system should be evaluated and then finally, process parameters defined on the basis of both sets of considerations.

Constraints

In the use of the terms system and subsystem, it is important to recognize that hierarchies exist; thus, although we consider mollusk aquaculture as a "system," constraints upon it represent the interface of higher level systems of which this particular problem (mollusk aquaculture) is one "subsystem." The validity for treating these constraints independently rests upon the ability to demonstrate that the subsystem objectives are compatible with the higher level objectives. If I may steal a line from many a textbook—for the purpose of my presentation, proof of this validity will be left to the listener.

*It remains for more detailed functional analyses to be prepared for all of the other elements of the aquaculture process in order to provide clear visibility into the total system requirements.

FIGURE 7. A second level expansion of aquaculture functions.



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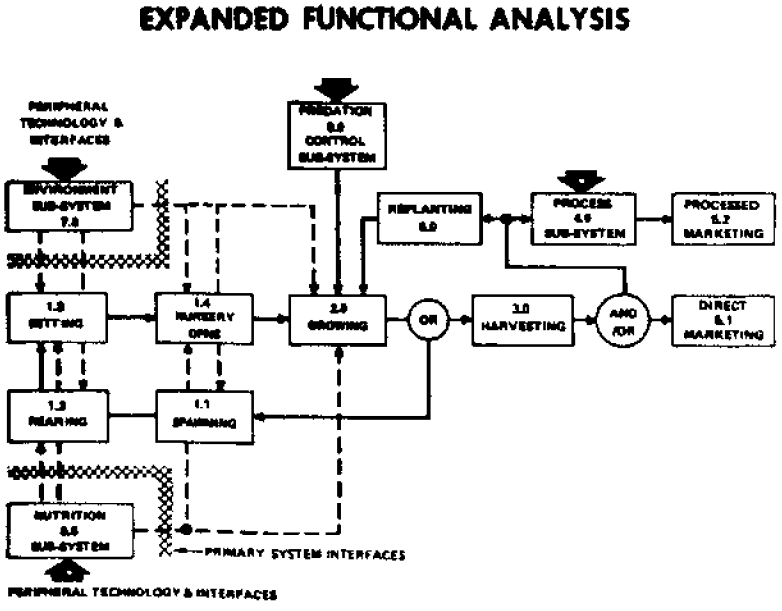
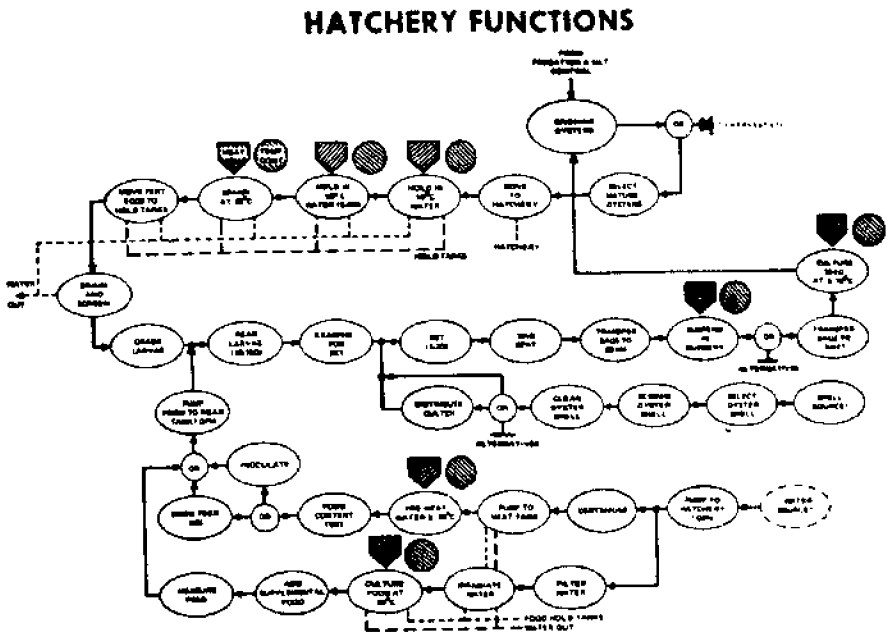
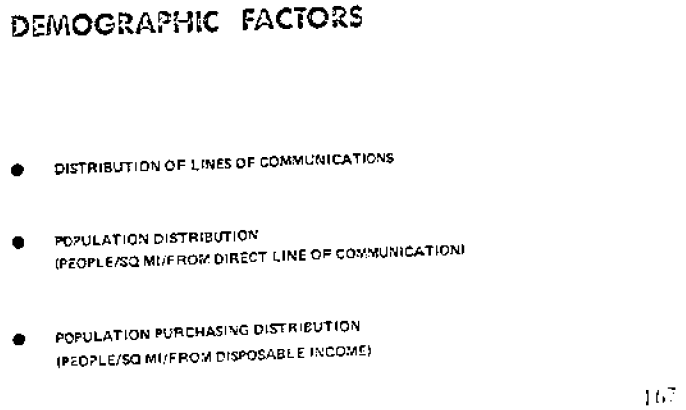


FIGURE 8. A detailed description of oyster hatchery functions.



Constraints upon the system are generally expressed as process, volume, or cost boundary conditions. They comprise those of a technical nature that are process-oriented, and those of a nontechnical nature that encompass everything else. In the case of ocean transportation, for example, demographic factors such as those described in Figure 9 would be considered constraints upon the system.

FIGURE 9. Demographic factors that influence the development of ocean transportation systems.



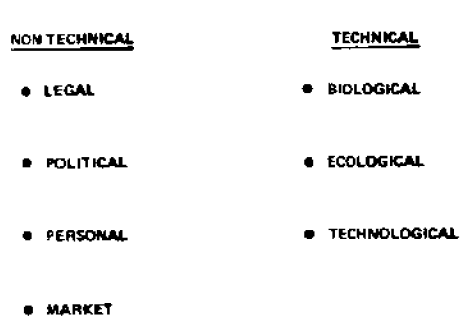
Similarly, in the case of aquaculture, Figure 10 lists classifications which appear to be germane to this analysis. In the area of legal and political constraints, the National Oceanography Association News (June–July 1969), commenting upon the politics of aquaculture, stated, "The real holdups (in the development of aquaculture) it appears, are legal and political, due primarily to conflicting uses (recreation, navigation, etc.) for public land (estuarine and shore areas) and other natural resources such as freshwater, unpolluted water, etc."

Jurisdictional problems are other legal and political constraints that require consideration because of potentially conflicting requirements imposed by state and federal agencies. The impact of these constraints would be felt in investment or operating costs necessitated by nonoptimum conditions (i.e., better oyster areas available only for recreational use, or added processes such as those necessary to remove excessive quantities of pesticides from process water) (See Ocean Industry, July 1969).

There are numerous constraints upon oyster aquaculture that are created by personal prejudices, ranging all the way from aesthetic considerations such as the effect of visible portions of culture devices upon the seascape to shell shaping, meat color and product texture. These would manifest themselves in the costs of additional handling, additional processing, and possibly beautification (i.e., THUMS project of Long Beach).

The last set of nontechnical constraints that I would like to comment upon are those associated with the market and in particular, keeping quality, demand, and packaging—all closely interrelated. The characteristics of demand for oysters appear to be primarily regional, dependent upon income, and strongly subject to personal prejudices (let's face it, some people wouldn't touch an oyster with a ten-foot pole based solely on appearance and/or texture). Since the implications of aquaculture are increased time of availability and larger quantities of product availability (and by inference, lower costs), the need exists for quantifying market boundary conditions based upon historical demand on the one hand, and potential demand derived from new preparation (home or preprocessed) and marketing methods; thus the expense of market research and process research must likely be

FIGURE 10. Factors that are constraints upon aquaculture systems.



added to an aquaculture program in order to determine the applicability of *economies of scale* constraints.

The consideration of technical constraints either results from research and development or identifies, through sensitivity analysis, likely areas for performing research and development. Thus, in the biological area, genetic selection, seed production, and larvae nutrition operationally constrain the process and thus warrant investigation. While in the ecological area, water chemistry and fertilization processes, process ecological balance, and process multiple utilization offer the opportunity for exploring new avenues for effecting economic feasibility. Similarly, the technology of environment control and machine design offer additional worthy avenues for investigation.

At this point in the presentation, please note that no system or component design drawings have yet been originated, nor have experiments been designed necessary to assure the timely and economic development of the system. In an S.E. sense, functions such as those are performed after the "feel" of the problem has been obtained.

Simulation Model

Visibility into a system gained by functional analysis and the identification of constraints as already discussed permits a preliminary description of variables to be developed and probable interdependencies to be noted—an analysis which must precede model development. Looking to the illustrations for guidance again, Figure 11 depicts a matrix that identifies a set of environmental variables and their

FIGURE 11. A matrix of National Environmental Conditions and Factors related to Oceanic Transportation Systems.

PREVAILING NATURAL ENVIRONMENTAL CONDITIONS AND FACTORS

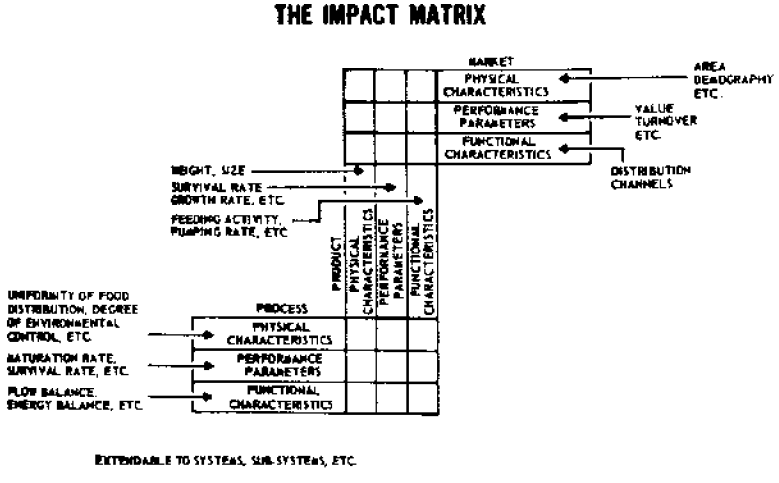
FACTORS / CONDITIONS	SEA-WORTHINESS	DRAFT	CARGO HANDLING	MOBILITY	ANCHORAGE
WIND	x		x		x
WAVES	x		x		x
TIDES		x	x	x	x
SILTING		x		x	x
DEPTH		x		x	x
PRECIPITATES			x		
CURRENT			x	x	x
RIVER NAVIGABILITY		x	x		x

probable interrelationships with a set of performance variables. Actually, this is the first step in the preparation of an "Impact Matrix," which is a useful tool for assessing not only the extent, but in some cases the strength, of variable interdependence. The extent of interdependence thus suggests the structure of the model and the strength of interdependence suggests the direction of quantification efforts.

In a typical commercial application, the impact matrix permits one to move from market to product to process to subsystems and then to components. In the case of mollusk aquaculture, as shown in Figure 12, for example, if the market value of oysters varies as a function of unit meat weight, we may trace this via those processes that influence meat weight, i.e., food concentration, extent of pollution, temperature, etc., directly back to subsystems such as filtration, process control, etc., and finally to components such as pumps, filters, metering devices, tanks, etc. Since it is likely that there will be numerous concurrent interdependencies, the matrix also usually reflects whether the relationship is estimated to be a first, second, or lower order effect. For example, market value could be a function of quantity, product meat weight, and overall size (length and breadth) with the variables being first-, second-, and third-order effects in that order. Effort in this case would just be directed to establishing market value vs. quantity relationships, or if resources permitted, multivariate relationships including first- or second-order effects, etc. Subsequent traces through the matrix would identify similar weighting relationships throughout the trace establishing priorities as functions of economic significance, commonality, or other measures of value. Needless to say, I cannot offer such a model for your inspection at this time. I do, however, feel safe in saying that significant strides are presently being made in developing one.

Professor Gaither has in his work already identified well in excess of 200 variables considered significant to the system, which I have taken some liberties with in Figure 13 where, for the sake of brevity, I have summarized them under three classifications: (1) product descriptors, (2) process descriptors, and (3) growth stage descriptors. It remains to interrelate these variables and to quantify them either by

FIGURE 12. Moving from process to market through an Impact Matrix.



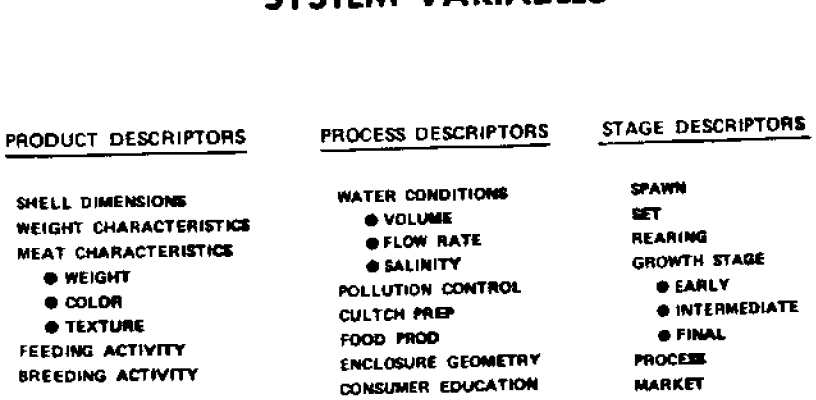
mathematical functions where direct relationships can be established, or by tabulated values based upon well-documented, rigorous, but perhaps poorly quantified judgments.

System Design Concepts

The next task to be considered, before the synthesis and evaluation steps of System Definition can be undertaken is the evaluation of sufficient system design concepts and supporting details to permit the derivation of performance-related implementation costs such as development, investment, and operating expenses. The previously described functional analyses, evaluation of constraints, and parameter definitions lend assurance to the completeness of the design concepts and thus diminish the possibilities for time and cost consuming technological surprise (process or hardware). Note, please, that it eliminates *surprise*, not increased cost or time. Hopefully, the process will identify areas that might require substantially greater resource allocations—but, by this method, one will be able to plan an approach to the problem rather than being precipitously cast into it.

The designer must frequently select one from among several design alternatives when developing a system design concept. In so doing, the selection rationale, if one existed, is frequently lost. Sometimes methods or designs are used because "that's the way they have always been done," or "my umpty-ump years of experience leads me to believe that's the way it should be done." In order to provide a better basis for describing and documenting design alternatives and intuitive selections, system engineering as a discipline has evolved the "Trade Study," which is simply a formalized selection procedure with the selection predicated upon the effect of the design on two quantifiable parameters—one related to system performance and the other to system cost. The concept is neither new nor novel. Virtually every time a choice is made between two or more possible actions, a trade-off has been conducted—even a child selecting between two candies has made one.

FIGURE 13. A summary of variables considered significant to oyster aquaculture systems.



It is essential in a technical trade-off study that *all* performance and cost effects be accounted for since quite frequently a second-or even third-order effect may significantly influence a design selection. As an example, let us consider the impact of crew size reduction upon the total system cost of a ship. First-order effects are *reduction* in payroll and living quarters and *increased* automation; second-order effects are reduction in support space, and reduction in topside weight (with consequent improved stability). The improved stability could result in a third-order effect of significant reallocation of space and possibly greatly improved cargo, fuel, or other material distribution with far-reaching cost or design implications. The quantification of these effects is the subject of the trade study, with the *functional analysis* and *evaluation of constraints* providing the roadmap that assures process and system accountability.

Figure 14 illustrates a trade-study tree developed for a ship system, and Figure 15 is the start of an analogous tree for oyster aquaculture—organized in terms of primary process (growth), logistic support (support), and the mission (market). As with any process or procedure designed to improve, it is possible to overdo a good thing—typically having to reinvent the wheel because of the rejection of educated intuition that says it should be round. It is even possible that quantifiably superior decisions be required to bow to mores and other nonquantifiable criteria. The process will be traceable, however, and thus if in the future conditions change, they will be readily amenable to reevaluation. The process of developing system design concepts can frequently best be started from an intuitively or empirically derived base line.

In the case of ocean transportation where, broadly speaking, the functions appeared to encompass movement, terminal operations, and distribution, the spectrum of possibilities envisioned (Fig. 16) ranged from integrated/independent to

FIGURE 14. An Ocean Transportation System Trade Study Tree.

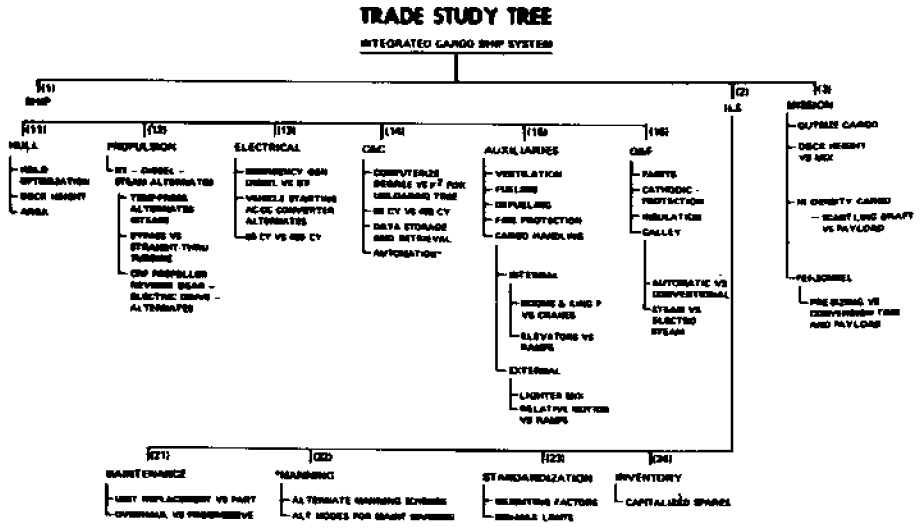


FIGURE 15. A Preliminary Trade Study Tree for an Oyster Aquaculture System.

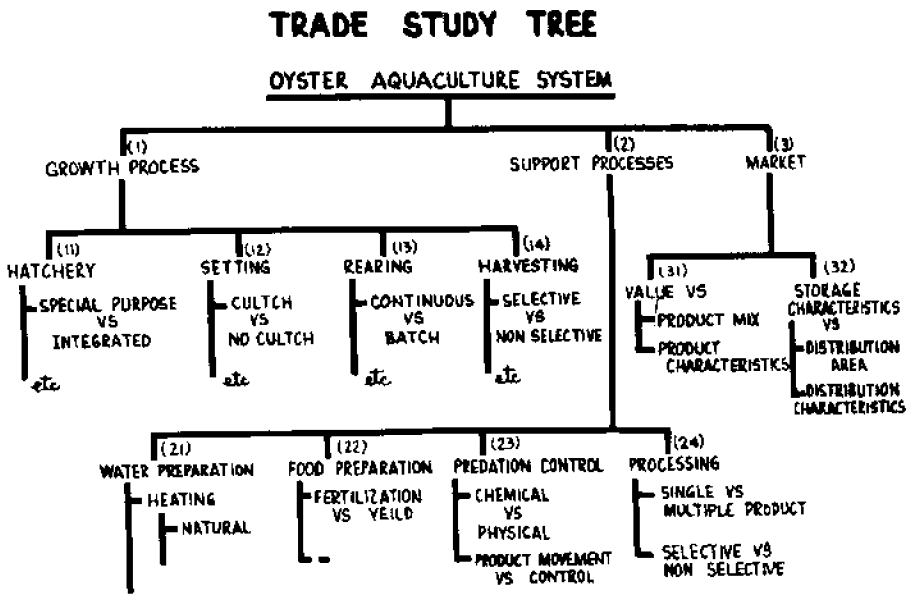


FIGURE 16. A schematic description of the limits of the spectrum of possibilities for an integrated bulk cargo transportation/distribution system.

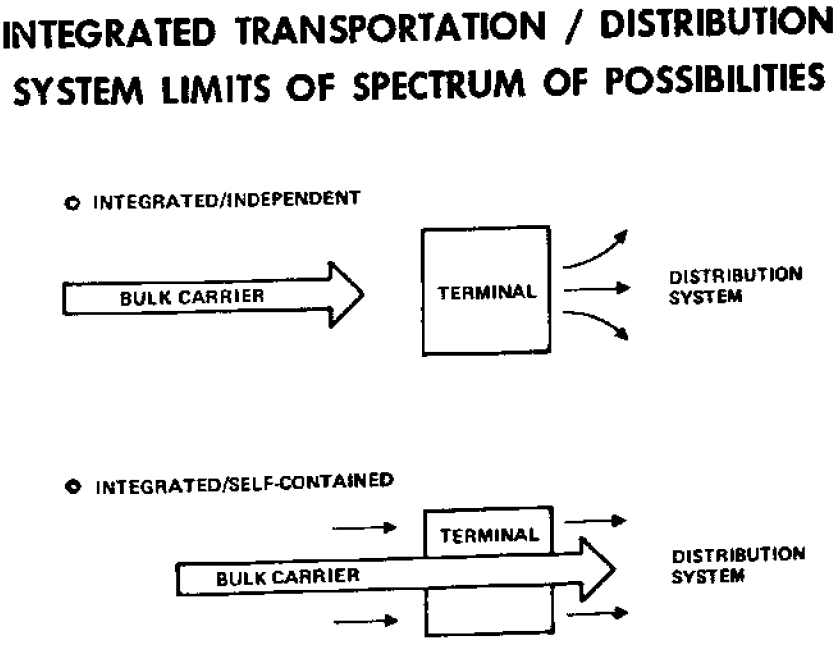
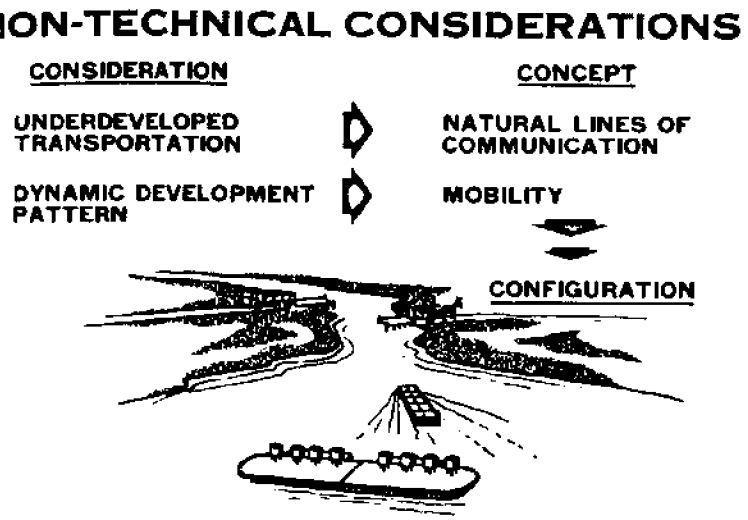


FIGURE 17. A configuration concept for an integrated bulk cargo transportation/distribution system based upon nontechnical considerations.



integrated/self-contained concepts shown in schematic form here—and interpreted more technically (and incidentally, artistically) in Figures 17 and 18.

In the field of mollusk aquaculture, it might be reasonable to start with the process described earlier for the Long Island environment. This basic concept would then be modified in a well-ordered way, considering alternative ways of performing major functions as shown on Figure 19 while applying basic engineering economic considerations that would be expected to influence economic feasibility of any design concepts. These would include: (1) economies of scale, (2) elimination

FIGURE 18. A configuration concept for an integrated bulk cargo transportation/distribution system based upon cargo handling considerations.

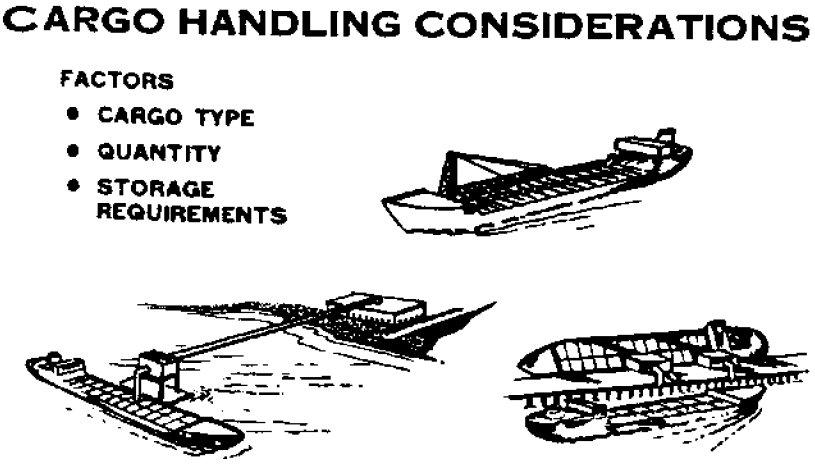
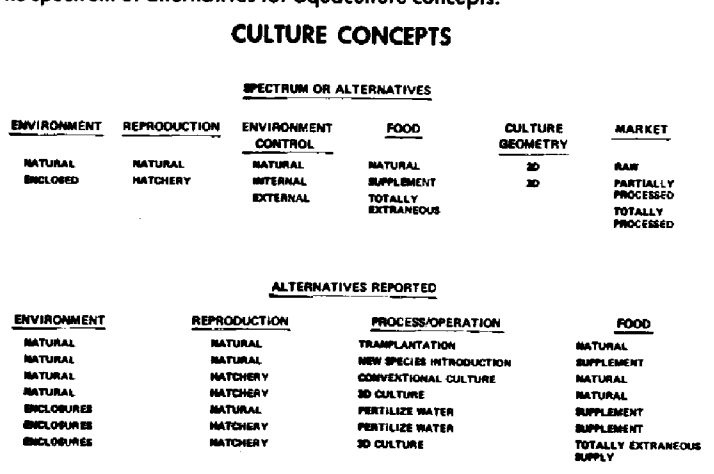


FIGURE 19. The spectrum of alternatives for aquaculture concepts.



of process steps, and (3) increased utilization by multiple usage and/or joint usage. It is the recognition and application of these considerations that initiated the trade-study activity.

The organization of all of the design data itself represents a problem since evaluation and selection rest upon both qualitative and quantitative considerations. Figure 20, concerning ocean mining systems, illustrates the manner in which en-

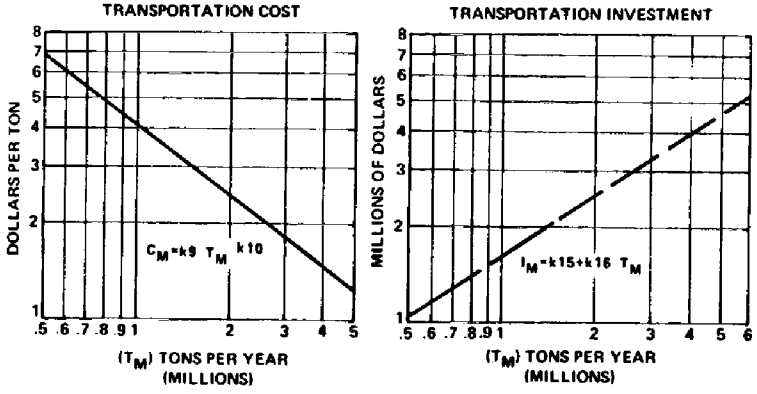
FIGURE 20. A comparison of nonquantitative characteristics for a group of alternative ocean mining systems.

COMPARISON OF CHARACTERISTICS OF ALTERNATIVE MINING SYSTEMS

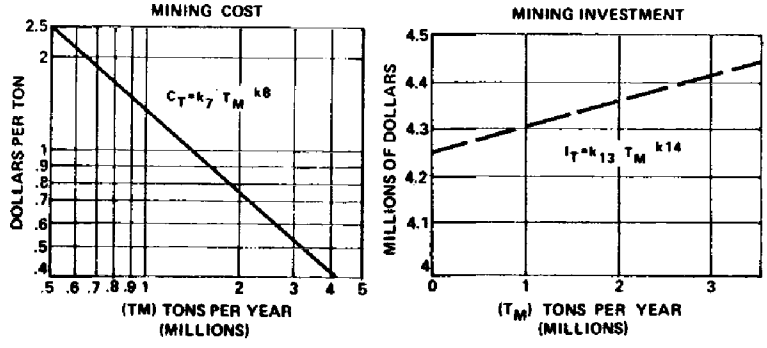
BOTTOM MINING SYSTEM	COMPATIBLE ORE TRANSFER SYSTEMS	PICKUP METHOD	NODULE ACQUISITION CAPABILITY			MOTIVE POWER	RELIABILITY	CAPACITY (5,000 LONG TON/ DAY MINIMUM)
			LOOSE SURFACE	EMBEDDED SURFACE	SUBSURFACE			
TOWED SUCTION SLED	PIPELINE	SUCTION	GOOD	GOOD	N/A	TOWED FROM SURFACE	GOOD	NOT ADEQUATE
SELF-PROPELLED MULTIPLE SUCTION SLEDS	PIPELINE OR MECHANICAL	SUCTION	GOOD	GOOD	N/A	SELF-PROPELLED – ELECTRIC	GOOD	GOOD
SELF-PROPELLED BUCKET DREDGER	PIPELINE OR MECHANICAL	MECHANICAL	GOOD	GOOD	FAIR	SELF-PROPELLED – ELECTRIC	FAIR	GOOD
SELF-PROPELLED ROTARY CUTTER DREDGE	PIPELINE OR MECHANICAL	MECHANICAL	GOOD	GOOD	FAIR	SELF-PROPELLED – ELECTRIC	FAIR	GOOD
SELF-PROPELLED ROTARY CUTTER DREDGE	PIPELINE OR MECHANICAL	MECHANICAL	POOR	FAIR	GOOD	SELF-PROPELLED – ELECTRIC	FAIR	NOT ADEQUATE
SUCTION HEAD	PIPELINE OR MECHANICAL	SUCTION	GOOD	POOR	N/A	SELF-PROPELLED – ELECTRIC OR TOWED FROM SURFACE	GOOD	NOT ADEQUATE

FIGURE 21. Functions that define the variation in procurement and operating costs with system capacity for principle ocean mining functions.

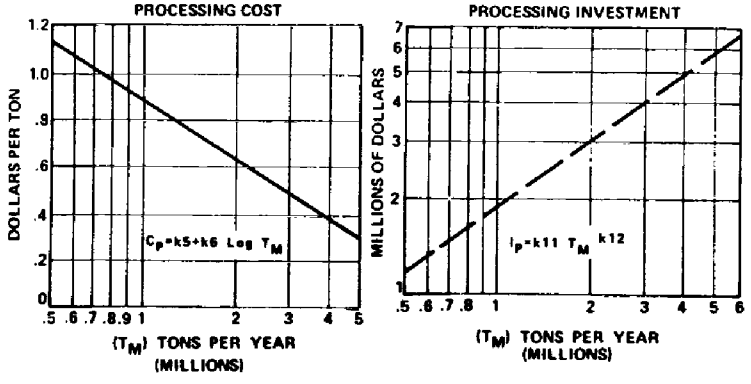
TRANSPORTATION



MINING



PROCESSING



gineering information can be organized to facilitate subsequent evaluation of some of the less quantifiable considerations, whereas Figure 27 illustrates, for the same area, the types of cost performance relationships necessary to perform an evaluation, utilizing a simulation model. The information required need not be detailed, but should be comprehensive.

Figure 22, on the other hand, depicts the need for similar information concerning mollusk aquaculture subsystems—the absence of functions reflects my assessment of the general adequacy of the information that I have found available concerning food supply subsystems. I have not investigated either of the other areas sufficiently to warrant comment.

System Synthesis and Evaluation

The last link in the System Definition process is that of synthesis and evaluation. It is in this phase of effort that models, constraints, and concepts are jointly "exercised" for the purpose of selecting a development path. The types of decision aids that we might expect to emerge are illustrated by the graphs in Figure 23 pertaining to ocean mining where both ROI and investment constraints have been specified, thus identifying minimum required ore values and allowable production rates.

A similar set of relationships would be desirable for mollusc production as portrayed in Figure 24 relating product value, quantity, investment, and returns. Although the specific functions are not known, we might expect functions with gross characteristics such as those indicated here to result. The preparation of a system

FIGURE 22. A preliminary estimate of the availability of cost-performance functions for aquaculture processes.

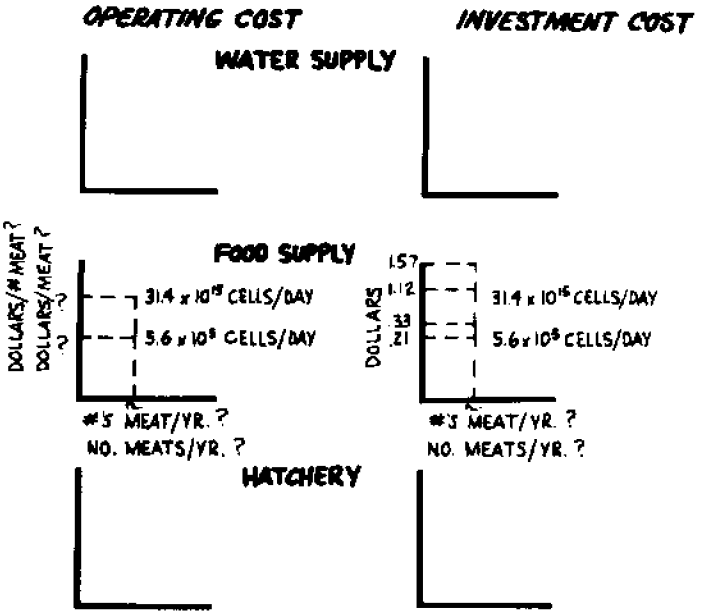


FIGURE 23. Typical decision aids available as a result of the synthesis and evaluation of ocean mining alternatives.

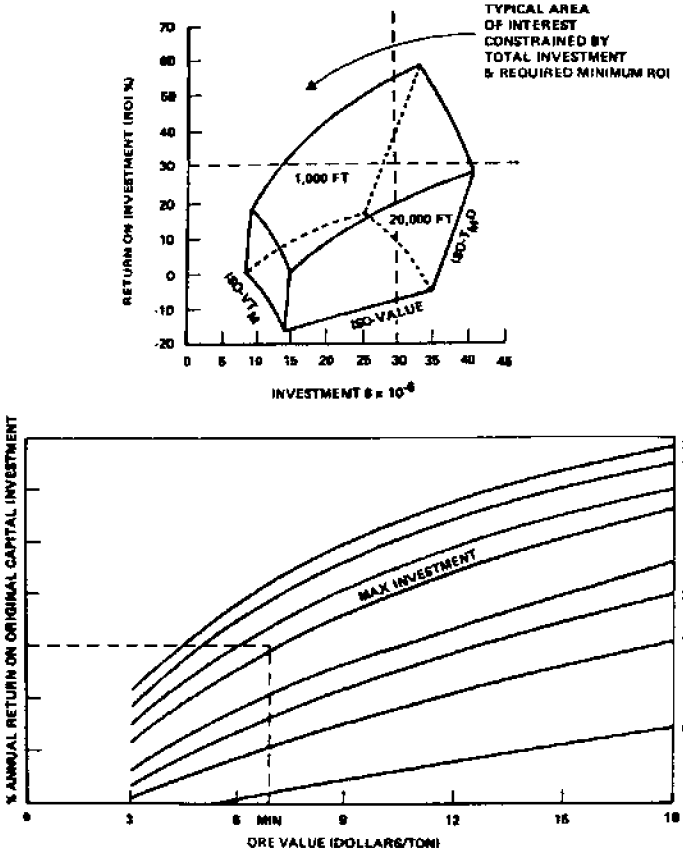


FIGURE 24. A preliminary estimate of the types of decision aids that could result from an evaluation of aquaculture systems.

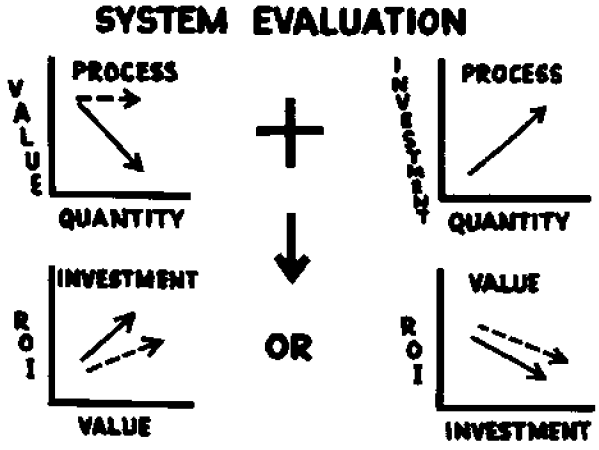


FIGURE 25. A preliminary development plan for a bulk cargo integrated transportation/distribution system.

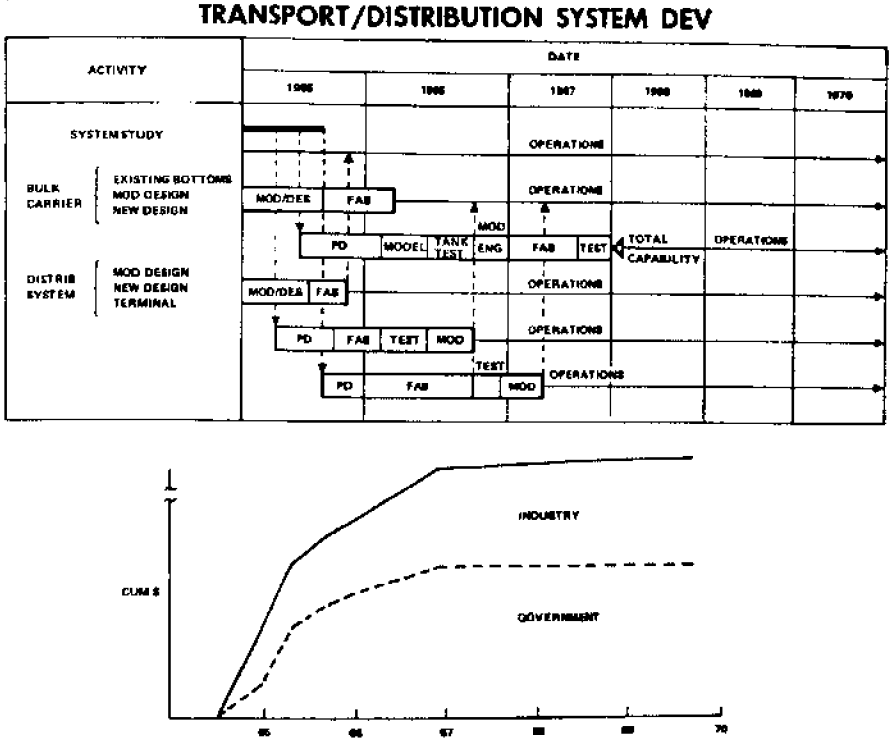
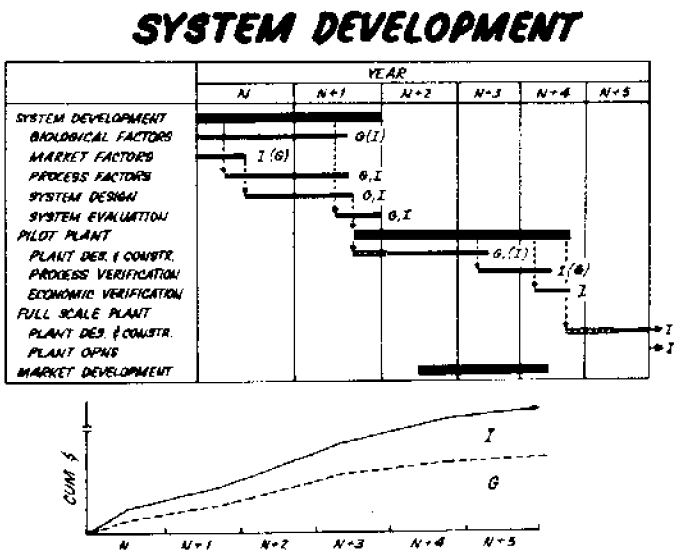


FIGURE 26. An example of a preliminary development plan for an aquaculture system.



development plan is also considered a step in the evaluation process since it affords a means for quantifying the time and cost phasing aspects of a technical development cycle, thereby exposing relative technological risks implicit in concept and configuration alternatives.

Figure 25 shows a preliminary development plan for the transportation system example, while Figure 26 is an example of one for aquaculture. The most significant differences between the two result from the latter being much more process oriented.

The System Job To Be Done

Literature concerning mollusc aquaculture indicates that some systems work of the type I have described has been done, particularly system design and trade studies. The most significant work reported treats the degree of environmental control, cultch type, and growth process configuration. The recent work of the American Cyanamid Company under contract to the Connecticut Research Commission represents the most formalized approach to system design that I have noted to date, there having been sufficient information developed to facilitate some system synthesis and evaluation. I have not been able to discover much information reported concerning the development of product/market characteristics or interrelationships which, I believe, represents one of the most significant problems to be faced in engineering and evaluating mollusc aquaculture. When one looks at oyster consumption, for example, one must inquire whether the purpose of oyster aquaculture in the United States is intended to preserve the industry from extinction, to increase the profitability for the current yield, to raise the level of output of meat to the high of 1908, or to substitute the oysterburger for the hamburger. For each of these objectives, there are associated implications concerning process type and costs, product mix, product quality, etc., etc.

It appears to me, therefore, that a system engineering approach to mollusk aquaculture can have the following salutary effects upon the process. First it would force a clear understanding of the objective, "Commercially Valuable." Second, more significant decisions could be made based on feasibility and desirability, if mollusk aquaculture were systematically explored from biology to market considering: (1) how to raise them, (2) how long to grow them, (3) how to use them, and (4) how to sell them. Thus, the approach to the problem would be interdisciplinary throughout, that is, not just artificial propagation, but also commercial and valuable—the biologist, engineer, economist, and businessman all having their contribution to make in solving the problem.

Third, and with adequate recognition being given to the complexity of the problems represented by data-poor biological processes, careful consideration given to the design of the analytical evaluation methodology could effectively reduce the problems inherent in dealing with a process of so many dimensions. A systematic approach to the definition of process alternatives, for example, will do much to avoid the pitfalls of suboptimization, and sensitivity analyses facilitated by simulation models will readily identify areas worthy of additional effort.

Last, and perhaps most important, design preconceptions can be much more rigorously questioned and the value of "far-out" concepts more readily established. Thus, a more effective bridge between technological innovation and business conservatism can result.

DISCUSSION

ZAHRADNIK: Let me compliment you on your presentation. I think you have made a very valuable contribution to the conference with your approach. The thought occurred to me that you have suggested an extremely useful tool for management, whether it is used for the management of a transport system that you referred to in your specific examples or the development of an agricultural production system. I wonder if this same tool would not be extremely useful on an interagency basis? By that I mean we have many different agencies making contributions to this area. We have input from the NSF Sea Grant Program and the Bureau of Commercial Fisheries together with state and private agencies. It seems to me that the approach or procedure you have presented would lend itself very well to the coordination of these activities on an interagency basis. Are you aware of any systems management scheme being used today?

GOODMAN: It so happens I am. Still it is in the planning stage and I hope we will have the opportunity to use it. May I refer you to Dr. Gaither to elaborate on this.

GAITHER: We believe this to be the intention of the Sea Grant Office, to ask Delaware to undertake the system study in conjunction with the work performed by the biologists. In other words, we had an opportunity to blend systems people with people knowledgeable in another area, in this case, shellfish. I think the Sea Grant people hope that when our model is completed that it can be used as an overall guidance and coordinating tool—a tool to identify gaps and holes that could be used both on a local and national basis. We hope we can provide them with something of that nature.

LOOSANOFF: Whatever your means of communication are, I do not consider them very efficient because the Sea Grant Program did not really suggest this approach first. About five years ago a representative of the Bureau of Commercial Fisheries gave a talk at New Orleans to a group of agriculture engineers. As a result, several schools including California Polytechnical Institute established departments of maricultural engineering. You may be interested in contacting Professor Lamorris, one of the leading men in the field at Cal Poly, and discuss the matter with him.

GOODMAN: Thank you, I appreciate the information.

Introduction to
TECHNICAL TRAINING AND EXTENSION SERVICES RELATED TO THE PRODUCTION OF SHELLFISH

SAMUEL M. GWINN
Director, Agricultural Extension Service
University of Delaware

The original objectives of our Marine Extension program were:

1. To train an extension specialist on an inservice basis to serve the marine resource interests of the State.
2. To provide technical information to persons interested in aquaculture.
3. To conduct extension programs that would enhance the marine resources of the State.

The principal accomplishments of the program, in addition to the basic one of inservice training, involved some investigation into the use of waste clam shells as cultch for oyster setting; the planting of seed oysters in the Delaware Bay; and the initiation of a study on the feasibility of catfish production in farm ponds.

The marine extension program is presently conducted on a part-time basis. Some progress has been made, but the program will never reach its full potential unless additional resources are committed to the job to be done. A real need exists for a broadened and more concentrated educational program. It is my opinion that this is so for several reasons:

1. There is an increased opportunity for transmitting improved technology to the marine industry. The planting, cultivating and harvesting of food from the sea is more archaic than farming was 50 years ago. There must be a more complete and efficient use of marine resources based on technological innovations and the ability of the environment to produce.
2. There must be a greater understanding and appreciation of the ocean and its resources on the part of the general public. A sound extension marine resource program could help develop this understanding and also serve as a vehicle for promoting public support of marine research as has been done with agricultural research.
3. There is an increased opportunity for improving the economic status of those who earn their livelihood in marine-related industries. Many are presently underemployed and there has been little growth in jobs on a full-time basis.
4. Finally, there is an opportunity for the development of human resources. The Agricultural Extension Service has proved that it is possible to train people

through informal educational programs. It can be readily seen that those associated with the marine industry can be taught to share in community leadership, community growth and community development.

A good extension program cannot be developed overnight. Fundamental to such a program is subject matter knowledge, organizational ability and, above all, the willingness to become involved with the people to be served. This is not a job that can be done by working on an eight to five shift. Two-way communication and feedback, so necessary for any good extension program, will never occur unless there is a willingness to work at the level of the groups to be served.

It is possible during the coming fiscal year that we may employ our first full-time County Agent of the Sea. Such a person is justified to continue the present on-going program, to extend new findings of research to the industry, to advise researchers on problem areas and to improve the utilization of marine resources through adult education.

The Extension Service is noted for its ability to establish and maintain channels of communication. The technological revolution in the agricultural industry is perhaps the most outstanding example of what can be done through the cooperation of education, government and industry. It is my feeling that many of the same educational techniques used to transform agriculture into a modern and progressive industry can be used to do the same for the marine industry. We should at least be willing to give them a try.

TECHNICAL TRAINING AND EXTENSION SERVICES RELATED TO THE PRODUCTION OF SHELLFISH

JAMES B. ENGLE*
Chief, Shellfish Advisory Service
U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Biological Laboratory, Oxford, Maryland

To a certain degree Mark Twain's comment about the weather, that, "everybody talks about it but nobody does anything," not too long ago could have applied to the fishery extension effort. Today talk is being replaced by tangible forms of extension work. Under one title or another, however, fishery extension efforts have been practiced for many decades. Its history, to a large extent, especially in its early periods, is a series of unrelated, unplanned attempts at meeting emergencies—"fire-fighting" in today's jargon. The intensity of the effort to meet fishery problems depended often on the energy, knowledge and persistence of the person or group of persons selected to meet and investigate the emergency. Funding frequently had to come from some other source already under-financed in a limited state or federal budget.

I am not sure that the current history of fishery extension is greatly changed. We live in hope however, that the problems of the fishing industry, the fisherman, and the consumer of the fine aquatic foods will have a well-coordinated, efficient program of safeguards; of gear improvement efforts; of elevated standards of living for fishermen's families; of maintenance of high quality food requirements; and of progressive improvement in methods of preparation, preservation and distribution of fishery products to the consumer. All segments of the fishing industry must be involved and aware of their responsibilities to improve the use of the resource. The many efforts in the fields of economics, biological research, marketing, gear development and consumer demands must be coordinated. A bona fide fishery extension service is a first step in meeting this fishery need. The U.S. Bureau of Commercial Fisheries' present service is an informational and educational activity to bridge the gap between scientific, technological and service programs and the needs of the fishing industry, allied industries and the public, which to all intents is a fishery extension effort.

I have been asked to review one segment of the nation's fisheries, the shellfish industry and the relationship of Technical Training and Extension Services to production. While this seems to be a simple and straightforward request, I have misgivings about attempting to present a simple answer acceptable to the shellfish

*Retired.

industry or the governmental management agencies. I would like to depart a little from the direct part of the assignment and briefly recount some approaches to extension work. This part of my discussion may be "old hat" to many of us here but it will set the background for my later remarks.

U.S. Department of Agriculture Cooperative Extension Service

Any general background of extension effort in the United States must include a statement on the very successful extension work supported by the United States Department of Agriculture. In 1914, Congress passed the Smith-Lever Act which formally launched the Department of Agriculture Cooperative Extension Service. The formative act was broadened in its scope by amendments through the years. The vehicle that made it possible for the implementation of agricultural extension development, however, was established long before the Smith-Lever Act when Congress passed the Morrill Act of 1862, during the Lincoln administration, establishing the land-grant colleges. Their major purpose was to provide instruction in agriculture and mechanic arts ("not to exclude other subjects"). These colleges, now numbering 67 with at least one in each state, have provisions for carrying instruction to adult and young out-of-school groups in rural areas. Counseling by extension personnel for farm youths in rural areas often is the incentive that keeps up or reinstills a desire to improve their opportunities through a more complete formal education. Vocational agriculture taught in rural high schools received government support in the Smith-Hughes Act passed by Congress in 1917.

The many services made available to agriculture through the cooperative extension program include information and demonstration for food production and marketing; community leadership and development; research-based data on crops, soil conservation, forestry, poultry and animal husbandry, farm machinery and buildings; and all aspects of producing, marketing, processing and using agricultural products. A popular and well supported activity is the 4-H program, which encourages young people to participate in agricultural experiment projects often at a highly sophisticated level.

The Department of Agriculture describes its cooperative extension service as ". . . a unique out-of-school learning opportunity—available to persons of every background, income and level of living. It is a self-help program made possible through the three-way partnership of the U.S. Department of Agriculture, the land-grant colleges and universities, and the county governments. Its name comes from 'extending' to the people the practical knowledge evolving out of research done by government agencies, colleges and universities and private industry."

Agriculture claims extension agents make 23 million personal contacts each year by a professional staff of specialists and agents of close to 30,000 persons. In addition to this, there are over one million volunteer leaders composed of farmers, farm women and other citizens that help the agents to assist more people. These volunteers serve under the general guidance of county extension agents as leaders in 4-H clubs and home demonstration clubs.

The Cooperative Extension Service and Agriculture Experiment Stations in many of the land-grant schools are conducting *Fishery Extension Services* relative

to catfish farming, trout farming, pond-fish culture, shellfish industry mechanization studies, and shellfish culture programs. All this is fine and undoubtedly needed, but aquaculture and the requirements for serving fishing industry needs are not as static as those in land farming. In the first place most of the products of the fishing industry are gathered by hunting or herding wild populations whose numbers are at the mercy of the vicissitudes of nature. True, in recent years aquacultural controls are under development for fish and shellfish "crops." Extension services to the fishing industry at this stage of development, if a comparison can be made, are lagging at least 50 years behind the extension work devoted to improvement of agriculture. I use that period of time on the basis of reasoning that the Department of Agriculture dates its birth and development of formal extension activities from 1914 with the passage by Congress of the Smith-Lever Act. As of today, the fishing industry has no *formal* extension service coordinated directly with the problems of the fishing industry.

U.S. Bureau of Commercial Fisheries' Extension-like Activities

As I mentioned at the very beginning of this discussion, an informal fisheries extension service has been functioning since the Bureau of Commercial Fisheries and its predecessors have been in existence. Established in 1871 and known as the U.S. Bureau of Fisheries, this agency in a comparatively short time had its operations extended so that there is scarcely a phase of "aquaculture," of the fishing industry, or of biological and physical science as connected with the waters that does not come within the purview of the Bureau. Prior to 1871 there was no branch of the United States Government especially charged with the consideration of fishery affairs, although fishery questions of greater or lesser import, some domestic, some foreign, had been arising ever since the achievement of national independence. Because fishery problems are often not entirely local, there arose an urgent demand from state officials and industry people for a national bureau devoted to fishery interests.

It was not until 1956 that Congress, in the Fish and Wildlife Act of that year, clearly recognized the government's "obligation" to develop basic knowledge about our nation's fishery resources and to make that knowledge available to permit an orderly exploitation and maximum use of these resources. Thus stated, this constitutes a national fishery policy and obligation. Congress specifically noted that, as with any other industry, the fishing industry has certain fundamental needs that the government has an obligation to satisfy by means that ". . . are convenient with the public interest and in accord with constitutional functions of Governments". Among the needs cited was "Assistance," and examples of the type of assistance that governments could render were listed as follows:

"(a) service to provide current information on production and trade, market promotion and development, and *AN EXTENSION SERVICE*,
(b) research services for economic and technological development and resource conservation, and
(c) resource management to assure the maximum sustainable production for the fisheries."

Congress also made it clear at that time that the Bureau of Commercial Fisheries should be the leading agency to provide this type of assistance to the fishing industry. Thus we have the legal authority to develop a *Fishery Extension Service*. The Director of the U.S. Bureau of Commercial Fisheries in March 1966 appointed a committee to "study fishery extension activities and to recommend ways in which the Bureau can increase the effectiveness of present extension activities as well as to recommend ways in which they can be expanded." Many of the statements included in this discussion were developed from the material included in the report of that committee.

The function of a fishery extension service is to get the results of scientific and technological breakthroughs into the hands of those who need to know, and to follow through to see that knowledge and skills are used to achieve fruitful results. A nation-wide fishery extension service, directed by the Bureau of Commercial Fisheries, will not only ensure that information flows throughout the nation, but also open channels of communication so that the individual fisherman or processor will know to whom he can turn for assistance.

The Bureau is already doing a great deal of extension work but it is a hodgepodge of loosely organized and inadequately coordinated programs. This is not to say that the Bureau is not doing any good work. In fact, the Bureau of Commercial Fisheries has 9 programs involving 32 items of service-orientation from biological and ecological information to information on economics, processing, harvesting, distribution and marketing, to name a few that belong in the extension category. Outstanding examples mentioned by the committee are Pacific mid-water trawl training, the work of the home economists and market specialists in the Branch of Marketing, and the Shellfish Advisory Service. A weakness in the Bureau's extension work in fisheries may be in its regional and area organization, each practicing extension efforts independently as local demands and vested interests may dictate. While there is virtue in regionalization to advise in local fishery management effort, the extension effort should be separate and directed by a trained extension specialist who can sort out the problems and determine the services and the laboratories with technical specialists available to supply needed information.

Can we learn from the experiences of the U.S. Department of Agriculture on the scope of activities of most importance for the improvement of our extension effort? Can a cooperative extension effort be spread too thin? Should we limit our effort to matters dealing directly on improvement of fishery production, harvesting, processing, marketing and consumer use and let the betterment of the fisherman's social lot and subsequent economic status be a function of his individual desires made possible through an improved fishery? We are planning a fishery extension program at a time when communications are in a highly developed state. Radio, television and travel means are making communications almost instantaneous. Indian smoke signals, Paul Revere's ride, the pony express, the stage coach, and even the railroads are surpassed by modern-day convenient ways of spreading information. The New Deal in dispensing knowledge of improved methods in aquaculture or agriculture is here and waiting. Agriculture has made use of the best of these means through its USDA Cooperative Extension Service. Aquaculture and

the whole fishing industry are still fumbling with the uncoordinated and only partly effective systems now in existence. Can a well-organized National Fishery Extension Service effectively put the accumulated knowledge of aquaculture and the fishery production techniques to work? I believe it can and the time is NOW!!!!!

U.S. Bureau of Commercial Fisheries' Shellfish Advisory Service

The U.S. Bureau of Commercial Fisheries Extension Service study committee mentioned, among the Bureau's successful extension efforts, the work of the Shellfish Advisory Service. This program was first suggested at a meeting of the Oyster Institute of North America in a speech by Dr. J. L. McHugh, Deputy Director to the BCF, in 1962. Again in 1963 he addressed this same organization in Washington, D.C. with the subject entitled, "An Advisory Service for the Shellfish Industry." The discussion of extension service for fisheries was reviewed before a subcommittee of the U.S. House of Representatives of the Committee on Merchant Marine and Fisheries at a hearing in October 1963 in Washington, D.C. on the state of the oyster industry and the means to be taken to improve it. Dr. McHugh, in a prepared statement, outlined a shellfish extension program entitled "Shellfish Advisory Service," a unit of the U.S. Bureau of Commercial Fisheries. In 1964 it was staffed and funded and began operations working out of the U.S. BCF, Biological Laboratory, Oxford, Maryland, as headquarters. The reason for locating at an active shellfish research unit had a psychological value. The fruits of research would be observed and evaluated first hand for direct transfer to the needs of the shellfish industry.

You may ask, "Why Shellfish Extension Service instead of a more inclusive Fishery Extension?" I am sure "Extension Service" will be all-inclusive one day and the planning in the Bureau has not overlooked this eventuality. The precedent for singling out a phase of the fishing industry was set when the Technical Advisory Service was established in the Bureau of Commercial Fisheries to assist the processing segment of the fishing industry. The Shellfish Advisory Service is another segment of the whole plan with a comparable goal for shellfisheries.

The future of the Shellfish Advisory Service, or for that matter of a wider potential Fishery Advisory Service, hinges on recruitment of a staff of dedicated and trained people. The prime requisite of the technical members of this team or staff would be a thorough background in a specialty of one or more of the disciplines involved in fisheries. The research laboratories of federal, state and private institutions have many of the experts well acquainted with phases of this technical know-how. The science departments of the universities and colleges have many well-informed, academically able persons available for consultation and research on fishery problems. The fisheries, unlike USDA Cooperative Extension Service, have no land-grant college system of institutions directed by law to furnish expertise in aquacultural matters. This is regrettable and should be remedied. The fishery extension program, therefore, must seek a different organization of ways to make the needs and knowledge available to the maintenance and improvement of the fisheries industry.

On paper a number of different plans are proposed. Under any plan or circumstance, cooperation of federal and state agencies of fishery management and research, of universities and colleges, and of the industry itself is required. The key personnel in an extension service are those with general training in some part of fishery technology; with a personality that is outgoing and sympathetic to fishery problems; with the ability to understand and impart technical knowledge; and with the facility to evaluate and delineate fishery needs so that the biological, ecological and many other phases of extension responsibilities can be directed to the specialists capable of producing helpful advice. In other words, among the key personnel in a fishery extension service is the extension specialist whose training should include knowledge of public relations techniques, professional teacher training; philosophy and concepts of extension work; and the ability to organize the efforts of both federal and state governments and industry to the solving of problems in the many phases of the fishing industry that deal with progress, economics, and the consumer.

The Commercial Fisheries Research and Development Act (PL-88-309)

In 1964 Congress enacted into law "The Commercial Fisheries Research and Development Act," commonly known as PL 88-309, in which matching funds with the states would be made available to improve commercial fisheries. Many states have applied "309" funds to establish fishery extension services. Among the first segments of the fisheries to utilize this opportunity was the shellfish industry. Maine, Massachusetts and Maryland formed, under this Act, shellfish extension services. Staff selection in each of these states was made from current members of established state conservation or fish and game departments. Each of the persons chosen to direct or manage the extension program had a professional rating or experience in shellfish research or management. As pioneers in the field of extension work they have made good progress in opening the way to a more inclusive fishery extension service and each of these programs was recently broadened to include other segments of the fishery industry.

Other coastal states under different titles have applied for these "309" funds to improve collection of fishery statistical information, marketing practices, and food preparation methods for fishery products. While these latter activities are not labelled extension functions *per se*, they undoubtedly belong under this title. Personnel to perform these services again will be recruited from programs already functioning in the specialties listed independently. We might assume that part of the Research and Development Program mentioned here can be the basis of a cooperative fishery extension program.

The Bureau of Commercial Fisheries Shellfish Advisory Service, financed directly from the BCF regular budget, was partly instrumental in encouraging state fishery management agencies to consider using some of their matching funds in extension-oriented programs. Whether this is the way to the eventual establishment of a cooperative fishery extension service, I am not prepared to say. It is, however, a definite application of a means to the ends being called for by the fishing industry and the political supporters of our fishery economy and also a need recognized by scientific and management agencies of federal and state governments.

National Sea Grant College and Program Act

In 1966 Congress passed into law on October 15 the National Sea Grant College and Program Act (PL-89-688). This act authorizes contracts and grants to suitable public or private institutions of higher education, institutes, laboratories and public or private agencies that are engaged in or concerned with the various fields related to the development of marine resources. The program authorized is administered by the National Science Foundation, which will: (a) initiate and support programs at Sea Grant Colleges and other institutions to educate participants in various fields involved in the development of marine resources; (b) initiate and support research programs related to the development of marine resources with preference for research aimed at practices, techniques and design of equipment applicable to development of marine resources; and (c) encourage and develop programs of instruction, practical demonstrations and publication by Sea Grant Colleges and other institutions to impart useful information to persons employed or interested in fields related to the development of marine resources, the scientific community and the general public.

Money was appropriated in 1967 and 1968 totaling $20 million to put this program into action. Many universities, including the University of Delaware, are embarked on programs under this act. The qualifications and program stipulations initiating the Sea Grant Colleges are not as precise as those that established the Land-Grant Colleges and the basis for the successful U.S. Department of Agriculture Cooperative Extension Service and Agriculture Experiment Station programs. They are, however, designed to improve the nation's ability to obtain and use natural resources of the marine environment. The Sea Grant College Act paves the way for expanding the capability of universities and other institutions of higher education to train scientists, technicians, engineers and others needed to locate, cultivate, harvest and use the marine resources. It will also encourage programs of practical demonstration on how to utilize these resources economically and efficiently. Public Law 89-688, the Sea Grant College and Program Act of 1966, looks like the "gimmick" that can be used as a vehicle for developing marine resources experiment stations and the companion Fishery Extension Service. This act will assist in research, development and applied work necessary to learn how to use the marine resources following the same concepts that have been applied to the land. Shellfish production benefits equally with other segments of the whole fishery industry.

The principal objective of an Extension Service is to make available progressive information to the industry that leads to increased production, improved quality and consumer acceptance of fishery products. To accomplish this objective with its multiple demands requires a broad program of education beginning early in the lives of the members of the fishing community. Learning skills by the sad experience of a series of mistakes is certainly not economical or encouraging. Vocational training in a planned program conducted as part of fishery community adult education or as is practiced in some of the vocational high schools in tidewater Maryland by demonstration oyster farming is a way. These programs should start with an orientation course for science and vocational teachers who will in turn conduct the

program of basic biology, ecology and field practice studies for the high school students. This is one way to introduce a refined and modern viewpoint that can be a part of the influence needed to break down traditional practices often handicapping progress in effective commercial utilization of fishery resources. The success of this method of technical and philosophical enlightenment depends on extension-like effort and resembles a 4-H approach sponsored by the established public youth education system.

The more sophisticated problems in management, mechanization, biology, processing and marketing are more closely related to advanced education in research institutions. Here again the communication medium is a well-coordinated extension program that has contact with the industry and the technical elements working on the recognized problems.

State Technical Services Act

Congress, through the passage of the State Technical Services Act of 1965, PL-89-182, again took action to get technical information out of the files in the cloistered halls of federal research agencies so that ". . . benefits of federally financed research, as well as other research, [can] be placed more effectively in the hands of American business, commerce and industrial establishments." This act is to be administered by the U.S. Department of Commerce. In defining the act, the term "technical services" is used, which is further explained and accomplished in the following three action statements of:

1) preparing and disseminating technical reports, abstracts, computer tapes, microfilm, reviews and similar scientific or engineering information, including the establishment of state or interstate technical information centers for this purpose;
2) providing a reference service to identify sources of engineering and *other* scientific expertise; and
3) sponsoring industrial workshops, seminars, training programs, *extension courses*, demonstrations, and field visits designed to encourage the more effective application of scientific and engineering information.

Here again is a special reference in an Act of Congress to "extension services." The stated objectives of the State Technical Services Act would seem to closely parallel those suggested in a proposed fishery extension service by the U.S. Bureau of Commercial Fisheries.

Manpower Development and Training Act

The Manpower Development and Training Act (PL-87-415) 1962, allows the U.S. Department of Labor to pay training and subsistence allowances for on-the-job training, institutional training, or a combination of the two. Training can be given to the unemployed, and the underemployed, or to persons who need to acquire additional skills in their jobs. The training may not exceed more than 104 weeks; and the allowance may not exceed $10 more than the average weekly unemployment compensation. The trainee may receive $5 a week for each dependent over

two up to a maximum of four additional dependents. Other expenses such as commuting and transportation as well as a subsistence of $5 a day may be paid. The Labor Department has money to support fishery training. Under this act the scarcity of oyster and clam shuckers, crab pickers and even some of the other skills of the fishing trade now might be helped through this program.

Preceding the Manpower Development Act was the Payne Act (PL 1027) 1956, which provided for grants to public and nonprofit private universities and colleges to promote the education and training of professional personnel available to schools in states that participate in active commercial fishing. Another section of the same act provides for vocational education in the fishery trades and industry on the same basis.

In the shellfish industry the U.S. trails Japan in fishery production, technical advancement and training of fishermen. Fishery extension services are well organized there to serve as a link between government and industry. Educational institutions specifically designated as fishery schools are widely distributed throughout the country to supply the research and training needed to keep its vital fishing program productive. Russia, England, the Scandinavian countries and Canada have strong fisherman-training programs at all levels of need. Whether there is formally organized extension services supporting these is not clear but the influence on fish production is evident. Perhaps a cooperative fishery extension or advisory service coordinating all the forces involved in our fishery program will help to stem the loss of our fishery products in our local markets to foreign imports that now supply about 70 percent of our local consumption. Something certainly needs to be done.

Summary and Conclusions

Fishery Extension Service is identified or proposed as the link between the accumulation of information by professional researchers and its application in the improvement of the fishing industry. At the moment there is no national fishery extension service comparable in any way with the U.S. Department of Agriculture Cooperative Extension Service. The U.S. Department of Agriculture is doing fishery work at some of the land-grant colleges and universities. The U.S. Department of the Interior, Fish and Wildlife Service has the Shellfish Advisory Service, Bureau of Commercial Fisheries, working closely with the Molluscan Shellfish Industry and the Bureau of Sports Fisheries and Wildlife working under the authority of Public Law 86-686, performing extension-type fishery education at 20 or more colleges and universities and State Fish and Game Departments. Under Public Law 88-309, state fishery conservation and management agencies are developing local fishery extension programs. And, last but not least, colleges and universities under the Sea Grant College Act are preparing to include extension-oriented programs for training and demonstration in fisheries activities.

All of these efforts need to be tied together to make an effective National Fishery Extension Service. Any proposal I make is simply an opinion that needs considerable refinement. With the number of federal agencies in different departments striving to organize an effective fishery extension service, there is bound to be

overlapping and duplication of effort. Consolidation of these several efforts, however, is certainly on the minds of the responsible leaders in the agencies, institutions and industry involved. I am hopeful that there will be a melding of these efforts into a strong and efficient national cooperative fisheries extension service. Many in the fishing industry and government are pulling for this. The fisheries extension-type work now being done will be strengthened by and made more effective through a single-purpose organization. The tendency of state-operated fisheries extension work developing through the Cooperative Research and Development Act PL 88-309 and its successor PL 90-551 brings this effort to put the fruits of fishery science in practical use closer to the benefit of the fishing industry. The Shellfish Advisory Service of the U.S. Bureau of Commercial Fisheries working with the state extension organizations and the shellfish industry has helped to solidify this liaison. What direct effect on shellfish production the recent extension activities has produced it is too early to say, but extension-like efforts such as:

1. Marketing—
   a) Sea scallop promotion, a joint effort of BCF, State of Massachusetts, the city of New Bedford and the industry,
   b) Maryland clam festival, a cooperative effort of the state, the federal government and the local soft-shell clam industry,
   c) New England campaign to promote the use of pollack, a combined program of New England states, the BCF, and the fishing industry to assist the fishing industry at a time of crisis in the haddock fishery.
2. Exploratory fishing and gear development and the effect to discover and develop for commercial use—
   a) The Calico scallop, *(Aequipecten gibbus)* off the south Atlantic coast,
   b) The deep sea red crab, *(Geryon quinquidens)* resource off the middle-Atlantic coast area is under investigation for potential exploitation,
   c) The royal red shrimp, *(Hymenopenaeus robustus)* in the deep waters of the south Atlantic and the Gulf of Mexico.
3. Gathering of statistics and economic information that will improve the grasp on the status of commercial resources, jointly the responsibility of state and federal management agencies.
4. Preparation and dissemination of scientific and technical information through:
   a) Seminars, workshops, demonstrations, and training programs for industry groups, and
   b) Providing sources of scientific and technological expertise for conferences on immediate problems facing the fishery, have all been available to aid and promote fishery production.

Shellfish production factors causing both upward and downward trends are often difficult to identify. Downward trends in many instances follow natural catastrophes but the recoveries, where they occur, are often the result of scientific research or practical experimentation and demonstration. A few examples of each are:

1. Downward Trends:
   a) Storm damage causing destruction of oysters in Long Island Sound and the Gulf of Mexico.

b) Disease in oysters, (MSX) in Delaware and Chesapeake bays and *Dermocystidium* in oysters in the Gulf of Mexico and Chesapeake Bay.
c) Predation by oyster drills, starfish, flatworms in Long Island Sound; by oyster drills and flatworms in Chesapeake Bay; by green crabs on soft-shell clams in Maine waters; and by some species of fish, i.e., drums, rays, tautog in the Gulf of Mexico, Chesapeake and Chincoteague bays; by oyster drills and flatworms in the bays of the West Coast.
d) Pollution—industrial, in almost every body of water capable of growing shellfish; domestic, somewhat the same as above but damaging because of the need for human health protection which prohibits the use of shellfish from affected areas.
e) Overfishing—undoubtedly a contributing cause of reduction in production in many shellfish producing areas on all coasts.

2. Upward Trends:
a) Rebuilding shellfish grounds that have been damaged by storms with reshelling and reseeding in Delaware Bay and the Gulf of Mexico.
b) Development of disease-resistant stocks of oysters in (MSX) disease-damaged areas and through results of scientific study which advised replanting to disease-free areas in Delaware and Chesapeake bays.
c) Control of predation by chemical and mechanical means has saved many shellfish through the cooperation of science and industry in all the areas mentioned.
d) At the present time pollution control, both industrial and domestic, is receiving strong public attention and efforts by all governments—federal, state and municipal—are called upon to remedy the causes of water contamination.
e) Where cooperation between science and industry is most needed is perhaps in the overfishing problem; its solution is in the hands of the ecologist, the biologist, the aquaculturist, the state and municipal management officials and of course, the shellfish industry members.

Considerable technical information concerned with shellfish has been accumulated over the years by federal, state and academic institutions. Its application to the welfare and improvement of the shellfish industry has not in many instances produced the desired results. Some of the reasons can be attributed to faulty communication; reluctance to change from traditional customs; adherence to outmoded and unrealistic local legislation; and a natural but obstinate clinging by fishermen in general to a traditional spirit of rugged independence. We can change these obstructions and encourage progress in the shellfish industry through a coordinated, cooperative National Extension Service which pulls together all of the efforts mentioned here and some I may have left out. The strongest tool we have to bring about the transfer of this knowledge to practical use is education and the proven medium as is demonstrated in agriculture is the Extension Service.

LITERATURE CITED

ANONYMOUS. 1968. Fish and Wildlife Act of 1956 as Amended. (An excerpt from Title 16 of the U.S. Code) U.S. Fish & Wildlife Service Circular 304. pp 1–11.

ENGLE, J. B. 1963. Shellfish Advisory Service-Cooperation with the Industry. *Proc. Gulf & Caribbean Fish. Inst.* 16th Ann. Session. Nov. 1963. pp 79–83.

ENGLE, J. B. 1964. Advisory Service for the Shellfish Industry. Minutes 23rd Ann. Meeting Atlantic States Mar. Fish. Comm. Appendix 12. pp 151 153 (mimeo).

MCHUGH, J. L. 1962. Research and the Oyster Industry. An address presented at the 54th. Ann. Meeting of the Oyster Institute of North America Baltimore, Maryland July 30, 1962. *Proc. Nat. Shellfish Assn.* Vol. 53. pp 1–10.

MCHUGH, J. L. 1963. An Advisory Service for the Shellfish Industry. An address presented at the 55th. Ann. Meeting Oyster Inst. of North America. Washington, D.C. July 24, 1963.

VANIMAN, P. E. (Chairman). 1966. Report of the B.C.F. Committee on Extension Activities. Report to the Director, Bureau of Commercial Fisheries, Washington, D.C. pp. 1–57 (mimeo).

DISCUSSION

RAY: Last summer Texas A & M established a fishery extension service in connection with its Sea Grant Program and now their first effort is specializing with the shrimp fishermen to induce them to modernize their techniques.

ENGLE: Thank you, Sam. Every institution and state that accepts this challenge is certainly gratifying to me.

DOWN: Through what agency are the Future Farmers of America organized and how were they created? Would a similar agency or some other unit be more appropriate for future sea farmers?

ENGLE: May I refer you to Dr. Gwinn.

GWINN: Programs of the Future Farmers receive federal funds through the Smith-Hughes Law. These funds are given directly to state departments of public instruction. The funds, in turn, are allocated to various schools throughout the state and then administered through the particular school district. This is different from the agricultural extension service where funds come from the Department of Agriculture to the University of Delaware which is a land-grant university. In addition, we receive county funds as well. Thus, the funds are directed to the university as a land-grant college and then are distributed by the university. But the FFA is conducted through the local school districts. Finally, any questions about 4-H work can best be handled through the University's Extension Service.

ENGLE: I hope someday we can do somewhat the same thing for the fisheries program.

CONFERENCE SUMMARY AND CLOSING REMARKS

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It is great to be back to what I might call "home grounds" again, as several years ago the University of Delaware approached our company to do some consulting work to assist them in setting up a molluscan program. We helped them start their oyster culture program. Now, after several years, I am amazed at the progress this university has shown in this very vital marine field. The administrators of the marine program for the University of Delaware deserve great credit for the strides they have made in this field and I wish them all much success in the future.

One thing this conference accomplished was to bring us up-to-date as to where we started and what we have achieved in molluscan development. To me, one of the most startling events was the pictures we saw the other night, taken 40 years ago, of artificially spawned oysters, and the remark made by Dr. Loosanoff that these are some of the best pictures we have of the actual spawning, fertilization, and growing of the egg. This struck me as very unusual because, as we all know, in the last 40 years there have been great technical developments in the field of photography and new microscopes have been developed. Apparently, we haven't progressed scientifically as fast as we think we have.

At many of the oyster conventions I have attended, I noticed a great deal of condemnation of industry for not being more progressive. Yet, when I looked at the outmoded boat which was shown in this picture from 40 years ago, with the steam power and the old-fashioned equipment, and compared it to some of our modern boats; when I looked at the laboratory and the growing of larvae, I am not so sure that industry has not grown fully as fast, if not faster than some of the scientific fields. This is worth noting because I do not think we have progressed scientifically to where we should be in the oyster industry. I think we might examine the reasons why before we go on to a discussion of the papers.

After the work of Prytherch, Wells, and Glancy in the 1920's, just to mention a few who really demonstrated the feasibility of mass growth of oyster larvae and oyster set, nature decided that it had better come back and show what it could do, and it gave us an overabundance of oysters. As a result, much to the disgust of the leading scientists at that time, their work was all but forgotten. It was not devel-

oped. It was not carried on as it should have been. For 30 years, research was being done but not to prepare for a commercial venture. Oysters were plentiful and cheap. The problem was to sell oysters, not to produce more, especially artificially which proved to be very expensive. However, no thought was given to the future regarding what might happen if the natural source dried up.

I'll never forget a remark Dr. Loosanoff made to me when I was first starting in the shellfish business. He said, "The oyster hatchery will be your bread and butter, and natural set will be the frosting on the cake." This may very well be a fact and we had better be sure we have the "bread and butter," even though until now there has been plenty of "cake" in all areas. I would like us to learn from the past, and maintain a progressive attitude toward the future.

In the northeast, there has been almost a complete lack of setting, and a serious decline in the entire industry in the last 15 years. In the Delaware and Chesapeake, MSX has nearly wiped out many of the most productive beds, and oysters have been at an all-time low. Down in the Gulf, violent storms have destroyed large quantities of oysters. We have had problems and these problems stimulate the demand, and the need, for artificial production of oysters.

One thing we must always do in research in this field is coordinate industry's needs with scientific development in the field. One very important breakthrough is that industry is now recognizing the value of science and the people in science are also recognizing the need for industry. This cooperation can help us reach the goals that this conference has set for us.

Some people say that the oyster is one of the most studied animals alive, and it might very well be because the oyster lends itself readily to study. But I think in order for our studies to be useful in nature, we have to have the guidance of some industry objectives. What does industry want and need? What is its timetable? With the present overabundance of oysters, my greatest fear is that the industry will allow scientific research to drift on to a different vein that would not be as productive in the long run for the shellfish industry. It is conceivable that the 1920's could repeat themselves, but I think that many of us in the industry have learned our lesson and will attempt to have adequate insurance to protect ourselves against the problems of nature in the future.

Some of the particular problem areas in aquaculture that I foresee are:

1. Farming of the sea is a widely used term. Freedom of the sea is another widely used term. The meeting of these concepts causes one of the greatest problems to the development of our industry.
2. Genetics, a field that was discussed here by a number of participants, can be very meaningful for artificial propagation of shellfish, but I think we must define what the goals are in a genetic program and what qualities we are looking for, and how to obtain them. I think we should learn from agriculture what has been done in this field.
3. Food for shellfish—what are the food requirements? Can it be grown? What is known about the foods?
4. What are the natural problems that might cause our industry to be lost for good? It has long been known that wetlands are the sources of nutrients and spawning areas for our shellfish but, to date, scientific work to justify these

beliefs has not been forthcoming. I think we should concentrate our future work in this direction.

5. Pollution, which could wipe out our industry completely, must be understood; not as scare newspaper items and reports designed to get headlines, but basic facts as to what oysters can take, what they cannot take, and what would be the best method of dealing with the pollution problem.
6. I find that many government agencies will listen to what we are saying. They are sympathetic to our problems. However, it is very disappointing to hear, "We *think* this because of such and such circumstances." This is not enough. In this day and age, industry needs hardnosed scientific facts from the laboratories and I think we can get them.
7. We are finding many diseases of the larvae and juveniles. How do we treat them? What do they mean to us? Do they occur in nature? These are big problems that are not going to yield to solution easily, but I do think answers can be found.
8. One thing I really learned from this conference was that we should examine the research that has been done in the past and see why we haven't progressed more in 40 years since there was a large amount of research being done and money spent. What can we do *now* to get this program into focus?
9. Why is the oyster industry so reluctant to accept scientific information? Why are the scientists so reluctant to go to the oystermen for advice? I think these two questions might very well answer themselves—because of lack of communication and lack of understanding of the values of both parties concerned. I think this is where we have failed in the past and hopefully will not fail in the future.

In conclusion, I feel this conference has provided the opportunity for people of many disciplines and interests to engage in the kind of dialogue that will eventually solve our problems.

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