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Oyster biology has benefited greatly from long continuous service from several famous scientists. At the 1957 Convention, three biologists, all retired from positions of authority, but all very actively engaged in research, were introduced as Honorary Members. Through teaching, research and students, these scientists have made a profound impression on shellfisheries activities in this country. Together, these three men have given approximately 157 years of service to shellfisheries.

It is our pleasure to honor these men in this volume of the Proceedings and to acknowledge their good works. At the Convention banquet, Dr. Trevor Kincaid was introduced by Mr. George D. Esveldt, Dr. Paul Simon Galtsoff by Victor L. Loosanoff, and Dr. Thurlow Christian Nelson by Dr. Melbourne R. Carriker. A brief biography of each follows.
For 69 years Dr. Trevor Kincaid has been observer, investigator and councilor to the oyster industry of the West Coast of North America. Born in Ontario in 1872, he is the son of a physician who moved in 1889 to Olympia, the center of the native oyster industry. Since that date Dr. Kincaid has seen native Olympia oysters decline, eastern oysters imported but also fail, and finally Japanese oysters become the basis for a prospering industry.

Although a professor at the University of Washington since 1901 with many other duties, Dr. Kincaid has always maintained a keen interest in oysters. For several years he was employed by the U. S. Bureau of Fisheries as a consultant to Olympia oystermen. He made surveys of state oyster reserves for the State Department of Fisheries and for eleven years directed the fisheries laboratory of the Department on Willapa Bay. He was intimately associated with the men who established the culture of Japanese oysters on the West Coast.

Dr. Kincaid was "fully" retired by the University in 1942 at the age of 70 with the title Professor Emeritus in Zoology and Research Consultant, but he is still active and maintains an office and laboratory at the University. Each summer he returns to Nahcotta on Willapa Bay where he has a series of covered tanks for experiments with larvae and breeding of Olympia oysters. As a hobby, he has installed a printing press in his basement and now prints his own research papers. The latest paper, on races and clines in the gastropod Thais lamellosa, contains 75 printed pages and 65 plates.

Dr. Kincaid organized the School of Fisheries of the University of Washington and graduated the first class before other hands began building it into the present extensive organization entitled the College of Fisheries. As founder and past director of the Puget Sound Marine Biological Laboratories at Friday Harbor, Dr. Kincaid's reputation is worldwide and his influence reaches far beyond the realm of oyster culture. We are proud to honor him as the father of oyster science on the West Coast and one of the "Grand Old Men" of biological science. Sir, we salute you!

--Jay D. Andrews
I consider it a distinct privilege to have the opportunity to talk to you on this occasion about a man who is an outstanding biologist and whose scientific efforts and contributions in the field of applied biology will constitute a remarkable chapter in the history of shellfisheries. This man is Dr. Paul S. Galtsoff.

It was in the early '20s that Dr. Galtsoff directed his chief attention towards shellfisheries. Soon after, a series of excellent articles offering comprehensive descriptions of oyster communities in different parts of the country began to appear. Parallel with that, numerous treatises on the ecology and physiology of oysters, representing original research, were published. Biologists soon noticed that in his experiments, Dr. Galtsoff stressed precise, quantitative approaches, skillfully separating scientifically established facts from certain old conceptions sometimes founded only on superstitions or even emotions. Studies of behavior of oysters in relation to various factors of environment became more and more stressed in Dr. Galtsoff's contributions and, later on, in those of his associates. In general, under his leadership oyster research ceased to resemble, in some respects, the fine arts and began to approach an exact science.

The results of Dr. Galtsoff's work, could not remain unnoticed and soon his name became familiar in every school, where the principles of aquatic biology were taught. His contributions were also eagerly studied by research workers. Because of this I consider that Dr. Galtsoff, indirectly, has had probably more students and has influenced more research workers than any of his contemporaries working in the same field.

In the past Dr. Galtsoff has held several offices in the National Shellfisheries Association. Here again he worked hard to attract the best men to work with us. During this period, as well as during his entire career as a biologist, Dr. Galtsoff was especially concerned with the preservation and development of oyster industries. From his earliest days as an aquatic biologist he accepted an uncompro- mising attitude towards pollution. In several cases, by precise scientific approaches, he and his associates demonstrated the many-sided effects of some pollutants. In some of these situations he stood virtually alone defending his principles. Broadly speaking, he believes that "there is no pollution that is good pollution" and that a means should be found to protect our rich aquatic resources from destruction. Most of us are familiar with his extensive studies on effect of various industrial pollutants directly on oysters and indirectly on their environments. These studies conducted in Louisiana and the York River will long be considered and, as a matter of fact, are at present used as standards in pollution studies.

--Victor L. Loosanoff
Professor Nelson's warm personality, keen fertile mind, broad training and interests in biological sciences, and his enviable gift of vigorous oral and written expression are well known. Over the years these rich assets have given direction, vitality, and characteristic "Nelsonian" color to his stimulating teaching, forceful speaking, lucid writing, productive research, and devoted public service.

This able scientist has energetically and effectively advanced research and training in the field of shellfish biology. Early in his professional career he became affiliated with our Association and soon assumed a leading role. Almost every Association Convention has been enriched by his scientific papers and discussions. He is quick to capture significance of scientific discoveries and to underscore potential practical applications. He is characterized by a unique capacity to ferret out profitable new lines of research and is generous in transmitting these discoveries.

Dr. Nelson has contributed much to popularization of technical reports in shellfishery biology by writing biologist's language in prose understandable and stimulating to general readers. As a member of the Department of Zoology at Rutgers University for 37 years and chairman for 28 years; as Biologist for the New Jersey Board and Council of Shell Fisheries since 1928; as a member of the New Jersey State Water Policy Commission and of its successor the Water Policy and Supply Council since 1929, and for the past 13 years its chairman; and as a member of the U. S. Public Health Service Committee on Shellfish Sanitation, he has rendered services in the fields of water supply and shellfishery biology to a degree which it is not yet possible to evaluate.

In American Men of Science his specialties are listed as biology of the oyster, estuarine ecology, marine biology, and limnology. Advancement of these disciplines has provided background for more than 125 contributions to knowledge of parasitology, water supply, and anatomy, physiology, and ecology of the oyster and associated organisms. On July 1, 1956 Professor Nelson was granted permanent leave from the Rutgers campus with the title of "The Julius Nelson Professor of Zoology". He retains active membership in the Graduate Faculty. With full devotion to scientific writing, he aims to revive voluminous unpublished researches --affectionately nicknamed by him "my morgue". We wish him every success!

--Melbourne R. Carriker
THE PAST AND FUTURE OF OYSTER RESEARCH

Paul S. Galtsoff

Shellfish Laboratory, Bureau of Commercial Fisheries

I wish to express my thanks to the officers of your organization for the invitation to address your opening session. This invitation gives me an opportunity to present some of the ideas which have developed in my mind during the 3-1/2 decades of my participation in oyster research in this country. As the title of my address indicates, I intend to speak of the past and future of oyster research. The omission of the present is deliberate because I am certain that all the members of this convention are fully aware of the current developments and achievements in oyster biology. Only occasionally, I shall refer to current research in relation to my discussion of future trends.

The beginning of the study of the oyster is shrouded in ancient history. We all know that oyster culture was practiced many thousands of years ago and that the oyster culturists of ancient times succeeded in developing the fundamental techniques of oyster farming which are used now. The Middle Ages hardly contributed anything worthwhile to the progress of zoology. The development of natural sciences during the Renaissance and the following period brought to light several problems in mollusk anatomy and physiology which are vitally important today. One example may suffice. In 1686 Anton de Heide described a gelatinous structure found in the stomachs of mussels and other shellfish. He called it "stylus cristallinus", a name retained ever since. Heide hazarded two guesses: that the crystalline style has something to do with reproduction, and that the structure is involved in the process of digestion. How prophetic was the latter surmise!

For more than two centuries the strange worm-like structure continued to puzzle biologists until in 1900 Coupin in France and Mitra in England, working independently, showed that the style is a protein rod saturated with digestive enzyme. During the intervening period at least seven different groups of theories were advanced to explain the nature and function of the style. The story of the style is given in detail by T. C. Nelson in his paper of 1918 in which he discusses the significance of this organ in lamellibranch physiology. This valuable contribution contains also the proof that the crystalline style is rotated inside its sac and in this mechanical way expedites the separation of food from sand in the stomach, while the dissolving style digests carbohydrates.
The last quarter of the 19th Century was the time of rapid advances in invertebrate anatomy and embryology. As a part of this general progress many facts regarding the structure of oysters and related mollusks were reported. At the beginning of the present century biologists began to pay more attention to life histories and mode of living of creatures of the sea. Aquatic biology, a new branch of science, was born and investigators began to emphasize the relationship between organism and environment, a new trend which found its full expression in modern ecological studies.

I want to limit my discussion today to the main phases of oyster research carried on in this country and abroad during the 30 years from 1921 to 1951. I know, of course, that breaking the continuity of historical events is arbitrary, but my reason for selecting this particular period is two-fold. First, during this time oyster research rapidly expanded and produced thousands of technical and popular papers. The second reason is a personal one. My association with oyster investigations in this country began in the twenties and has continued without interruption until the present. I believe, therefore, that I am in a position to add a little color to my technical presentation.

In 1921, when I joined the U. S. Bureau of Fisheries, the post of the Commissioner of Fisheries was occupied by Dr. Hugh Smith, a man of extraordinary scientific ability and broad intellectual interests. He was an inspiration to the young members of the Bureau and his collecting trips to the waters of Cape Cod, which he made regularly during the summers he spent at Woods Hole, gave us an opportunity to acquire a wealth of information about the local fishes and their habits. In his office he seemed to be reserved and rather formal. But we soon realized that under this appearance was a human heart of great kindness and understanding. An M. D. by education, he forsook the medical profession for ichthyology and became a world authority on fishes.

The position of Deputy Commissioner of Fisheries was occupied by Dr. Henry Frank Moore, whose name is familiar to many of us attending this convention. Dr. Moore's extensive work on the biology of oysters, his surveys of oyster grounds in Texas and Louisiana, and his penetrating study of the problems related to oyster feeding and oyster culture form a chapter in oyster studies of this period. Many investigations conducted after he finished his work started from the point where Dr. Moore left them. I specifically refer to his method of collecting the material discarded by the oyster and his quantitative studies of the food and feeding habits of this mollusk. The application of Moore's technique made it possible for Dr. Thurlow Nelson to develop the method for accurate determination of the amount of water filtered through the oyster gills. This technique has greatly advanced our knowledge of the physiology of the oyster.
In the early twenties scientific circles in the United States showed very little interest in marine sciences in general. Persons trained in this discipline were scarce. No courses in oceanography and fishery biology were given at any college, and consequently the Government positions which required training in this field remained vacant. My appointment to the staff of the Bureau of Fisheries as a naturalist of the Albatross was made on the basis of Section 10, Rule 2, of the Civil Service Commission which gives a Commissioner authority to fill existing vacancies without competitive examination. The position of naturalist of the Albatross remained vacant for nearly two years before my appointment on December 20, 1921.

Oyster research at this time centered in New Jersey where Dr. Thurlow C. Nelson continued the work started by his father, with special attention to behavior of oyster larvae, feeding of oysters, and shell movements. He made the first attempts to record behavior of the mollusk in its natural habitat; by using a kymograph he observed the peculiar contractions of the adductor muscle during spawning.

The importance of filtration of water by oysters and other pelecypods was already understood at that time, especially in its sanitary aspects. The question of obtaining pure oysters, devoid of contamination by pathogenic bacteria, became an acute and vital issue to the industry. The consuming public had no assurance that shell-fish sold on the market came from unpolluted water and therefore was not dangerous to eat. A disastrous crisis in the oyster industry caused by the epidemic in Chicago in 1924-25 left the leaders of the industry disorganized and in great doubt concerning the future of their business. I clearly remember the great tension felt by everybody present at the meeting called in 1925 by the Commissioner of Fisheries to find a practical solution for a difficult situation. Sales dropped almost to zero; hundreds of letters were received every week by the Bureau asking if all oysters were contaminated, and several housewives had written us that they discontinued buying chicken because poultry was fed on oyster shells. The Government officials, members of the state and federal public health services, two or three biologists, and many oyster growers were present at the meeting. The main scientific questions under discussion were the rate of water filtration and the effect of temperature on feeding and so-called hibernation of oysters. I was asked how temperature affects the rate of feeding of the oyster—and was not able to give an answer because the problem had not been studied. From bacteriological data it became evident that the Bacterium coli (now Escherischia coli) score in oysters is lowest in winter and highest in summer. Bacteriologists (Gorham, Gage and others) concluded from these observations that the difference must be due to suppression of vital activities by low temperature and called it hibernation. As a consequence of the conference I started a study of the ciliary motion of the gills and found that this motion is suppressed at temperatures
below 48°F. In the course of this study two methods were developed for measuring the volume of water passed through the gills. In the first method rubber tubing was introduced into the cloaca and made fast by cotton paddings. The free end of the tubing was attached to a horizontal glass tube of known diameter and graduated in centimeters. The velocity of the current inside the tube was measured by adding, through an inverted T tube, small quantities of carmine suspension which formed a clearly visible cone moving along the entire length of the tube. The so-called "carmine cone" method proved to be useful for rapid bioassays in cases when it was necessary to demonstrate the effects of toxic substances on ciliary mechanisms.

The other method involved the use of one large and one small connecting vessel in which the level of running water was kept constant. An oyster with the rubber tubing inserted into the cloaca was placed into the large vessel; the free end of the tubing was connected to a glass tube inserted in the wall between the two vessels. Water pumped by the oyster into the small vessel was collected through an overflow, measured and analyzed. With modifications added by various investigators the method became generally used in studies of feeding and respiration of oysters.

Determination of the rate of water pumping was much improved by Thurlow C. Nelson’s apron technique which consisted of wrapping the oyster in plastic in such a way as to intercept the excurrent water. At the same time Nelson called our attention to the existence of the promyal chamber in the species of oysters which are now grouped under the generic name Crassostrea. The promyal chamber was depicted in earlier work of J. L. Kellogg (1892) but the significance of this asymmetrical area on the right side of the body was overlooked. The importance of the promyal chamber fully justifies the splitting of the genus Ostrea into two genera, Crassostrea with the chamber and Ostrea without it.

Almost every oyster biologist of the last quarter of a century was more or less involved in a study of sex, spawning, and setting of oysters. In a series of carefully documented papers W. R. Coe (1936, 1938) showed that along the coast of the United States from New England to the Gulf of Mexico young oysters, less than one year old are predominantly males. The sex ratios are, however, highly variable. In northern localities there may be only 8 females to 100 males while in southern waters the ratio increases to an average of 40 females to 100 males. The reasons for the diverse sex ratios are unknown. The differences may be attributed according to Coe to local races with differing genetic factors and to environmental conditions. During the second year of their life some of the males change sex and become females. In this way a normal 1:1 sex ratio is restored.

A. B. Needler (1932) found that adult C. virginica also change their sex. Her observations were fully confirmed by my experiments.
with about 200 five-year-old oysters which were kept in the bay near the laboratory for six years. Every summer each of these oysters was induced to spawn and its sex ascertained. About 10 percent of them changed sex annually either from male to female or vice versa. One oyster changed its sex five times. Sex changes in adult Japanese oysters, C. gigas, were described by I. Ameniya (1929). Rapid sex changes in the hermaphroditic O. edulis and O. lurida were reported in considerable detail by J. H. Orton (1927-33, 1936), R. Sparck (1925) and W. R. Coe (1932).

Hundreds of papers in oyster literature deal with the effect of temperature of water on spawning. From purely descriptive data it is difficult to determine the nature of this relationship. My experimental studies of the physiology of reproduction of C. virginica (Galtsoff, 1938) showed the fundamental differences between male and female spawning. The discharge of ripe eggs by the female is accomplished by a complex process in which eggs are first discharged into the suprabranchial chamber, then forced through the gills into the mantle cavity, and finally are thrown off by violent movements of the valves. Spawning of the male is much simpler; sperm is discharged into the cloaca and is dispersed in water by the outgoing current. No definite temperature of spawning can be established for a given population of oysters. In my experiments specimens at different degrees of ripeness spawned at any temperature between 21° and 32°C. Some of them were able to spawn only under the combined influence of temperature and chemical stimulation by sperm. I would prefer to think of a critical condition of a fully ripe organism ready to discharge its sexual products rather than the existence of critical temperatures. Experimental evidence is against the latter view. The trigger-like effect in releasing sex products from ripe gonads may be produced by slight changes in temperature at different levels, by chemical stimulation, and by a combined effect of both agents. An attempt to explain the differences in the temperatures of spawning of C. virginica living at different latitudes was made by L. A. Stauber (1947) by assuming the existence of "physiological races". This view is shared by some biologists (V. L. Loosanoff and C. A. Nomejko, 1951) but in my opinion the final test should come from study of the genetics of the oyster. The existence of a "physiological race" can be proved only by breeding experiments and by testing under controlled conditions the spawning reaction of specimens of known genetic origin.

Spawning of oysters has been associated frequently with lunar phases. The work of the Milford Laboratory under Loosanoff demonstrated that such relationship does not exist in C. virginica in Long Island Sound, although according to P. Korringa (1947) it is present in O. edulis.

Practical considerations demanded that oyster biologists predict time of spawning and setting. Based on many years of observations the laboratory at Milford arrived at a general empirical
formula which has proved to be useful. A slightly different but also empirical method is being used by the oyster biologists of the Pacific Coast in predicting spawning and setting of C. gigas. There are so many factors affecting the life and survival of oyster larvae that probably many years of intensive research will pass before the prediction of time and intensity of setting can be placed on a sound scientific basis.

The oyster larva still remains a very elusive little creature. Correct identification of larvae of oysters and other pelecypods presents great difficulty. Many so-called quantitative records of the abundance of larvae in various estuaries and sounds are unreliable because of probable errors of identification. One incident, which I remember very clearly, illustrates this point. An oyster culturist employed by the Conservation Commission of one of the coastal states reported faithfully every year the number of oyster larvae found in July and August in the waters under the jurisdiction of the state. One day he showed me his samples which supposedly contained many thousands of larvae in advanced stages of development. I saw under a microscope numerous round diatoms of the genus Coscinodiscus but no pelecypod larvae. The man was quite indignant when I tried to explain the difference between the two forms; he told me that what he was doing was the established practice of his office and, "anyway," he added, "we do not want to be troubled with scientific technicalities".

In the last ten years the problem of identification of larvae was greatly facilitated by the work of the Milford Laboratory which distributed microscopic preparations of larvae, the identity of which was beyond any doubt because they were all produced from eggs fertilized and developed in the laboratory.

A classification of lamellibranch larvae, based on the structure of hinge and detailed descriptions of larvae of several species of American and European oysters greatly facilitates identification (G. Ranson, 1948; C. B. Rees, 1950).

Although we have at present a fair idea of the time and place of setting of oysters in various localities, the questions how and why larvae select definite vertical levels for attachment, and what causes their aggregation in one place in preference to another, remain unanswered. Hydrographical studies conducted in Chesapeake Bay, jointly by D. W. Pritchard (1953) of the Chesapeake Institute, the Virginia Fisheries Laboratory, and the U. S. Fish and Wildlife Service, show that the pattern of circulation in a tidal estuary is a major factor in the transport and retention of oyster larvae. This factor is greatly influenced by prevailing winds. In this situation the density of water may play a predominant role in influencing vertical distribution of larvae, an idea developed long ago by New Jersey biologists who studied the setting of oysters in Barnegat Bay.
Correct interpretation of ecological factors which control setting is difficult because of the insufficiency of experimental data concerning the physiology and behavior of oyster larvae. So far, the experimental approach to the problem has been impossible because of the uncertainty in obtaining sufficient numbers of healthy larvae of known age for this type of study.

The need for raising oyster larvae in tanks was recognized long ago. In 1920, Dr. W. F. Wells developed a technique for growing larvae in large jars in which sea water was changed by centrifuging. For several years he conducted an oyster hatchery at Oyster Bay and later transferred his operation to the plant of the Blue Point Oyster Company at West Sayville. Although a fairly large number of young oysters was produced in this way, the method was too complex and uncertain to be of practical value.

Food and feeding is the main problem in raising oyster larvae. Great advances in this problem were made in 1940 by J. R. Bruce, M. Knight and M. W. Parker (1940) in England. They definitely established the feasibility of rearing larvae of *O. edulis* by feeding several species of flagellates. In 1949 and 1950 Imai and Hatanaka in Japan were successful in raising *C. gigas* and other species by feeding larvae with naked flagellates which they cultured in tanks. In this country V. L. Loosanoff (1954) and H. C. Davis (1953) at the Milford Laboratory developed an excellent technique of raising pelecypod larvae by feeding them several species of naked flagellates and by determining the nutritive value of these microorganisms. The results open up new and broad possibilities for scientific research on larval physiology and ecology.

Ecological studies conducted during the past 25-35 years failed to answer the most important question—why certain grounds produce an excellent set but are not suitable for rapid growth and fattening of marketable oysters, while other grounds, not far away from the first ones, are good for growing marketable stock but cannot be used to catch spat. Statistical study of associations or correlations between the setting and various factors of the environment so far have failed to produce tangible results. I am certain that the solution of this problem eventually will be found through a well planned study of the physiology of feeding and food requirements of larval and adult oysters.

The knowledge of feeding requirements is equally essential for understanding the reasons for differences in the productivity of oyster bottoms. There are many places along the coast where oyster beds located only a couple of miles apart are markedly different in their productive capacity and in the flavor of oyster meat. Routine biological and chemical analyses show no significant differences between the waters of the adjacent bottoms. Apparently other components of oyster environments which are not disclosed by present methods of study are responsible for differences.
It has been well established by numerous investigations conducted in this country and in Europe that chemical composition of oyster meat does not remain constant throughout the year. Particularly spectacular is the seasonal cycle of glycogen. The accumulation of this carbohydrate usually takes place in the autumn when oysters become fat. We still know very little about the carbohydrate metabolism of lamellibranchs. This consists of complex biochemical reactions in which several enzymes are involved. Likewise, the breaking down of carbohydrate in the oyster has not yet been studied. It is reasonable to assume that an insulin-like substance may be present in the oyster but so far attempts to extract insulin from oysters have given negative results. Work along these lines appears to offer interesting possibilities for further research.

Digestion in the oyster and various enzymes involved in this process were studied by C. M. Yonge (1926) in England. The continuation of these investigations using modern techniques appears to be very promising, since the question of the ability of oysters to digest certain types of food has not yet been settled.

Pathology of the oyster is a relatively new science. Most parasites and commensals found in the oyster, such as protozoa, sponges, crustacea and worms, are not pathogens in a true sense of the word although they may inflict damage to the tissues of the host. The discovery of the fungus-like microorganism Dermocystidium marinum a few years ago by J. G. Mackin, H. M. Owen and A. Collier (1950), and the studies by S. M. Ray (1954), focused the attention of oyster biologists on this dangerous pathogen as a probable cause of extensive mortalities among southern oysters. It is reasonable to expect that further pathological studies may disclose other types of infection including disease-producing viruses.

Many research studies conducted in the United States were concerned with the problem of the decline in oyster production. The question is of great complexity because the productivity of shellfish grounds depends on interaction of many physical and biological factors. The annual production of oysters in the United States has declined from an average level of 165 million pounds of meat during the decade 1893-1902 to 77 million pounds per annum in 1943-1952 (P. S. Galtsoff, 1956). Several factors may be responsible for the decrease, but the primary cause is the destruction of public grounds especially in the Middle Atlantic and South Atlantic States. The acreage of productive oyster bottoms in these states has been steadily decreasing through overfishing, dredging of coastal waters for navigation, sedimentation, and pollution. Lack of management or ineffective management of public grounds has also contributed to the general trend and may be considered as a major factor in the depletion of oyster resources. Numerous studies conducted by various states and federal organizations demonstrated that the production of oyster bottoms cannot be maintained on a sustained basis without applying
oyster-farming methods. Several years ago the problem of state management was investigated in Maryland by J. B. Engle of the U. S. Fish and Wildlife Service, and by our present director of the Oyster Institute. The cost of rehabilitation of the depleted oyster grounds was determined and several possibilities were found for developing seed-producing areas of the state. Because of high initial cost of this type of work, state governments are not in a position to undertake large scale projects of rehabilitation. This could be accomplished by private oyster farmers, but strong opposition against private leasing prevails in many southern states and usually succeeds in preventing passage of necessary legislation.

About 25-30 years ago the feeling against private oyster farming in some communities along the Atlantic seaboard was so strong that anybody who publicly advocated this course could have met with physical violence. On one occasion I was threatened with this possibility after a discussion of local oyster problems at a public meeting in a small town.

At present the low level of productivity of public grounds is maintained by administrative measures designed to restrict the efficiency of harvesting. The use of inefficient and therefore expensive methods of fishing is, however, against the general trend in other industries which try to attain the highest efficiency through mechanization and automation.

No oyster biologist in the employ of a state or federal agency can escape being involved in the problem of pollution. Increased discharges of domestic sewage from municipalities and of trade wastes from industrial centers greatly damage shellfish bottoms. According to the Public Health Service statement in a publication entitled, "Environment and Health" (1951), there were in 1950-51 over 22,000 sources of pollution in the United States, 11,800 municipal sewer systems and 10,400 factory waste outlets. Only 9,300 treatment plants were in operation; the remaining sources discharged their wastes without even a partial treatment. Pollution of inshore waters became so great that at least 400 shellfish growing areas were condemned by state authorities. Damages caused by pollution are extensive. For instance, in New York-New Jersey harbor, water pollution has caused an estimated 100 million dollars of damage in 20 years to fisheries, recreation, and property values.

Biological aspects of water pollution are well understood. But pollution is primarily a social problem, the solution of which can be attained through awareness of its great dangers and education of the general public. As Representative Karl E. Mundt stated in his defense of the Anti-Pollution Bill in 1945, "Of all the natural resources, water today suffers from the greatest amount of public neglect."
Numerous investigations conducted in Europe and in this country have demonstrated the toxicity of various chemical compounds that are discharged with industrial wastes. The deleterious effect of pollution is frequently difficult to prove because in a complex ecological environment the injury caused by poison may be masked. Pollution frequently weakens organisms, makes them more susceptible to changes in the environment, and interferes with reproduction and feeding. Mortality may be due to combined effect of these conditions.

Virtually every state and federal laboratory of this country has been engaged in the problem of control of predators. Gastropod snails are undoubtedly the most destructive enemies of the oyster. Partial success in controlling predators has been achieved by trapping, dredging, barrier construction, and other physical means. In the past, careless bedding of native oysters or introduction of foreign species were the main causes of the wide distribution of carnivorous drills. These snails have only limited powers of locomotion but are brought to new territories with shipments of infested oysters. Their inroads are especially destructive in a new environment in which natural enemies are absent. Suffice to mention the introduction of Urosalpinx into European waters and the spread of Tritonalia japonica in Puget Sound.

The future welfare of the oyster industry calls for the solution of the following problems: reliable production of seed oysters; production of rapidly growing oysters with high-glycogen and high-solid contents; selection of disease-resistant races; improvement of methods of self-purification of oysters; scientific evaluation or appraisal of oyster bottoms based on their productive capacity; and development of oyster pond culture in tidal marshes and other areas not used at present for oyster farming.

Solution of these problems presents a challenge to young oyster biologists who are just entering this field of research. From the background of heretofore accumulated studies they may proceed with critical and basic research intended to answer the following questions. How oyster larvae feed and grow, how they react to physical and chemical changes of the environment and why they set in certain places and levels and not in others. To answer these questions elaborate physiological studies should be conducted simultaneously with ecological observations.

Production of oysters of highest quality, judged not by their size alone, but by flavor and high content of solids and glycogen is essential for the future of the industry. The problems of carbohydrate metabolism, feeding, and digestion should be studied and understood in order to attain this goal. It is futile to enumerate the various organisms that may be found in the stomachs of the oyster without knowing their nutritive value. Advance in physiology of nutrition in shellfish will lead to establishment of feeding or fattening basins or ponds in which shellfish may be rapidly conditioned for market.
The progress in methods of raising oyster larvae gives us assurance that the study of oyster genetics may be undertaken with reasonable expectation of success. Modern genetics tells us that variability and adaptability of a species depend on gene combinations and chromosome changes. The oyster is an extraordinarily adaptable and plastic organism. It can be assumed on theoretical grounds that wild populations contain many mutants which can be selected by proper breeding techniques. Races having desirable characteristics can be bred and used for establishing breeding stocks of particular qualifications needed for local conditions. The work along these lines requires the establishment of oyster breeding centers with adequate numbers of tanks or ponds, adequate water supply and culture houses for mass production of microorganisms needed as food for larval and young oysters. Experiments on hybridization can be conducted in these places in order to obtain the most desirable hybrids.

Ecological studies in the future should consider the oyster in relation to its physical and biological environment. Emphasis should be placed therefore on the factors which control the entire community of an oyster bottom. It seems erroneous to concentrate on a study of the biology of a single species without paying attention to other organisms which form an integral part of an ecological system.

The struggle for protection of our inshore waters and oyster grounds from pollution is one of the major tasks of the conscientious biologist who feels moral responsibility towards his country. Ample scientific evidence permits us to state that any pollution of water is bad and that no harmless pollution ever existed in spite of the claims made to the contrary by some industrialists. All types of pollutants which existed in the pre-atomic age have one common characteristic; after being discharged into natural waters they are oxidized and undergo other chemical changes which eventually render them harmless.

At present we are facing a more serious problem of safe storage and elimination of radioactive wastes which cannot be destroyed by ordinary methods known to us. These wastes can be only isolated in special containers or dispersed in water or air in order to reduce the level of their radioactivity. There are at present more than 4,000 establishments in our country using radioactive isotopes which have to be disposed of without endangering life. With rapid industrial development of nuclear energy the problem will soon reach a critical stage. The ability of shellfish to accumulate metals in their bodies and shells makes them particularly susceptible to certain radioactive elements. This new situation confronting the oyster industry should be given serious consideration. I trust that the realization of dangers involved in the radioactive pollution of sea water will stimulate intensive research and that a satisfactory solution of the problem will be found in the nearest future.
It is clear from my presentation that considerable investment of public and private funds is necessary to implement the outlined program. No doubt the oyster industry will bear a fair share of this burden. I believe the industry should do it if it wants to survive.

Centuries ago oysters constituted a cheap food, both in Europe and in this country. The following quotation from Dickens' "Papers of the Pickwick Club" expresses this idea. "It is a very remarkable circumstance, sir," said Sam, "that poverty and oysters always seem to go together--the poorer a place is, the greater the call there seems to be for oysters."

Very rapidly the oyster became a luxury in England while in this country it continued to be inexpensive and available to persons of low income groups. The situation changed during the last decade and the American oyster moved into a class of luxury food which poor people can no longer afford. Being a low energy food, valuable for its nutrient quality, but providing much less calories than other types of food, they oyster probably will remain in this category. It is imperative, therefore, that greater consideration be given by oyster producers to the attractiveness, the flavor, the content of solids and the purity of their product. To solve these problems they have to rely on the scientific resources of modern science and the skill and imagination of young biologists.

References


SANITARY CRITERIA FOR SHELLFISH BY SPECIES AND BY AREA

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Abstract

Recommendations to establish shellfish sanitary criteria by species and areas were approved by the National Shellfish Sanitation Conference in 1954 on the basis of laboratory and field observations and experiments reported by the writer.

To implement these recommendations (1) further studies were conducted in Maine to evaluate the relative importance of hydrographic, geological and biological factors having actual or potential influence on the sanitary qualities of shellfish growing areas and (2) cooperative experiments among the several northeastern states (Maine, Massachusetts, Rhode Island, Connecticut and New York) have been carried on to establish standards for (a) blue mussel (Mytilus edulis) and (b) (still in progress) soft clam (Mya arenaria) shell stock.

Introduction

Traditionally the problem of sanitary control of shellfish-growing areas has been the responsibility of chemists, bacteriologists and sanitarians. With the development of biological knowledge of the several shellfish species and greater understanding of factors influencing the growing area, it has become apparent that problems of hydrography, of geology and of biology must also be given consideration in the establishment of criteria for adequate sanitary control of shellfish-growing areas.

To establish sanitary standards for shellfish-growing areas without taking into account biological differences among the several shellfish species, or to conduct sanitary surveys without benefit of hydrographical and geological study, is to assume that all shellfish species behave alike physiologically and that all growing areas have the same hydrographical and geological conditions. These assumptions, applied indiscriminately, could be disastrous to the industry as well as to public health.

Laboratory and field observations and experiments of the Department of Sea and Shore Fisheries indicating the need for a
review of shellfish sanitary criteria were reported by the writer to the National Shellfish Sanitation Conference in 1954. As a result of this report, the Conference amended its resolution on shellfish sanitation to include two recommendations:

(1) Establishment of sanitary requirements by shellfish species, and

(2) Establishment of sanitary requirements on an area basis.

Clarence Sterling and Leo Fox of Massachusetts, Leslie Sherman of Connecticut, Harold Udell of New York, and the writer arranged for a northwestern area (New England and New York) meeting to work out a cooperative research program to implement these proposals.

In addition to the cooperative program, the Department of Sea and Shore Fisheries undertook an investigation of problems peculiar to representative shellfish growing areas of Maine.

Factors Influencing the Growing Area

Results of these and previous studies indicate that factors associated with growing areas and having actual or potential influence upon sanitary requirements fall into three major categories: hydrographical, geological, and biological. It was further suggested that the relative importance of each varies seasonally by area and by species.

I. Hydrographic factors

A. One of several coves which make up Quahog Bay, Harpswell, Maine, serves to illustrate the complex influence of inshore hydrography. This serpentine cove is shallow at its head but slopes to about sixty feet in depth at its constricted mouth.

1. A survey was made to enumerate sources of pollution and to estimate the pollution load and its probable distribution. Results suggested that the cove might be a borderline area as far as satisfactory sanitary conditions were concerned.

2. Scores of bacterial water samples, ranging from zero to 3.6 MPN, compared favorably with those from the most isolated growing areas.

3. A hydrographic survey of the cove indicated that: (a) water currents moved independently of wind direction, with large homogeneous water masses shifting with the direction of the tide; (b) buoyant material at or near the surface drifted with the direction of the wind; (c) the volume of fresh sea water moved into the cove with each eight-to-twelve foot tide could account for low bacterial scores; and (d) inferences derived from the shoreline survey that the overlying waters might be excessively contaminated were not supported by hydrographic studies.
B. Similar studies have been made in other areas. Results suggest evaluating parts of a sanitary survey in the order: hydrographic studies, bacteriological analysis of water quality, and other components.

C. Additional experiments during the last several years indicate that complexity of inshore hydrography is seriously underestimated.

1. In one study area it was repeatedly observed that a water mass moved through another water mass of similar temperature and salinity. Water pressure created by the tide in combination with geological features appeared to be the causative factor. Direction of movement was related to conformation of the bottom.

2. In a second study area, current velocity during the tidal cycle varied from nearly zero to more than six miles per hour.

3. Even in areas where land mass constrictions or other obstacles to laminar flow induced turbulence, stratification regularly occurred with respect to salinity, temperature and bacteriological water quality.

II. Geological factors

A. Studies of periodic changes in surface hydrography and in geological modification of intertidal growing areas were carried on jointly by the Maine Geological Survey and the Department of Sea and Shore Fisheries from 1948 through 1954. Although conclusions are not final, results of these investigations show that geological factors with their influence on hydrography must be considered if a valid appraisal of pollution conditions is to be made.

1. Photographic (both still and motion) records of minor geological changes were made by the writer while working with Dr. Joseph M. Trefethen, State Geologist and Dr. Wilmot H. Bradley of the United States Geological Survey. Redistribution of marine sediments and other debris by surface tension, sub-surface vortices, tidal run-off, and ice floes was observed and photographed.

2. Major changes were observed to take place under extraordinary meteorological conditions. Flats in some growing areas were drastically altered in elevation, conformation and compaction.

3. More permanent geological features, including sub-surface bedrock, were found to retard distribution.

4. Observations suggest that geological factors including surface texture, particle size, compaction, permeability and gradient influence the distribution or redistribution of pollutants.
III. Biological factors

A. There are several species characteristics which have bearing upon sanitary criteria.

1. Examination by the Department of Sea and Shore Fisheries of shell liquors of clams (Mya arenaria) from commercial areas of Maine indicates that clams did not siphon or feed when salt concentrations of the water dropped below twenty-four parts per thousand. Some clams have been found to survive in water that, on occasion, dropped below one part per thousand. That clams siphon overlying waters of much lower salinity than twenty parts per thousand in other geographical areas has been verbally reported to the writer by marine biologists working in Massachusetts and in Maryland.

2. When the range of salinities is wide, clams siphon only during the higher concentrations of that range.

3. Grossly-polluted clams repeatedly cleansed themselves at temperatures as low as 41°F., approximately nine degrees lower than the temperature reported in the "Manual of Recommended Practice for Sanitary Control of the Shellfish Industry".

4. In shellfish growing areas of Maine where both clams and quahogs (Mercenaria mercenaria) occur in commercial concentrations, quahogs invariably will be found in those portions of the area where the overlying waters are of higher salinity. Since, under normal conditions, contaminated waters are those of lower salinity, species that tolerate lower salinity are more likely to be exposed to contamination than are those that require more saline water. Quahogs are less subject to contamination than clams in the same growing area.

5. Shell form can contribute to contamination. The quahog, with ability to close its valves completely, is less exposed to contamination from brief periods of polluted fresh water than the clam which can neither completely retract its siphon nor close its valves. Although the clam may not feed during these periods, the external parts of the siphon and the mantle may become contaminated.

6. Differences in viability among shellfish species is an important health consideration. Experience in holding shellfish alive under varying conditions indicates that clams will live more than twice as long as blue mussels, but only from one-half to one-fifth as long as quahogs. It is apparent that because of biological differences the same sanitary requirements should not apply to these three shellfish species.
Standards for Blue Mussel (Mytilus edulis)

Blue mussel shell stock has for years been a quality problem for producers, distributors and wholesalers as well as for consumers. Although the magnitude of production is not comparable to that of the oyster, clam, and quahog fisheries, the lack of essential information on quality control is considered by research personnel of the northeastern states to be a major deterrent to market development and production increase. The group selected the blue mussel for its initial research effort because of industrial considerations.

Minutes of the meeting held in Lawrence, Massachusetts, on April 18, 1956 contain the results of experiments carried on cooperatively by research personnel of New York, Connecticut, Rhode Island, Massachusetts and Maine. Recommendations on sanitary requirements covering harvesting, cleansing, shipping, refrigeration, seasonal harvesting and shipping problems, and bacterial limitations for mussel shell stock in interstate commerce were prepared from these findings. After further study by the regulatory and advisory agencies of the several states, these recommendations were adopted as sanitary criteria.

Clam (Mya arenaria) Shell Stock

Currently the same group of researchers is cooperating on a program to establish sanitary requirements for clam shell stock. Experiments were outlined by the writer and Phillip L. Goggins, bacteriologist of the Department of Sea and Shore Fisheries.

The purpose of the program is to enhance quality of product in order that general economy of the industry may be improved. The primary objective is to evaluate quality of clams from growing areas to receiving markets and handling and shipping in various containers at different temperatures. The secondary objective is to relate these evaluations to biological and conservation problems of the species.

1. Pre-shipment experiments carried on at Boothbay Harbor by the Department of Sea and Shore Fisheries include:

   a. Harvesting -
      (1) From approved areas only, unless otherwise specified.

   b. Handling -
      (1) A series of duplicated experiments carried out under the following conditions:
(a) washed versus unwashed.
(b) refrigerated versus unrefrigerated.
(c) broken versus unbroken.
(d) stimulated shipping conditions versus undisturbed storage (room temperature, incubator temperature, and refrigeration at 40°F.)

2. Receiving experiments carried on by agencies of states concerned include:

a. Packaging (three-month trial with each method)
   
   (1) unprotected box and basket (no insulating or padding material).
   
   (2) protected box and basket.
   
   (1) various degrees of protection using various materials.
   
   (3) clams from approved versus moderately polluted areas. Variations of receiving experiments will depend upon results at Boothbay Harbor.

Summary

For administration and enforcement convenience, it would be desirable to establish specific sanitary criteria for all species of shellfish from all growing areas. Biological differences within the same species from different growing areas and hydrographical and geological differences for all species from different growing areas preclude the application of a single index of quality.

Recognizing these problems, researchers from New York and New England are presently engaged in a cooperative program to develop applicable standards of quality for the shellfish industry.

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Goggins, Phillip L. and John W. Hurst, Jr. 1949-1957. Bacteriological surveys; files, Dept. of Sea & Shore Fish., Augusta, Me.


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BACTERIOLOGICAL STUDIES OF HARVESTING AND PROCESSING OF OYSTERS IN VIRGINIA


Virginia Department of Health

In 1939 and 1940 many major improvements were made in the construction and equipment of oyster shucking plants to meet revised standards. The standards were again revised in 1946 to include, among other things, requirements for concrete floors, removable blower tank pipes, and the present type of shucking pail. During this 1939-46 period, as now, improved sanitary practices were emphasized. These changes accomplished some reduction in bacterial content of shucked oysters examined in the laboratories; however, most samples continued to show coliform bacteria in excess of the suggested limit of 230 MPN (most probable number of coliforms per 100 milliliters of sample). Consequently, it appeared that the shucking plant operation was not the all-important source of contamination it was previously thought to be.

It had been known for some time that shell oysters of good bacteriological quality would show an increase in bacteria when shucked. There was found to be little difference in this increase between oysters shucked under controlled commercial conditions known to be clean and those shucked under regular commercial practice (Kelly and Arcisz 1954). Preliminary work in the White Stone laboratory was conducted from December 1954 to June 1955 to determine what steps in oyster handling would be investigated to obtain data on this increase. Four sampling stations were selected for tracing single lots of oysters through the plants:

1. "Shell Oysters". A composite sample from shellstock on shuckers' benches or in storage bins.

2. "Shell Oyster Washings". Washings of mud and detritus from the outside surfaces of a composite sample of "shell oysters".

3. "Meats As Shucked". A composite sample of meats as they are shucked commercially. Shuckers to shuck one oyster each directly into the sterile collecting jar until the quantity of oyster meats required for examination has been accumulated.

4. "Meats As Packed". Sample of oysters as packed in plant.

From June 1955 through April 1956, 121 lots of oysters were sampled and examined in the White Stone and Norfolk laboratories. With few
exceptions these oysters were taken from beds in the lower Chesapeake Bay, in Mobjack Bay, and in the York and Rappahannock Rivers near their mouths; these beds are remote from ship channels and on-shore pollution. The samples were collected from well-constructed, well-equipped and well-operated plants.

METHODS OF EXAMINATION

Procedures for the bacteriological examination of shell and shucked oysters, except for present accepted modifications, followed those recommended by the American Public Health Association (1947). The program was planned with the cooperation of the Public Health Service Shellfish Sanitation Laboratory and the Service's Region III office, and in conjunction with them written procedures were developed; frequent consultations were held during the project. These procedures were generally followed by the Service's Shellfish Sanitation Laboratory and laboratories in other areas making similar studies.

In preliminary work at the White Stone laboratory need for a method of examining bacteriologically mud and detritus on the outside of shell oysters became evident. To fill the need this laboratory developed uniform procedures for removing this material, submitting it to examination, and expressing the results in MPN's, as follows:

Sampling. Place in a sterile one-gallon, water-tight oyster-shipping container at least 12 oysters of uniform size and shape; these should be representative of the lot to be sampled, and as nearly as possible between three and four inches in length with deep bowls. If more than two hours in transit, dry-refrigerate the sample.

Washing. Transfer ten oysters to a sterile one-gallon oyster-shipping container in good condition with tight-fitting lid. Add to the container one liter of sterile phosphate-buffered dilution water. Agitate the container and contents at least 50 times with a continuous circular motion. The number of agitations will depend on the thickness of the mud, but should be limited to that required to free the shells of clots of mud. Care should be taken to avoid violent motions that might break the "bills" of the oysters and consequently allow incorporation of shell liquor with the washings. Immediately after agitation transfer approximately 100 ml. of the washings to a sterile bottle.

Examination. Inoculate suitable dilutions into lactose broth. The sub-sample should be thoroughly shaken prior to each withdrawal of measured portions. Proceed with incubation and confirmation as in the examination of samples of water or shellfish, paying due attention to the necessity of submitting questionable partially-confirmed tubes to the completed test. This might be indicated more frequently in muds because of the greater likelihood of spore formers and other lactose-fermenting non-coliforms.
Expression of Results. Express results as coliforms per 100 ml. of washings. If the oysters are of uniform size, and within the standard size limits, it may be assumed that the MPN is roughly equivalent to coliforms per shell oyster.

The data were forwarded to the Public Health Service Shellfish Sanitation Laboratory and statistical analysis was made by Dr. E. K. Harris, Analytical Statistician, Robert A. Taft Sanitary Engineering Center. Interpretations and comments were made on the data by the aforesaid laboratory. Results discussed hereafter developed from analyses and interpretations.

RESULTS

Changes in Bacterial Quality of Oysters During Processing

Each lot of oysters from shellstock to final pack was followed through the plant for changes in bacterial quality. The identity of each lot was maintained. As a base line for comparison, the sum of MPN's of "shell oysters" and "shell oyster washings" was assumed to represent unwashed oysters as presented to shuckers. This base line is an estimate of the total potential coliform MPN which could be incorporated in meats during shucking--exclusive of contamination by hands, utensils, and equipment in use during the operation.

To show the changes from this base line, "shell oysters + washings", oysters "as shucked", and oysters "as packed" were assigned to various groups by MPN's. Group 1 was less than 230, Group 2 from 230 to 2400, Group 3 from 2401 to 24,000, Group 4 from 24,001 to 160,000, and Group 5 over 160,000 MPN. Oysters in one bacterial range prior to shucking may fall into another range after shucking and perhaps still a different range when packed. We assigned zero to samples remaining in the same group, +1 or -1 to those which rose or fell one group, and +2 or -2 to samples changing two groups between sampling points. The changes were averaged at each sampling point.

We find (Table 1) that at White Stone the average change in group units between "shell oysters + washings" and oysters "as shucked" was in October and November +0.52, in December and January +0.39 and in February and March +0.39. This shows that shucking increased bacterial content significantly above the potential from unshucked oysters. This is a conservative statement for not all detritus and mud on the outside of shells gets into shuckers' pails. At White Stone, we found a consistent decrease in the average change from "as shucked" to "as packed" in all months. From "shell oysters + washings" to "as packed" there is a slight increase in bacterial content but the rise is not significant. Interestingly, at Norfolk shucking had little or no effect on changes in group units.

-32-
Table 1. Average changes in group units during processing

<table>
<thead>
<tr>
<th>Location &amp; Period</th>
<th>From &quot;shell oysters + washings&quot; to &quot;as shucked&quot;</th>
<th>From &quot;as shucked&quot; to &quot;as packed&quot;</th>
<th>From &quot;shell oysters + washings&quot; to &quot;as packed&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Stone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct-Nov (23 samples)</td>
<td>0.52</td>
<td>-0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>Dec-Jan (18 samples)</td>
<td>0.39</td>
<td>-0.28</td>
<td>0.11</td>
</tr>
<tr>
<td>Feb-Mar (18 samples)</td>
<td>0.39</td>
<td>-0.33</td>
<td>0.06</td>
</tr>
<tr>
<td>Norfolk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan-Feb (8 samples)</td>
<td>-0.12</td>
<td>-0.38</td>
<td>-0.50</td>
</tr>
<tr>
<td>Mar-Apr (8 samples)</td>
<td>0</td>
<td>-0.38</td>
<td>-0.38</td>
</tr>
</tbody>
</table>

Evaluation of Bacterial Densities

The data were analyzed by season for each of the four sampling stations. Probability graphs were developed for each station by plotting on logarithmic paper the MPN values against their occurrence percentage-wise (Velz 1951). For each season there is a distinct curve on each of the graphs, with the exception of "shell oyster washings" for Jun - Sep which could not be plotted as all results were over 1100+. These graphical summarizations allow immediate estimations of the medians and of the percentages of "shell oysters", "shell oyster washings", oysters "as shucked", and oysters "as packed" which fall within any specified MPN range.

Assuming we are interested in what ranges 25% of the "shell oysters" fall, from season to season, we find in Figure 1 that in Jun-Sep the range is 2300 MPN or less; Oct-Nov 600 MPN or less; Dec-Jan 26 MPN or less; and Feb-Apr 31 MPN or less. Selecting the median or 50% to see into what ranges "shell oyster washings" (mud and detritus) fall, we find from Figure 2 that in Oct-Nov the range is 9000 MPN or less; Dec-Jan 1700 MPN or less; and Feb-Apr 2100 MPN or less. From Figure 3 it can be seen that in Jun-Sep 75% of the "as shucked" oysters fall into the range of 56,000 MPN or less; Oct-Nov 50,000 or less; Dec-Jan 14,000 MPN or less; and Feb-Apr 14,000 MPN or less.

The "as packed" oyster is the product as it goes on the market. We would like to know, by seasons, into what MPN ranges 90% of the product will fall. Referring to Figure 4, in Jun-Sep the range is 400,000 MPN or less, in Oct-Nov 100,000 MPN or less, in Dec-Jan the range is down to 17,000 MPN or less, and in Feb-Apr it rises to 30,000 MPN or less.
It can be seen from Figures 1-4, in the case of each sampling station, that the curves group together naturally in pairs. On investigating the basic data it was found that during the latter part of November air temperatures dropped abruptly to below 50°F, and seawater temperatures took a corresponding drop. Temperatures remained in this category into April. The Jun-Sep and Oct-Nov curves with temperatures above 50°F, form a natural pair, and the Dec-Jan and Feb-Apr curves with temperatures below 50°F, from another natural pair. This indicates a relation between air-water temperature and bacterial content of oysters.

A probability graph (Fig. 5) was made for Oct-Nov with curves plotted for each sampling station. A similar graph (Fig. 6) was made for Feb-Apr. It is apparent that at all four stations lower bacterial content were obtained during Feb-Apr than in the warmer months of Oct-Nov. Also the rise in bacterial content from "shell oysters" and "shell oyster washings" to oysters "as shucked" and the subsequent drop to oysters "as packed" can easily be estimated. It can be seen that the "shell oysters" are relatively low in bacteria as compared with "shell oyster washings"; however, these two combined contain less than oysters "as shucked", and oysters "as packed" contain less than the product "as shucked".

The United States Public Health Service (1950) suggests a limiting coliform MPN of 230 in oyster shell stock in growing areas or in shell stock or shucked oysters at point of shucking; MPN's of higher values should be interpreted as indicative of unfavorable conditions or practices surrounding the production and handling of the product, however, in occasional samples, an MPN value of 2400 may be tolerated. Kelly and Arcisz (1954) utilized the additional MPN classification of 24,000. The MPN of 16,000 has been included as an intermediate. Ranges used in following tables are based on these MPN values.

Table 2 was compiled from the "shell oysters" probability graph and shows by season the percentage of shellstock that can be expected to fall within the bacterial ranges. It can be seen that during Dec-Apr about two-thirds of shell oysters can be expected to be below 230 MPN and approximately 90% below 2400 MPN. It is also apparent that, with no increase in pollution, oysters from the same areas will increase in bacterial content during warm months. During summer no shell oysters can be expected to be within the range of 230 MPN or less, and only about 25% within 2400 MPN or less.
Fig. 1. Seasonal variation in coliform content of shell oysters.
Fig. 2. Seasonal variation in coliform content of shell oyster washings.
Fig. 3. Seasonal variation in coliform content of shucked oysters.
**Fig. 4.** Seasonal variation in coliform content of packed oysters.
Fig. 5. Change in coliform content as oyster is processed through plant; air-water temperature above 50° F.
Fig. 6. Change in coliform content as oyster is processed through plant; air-water temperature below 50°F.
Table 2. Percentages of shellstock falling below certain MPN in various seasons

<table>
<thead>
<tr>
<th>MPN</th>
<th>Jun-Sep</th>
<th>Oct-Nov</th>
<th>Dec-Jan</th>
<th>Feb-Apr</th>
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<tbody>
<tr>
<td>230</td>
<td>0</td>
<td>11</td>
<td>67</td>
<td>62</td>
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<td>2,400</td>
<td>26</td>
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</tr>
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<td>16,000</td>
<td>72</td>
<td>89</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>24,000</td>
<td>82</td>
<td>93</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3 was compiled from the "as packed" probability graph and shows the percentage of packed oysters by season that can be expected to fall within certain bacterial ranges. These figures indicate again that packs during cold seasons have lower bacterial content than those processed during warm seasons. None of the packs met the standard 230 MPN during warm months and less than 10% during cold months.

Table 3. Percentages of packed oysters attaining certain MPN levels by seasons

<table>
<thead>
<tr>
<th>MPN</th>
<th>Jun-Sep</th>
<th>Oct-Nov</th>
<th>Dec-Jan</th>
<th>Feb-Apr</th>
</tr>
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<tbody>
<tr>
<td>230</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>2,400</td>
<td>7</td>
<td>10</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>16,000</td>
<td>33</td>
<td>50</td>
<td>89</td>
<td>83</td>
</tr>
<tr>
<td>24,000</td>
<td>42</td>
<td>62</td>
<td>93</td>
<td>88</td>
</tr>
</tbody>
</table>

Summary

It has been known for sometime that shucking causes an increase in the bacterial content of oysters. There is little difference in this increase between oysters shucked under controlled commercial conditions known to be clean and those shucked under regular commercial practice. In the Virginia shellfish laboratories 121 lots of oysters were examined from June 1955 through April 1956 to obtain data on this increase. Each lot was sampled at 4 sampling stations, i.e., "shell oysters", "shell oyster washings", oysters "as shucked", and oysters "as packed". With few exceptions the oysters were caught from beds in the lower Chesapeake Bay, in Mobjack Bay, and in the Rappahannock and York Rivers near their mouths--these beds are remote from ship channels and on-shore pollution. The oysters were processed in well-constructed and well-operated packing plants. The data were
submitted to statistical analysis and it is concluded that shucking contributed bacteria in significant numbers over and above the total potential due to incorporation of detritus and mud from the outside of the shell into the shucked oyster. During packing this bacterial content decreased and although it did not return to the base line ("shell oysters" + "shell oyster washings"), the overall average rise is not considered significant.

Probability graphs, prepared from the data, show the percentage of samples by season, for each sampling station, which fall within any selected MPN (most probable number of coliforms per 100 milliliters of sample) range. The United States Public Health Service suggests a limiting coliform MPN of 230 in oyster shell stock or shucked oysters at point of shucking; MPN's of that value or greater should be interpreted as indicative of unfavorable conditions or practices surrounding the production and handling of the product, however, in occasional samples, an MPN value of 2400 may be tolerated. In 1954 Kelly and Arcisz, studying bacteriological control of oyster processing, utilized the additional classification of 24,000 MPN. The MPN value of 16,000 is included as an intermediate. Ranges used in following examples are based on these MPN values.

From the "shell oysters" graph it can be seen that during Dec-Apr about 2/3 of the shell oysters can be expected to be within the range of 230 MPN or less and approximately 90% within 2400 MPN or less. However, oysters from the same good areas, with no increase in pollution to these areas during the warm months to justify an increase in the bacterial content of the oysters, will show an increase. We find that during the summer no shell oysters can be expected to be within the range of 230 MPN or less and only about 25% within 2400 MPN or less.

From the probability graph for "as packed" oysters it can be seen that during Dec-Apr about 10% of the packed oysters can be expected to fall within the range of 230 MPN or less: 50%, or the median, within 2400 MPN or less; 85% within 16,000 MPN or less; and 90% within the range of 24,000 MPN or less. During the warmer months of Jun-Nov, none of the packed oysters can be expected to be within the range of 230 MPN or less and only about 8% within 2400 MPN or less; 40% within 16,000 MPN or less; and 50% within 24,000 MPN or less.

Literature Cited


UNUSED OYSTER SHELL IN SOUTH CAROLINA
SUITABLE FOR SEED OYSTER PRODUCTION

G. Robert Lunz

Bears Bluff Laboratories, Wadmalaw Island, S. C.

Abstract

Although South Carolina now has a surplus of cultch from the steam canneries, this may dwindle as an export seed oyster industry develops. A possible substitute is to be found in old oyster shell thrown up on the banks of many South Carolina creeks and rivers by wave action and storms.

Experimental plantings indicate that this washed shell is not very suitable as a spat collector when broadcast on the normally soft oyster-producing bottoms of South Carolina, but when screened to remove small pieces it is useful as cultch in suspended tray, bag or basket planting. Series of test plantings made in different years at different times of the year show that washed shell catches about half as many spat as steamed shell from canneries. Since spatfall in South Carolina waters is intense and long-continued, this reduction in catch may be a valuable attribute.

Washed shell is available in large but as yet unmeasured volume. One stretch of the North Edisto River is estimated to have a half-million bushels of washed shell on its banks. This is a "renewable" resource in the sense that every period of bad weather washes new shell up on the banks.

Fine and small shell from screening amounts to 50 per cent of the volume of the material as it is taken from river banks. In Low Country South Carolina where rock is non-existent, fragments of washed shell not suitable for oyster cultch may be used as aggregate in concrete mix. This has been done successfully at Bears Bluff Laboratories.

South Carolina waters are admirably suited to produce small seed oysters. Naturally one of the principal requirements for seed oyster production is suitable cultch. After the oyster industry in South Carolina has satisfied its legal demands for shell planting there is still approximately one-quarter million bushels of shell remaining annually. In the past much of this shell has been sold for
purposes other than oyster cultivation. This surplus shell could be used for the production of seed oysters. However, those companies with surplus shell piles have sometimes shown an unwillingness to release this shell at prices which would make it usable by the seed oyster industry.

Various types of substitute culch have been tried by Bears Bluff Laboratories as well as by other laboratories in oyster producing areas. It is well known that oysters will set and survive on many different materials, but none as yet has been found which can satisfactorily compete with the shell of the steam oyster canners.

A possible substitute is to be found in old shell thrown up on the banks of many South Carolina creeks and rivers by wave action and storms (Fig. 1). As culch this "washed" shell is about as effective as reef shell of the Gulf Area.

South Carolina washed shell seems to lack resilience. It feels chalky and it is easier to scratch with a knife than steamed culch. Microscopically, washed shell is pitted and eroded.

Steamed culch seems thicker, is not as soft, and the inner face is smoother and generally shiny. Microscopically the inner shell surface does not show the pits and eroded areas seen in washed shell.

Reef shell, or mud shell - at least the sample obtained from the Corpus Christi Bay area of Texas - is generally coated with a marl or clay-mud mixed with small shell flakes. It is thicker than washed shell and is more riddled with sponge. The Texas sample contained a higher percentage of small broken shell than does the average sample of South Carolina washed shell.

The specific gravity of the three types of shell does not differ greatly. South Carolina washed shell (5 samples) has a specific gravity of 2.33; steamed culch (4 samples) 2.40; and Texas reef shell (3 samples) 2.37.

In the Gulf Area, Gunter (1938), St. Amant (1956), and Ingle (1956), arrived at the conclusion that reef shell was suitable for culch, but found that generally it did not catch as many spat as steamed culch.

From time to time Bears Bluff Laboratories has attempted to use washed shell as a substitute for steamed shell. Although washed shell is useful for hardening soft bottoms, it settles too rapidly to give good results as culch. On bottom firm enough to hold up washed shell it is too susceptible to wave action and movements by currents. Generally, washed shell broadcast on oyster grounds is not worthy of use.
Fig. 1. Washed shell is deposited along the edge of most South Carolina coastal rivers.
Fig. 2. Washed shell (right) catches about half as many spat as does steamed cultch (left).
Fig. 3. Cultch can be suspended in wire troughs as well as wire bags.
For seed oyster production in the manner described by Lunz (1952) however, the results are more promising. In view of the increased interest in seed oyster production for out of State markets, and the possible shortage of steamed shell as the seed oyster industry develops, it seems worthwhile to present some comparative data on the spatfall on washed and steamed shell.

On June 1, 1955, two half-bushel shell bags, one with washed shell cultch and one with steamed shell cultch, were hung on cables just above low water in We Creek at the laboratory. The steam cultch was the usual cluster type with several lower valves attached together. The washed shell was made up of selected deep-cupped shells, largely individuals. On June 20, 1955, spat on ten inner faces from each type of cultch were counted. The spatfall ratio was 7 to 5 in favor of the steamed shell cultch.

In August 1956, fifty half-bushel bags of shell were suspended on cables in We Creek above low water. The bags of steamed and washed shell were alternated. The washed shell had been screened through a 1 x 1 inch wire to remove the fine shell. This removed approximately 50 per cent of the bulk of the washed shell as it was taken from the banks. In November detailed studies showed that the intensity of setting was 1.0 spat per square inch for the steamed shell and 0.51 spat per square inch for the washed shell.

Although it had been screened, the washed shell contained a higher percentage of small and flat pieces of shell than the steamed cultch. The bulk of the steamed shell was made up of cupped and cluster shells. The washed shell was 65 to 70 per cent flat upper valves of which 60 per cent had only one spat. Only 20 per cent of the steamed shell had one spat per shell and 70 per cent had 3 or more spat per shell.

At first inspection the spat on the steamed shell seemed larger than those on the washed shell. However, a series of measurements showed little difference. Measurement of 93 spat selected at random showed that the mean size was 25 x 19 mm on the washed shell, and of 138 spat on steamed cultch the mean was 29 x 19 mm. The largest spat found on steamed shell was 55 x 26 mm while on washed cultch the maximum size was 46 x 25 mm. Thus the difference in size of spat on the different types of cultch seems insignificant.

In early May 1957, before setting began, bags of washed shell and steamed shell again were hung on cables in We Creek above low water mark. The bags were so hung that every other bag was washed shell. The washed shell had been screened as before. The small screenings were discarded.

By May 21, both types of cultch had accumulated a fair catch of spat. The steamed shell had 4.4 spat per square inch; the washed shell 2.8 spat per square inch. As roughly translated (Lunz 1954),
this is 20 spat per shell for steamed cultch and 12 spat per shell for washed cultch. On June 6, 1957, shells from the two types of cultch bags showed a catch and survival of 8.5 spat per square inch for steamed shell and 9.5 for washed shell. By July 2, 1957 the count was 10.2 for steamed cultch and 5.0 for washed cultch.

These experiments indicate that washed shell, even though it is only about one-half as efficient as steamed shell, is satisfactory for use in trays, baskets, or suspended bags for collecting seed oysters (Fig. 2 and Fig. 3). Because of the lower set and survival rate on washed shell it is in some ways superior to steamed shell. The use of washed shell for cultch would tend to eliminate overcrowding - a tendency of South Carolina seed criticized by Andrews and McHugh (1956). Thus, although washed shell is not the perfect substitute for steamed shell it can be used as cultch, particularly as steamed shell becomes unavailable.

If the seed oyster industry does adopt the use of washed shell as a substitute for steamed shell, how much washed shell is available?

Along the edge of most of the larger coastal rivers in South Carolina, particularly where the long reach of the river runs northwest to southeast or northeast to southwest, fairly large seas are built up at the time of moderate to fresh winds. Maximum tidal current velocities in these coastal rivers and inlets exceed 2 knots. When wind and tide oppose, the seas build up and shell from dead oysters is piled up along the edge of the river. There are many thousands of piles of shell along the banks of the creeks and rivers of South Carolina.

Across from Bears Bluff Laboratories on the northern shore of Wasmalaw River is a series of small but typical washed shell banks. Moderate to fresh southwest winds driving against a 3-knot ebb tide piled the shell up in the marsh. One of the series of shell banks across from the Laboratories now is 125 feet long by 35 feet wide and has a thickness or depth of approximately two feet. This bank contains about 7,000 standard U. S. bushels.

Although no attempt has been made to calculate the volume of washed shell available in all of South Carolina, the North Edisto River system which begins at the ocean and extends up to Bears Bluff Laboratories was surveyed to estimate the amount of available washed shell. In the five statute miles from Bohicket Creek on the east bank and South Creek on the west bank of the North Edisto River, on up the river to Dawhoo entrance near Bears Bluff, there are an estimated 525,000 bushels of raw washed shell which could be taken from this one of the many river systems in the State having washed shell along their banks.
It was noted before, however, that approximately 50 per cent of the washed shell as taken from the banks of the river would be removed if the shell were screened through a 1 x 1 inch square mesh wire screen. Thus, the North Edisto River would produce only slightly more than 260,000 bushels of washed shell suitable for cultch.

No attempt has been made as yet to determine the cost of removing this washed shell from the banks of the river. There is a possibility that a sizeable part of the cost of removing the washed shell from the banks could be paid from the sale of the screened shell. This screened shell has a valuable use as aggregate. For over ten years now, much of the concrete mixed and used at Bears Bluff has been made from shell aggregate. The use of shell aggregate is age-old in Low Country South Carolina, and prior to the availability of cement a mixture of burned shell, unburned shell, and sand was used as a fore-runner of present day concrete. A few ruins of structures made from this material, called "tabby" in the 16th and early 17th Century, are still in existence in coastal Carolina today. The manufacture of tabby is a lost art, but screened shell with cement makes a perfectly usable concrete.

It is of interest to note that washed shell is a "renewable" resource. In 1948 and 1949 across from Bears Bluff, approximately 6,000 bushels of shell were removed from one bank now estimated to contain 7,000 bushels of shell. Since 1949, between 500 and 600 bushels of shell have been removed annually, but the pile recovers with each new fresh southwest wind and seems today to be as large as it was in 1948.

Literature Cited


SURVIVAL OF SOME JUVENILE BIVALVES IN WATER OF LOW SALINITY

Paul E. Chanley

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Abstract

The minimum salinities at which juvenile clams, Venus mercenaria, Ensis directus and Mya arenaria, survived were 12.5, 7.5 and 2.5 parts per thousand, respectively. Juvenile oysters, Ostrea edulis and Crassostrea virginica, survived in salinities as low as 12.5 and 5.0 ppt, respectively, and juvenile mussels, Brachidontes recurvus, survived at 2.5 ppt. E. directus and M. arenaria survived at 7.5 and 2.5 ppt only after first becoming acclimated to intermediate salinities. They did not survive if transferred directly to these salinities from 26.0 - 27.0 ppt. V. mercenaria ranging in size from 10.0 to 21.5 mm survived at 12.5 ppt, but 15.0 ppt was the minimum salinity at which those ranging in size from 1.8 to 3.6 mm could survive. Growth was retarded at salinities of 22.5 ppt and lower. The optimum salinity for growth of recently set C. virginica was 15.0 to 22.5 ppt.

Introduction

Many commercially important bivalves occur in estuarine environments where considerable fluctuations in salinity are common. Some are found in areas where water may be almost fresh, while others require a more saline environment. Intelligent management of these fisheries, as well as proper evaluation of harbor improvements and other engineering projects, requires a knowledge of the effects of salinity on growth and survival of these animals. Such information is also necessary for effective management of future commercial ponds and hatcheries.

Some effects of low salinities and of changes in salinity on adaptation and survival of juvenile bivalves are given in this report. The term juvenile has been defined by Carriker (unpublished manuscript) for Venus mercenaria as "very young clams in which the byssus gland is no longer functional and the animal maintains its position in the bottom by means of its foot alone". In this report a broader definition is necessary to include the several species of bivalves studied. Juvenile bivalves, then, are small mollusks, generally less than one year old, that have adult rather than larval characteristics and are capable of either digging or attaching to substrates. The species
included in this report are the hard clam, Venus mercenaria, the soft clam, Mya arenaria, the razor clam, Ensis directus, the American oyster, Crassostrea virginica, the European flat oyster, Ostrea edulis, and the hooked mussel, Brachidontes recurvus.

Methods

In addition to our laboratory sea water (salinity 26.0 to 28.0 parts per thousand, which will be referred to as full salinity), the salinities used in these experiments were 22.5, 20.0, 17.5, 15.0, 12.5, 10.0, 7.5, 5.0, 2.5 ppt and fresh water. The lower salinities were obtained by diluting laboratory sea water with demineralized water or with water from the Wepawaug River which empties into Long Island Sound in Milford. The demineralized water was obtained from a Barnstead BD-2 demineralizer and stored in either polyethylene or pyrex containers.

Experimental animals were kept in either enamel or plastic containers of 3-, 4- or 9-liter capacity and water was changed at two-day intervals. Clams were provided with sand substrates to permit burrowing. Food, generally a mixture of algae, flagellates and diatoms, was added only when water was changed and before salinity was adjusted. Temperatures ranged from 15.3°C to 21.9°C but were uniform for all animals within an experiment. Feeding, ability to dig in, growth, and survival were considered evidences of adaptation to reduced salinities.

Results

Venus mercenaria

Our first experiment was designed to find the minimum salinity at which hard clams can survive and also to determine how well they recover after exposure to abnormally low salinities. Three sand-filled plastic boxes, each containing 25 hard clams reared in the laboratory and ranging in length from 10.0 to 21.5 mm, were transferred directly from full salinity to each of the experimental salinities. When 10 per cent of the animals at any salinity had died, one box was returned to full salinity. The second box was returned to full salinity when 50 per cent of the remaining clams were dead. The third box remained at the experimental salinity until all died or until the survivors had become adapted to the salinity.

At full salinity and at 22.5 ppt, over 90 per cent of the clams dug in and cleared the water of food during the first day. At salinities of 20.0 and 17.5 ppt it required 7 days for 90 per cent of the clams to become adjusted sufficiently to dig in and clear the water of food. At 15.0 ppt none opened until the seventh day, but by the 19th day over 90 per cent had dug in, were feeding and had apparently adjusted to this salinity.
<table>
<thead>
<tr>
<th>Species</th>
<th>Origin and length in mm</th>
<th>Minimum salinity in ppt</th>
<th>Per cent surviving</th>
<th>Days exposed</th>
<th>Minimum salinity in ppt</th>
<th>Per cent surviving</th>
<th>Days exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venus mercenaria</td>
<td>Milford, Conn. 10-22</td>
<td>15.0</td>
<td>100</td>
<td>75</td>
<td>12.5</td>
<td>100</td>
<td>49</td>
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<td>1.8-3.6 Narragansett Bay</td>
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<td>100</td>
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<td>Milford, Conn. 14-29</td>
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<td>100</td>
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<td>Sheepscot River, Me. 5-10</td>
<td>2.5</td>
<td>94</td>
<td>14</td>
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<td>Crassostrea virginica</td>
<td>Milford, Conn. .5-2.2</td>
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<td>100</td>
<td>28</td>
<td>2.5</td>
<td>19</td>
<td>28</td>
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<td></td>
<td>.3-1.3</td>
<td>5.0</td>
<td>39</td>
<td></td>
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<tr>
<td>Ostrea edulis</td>
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<td>15.0</td>
<td>84</td>
<td>35</td>
<td>12.5</td>
<td>92</td>
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<td></td>
<td></td>
<td>12.5</td>
<td>4</td>
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<td></td>
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<td>Ensis directus</td>
<td>Milford, Conn. 25-48</td>
<td>17.5</td>
<td>100</td>
<td>35</td>
<td>7.5</td>
<td>88</td>
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<td>Brachidontes recurvus</td>
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</table>
There was little or no feeding at 10.0 ppt or lower, and in these salinities all clams eventually died. At 10.0 ppt several clams opened and one actually dug in on the 69th day, but at 5.0 ppt and lower they neither opened nor dug in.

Although salinities of 10.0 ppt and lower eventually caused the death of all clams, they withstood these salinities for several weeks (Table 1). Thus, at 10.0 ppt no deaths were observed until the 28th day, and 32 days were required before 10 per cent died (Table 2). After nine additional days of exposure to this salinity 50 per cent were dead. Two individuals, however, survived until the 82nd and 90th days of the experiment. At 5.0 ppt the first death occurred on the 18th day, and all were dead by the 54th day. Even in fresh water clams survived for 22 days before the first death, but all were dead by the 45th day.

Thirteen per cent of those clams returned to full salinity from salinities of 0.0, 5.0 and 10.0 ppt after about 10 per cent had died, eventually died. If they remained at these low salinities until approximately fifty per cent were dead however, 51 per cent of the survivors died eventually.

Table 2. Comparison of the length of exposure required to cause a 10 per cent and a 50 per cent mortality of juvenile 
mercenaria in three different salinities and the percentages of the surviving clams that died when returned to full salinity.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Number of days exposure before a</th>
<th>Per cent of surviving clams that died when returned to full salinity after a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 per cent mortality</td>
<td>50 per cent mortality</td>
</tr>
<tr>
<td>10.0 ppt</td>
<td>32</td>
<td>41</td>
</tr>
<tr>
<td>5.0 &quot;</td>
<td>29</td>
<td>37</td>
</tr>
<tr>
<td>Fresh water</td>
<td>24</td>
<td>33</td>
</tr>
</tbody>
</table>

At the conclusion of this experiment 25 clams that had survived at 15.0 ppt for 75 days were kept in water at 12.5 ppt for an additional 49 days without mortality. At this salinity, they dug in normally and fed, but did not clear the water of food as rapidly as in full salinity.

In a second experiment, 25 clams were transferred directly from full salinity to 12.5 ppt and kept at this salinity for over
100 days. Only three died, but those that survived did not feed normally.

Conditioning juvenile *V. mercenaria* at intermediate salinities did not enable them to survive at lower minimal salinities. Most survived at 12.5 ppt either after direct transfer from full salinity or after conditioning in intermediate salinities. None adapted to a salinity of 10.0 ppt regardless of whether they had been conditioned at intermediate salinities or transferred directly from full salinity. In another experiment it was found that smaller hard clams, ranging in length from 1.8 to 3.6 mm, reacted to the lowered salinities more rapidly than did larger juveniles used in previous experiments. At 15.0 ppt, for instance, 70 per cent of the small ones dug in by the seventh day, while 17 days were required for the larger ones (Fig. 2). Moreover, at 10.0 ppt, the smaller clams all died within 15 days although none of the larger ones died at this salinity until the 28th day. In addition, all of the smaller group died at 12.5 ppt even though this salinity was not lethal to larger clams (Table 1).

Because of the possibility that hard clams from different areas have different salinity requirements, a similar experiment was conducted with specimens from Narragansett Bay. Like the large Milford clams, those from Narragansett Bay dug in rapidly, adjusted to higher salinities almost immediately and required much longer to adjust to salinities of 17.5, 15.0, and 12.5 ppt. They survived at 12.5 ppt and all died at salinities of 10.0 ppt and lower.

Narragansett Bay clams did not survive in salinities of 10.0 ppt and lower as long as large clams from Milford. Thus, at 10.0 ppt all from Narragansett Bay died within 34 days, while only 50 per cent of those from Milford were dead after 41 days of exposure to this salinity. This may have been due to the smaller size of the Narragansett Bay animals and slight differences in the temperature of the two experiments.

Although juvenile hard clams survived in salinities as low as 12.5 ppt, it seemed probable that growth would be retarded or prevented at low salinities. Turner (1953) reported that adult hard clams did not grow when the salinity was lowered to 19.0 - 21.0 ppt and that the optimum salinity range for growth was 24.0 to 28.0 ppt. Pratt and Campbell (1956) reported that hard clams grew at salinities ranging from 21.4 to 31.9 ppt in Narragansett Bay and that growth was apparently not affected by salinity within this range.

To determine how reduced salinities affect growth of juvenile hard clams, twenty-five of them were placed in each of five 9-liter polyethylene containers of water ranging from 15.0 ppt to full salinity. Water was changed every second day but no food was added during the first three weeks to prevent growth of clams at higher salinities.
Fig. 1. Number of days needed by each of two size-groups of juvenile *V. mercenaria* to dig in at salinities of 15.0 and 27.0 ppt.
before those in lower salinities opened or dug in. After three weeks, at least 70 per cent of the clams had dug in at each salinity and food was then added whenever water was changed. At that time the clams ranged from 4.9 to 9.9 mm and averaged from 7.3 to 7.6 mm in length. They were measured at two-week intervals for 6 weeks. The temperature during the experiment ranged from 16.5 to 22.7°C.

The increase in average length was similar for each of the two-week growing periods, which indicates that the animals had adapted to the experimental salinities before feeding was started (Table 3). Otherwise those in low salinities would have grown more rapidly late in the experiment, as they became adapted to the low salinities. Growth was reduced progressively with decreases in salinities. At 15.0 ppt growth was negligible. Apparently, hard clams can survive in salinities slightly lower than those in which they are capable of growing.

Table 3. Average growth increments of juvenile V. mercenaria kept at various constant salinities.

<table>
<thead>
<tr>
<th>Salinity in ppt</th>
<th>Average increment in millimeters from</th>
<th>0-2 weeks</th>
<th>2-4 weeks</th>
<th>4-6 weeks</th>
<th>Total average increment</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.0</td>
<td></td>
<td>.2</td>
<td>-.1</td>
<td>.0</td>
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Mya arenaria

When 25 soft clams, ranging in length from 5.0 - 34.0 mm, from each of five different areas were transferred directly from their normal salinities to lower experimental salinities, it was found that the minimum salinity for survival varied (Table 1). Soft clams from Chesapeake Bay, where salinity is low, survived direct transfer to 2.5 ppt but soft clams from Milford Harbor and Narragansett Bay, where salinity is higher, were killed by such a change. To determine whether this variation was caused by conditioning or was evidence of inherent differences, soft clams from Chesapeake Bay that had been kept at full salinity for three weeks were then transferred directly to a salinity of 2.5 ppt. Under these conditions all died
within three days as did those from Milford Harbor and Narragansett Bay. On the other hand, when soft clams from Narragansett Bay and Milford Harbor were adapted to a salinity of 12.5 ppt and then transferred to the lower salinities by steps of 2.5 ppt per week, they dug in and fed at 2.5 ppt. Furthermore, after such gradual conditioning, Milford clams which were transferred directly from 2.5 ppt to full salinity all dug in and were feeding by the second day. After 8 days at full salinity these same clams were again returned directly to 2.5 ppt. Two days later 9 out of 10 had dug in and were feeding. Another group of 25 Milford soft clams that had been conditioned similarly to survive at 2.5 ppt, also survived direct transfer to full salinity and all but two dug in within 48 hours.

These experiments indicate that the minimum salinity at which soft clams survive is determined by the salinity to which they have become adjusted and not by inherent differences in clams from different areas. Unlike V. mercenaria, the minimum salinity at which soft clams survived was lowered by gradually conditioning the animals in intermediate salinities. If conditioned properly, soft clams from any of the areas reported here survived at all salinities ranging from 2.5 to 28.0 ppt.

**Ensis directus**

Eighteen juvenile razor clams, ranging in length from 25.0 to 47.5 mm, were transferred from full salinity to each of the experimental salinities.

All clams transferred directly from full salinity to 15.0 ppt or higher dug in rapidly and fed normally almost immediately (Table 1). Seven of the 18 placed in 12.5 ppt dug in, but all were dead by the fifth day. At 10.0 ppt they were gaping widely one day after the transfer and, except for feeble movements of the foot after stimulation, were not able to move. Although three had partially dug in at this salinity, all were dead by the third day. At 7.5 and 5.0 ppt there was no evidence of digging in and all died within two days. All kept at 2.5 ppt or in fresh water died in less than 24 hours.

Razor clams kept at 15.0 ppt and higher for 35 days were later subjected to successively lower salinities at the rate of 2.5 ppt per week. They dug in and fed normally at salinities down to and including 7.5 ppt. When transferred from 7.5 to 5.0 ppt all but two dug in. Nevertheless, they began to die after 24 hours and within a week all were dead.

Even though razor clams survived from 7.5 to 28.0 ppt, when the salinity change was gradual, sudden changes were quickly lethal. Those that had survived for 13 days at 7.5 ppt all died within 48 hours after they were returned directly to full salinity. Apparently, these clams are incapable of adjusting to a rapid salinity change of this magnitude in either direction.
Crassostrea virginica

The American oyster occurs naturally in areas of widely varied salinities. Ingle and Dawson (1950) reported commercial production of oysters in an area where the salinity ranged from fresh water to 42.5 ppt annually. Loosanoff (1932) found commercial sets of oysters in an upper James River area where the salinity ranged from 4.29 to 10.66 ppt and in 1950 he demonstrated that oysters from Long Island Sound could stand rapid transfer from 3.0 to 27.0 ppt with no apparent ill effects.

Our salinity work with juvenile oysters has been limited to two growth experiments. In the first, groups of 50 recently set oysters, averaging from 0.3 to 0.5 mm in length, were placed directly in experimental salinities from 2.5 ppt to full salinity. They were measured after two weeks and again after four weeks.

All spat kept in 2.5 ppt at a temperature of 21.0\(^\circ\) to 24.0\(^\circ\)C died before the end of the first two-week period. Slightly over half of the spat kept at 5.0 ppt died before the final measurements were taken, but those that survived did show a slight but measurable amount of growth. Oyster spat kept at 7.5 and 10.0 ppt showed substantial but slow growth. Their average size at the end of the experiment was only 1.94 and 1.85 mm, respectively, while the spat kept at salinities of 12.5 ppt and higher showed much faster growth and averaged from 3.69 to 4.91 mm at the end of the experiment.

In another experiment 29 to 37 recently set oyster spat averaging from 1.0 to 1.4 mm in length were transferred gradually to the experimental salinities over a period of seven days. They were measured again after two and four weeks of exposure to salinities ranging from 2.5 ppt to full salinity.

Once again oysters kept in salinities from 15.0 to 22.5 ppt grew well with an average four-week increment of from 7.0 to 8.2 mm in length. Those kept at 10.0, 12.5 ppt and full salinity grew less with average increments of from 3.8 to 5.0 mm, while those at 5.0 and 7.5 ppt had average growth increments of only 1.2 and 2.8 mm, respectively. Spat kept at 2.5 ppt decreased in average length .3 mm during the experiment. This may have been the result of selective mortality. However, the shells of the spat may have been eroded at this salinity since the shells of even the survivors were papery and soft. Eighty to 100 per cent of the spat survived in all of the experimental salinities except at 2.5 ppt where only 19 per cent survived and at 5.0 ppt where 66 per cent survived.

These results indicate that juvenile oysters differ somewhat from larvae in their salinity requirements. Davis (personal communication) found that oyster larvae showed negligible growth at 7.5 ppt and eventually died. Even at 10.0 ppt growth was slow and no larvae reached the setting stage. However, both larvae and juveniles grew
normally at 12.5 ppt and higher. Juvenile oysters responded to reduced salinities in a manner similar to that reported for adults (Loosanoff 1952), that is, they failed to grow at salinities below 5.0 ppt, grew slowly at salinities below 12.0 ppt, and grew normally from 12.0 to 27.0 ppt.

**Ostrea edulis**

The minimum salinity at which most 6-month-old European oysters survived following direct transfer from full salinity was 15.0 ppt (Table 1). One oyster, however, survived at 12.5 ppt, fed normally and appeared to thrive throughout the 35 days of the experiment, although all other oysters at this salinity quickly perished. After 35 days the oysters kept at 15.0 ppt were transferred to 12.5 ppt. They appeared to adjust to this salinity and only two died during the 31 days of additional exposure.

**Brachidontes recurvus**

Twenty hooked mussels from Chesapeake Bay, ranging in length from 17 to 42 mm, were placed in each of 11 experimental salinities from fresh water to full salinity. They were kept in 3-liter polyethylene containers at temperatures ranging from 17.60 to 24.0°C. They were feeding normally in each salinity from 2.5 ppt to full salinity four days after direct transfer and no appreciable mortality occurred in these salinities during the 50 days of the experiment (Table 1). In fresh water, however, the first mussel died after four days of exposure and all were dead within 30 days.

After 50 days, five animals from each salinity were opened and the gonads examined. In general, gonad condition was poor, presumably because of the limited amount of food and water available during the experiment. However, except at full salinity, at least one mussel in each sample had recognizable gametes.

**Discussion**

Several factors must be taken into consideration before efficient management of shellfish resources can be achieved with respect to salinity. The minimum salinity at which bivalves can survive is determined by several conditions that must be understood. In addition, it is also important to determine how long unfavorable salinities can be tolerated and what factors affect the length of time a species can survive in unfavorable salinities. We shall first discuss conditions that determine the minimum salinity at which survival is possible and, finally, conditions that affect the length of time bivalves can survive in unfavorably low salinities.
The minimum salinity at which survival is possible varies with the size and/or stage of development of bivalves of the same species. For example, in these experiments, large juvenile hard clams (10.0 - 21.5 mm) survived for over 100 days at 12.5 ppt but smaller juveniles (1.8 - 3.6 mm) did not survive below 15.0 ppt. Moreover, growth of even the larger clams was retarded at lower salinities and virtually stopped at 15.0 ppt. Larvae of the same species have even higher salinity requirements. Davis (personal communication) found that larvae grew slightly at 15.0 ppt but that none reached setting stage at salinities below 17.5 ppt. Even at this salinity the larvae were so sluggish and weak that they perished during the setting and immediate post-setting stages. Turner (1955) also found that although larvae grew at 20.0 ppt, one-day-old larvae, which normally swim upward, were unable to do so between 15.0 and 20.0 ppt. Apparently, then, a population of hard clams cannot be sustained below 20.0 or 17.5 ppt, although individuals might survive in salinities as low as 12.5 ppt.

Geographically separated populations of bivalves may also have inherently different salinity requirements. Races, physiologically different in other respects, are known to exist and although little supporting evidence was found in these experiments the same species of bivalve from different areas may require different salinity ranges.

Another factor affecting the minimum salinity at which marine bivalves survive is the source of fresh water. We found that hard clams died at 20.0 ppt when the salinity was reduced by fresh water very high in copper content, yet, they survived at 12.5 ppt when the salinity was reduced by demineralized water.

The rate of salinity change is also important. Razor clams, for example, were killed readily by rapid salinity changes of 15.0 ppt, while gradual changes over the same range were tolerated. Bivalves capable of withdrawing all organs into a water-tight shell, such as the hard clam, are not nearly as susceptible to rapid changes of salinity.

In addition to a knowledge of the minimum salinity at which a species will survive, grow, and reproduce, it is important to know how long unfavorable salinities can be tolerated. Several conditions affect the length of time bivalves can exist in salinities too low for indefinite survival.

Water temperature, for example, is of primary importance. Loosanoff (1948) found that oysters could survive in fresh water or at a salinity of 3.0 ppt for 70 or 115 days, when the temperature ranged from 8.0° - 12.0°C. At these same salinities all oysters died within 15 days when the temperature ranged between 23.0° and 27.0°C.

Size is also important in determining how long unfavorable salinities can be tolerated. As previously noted, small hard clams
Fig. 2. Comparison of the mortality rates of five species of mollusks in salinities which were 2.5 ppt lower than the minimum salinity at which they survived.
(1.8 - 3.6 mm in length) all died within 15 days at 10.0 ppt but none of the larger ones (10.0 - 21.5 mm) died at this salinity until the 28th day of exposure.

Finally, some species are capable of enduring longer periods of unfavorable salinity than others (Fig. 2). Hard clams survived in unfavorable salinities for several weeks probably because they could withdraw all organs into a water-tight shell and prevent exposure of vital parts to unfavorably low salinities. However, other bivalves, such as soft clams and razor clams, did not survive exposure to lethal salinities for more than a few hours or days because their shells were incapable of forming a water-tight cavity to protect the clams from unfavorable salinities.

Acknowledgments

I would like to express my gratitude to Dr. V. L. Loosanoff and Mr. H. C. Davis for their advice and cooperation in these experiments, and for their assistance in preparing this report. I am also indebted to Mr. C. A. Nomejko for preparing the graphs, and to our associates in the Clam Investigations for supplying the experimental animals from Maine, Narragansett Bay and Chesapeake Bay. I am also grateful to the Office of River Basin Studies, U. S. Fish and Wildlife Service, for the financial support that made these studies possible.

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CAUSES OF DEPLETION OF OYSTERS
IN ST. VINCENT BAR, APALACHICOLA BAY, FLORIDA

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Abstract

During the period from June 1955 through May 1957 an ecological study was made of oyster reefs in Apalachicola Bay, Florida. This report covers a phase of the study on St. Vincent Bar. This reef is entirely depleted although it has been very productive in former years. Salinity over this reef has been high and several enemies of the oyster have become established here, especially the southern oyster drill, Thais haemastoma, and the stone crab, Menippe mercenaria. There was an abundant spatfall on the old shells during both years of study, but no oyster was found on the reef larger than 50 mm in length in the first year and the maximum size was less than 40 mm in the second year. An experiment was made during the second year in which the oysters were protected from predation of both drills and stone crabs, and not only was the mortality less for the protected oysters, but a maximum size of between 70 and 80 mm in length was reached before the end of the observations. Circumstantial evidence indicated strongly that the predators, especially drills and stone crabs, have caused depletion of the reef.

Introduction

An ecological study of oyster reefs in Apalachicola Bay, Florida, was begun in June 1955. During the first year's study four oyster reefs were sampled at approximately monthly intervals for oysters and associated organisms, especially predators. The method of sampling involved removal by hand of all shell from a measured area, usually one to three square meters. Aqua-lungs were used in deep water. Samples were brought back to the Oceanographic Institute, Florida State University, Tallahassee, for examination. All large animals

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were either counted and recorded as number per square meter, or were estimated as to abundance. The second year was devoted to a more intensive study of one of the reefs, St. Vincent Bar, while the other reefs were sampled seasonally. This is a report of part of the study; a more detailed report will appear later.

This report deals only with St. Vincent Bar, which was sampled from September 1955 through May 1957. Only a small portion of this reef was systematically sampled, but numerous observations indicate that it was entirely depleted of large oysters. There were many old shells and fragments of shells, but large oysters were never seen. Swift (1898) shows dense growth of oysters in this area in his survey of 1895-1896. Danglade (1917) stated that St. Vincent Bar was showing signs of depletion and was closed by the State to recover. During the investigation of Pearse and Wharton (1938) there was an indication that St. Vincent Bar was still productive. Ingle and Dawson (1953) list the production of this bar as "none". Mr. Joseph Martina, Jr., agent of the Florida State Board of Conservation, has stated (private communication) that the last commercial oysters were taken from this reef more than five years ago, and that it has been depleted since then with only sporadic tonging by oystermen. Many of the oysters taken in recent years probably came from isolated lumps in adjacent areas, and not from the bar itself.

Results

St. Vincent Bar was first sampled 21 September 1955. At this time over 500 live oysters were found per square meter. Nineteen percent of the oysters were of the species Ostrea equestris Say, but nearly 400 were of the commercial species, Crassostrea virginica (Gmelin), and the density was considered fairly heavy. No oyster was over 50 mm in length, and it is fairly certain that all had attached during the summer of 1955. When the next examination was made, less than one month later (14 October 1955), only 165 live oysters of both species were found per square meter, and the largest oyster was less than 20 mm in length. By the following 9 May 1956 examination, 72 oysters of both species were recorded per square meter and all were under 40 mm in length. The percentages of Ostrea equestris decreased from 32% of the total number of oysters found in the October 1955 examination to a low of 6% in the May 1956 examination.

It is evident that the oyster mortality was high. The most serious aspect of the mortality was that the oysters died before they attained market size. Sometimes mortality was so rapid among large oysters that the size of the largest oysters decreased with each succeeding month.

It appears certain that an increase in salinity, caused by rainfall below normal over the past four or five years, has occurred on many of the outlying reefs in Apalachicola Bay. This has allowed
the invasion, often in large numbers, of many animals that cannot tolerate salinity as low as can the Virginia oyster. The small non-commercial species, Ostrea equestris is one example. The southern oyster drill or conch, Thais haemastoma Conrad, is less tolerant of low salinities than the commercial oyster, and the same is true for the stone crab, Menippe mercenaria Say. Both the drill and the stone crab are oyster enemies of importance in the Gulf area (Butler, 1954; Chapman, 1955; Menzel and Hopkins, 1955.) Drills occurred in numbers as high as six per square meter and averaged 2.75 per square meter for all of the 20 examinations during the period of the study. No young drills were seen, although egg capsules were deposited during the spawning season of 1956. The size of the adult drills ranged from 52 to 84 mm. The abundance of stone crabs, 50 to 100 mm wide, was estimated to be about one per square meter; no satisfactory quantitative sampling method was developed for stone crabs in the subtidal area. It is not possible to determine the mortality of oysters caused by T. haemastoma on a reef because of the method of attack, however, some drill "marks" were seen. Many broken shells were seen and these are evidence of damage by stone crabs. Because of the abundance of these two predators, it is assumed that they were responsible for much of the mortality.

Other enemies occur in this area also, but the effect of salinity on their distribution apparently is not as critical as with the two predators mentioned above. There were many snails of the genus Odostomia (O. impressa Say), but no correlation was possible with oyster mortality, e. g. they were as numerous in other areas where oyster mortality was much lower. Also present in large numbers on four occasions was the "oyster wafer", Stylochus frontalis Verrill. At the December 1955, and the January, February and April 1956 examinations they numbered up to 50 per square meter. From one to four worms were found per square meter in four other examinations of the natural bottom. The mortality rate did not reflect their presence or absence.

On 9 May 1956, wire baskets of one-half inch mesh hardware cloth were made. These baskets measured 26 by 51 cm, and were 9 cm high. Each basket held 12 liters of shell, which was tonged from St. Vincents Bar. All large organisms, especially drills and stone crabs, were removed before the material was placed in the baskets. It has been noted above that sampling showed 72 live oysters per square meter and therefore some live oysters were included in the baskets. Enough baskets were made so that two could be examined at approximately monthly intervals from the first examination on 27 June 1956 to the termination date on 11 May 1957. Eleven examinations were made during the period. Meter square samples of the bottom were taken concurrently with each examination of the baskets.

The volume of shell in the two baskets was approximately equal to the amount usually taken from one square meter of bottom, but the numbers of living organisms were not comparable. The baskets were
raised 9 cm from the bottom and hence provided more surface for the attachment of sessile organisms, especially oysters, and also seemingly provided a better habitat for many of the small sedentary organisms.

On the first examination in June 1956, there were 605 live oysters in the two baskets, ranging up to 40 mm in length. The majority, however, were below 20 mm and 51% were Ostrea equestris. The number of oysters in the two baskets was over 1500 by October and November 1956, but had decreased to 794 by the last sample taken 11 May 1957. The number of oysters per square meter of bottom ranged from a high of 363 in November to a low of 117 when the last sample was taken in May.

Previous to the May 1957 sampling, the salinity ranged from 20 to over 30 ppt. Occasionally the salinity was lowered to about 10 ppt for a short period of time as a result of freshets. These lowered salinities had no observable effects on the fauna of the reef. During the late spring of 1957, rainfall increased considerably lowering the salinity to below 10 ppt. On the May 1957 examination the lowered salinity (7.5 ppt) had considerable effect on some of the fauna. No living Ostrea equestris were found. Two Thais haemastoma were found but they were inactive, buried in the substrate.

There were always considerable oysters per unit area, but none of the oysters on the bottom exceeded 40 mm in length during the second year's study. Ingle and Dawson (1952) have shown that growth of the commercial oyster is rapid and continuous throughout the year in Apalachicola Bay. Yet on St. Vincent Bar the maximum size of the oysters on some sampling dates was less than the maximum of the month before. On the other hand, some of the oysters in the baskets during the latter part of the investigation reached a length of between 70 and 80 mm. These oysters were deep-cupped and fairly thick-shelled.

Although conditions were not the same in the baskets as on the bottom, all known enemies of oysters, except large stone crabs and drills, were present. In fact some animals were more abundant in the baskets than on the bottom. The "oyster wafer" was found in the baskets in seven of eleven examinations, and only four times on the bottom during the same period. In no instances were many worms found, the maximum at any examination being five in the two baskets and four per square meter of bottom. Small stone crabs in the baskets rarely measured as much as 30 mm in width of carapace, but even crabs this small are capable of killing oysters.

Dermocystidium marinum Mackin, Owen and Collier was known to be present in the area (Dawson 1955). Dr. J. G. Mackin, Texas A and M Research Foundation kindly examined slides of eight survivors from a mortality experiment conducted on the bar. These were large oysters that had been transplanted from another reef in the Bay and were placed in wire baskets to protect them from large predators. These oysters were taken in October 1956 and had a mortality of over 50%
during the previous month. Examination showed two oysters to be negative, and six others to have light to moderate infections. It is thought that the fungus caused the heavy mortality of the large oysters in this particular experiment. Mortality on the bottom, however, was not characteristic of that caused by the fungus (Mackin 1951, Ray 1953). The oysters were small and the mortality proceeded rapidly during the winter months. The best evidence against the mortality being caused by D. marinum is that the oysters lived better and were of a larger maximum size in the baskets than on the bottom.

The results of the basket experiments from which drills and stone crabs were eliminated, confirmed the assumption that predation by these animals was the major factor in the oyster mortality and the lack of any large oysters.

Summary

St. Vincent Bar, Apalachicola Bay, is a depleted oyster reef that was formerly very productive. For the past four or five years rainfall has been below normal in the area, with the result that salinity has increased and has allowed the establishment of several important oyster predators on the reef. Beginning in the spring of 1957, rainfall increased. In May 1957 the salinity over the reef was low (7.5 ppt) and there was a scarcity of predators. There was abundant spatfall during the two years of the study, but mortality was very high, although the numbers of oysters remaining were probably adequate to replete the reef. Even though the numbers of oysters per unit area were always considerable, no oyster was ever found over 50 mm in length. Shells from the bottom were placed in wire baskets in June 1956, and two baskets were examined monthly; the last examination was in May 1957. Drills and large stone crabs were effectively eliminated from the baskets and mortality of the oysters was considerable less than on the unprotected bottom. More important, some of the oysters in the protected baskets reached a length of 70 to 80 mm before the end of the period. This is strong circumstantial evidence that predation, especially by drills and stone crabs, was the primary cause of mortality and the lack of large oysters. Thus with increased rainfall and decreased salinity, and with elimination of predators, there is hope that St. Vincent Bar will recover.

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THE SEASONAL SIGNIFICANCE OF TOTAL SOLIDS OF OYSTERS IN COMMERCIAL EXPLOITATION

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Abstract

Per cent dry weight and total solids of oyster meats were determined from 1,000 samples taken monthly or more frequently for 10 years. Seasonal fluctuations are shown by monthly averages of 11 stations for all years. Lowest solids occurred regularly in August and highest solids in November and December. The average monthly low was 13-14% solids and the average monthly high was 19-20%. The lowest solids content was 6.1% in a sample taken during a period of abnormally low salinity in August 1946. The highest solids content was 26.5% in February 1947. Solids, seasonally were low in summer and early fall, high in late fall and early winter, relatively low in late winter and high in late spring. This indicates that for optimum quality of oysters, the best seasons for harvesting are late fall and late spring.

The quality of oyster meats is a factor of foremost consideration to consumer, producer and scientific personnel of State and Federal conservation departments, pure food agencies and health departments. From the commercial aspect, quality of oysters is in part yield and in part appearance. Each of these is related to a so-called fatness. "Fatness" in this instance is a misnomer because most of the accumulated material is glycogen, a carbohydrate, and not a fat.

The accumulation and utilization of glycogen are seasonal phenomena of great importance in determination and evaluation of quality in oyster meats. Fluctuations in quality and condition of oysters may be attributed to gonad development, discharge of gametes, type and availability of food, and effects of extreme changes in environment. Adverse environmental influences such as (1) rapid salinity depression caused by hurricane storms, freshets following wet seasons and melting ice and snow in the water shed (Engle 1946, Beaven 1946), and (2) accumulation of H2S and accompanying depletion of oxygen (Ito and Imai 1955) inhibited growth and fattening of oysters. Industrial pollutants, such as pulp mill effluents, may
change the local environment sufficiently to interfere with normal fattening of oysters (Galtsoff et al. 1947). These factors are linked and overlap and a summation of their effects on the condition of oysters can be measured.

Indices of condition of oysters such as per cent glycogen, per cent dry weight or total solids, and "condition factor" are acceptable means of measuring quality in oyster meats. Chipman (1947), Engle (1950) and Engle and Chapman (1951) described in detail the methods of determining condition.

The dry weight or per cent solids is the simplest of these methods of determining condition or quality of oyster meats. It is used here for that reason and because pure food agencies use it as an index of adulteration with water. It may be defined briefly as the dry weight or solids per unit quantity of wet meat determined by the following procedure:

1. Shuck oysters without cutting the body except at adductor muscle.
2. Drain meats on a stainless steel grid for 12 minutes after shucking.
3. Weigh drained meats and homogenize in Waring blender.
4. Weigh two aliquots of blended meats and dry for 72 hours in an oven at 90°.
5. Per cent dry weight is the dry weight of the aliquot divided by the wet weight of the aliquot x 100. Total solids are calculated by multiplying the wet weight of the whole sample of meats by the per cent dry weight. Per cent dry weight and per cent solids are synonymous in this discussion.

Condition or quality of oysters is far from stable. Individual oysters vary within a bar or bed and oysters from one bar or community may differ from those in another. These differences in quality are seasonal also, but within a geographical area seasonal changes are often similar. If oysters from widely separated geographical areas are compared, differences in seasonal patterns of fattening are observed. For instance, oysters in Long Island Sound appear to "fatten" earlier in the fall than do those in Chesapeake Bay. Variations in seasonal temperatures of waters affect the condition cycle. Continued high temperatures in the fall of the year usually delay fattening of oysters. Hopkins et al. (1953) indicated that Louisiana oysters reach peak fatness in late winter and early spring. The present studies are confined to seasonal changes in condition or quality of oysters in Chesapeake Bay and the influence on commercial use.
Fig. 1. Per cent total solids of oysters, salinity and temperature in Chesapeake Bay on monthly average basis, 1949 to 1955.

Fig. 2. Per cent total solids of oysters, salinity and temperature in Eastern Bay on monthly average basis 1949 to 1955.
Condition of oysters, expressed as per cent dry weight or solids, has been observed in oysters from several locations in upper Chesapeake Bay during the past ten years. Emphasis has been focused on seasonal changes with respect to cyclic occurrence and to annual amplitude. More than 1,000 determinations of per cent solids were made from 1945 to 1955. Samples were taken from 17 stations in upper Chesapeake Bay and its tributaries.

The solids content of oysters ranged from a low of 6.1% at Tea Table in upper Chesapeake Bay in August 1946 to 26.5% at Thomas Bar in the Patuxent River in February 1947. For oysters in Chesapeake Bay proper, the average annual low, based on seven years of continuous observations from 1949 to 1955 inclusive, was 14% and the average annual high for the same period was 20%. Eastern Bay, a major oyster-producing tributary of upper Chesapeake Bay, which was under observation for the same period, produced an average annual low of 13% and a high of 19%. Excessively wet seasons were included during 1945 to 1947 which accounted for the extremely low figure of 6.1% solids. Per cent solids is the complement of water content, hence, an oyster with a solids content of 6.1% is 93.9% water and an oyster with a solids content of 26.5% is 73.5% water.

Monthly averages of per cent solids taken over the seven years indicate a seasonal periodicity as follows: (1) The lowest level of solids occurred regularly in August at all stations. Almost as regularly the highest level of solids occurred in December in Chesapeake Bay and in November and December in Eastern Bay and other tributaries. High solids occurred regularly in May and June following a short period of decreasing solids in February, March and April (Figs. 1 & 2).

The principal cause of seasonal fluctuation in solids is changes in glycogen. In June glycogen is converted to gonadal products (Engle 1950). By August, glycogen and gametes are most expended and oysters are in their poorest state. The annual cycle of condition can be seen in Fig. 3 in which the points for each month represent the average for seven years.

Commercial utilization of oyster meats requires that the product contain as much meat and as little water as possible. A pure food measure of adulteration is the per cent solids and a tentative level of solid content proposed as the dividing line between good and poor oysters is 10.4 per cent. To have oysters go below that percentage in nature took a long exposure to low salinities such as prevailed in upper Chesapeake Bay in 1945-46. Salinities at that time, from March 1945 to August 1946, did not exceed 10 parts per thousand except for several weeks in September 1945 while for at least half this period the salinity was less than 5 parts per thousand and oysters were poor. During these unusual conditions from September 1945 to August 1946 solids were under 10 per cent. Long exposure to reduced salinity achieved this but it can be accomplished by over exposure to fresh water during shucking house cleaning (Pottinger et al 1941).
Fig. 3. Composite curves showing monthly averages of per cent total solids on basis of seven years, 1949 to 1955, to indicate general seasonal changes.
In September oyster meats are thin on most bars in upper Chesapeake Bay because little glycogen has accumulated since spawning (Fig. 3). At this time yield of shucked meats is low and plumpness and esthetic appeal, which in large measure are reflections of glycogen content, are lacking. The curve of seasonal condition indicates that oyster meats are prime in November. Yield in pints of meats per bushel of shell oysters may increase from 4 in August to 8 in November.

It would seem logical to postpone harvesting of oysters until October to obtain higher yields of a superior product. Oysters for September and summer use could be quick frozen in May when they are usually in prime condition. This is a departure from precedent and in upper Chesapeake Bay (Maryland) would require changes in laws to extend the harvesting season beyond April 15. There is no regulation that prohibits sale of frozen oysters in summer months.

With oyster production declining in many parts of the Atlantic seaboard, the necessity of getting the most out of nature and the oyster is imperative. One way of doing this is by applying the knowledge gained by studies such as these to the harvesting and processing of this food. The data disclosed by these observations show seasonal or cyclic variation in the quality of oyster meats. The higher level of solids and glycogen in oyster meats occurs in late fall and late spring and the lowest in late summer. Harvesting and processing oysters at these periods of peak quality would benefit the consumer by offering the food at its best and the producer by offering the food at its maximum yield.

Literature Cited


AN AUTOMATIC WATER SAMPLER FOR MARINE SHORE STATIONS

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Abstract

An automatic machine was designed which takes hourly samples of natural waters for a week. The machine was housed on shore with a pump located near high tide level and an intake hose buoyed at proper depths. The apparatus is suitable for measurement of salinities, some pollutants, and possibly bacteriological or plankton samples subject to the particular requirements of storage, evaporation and contamination of samples.

In many investigations of inshore marine environments it is necessary to measure changes in waters that occur during short spans of time. It has been determined that significant changes in chemical and physical properties of sea water may occur even in the space of an hour and that these changes may have a decided influence on the biological populations present in an area. Thus, in an investigation it is necessary to ascertain if such changes occur and to determine their magnitude to evaluate observed biological phenomena.

Some of the properties of sea water can be determined directly and automatically with recording instruments and these present no major problems. The properties that can be measured only by chemical determinations from water samples are quite a different matter. To station men in an area to collect the necessary samples is expensive and wasteful. For example, to collect hourly samples for a week would require 4 man-weeks of work. Usually it has not been possible to do this and continuous data have not been available.

During the past few years the Washington Department of Fisheries has been investigating unexplained mortalities of the Olympia oyster (Ostrea lurida) in the bays of south Puget Sound, Washington. In the course of these investigations a pulp mill located in the area became one of the suspected causes of mortalities. It was then deemed advisable to collect continuous data on pulp mill effluent and salinity values. To gather this information the University of Washington Department of Oceanography, working on contract for the Department of Fisheries, designed and built an automatic water-sampling machine which collects a sample of sea water each hour for a week. The machine was designed by Dr. R. G. Paquette and Mr. E. L. Scott, and constructed by Mr. Scott. The University of Washington is applying for commercial patent rights on the machine.
Fig. 1. Automatic machine for taking hourly samples of natural waters for a week.
The basic design of the machine is as follows. The 180 citrate bottles are held in exact position in rows by metal separators in a large, flat tray. The tray also serves as a large sink to carry off excess water. Mounted on the top of the tray, on a track, is a miniature, walking-gantry crane which holds the fill pipe, which in turn is connected by flexible tubing to the sea-water pump. The operation of the machine is governed by an electrically-timed motor operating a series of cams and micro-switches which in turn initiate and terminate the various functions of the machine.

Operating sequence is as follows: The walking crane motor starts, moving the crane and fill pipe to a point halfway between two bottles. The sea-water pump starts and flushes all the water in the system from the previous sample. The crane then moves forward again, stopping when the fill pipe is directly over the bottle to be filled, and after the bottle is filled the pump shuts off. From start to finish this operation requires about ten minutes. The complete cycle is repeated once an hour, filling one new bottle each hour. After walking crane moves forward to the end of one row of bottles a wedge on the traveler comes in contact with a fixed roller which causes the fill pipe to slide over to the next row of bottles. In this same motion a switch is activated, the direction of movement of the walking crane is reversed, and it moves backward over the next row of bottles.

Exact physical location of such a machine is governed largely by the information needed, tidal currents of the area, and special considerations of the specific problem. In the particular situation being investigated in south Puget Sound, the machine was located on Hammersley Inlet, a narrow channel connecting the pulp mill with the oyster areas. Complete mixing of water occurs in this restricted channel, and enables the machine to obtain a representative sample of everything leaving the mill estuary.

Installation of the present model must conform to a few basic requirements. The machine must be located near a source of electric power, and because of the number of samples collected, it should be readily accessible. The machine itself is housed in an 8 x 12 ft. building set on the shore. The sea-water pump is located in a waterproof casing at extreme high tide level on the beach. Care must be taken that vertical distance from the pump to extreme low water does not exceed the ability of the pump to lift water. From the pump a chemically-inert plastic line runs to an anchor placed in about 10 feet of water at low tide. From the anchor the hose is attached to a buoy floating on the surface which suspends the intake one foot below the surface of the water. A readily-accessible check valve is located at the buoy.

Operation of the machine proves to be fairly simple and trouble-free. The greatest source of trouble is the check valve; it is imperative that the check valve be reliable, resistant to the action of sea...
water and capable of complete closing even under low water pressure. The only remaining source of trouble with the check valve arises through jamming by small particles of foreign material entering the line through the intake screens. To avoid freezing, lines are buried in the ground and the building housing the machine is insulated and provided with a small, electric heater.

A large number of sample bottles is required to keep the machine in constant operation. In practice, about four sets of bottles are needed to insure against shortages caused by delays in sample analysis and bottle washing. The large number of samples collected, and the frequency with which each bottle is reused, requires that bottles be numbered in sequence. Extreme care in recording data is necessary to avoid confusion.

Analysis of samples is a major item. In this investigation a method of screening samples was worked out. Initially, all samples were run to determine expected variation; later, since our interest was primarily in the quantities of sulfite waste liquor leaving the mill, we were able to consider mainly ebb-tide samples. After determining the time period involved in concentration changes, we decided to run every other sample on the ebb tide, plus one sample on the start of the flood. If the values derived from these samples fall within the expected range no additional samples for the time period are analyzed. However, if variance is encountered, the rest of the samples are analyzed until the extent of the variation is determined.

With a little ingenuity, the basic principles of this machine could be applied to many different types of investigations requiring frequent observations. In situations where samples are desired with greater or less frequency, it would be a simple matter to change the electric-timing device which regulates the sampling period. If samples are desired only at certain stages of the tide, a pressure switch activated by increasing tidal height could be installed. In areas where tidal range exceeds the ability of the pump to lift water, the pump could be located on a float at water level. Some considerations necessary for adaptation of the machine to other investigations are:

1. Length of time samples can be stored.
3. Prevention of contamination of the sample by components of the machine or extraneous sources.

In addition to various types of samples that can be collected for chemical analyses, it is believed that modifications of the basic setup would permit collection of bacteriological or plankton samples.

In summary, it has been found possible with this machine to obtain data with a minimum of effort and expense. Servicing and repairs to the machine required about one man-day per week (an average during six months operation). Overall cost of the machine, building and installation was about $4,500.
BURIAL AS A METHOD FOR CONTROL OF
THE COMMON OYSTER DRILL, UROSALPINX CINEREA,
OF LONG ISLAND SOUND

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Abstract

A method is suggested by means of which the oyster drill, U. cinerea, an important enemy of oysters, may be controlled. The method consists of burying drills under several centimeters of bottom material. This can be accomplished by using modified agricultural plows, oyster and clam dredges, etc., to turn over layers of bottom deposit. Laboratory experiments demonstrated that when buried at a depth of 3 cm 40 per cent of the drills could not reach the surface and eventually died; at 4 cm 75 per cent died; and at a depth of 6 cm 92 per cent did not reach the surface. Additional experiments showed that all drills buried under a 6 cm layer of bottom material at 25°C. died in five days; at 20°C., in seven days; at 15°C., in 12 days, and at 10°C. 19 days were needed to kill all the drills. Approximately 52 days were necessary to cause complete mortality of the group which was buried in the mud when the water temperature ranged from 0° to 5°C.

Biologists and oyster growers have thought for some time that the oyster drill, Urosalpinx cinerea, is not able to emerge when buried beneath several inches of substratum (Carriker 1955). Since reliable field observations and studies under controlled conditions were not available to support this conception, we decided to determine the mortality of drills when buried at different depths beneath a mixture of mud and sand.

Preliminary experiments were undertaken during the summer of 1956 and have already been described (Loosanoff 1956). In general, it was found that when drills were buried beneath 2 cm or less of bottom material there was virtually no mortality because all of them emerged within a short time. When buried at a depth of 3 cm, however, approximately 40 per cent could not reach the surface and eventually died; at 4 cm, 75 per cent died; at a depth of 6 cm, 92 per cent did not reach the surface.
It was found that the rate of emergence from soft mud was higher than from harder substratum. Drills experienced no difficulty in moving along mud surfaces; both large (25 mm) and small (8 mm) drills moved through mud, even though submerged to one-half their height. The experiments also indicated that small drills emerge in approximately one-half the time needed by large individuals.

Since preliminary results appeared promising, studies were continued at Milford Laboratory during the winter of 1956-57. Two principal aspects interested us. First, we wanted to determine the best time of year to kill drills by covering them with bottom deposit. We thought that one such period is late fall or early winter when temperatures are low enough to render drills inactive. Under such conditions, drills would not be able to emerge from even a thin layer of bottom deposit and, regardless of the lowered metabolic activities would eventually suffocate and die. Nevertheless, it was also possible that burying of drills could be done most efficiently when water temperatures were high, because under such conditions the mud-covered drills would suffocate more quickly. To find the most favorable time for burial, a series of experiments was conducted to determine the depth from which oyster drills cannot emerge from a mixture of mud and sand at different temperatures.

These experiments were conducted during the winter months when the temperature of the water in our laboratory can be rigidly controlled. Drills were placed in a mixture of eight parts of sand and one part of heavy mud, which closely resembles the soil composition of the Long Island Sound oyster beds. The drills selected for this experiment were attached to the walls and bottom of the aquarium, which indicated that they were healthy. Before being used in these experiments, the drills were conditioned for one week at the temperature at which they were to be buried.

At the end of the conditioning period the drills were placed in special boxes filled with a mixture of mud and sand. These boxes were placed in larger ones through which water of the desired temperatures ran continuously. The temperatures were 5°, 10°, 15°, 20° and 25°C. To be certain that the mixture in the boxes was of proper temperature, the water was permitted to run over them for about three days before the drills were introduced.

Twenty drills were used for each temperature. They were of approximately the same size, measuring from 20 to 22 mm in length. They were pressed into the substratum by means of lucite forceps, to which a plastic ruler was attached for measuring the proper depth. The depths were measured from the top of the drill shell (the drill was held horizontally with the operculum down) to the top of the mud-sand layer. The drills were considered to have emerged when their siphons were exposed above the bottom. A period of three days was allowed because earlier experiments showed that if the drills did not emerge within this period, they would never emerge and would eventually die. The experiment was repeated, and the results were in close agreement.
Fig. 1. Percentage of *U. cinerea* of Long Island Sound, which were able to emerge within three days when buried at different temperatures at depths of 2, 4 and 6 cm in a mixture of mud and sand. Average of two experiments.
Fig. 2. Number of days needed at different temperatures to cause complete mortality of *U. cinerea* of Long Island Sound, buried at a depth of 6 cm in a mixture of mud and sand. Average of two experiments.
All drills buried beneath 2 cm of bottom material at temperatures of 25°C, 20°C and 15°C emerged (Fig. 1). At 10°C, however, only 85 per cent of the drills in the first experiment and 80 per cent of those in the second experiment reached the surface. At 5°C there was no emergence in either experiment.

Drills buried at a depth of 4 cm showed a considerably lower percentage of emergence than those at 2 cm. At 25°C only 10 per cent of the drills in the first experiment and 5 per cent in the second experiment were able to escape. At 20°C 5 per cent emerged in each experiment. Five per cent of the drills in the first experiment and none in the second experiment emerged when kept at 15°C. In the 10° and 5°C groups none of the drills emerged.

Of all the drills buried beneath 6 cm of bottom material and kept at different temperatures the only individual that emerged belonged to the group kept at a temperature of 25°C (Fig. 1).

The second series of experiments was conducted to determine the time needed to suffocate drills buried under a thick layer of bottom deposit. Groups of 100 drills were buried at a depth of 6 cm in large boxes measuring 32½" long, 16½" wide and 4½" deep. A mixture of mud and sand, as described above, was used. These boxes were placed in even larger boxes measuring 36" long, 20" wide and 8" deep through which a continuous flow of water of the desired temperature was allowed to run. The same temperatures as in the previous series of experiments, namely, 25°C, 20°C, 15°C and 10°C, were used. The temperature of the flowing water in the fifth box was not maintained strictly at 5°C, as in the previous series of experiments, but fluctuated between 0° and 5°C.

Samples of drills, consisting of ten individuals, were taken out and examined periodically; the time between the examinations was dependent upon the temperature at which the drills were kept. Complete mortality was determined in this manner: if ten drills constituting the sample were found dead, ten more were removed and examined a day or two later. If the latter were all dead, then the time when the first ten were found dead was taken as the time of complete mortality. After the second record of complete mortality was obtained the remainder of the population was removed and examined as an additional check.

The results of these experiments were as follows: at 25°C it took five days for all drills to die (Fig. 2). At 20°C all drills buried under 6 cm of bottom material died in seven days. Complete mortality of all the drills kept at 15°C was recorded in 12 days, and in the 10°C group 19 days were required. In the final group, kept in water the temperature of which varied between 0° and 5°C, approximately 52 days were needed to kill all the drills.

In these, as well as in the previous experiments already described (Loosanoff 1956), it was noted that if the drills did not
emerge, they were usually found at the termination of the experiment at the same depth as originally placed. This indicates that there was little or no movement after the drills had been placed in position. It also appeared that those U. cinerea, which started to ascend to the surface, were always successful in reaching their goal. In other words, they never stopped at intermediate depths.

The results of our experiments suggest a simple method which may be practical for controlling the common oyster drill in many areas. The method consists of burying drills under several centimeters of bottom material. Usually a layer 5 to 6 cm deep kills most drills. This depth of burial might be accomplished by using modified agricultural plows, oyster and clam dredges, etc., to turn over layers of bottom deposit. We have in mind several types that may be inexpensive and efficient. It may be necessary to remove from the bottom accumulations of shells heavy enough to hinder operations of the plow or other device used.

If the mechanical aspects can be properly developed, the method should help oyster growers in many sections of this country and abroad, where the bottom is soft enough for plowing and where drills or closely related species are abundant.

Construction and use of plows should be considerably cheaper than other devices such as suction dredges. It is also probable that their efficiency will be greater than that of other methods. For example, Carriker's 1957 observations indicate that as many as 49 percent of newly hatched drills may attach to plant fragments and be transported by tidal currents to new areas. Carriker thinks that this type of passive emigration accounts for greater dispersion of the drills than does phoresis. It may also explain sudden appearances of heavy concentrations of drills in certain areas where parent drills were virtually absent. Obviously, barriers built to protect oyster beds from drill invasions may be inadequate under such conditions, and either plows or suction dredges or both may be needed to combat drills.

In conclusion we want to emphasize that our studies have been conducted only with the common drill of Long Island Sound. Therefore, results obtained with drills in other geographical regions may be different.

We wish to express our thanks to E. F. Snoek, formerly of this laboratory, who participated in the early stages of this work.

References


THE VERTICAL DISTRIBUTION OF OYSTER LARVAE IN DELAWARE BAY

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Abstract

During the summers of 1954, 1955, 1956, and 1957 an extensive larva sampling program was conducted by this laboratory. The objective of the program was to gather as much information as possible about the distribution and movements, both vertical and horizontal, of oyster larvae in Delaware Bay. Since a thorough knowledge of vertical movements of larvae is essential to eventual understanding of horizontal movements, only the former aspect will be discussed here.

This report is based upon an analysis of larva counts made during the first three years. Surface and near bottom samples were counted from 636 stations distributed throughout the oyster-growing area of Delaware Bay. Samples were grouped according to tide current phases at times of collection, i.e., low slack, early flood, maximum flood, late flood, high slack, early ebb, maximum ebb, and late ebb.

Five stages of larval development -- straight hinge, early umbo, late umbo, mature, and eyed -- were recognized by size and physical characters; each was treated individually in the analysis.

Totals of each larval stage at each tide current phase were obtained. By plotting the percentages of these totals at surface and bottom, a vertical distribution pattern for each stage was derived. Mature and eyed larva exhibited an almost identical pattern whereas the three earlier stages showed no consistent pattern. The first three stages were then combined and termed "early stages", while the mature and eyed were combined as "late stages". The combined early stages showed almost uniform vertical distribution throughout the tide current cycle which is in agreement with the findings of most investigators.

The late stage larvae showed a marked tendency to congregate on or near the bottom during both slack water periods and throughout the ebb tide as well. The early flood and maximum flood periods revealed a nearly uniform vertical distribution, while the late flood again showed a trend toward the bottom. These results are closely in accord with M. R. Carriker's (1951) findings in Barnegat Bay, and further strengthen his conclusion that late stage larvae move toward the headwaters of an estuary by alternately riding the flood tide and remaining directly on the bottom throughout the ebb. The latter theory is further advanced by the fact that 143 flood tide stations averaged 20.8 late stage larva (surface plus bottom) per station whereas 122 ebb stations averaged 10.5 per station. Since we could not sample directly on the bottom, this 50% reduction in larva on the ebb is our best evidence that many larvae were directly on the bottom.
It appears obvious that oyster larvae could not perform any significant movements by their own means in any but weak current velocities, and that all horizontal and most vertical movements are the result of tidal currents and turbulence. The laboratory observation by T. C. Nelson and E. B. Perkins (1931), that eyed larvae are stimulated to active swimming by the introduction of higher saline water and, conversely, to inactivity by lower saline water, could explain the reaction of late stage larvae following each slack water period. However, the rate and minimal salinity change required for such stimulation is still unknown.

This study is being continued in an attempt to further establish these observations as well as to evaluate the factors of wind-driven currents, localized current variations, salinity sensitivity, and other influences on the distribution of oyster larvae.

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STUDIES ON THE COMPARATIVE UTILIZATION OF OXYGEN
BY LIVING AND DEAD OYSTERS

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Marine Laboratory
Grand Isle, Louisiana

Abstract

Studies were conducted using sealed mason jars and blackened paraffin-sealed battery jars. In each experiment one jar contained a live oyster, one a dead or dying oyster of the same weight and one jar contained only sea water. "Dead" oysters were killed by removing one valve and severing the heart. Disintegration of dead oysters did not require appreciably greater amounts of oxygen than that used by living oysters in respiration. Oysters survived for several days in water containing less than 1.0 ppm dissolved oxygen.

Introduction

Within the scope of responsibilities of the authors is the investigation of claims of damages by oystermen of Louisiana tidewater against various oil companies operating in the same area. In recent years there have been a number of such claims which allege that mass mortalities of oysters result from oxygen depletion caused by some operation of the industrial concern involved. These claims are all alike in that the mortalities are alleged to occur in two steps. First, there is a mechanical or chemical destruction of a few oysters on a bed. Second, these few, as they disintegrate, use oxygen from the surrounding water which results in suffocation of adjacent oysters. These, in turn, spread the area of oxygenless water until finally all oysters on the bed are dead. An example of this type of allegation was that of Mr. August Pitre (Petition, August R. Pitre vs. The Texas Company, May 3, 1954, number 32013, 24th Judicial District Court, Parish of Jefferson, State of Louisiana) which stated that "upon information and belief petitioner further avers that there was a sufficient burial (under a few drilling mud sacks) of the live oysters so as to initiate a successful oyster mortality, in that as the live oysters died it thereby caused an
added increase in oxygen demand, which resulted in a progressive mortality to the live oysters from one end of the lease to the other".

The authors know of no data which substantiate such claims, but there were no data which directly contradict the theory of progressive deoxygenation of a section of a bay beginning with a small nidus of dead oysters. In view of the persistency of the complaints and the prospect that it would some day be necessary to provide the court with something more substantial than opinion, it was decided to test the theory experimentally by determining whether or not the oxygen required by a dead oyster in disintegration exceeds materially that required by a live oyster for respiration.

Materials and Methods

Six experiments were conducted using mason jars or battery jars as aquaria. In each experiment groups of aquaria were set up as follows: a live oyster was placed in an aquarium containing sea water of known salinity, temperature, and oxygen content. In an adjacent aquarium an oyster, matching in weight, which had had one valve removed and the heart severed, was placed in sea water of the same content. A third aquarium contained sea water only. All three aquaria in each group were sealed, oxygen consumption was allowed to continue for a predetermined period of time after which a sample of water was siphoned from each aquarium and titrated by a modified Winkler technique for oxygen content. Each aquarium was sampled only once for oxygen content hence each figure in the tables represents the measurement of oxygen consumption of one live or dead oyster for a given period of time.

Experiments and Results

Experiment I. This experiment was set up as follows:

1. Fifteen sealed mason jars each containing 800 ml of sea water of 21.8 parts per thousand salinity taken from the running salt water system at the Texas A & M Research Foundation Marine Laboratory at Grand Isle, Louisiana were used as aquaria.

2. Water temperature was 24°C when the experiments began.

3. Five jars contained live oysters. Five jars contained oysters killed by removing one valve and destroying the heart. These oysters were designated as experimental "dead oysters", although ciliary activity continued for a considerable time. The five remaining jars contained only sea water and were designated as controls.
Table 1. Studies of utilization of oxygen by live and dead oysters. Experiment 1.

<table>
<thead>
<tr>
<th>Time</th>
<th>Set 1</th>
<th>Set 2</th>
<th>Set 3</th>
<th>Set 4</th>
<th>Set 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\frac{1}{3}) hr.</td>
<td>4.72</td>
<td>2.81</td>
<td>2.02</td>
<td>1.33</td>
<td>0.00</td>
</tr>
<tr>
<td>1(\frac{1}{3}) hr.</td>
<td>3.87</td>
<td>3.35</td>
<td>2.26</td>
<td>1.29</td>
<td>0.00</td>
</tr>
<tr>
<td>Controls</td>
<td>4.68</td>
<td>4.60</td>
<td>5.40</td>
<td>5.77</td>
<td>5.89</td>
</tr>
</tbody>
</table>

Discussion: The data from this experiment is contained in Table 1. Reduction of oxygen in the live- and dead-oyster jars in this experiment was approximately the same, and oxygen was completely exhausted in both "live" and "dead" jars within 22 hours. In Set number 5 the dead oysters, which had been killed 22\(\frac{1}{2}\) hours previously, had a strong odor. The oxygen in the control rose during the experiment, apparently as the result of photosynthetic activity of phytoplankton.

Experiment 2. This experiment was like Experiment 1 with the following exceptions:

1. The dead oysters were killed and held out of water for 18 hours before the experiment was begun.

2. Water temperature was \(26.0^\circ\)C and salinity 21.3 ppt.

3. Different periods were used.

Discussion: The data from this experiment are presented in Table 2. The dead oysters utilized oxygen at a slightly faster rate than the live oysters; the dead oyster in Set 11 exhausted the oxygen in its aquarium while 0.24 ppm remained in the live oyster jar. All dead oysters had a strong odor at the end of the experiment and were too "soupy" to retain their shapes.
Table 2. Studies of utilization of oxygen by live and dead oysters.

<table>
<thead>
<tr>
<th>Time</th>
<th>O$_2$ content in ppm after lapse of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Set 6</td>
</tr>
<tr>
<td>1 hr.</td>
<td>2.58</td>
</tr>
<tr>
<td>2 hr.</td>
<td>2.38</td>
</tr>
<tr>
<td>Controls</td>
<td>4.04</td>
</tr>
</tbody>
</table>

Experiment 3. This experiment was like Experiment 1 except that:

1. Longer time intervals were used.

2. Water temperature was 23.0°C and salinity was 24.46 ppt.

Discussion: There was no essential difference in the utilization of oxygen in this series of experiments (Table 3). Oxygen was exhausted in jars containing dead and live oysters between 8 and 24 hours after the experiment started.

Table 3. Studies of utilization of oxygen by live and dead oysters.

<table>
<thead>
<tr>
<th>Time</th>
<th>O$_2$ content in ppm after lapse of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Set 12</td>
</tr>
<tr>
<td>2 hr.</td>
<td>2.18</td>
</tr>
<tr>
<td>4 hr.</td>
<td>2.98</td>
</tr>
<tr>
<td>6 hr.</td>
<td>Controls</td>
</tr>
</tbody>
</table>

Experiment 4. The following changes were made in Experiment 4:

1. Battery jars painted black were substituted for mason jars. This was to prevent photosynthesis which apparently had occurred in the earlier studies (Fig. 1).

2. 3100 ml of sea water were used in each jar.
3. For siphoning samples, a pair of glass tubes were placed in each jar, and melted paraffin was poured on the surface of the water around the tubes, sealing the jar.

4. The oysters in these experiments were smaller, averaging 57 gms.

5. Water temperature was 22.0°C, salinity was 29.8 ppt, and oxygen in all aquaria was 3.71 ppm.

6. Water samples were siphoned for determination of oxygen content after 6, 7, 8, 9, 10, 11 hours.

Discussion: In this series, with more water and smaller oysters, oxygen was not exhausted in any aquaria containing live or dead oysters (Table 4). Oxygen was reduced at about the same rate in both sets of aquaria. As considerable quantities of oxygen remained in all jars after 11 hours, it was decided to repeat the experiments but to wait longer before sampling.

Table 4. Studies of utilization of oxygen by live and dead oysters. Experiment 4.

<table>
<thead>
<tr>
<th>Time</th>
<th>0₂ content in ppm after lapse of</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero hr.</td>
<td>Set 18 hr.</td>
<td>Set 19 hr.</td>
<td>Set 20 hr.</td>
<td>Set 21 hr.</td>
<td>Set 22 hr.</td>
<td>Set 23 hr.</td>
</tr>
<tr>
<td>Live oysters</td>
<td>3.71</td>
<td>2.02</td>
<td>1.61</td>
<td>2.10</td>
<td>2.14</td>
<td>1.17</td>
<td>1.93</td>
</tr>
<tr>
<td>Dead oysters</td>
<td>3.71</td>
<td>2.98</td>
<td>2.90</td>
<td>2.94</td>
<td>2.42</td>
<td>2.10</td>
<td>1.77</td>
</tr>
<tr>
<td>Controls</td>
<td>3.71</td>
<td>3.51</td>
<td>3.51</td>
<td>3.83</td>
<td>3.80</td>
<td>3.39</td>
<td>3.55</td>
</tr>
</tbody>
</table>

Experiment 5. This experiment was like Experiment 4, with the following exceptions:

1. The time intervals for sampling oxygen consumption were from 15½ to 20½ hours (see Table 5).

2. The oysters used in this series averaged 61 gms.

3. At the beginning of the studies the water temperature was 23.5°C, the salinity was 30.65 ppt, and the oxygen measured 5.00 ppm.

Discussion: After 15½ hours the dead oysters had reduced the oxygen to a point below 1.0 ppm while the oxygen in live oyster aquaria remained above 2.0 ppm (Table 5). By seventeen hours the live oysters reduced the oxygen to less than 1 ppm. In set 28 and set 29, the oxygen was above 2.0 ppm after 19½ and 20½ hours. It is uncertain that
Fig. 1. Battery jars painted black and sealed with paraffin.
Table 5. Studies of utilization of oxygen by live and dead oysters. Experiment 5.

<table>
<thead>
<tr>
<th>Time</th>
<th>O&lt;sub&gt;2&lt;/sub&gt; content in ppm after lapse of</th>
<th>0.2 hr</th>
<th>0.4 hr</th>
<th>0.6 hr</th>
<th>0.8 hr</th>
<th>1.0 hr</th>
<th>1.2 hr</th>
<th>1.4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live oysters</td>
<td>5.00</td>
<td>2.02</td>
<td>2.26</td>
<td>0.64</td>
<td>0.60</td>
<td>2.18</td>
<td>2.24</td>
<td></td>
</tr>
<tr>
<td>Dead oysters</td>
<td>5.00</td>
<td>0.32</td>
<td>1.17</td>
<td>0.36</td>
<td>0.85</td>
<td>0.00</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5.00</td>
<td>4.60</td>
<td>4.44</td>
<td>4.96</td>
<td>4.28</td>
<td>4.35</td>
<td>4.60</td>
<td></td>
</tr>
</tbody>
</table>

the latter is the case, since rises occurred at approximately the same time in the dead oyster aquaria and in the control aquaria. Because the living oysters had failed to utilize all the oxygen within the time limits of these experiments and because the oxygen appeared to be rising in the dead oyster and control aquaria, it was decided to continue the experiments, but to sample at longer intervals. All dead oysters in this series had a strong odor and were beginning to lose their shape and become "soupy".

Experiment 5. These studies duplicated the previous series with the following exceptions:

1. The oysters used ranged from 12 to 30 grams in weight, averaging 21.6 grams.

2. Samples were taken 24, 48, 72, 144, 168 and 192 hours after the experiments were started.

3. At the beginning of the studies the water temperature was 17.0°C, the salinity was 25.0 ppt, and the oxygen measured 7.32 ppm.

Discussion: During the experimental period the dead oysters reduced the oxygen more quickly than did the live oysters (Table 6). Within 72 hours, however, the live oysters had reduced the oxygen to less than 1 ppm. It is of interest that there was a decided loss of oxygen in the control vessel, due, it is presumed, to respiration and bacterial decomposition of the plankton. The live oysters in this series were all alive at the end of each period; the oyster in Set 35 presumably survived at least 120 hours in water containing less than 1.0 ppm of oxygen. The dead oysters had a strong odor and were almost completely disintegrated at the end of the series.
Fig. 2. Utilization of oxygen by live and dead oysters.
Table 6. Studies of utilization of oxygen by live and dead oysters.

<table>
<thead>
<tr>
<th>Time</th>
<th>O₂ content in ppm after lapse of</th>
<th>Experiment 6.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero hr.</td>
<td>Set 30 hr.</td>
</tr>
<tr>
<td>Live oysters</td>
<td>7.32</td>
<td>4.15</td>
</tr>
<tr>
<td>Dead oysters</td>
<td>7.32</td>
<td>4.19</td>
</tr>
<tr>
<td>Controls</td>
<td>7.32</td>
<td>7.24</td>
</tr>
</tbody>
</table>

Summary

The principal difference between the living and dead oysters in these experiments is that use of oxygen by living oysters is affected by opening and closure of the shell, while dead oysters utilize oxygen at a rate determined by the type and load of bacteria present at the beginning of the studies. Because of reactions to the stimuli of handling and moving when the experiments were set up, oysters remained closed for considerable lengths of time and apparently failed to remove any oxygen from the water. Mitchell (1914), working on oxygen requirements of shellfish, found that "a light tap on the table or water bath, a heavy step in the room, the slamming of the door in a neighboring part of the building was surely registered by some movement of the shell."

A graph of the first three series of experiments (Fig. 2) shows that, in general, the oxygen utilization of the live oysters and the dead oysters follows the same trend. The control jars in each of these series showed that a factor other than utilization by oysters was influencing the oxygen content. It was assumed that this factor was photosynthetic activity of phytoplankton. For this reason the remaining experiments were carried out in battery jars painted black and sealed with paraffin.

Experiments 4 and 5 are plotted in Figure 3. Experiment 4 indicates a more rapid utilization of oxygen by the living oyster for the first ten to eleven hours. The increase in oxygen in all battery jars at the eighth to ninth hour may be due to light penetrating the paraffin seal. The increase in oxygen occurred at 1:00 p.m., a time of maximum light in the laboratory.

Oxygen in the battery jars was not exhausted by live or dead oysters within the eleven-hour duration of Experiment 4. Subsequently, another series of experiments was set up in which the first samples were taken after 15 1/2 hours. The results of these experiments, plotted on Figure 3, appear to fit rather well on the end of the previous
Fig. 3. & 4. Utilization of oxygen by live and dead oysters, groups 4 to 6.
curves. The peaks in these curves appear at 10:30 a.m. and 1:30 p.m., again periods of brightness in the laboratory. These experiments indicate that dead oysters continued to utilize oxygen in decaying, but live oysters ceased to use oxygen before it was completely exhausted.

To determine if this were true, a sixth experiment was conducted, with the first samples taken after 48 hours. The results of these experiments are shown in Figure 4. Presumably, the oyster in the sample taken at 48 hours had remained closed for a considerable time and had not used oxygen at the same rate as the live oyster in the 24-hour sample. The curves indicate that the live oyster reduced the oxygen to less than 0.5 ppm, then no longer used oxygen. From 144 hours to 192 hours apparently no oxygen was used by the living oysters. It can be seen from the graph that the live oysters reduced the oxygen in their containers to considerably less than 1.0 ppm within three days, then survived five additional days at the low oxygen level. Oxygen was exhausted in the jars containing dead oysters sometime between 72 and 144 hours, probably shortly after 72 hours.

It is presumed that the oxygen depletion in the control of Experiment 6 is due to bacterial decomposition of plankton.

Conclusions

It has been demonstrated that dead oysters in disintegration do not use appreciably greater amounts of oxygen than living oysters of the same size use in respiration. It has also been shown that oysters are able to survive for several days in water containing less than 1.0 ppm dissolved oxygen.

Literature Cited

Abalone are widely distributed throughout the world in tropical and temperate waters, and are much sought after for their shells and as food. There are fisheries for these mollusks in California, Japan, Mexico, South Africa, and on a smaller scale, in the Channel Islands off England.

History of the California Fishery

Until the Chinese came to California during the gold rush days, local Indians were the only ones to utilize abalone to any extent; these people had been using the meats for food and the shells for ornaments, fish hooks, and money, since pre-Columbian times. Descendants of the Spaniards and immigrants from the United States did not use abalone, presumably because of the plentiful supply of other foods. Chinese, however, were happy to find this mollusk which they knew in their homeland as a great delicacy. They at first gathered abalone only for their own consumption but in 1864 they began to dry the meat for shipment to the Orient. Collections were made only from rocks that could be reached at low tide, and soon this rather limited area was over-exploited. Various seaboard counties soon began imposing legal restrictions (See Laws and Regulations).

At first San Diego was the center of operations and when nearby rocky shores became unprofitable, camps were established in Lower California and on several of the Channel Islands off southern California.

About 1900, after gathering of abalone on shore was no longer profitable, the Japanese came into the industry, introduced diving suits, and the fishery moved into deep water. Japanese divers worked out of Monterey from Pacific Grove south to San Simeon. Nearly all operations were carried on between Pt. Lobos in Monterey County and Picos Creek in San Luis Obispo County, the abalone being landed at Monterey. In 1929 the area from Picos Creek to Pt. Buchon was opened and a number of Caucasian divers started operating out of Morro Bay. The center of the industry gradually shifted to this more southern port and to this more southern fishing area, which extends roughly from Cayucos (Morro Bay) to a few miles above San Simeon, a distance of approximately 30 miles.

This area has been in continuous production since 1929 and is the source of most red abalone, (*Haliotis rufescens*) landed in
Fig. 1. Location of abalone fisheries along California coast.
California today. Red abalone produced in this area are of the highest quality and bring a higher price than the pinks (*H. corrugata*), which come from southern California.

During the first years of World War II, few abalone were landed. The Japanese were forbidden to dive and other divers were aiding the war effort by working in southern California gathering the marine alga, Gelidium, from which agar-agar is manufactured. Prior to the war 90% of our agar had been imported from Japan, and it was necessary to develop a local supply of this product which is used extensively in bacteriological work.

In 1943, the north coastal area from Mendocino County to Pigeon Point, San Mateo County, was opened to commercial diving. It is not generally known but most of the north coast was open to commercial diving from 1909 to 1937 and again from 1943 through 1945 (See Laws and Regulations). Among difficulties encountered by the few commercial divers attempting to operate along the North Coast, not the least was the attitude of some local residents. In the 1920's a group of citizens loaded an old brass cannon that was on the bluff overlooking Mendocino Bay and fired at the abalone boats that were at anchor. The boats left and did not return. Again, during World War II, abalone boats attempting to work along the Marin County coast were fired on. This opposition, combined with the characteristic rough weather and rugged bottom of the northern California coast, has tended to restrict the numbers of divers attempting to work in this area. As a result, only a very small amount of abalone was taken from the area during the periods when commercial abalone fishing was permitted in Northern California.

Due to wartime demand for additional protein foods, the mainland from Santa Barbara south to the Mexican Border and the Channel Islands were opened to commercial diving in 1943. In 1955 the mainland from Santa Barbara to San Diego was closed again and at the present time diving in this area is carried on almost exclusively at Santa Cruz, Santa Rosa, San Miguel, and Santa Barbara Island. Most abalone are pinks, a few reds are brought in, but greens (*H. fulgens*), which at one time were an important constituent of the catch, are no longer wanted by markets and consequently are seldom taken by divers.

Among the fisheries of California, the commercial abalone ranks 12th to 15th in importance both as to total poundsage landed and to monetary value. It is a luxury item, however, and as such invites attention and efforts to increase production. Although it is a unique fishery in the United States, abalone are found throughout the world and are taken for commercial purposes in several countries, but only in California are abalone taken by both commercial and sport fishermen.

The commercial fishery has had a varied and stormy history and even today all is not serene along the Pacific's shores. The areas open to commercial fishing are changed from time to time and
today abalone are taken from only two general areas. The red abalone, which at the present time is providing the major portion of the catch (2.35 million pounds in 1956), is taken almost entirely in Central California. The pink abalone, which produces the balance of the catch (1.88 million pounds in 1956), is taken in southern California exclusively from the Channel Islands. No commercial abalone fishing is permitted north of San Francisco. Although there has been at various times considerable pressure by commercial interests to open this region, recent findings of the Department of Fish and Game's abalone investigation indicate that the area would not support an abalone industry and that commercial abalone fishing is impractical.

Abalone are taken by divers who operate under a revocable permit issued by the Department of Fish and Game. Equipment must meet specifications and size limits must be observed. Present rules require that the diver must have a surface air pump operated from the boat, 100 feet of air hose, two baskets, and a measuring device. Divers must operate only in certain areas and must keep 150 feet offshore in water not less than 20 feet deep. Present minimum size limits for abalone are as follows: for commercial operations -- red 8", green 7-1/4", black 5", all other species 6"; for sport fishing -- red 7", green 6-1/4", pink 6", and black 5".

The commercial size limits, originally arrived at by a fortunate combination of economics and compromise, have proved adequate to protect the supply of abalone. If the market had been based on canned or dried abalone, it is debatable whether there would be a fishery in California today. The present fresh frozen market is built upon large abalone; labor costs automatically prohibit the utilization of abalone 6" in diameter.

There are no bag limits other than those imposed by the processors. At the present time there are approximately 60 boats operating in the industry. Each boat carries a diver, a line-tender and a boat operator. There is a $40 boat fee and each member of the crew and the diver pay a $10 commercial license fee. There are 15 processing plants which employ from 5 to 30 workers who prepare the abalone for market. Almost the entire catch is packaged quick frozen in 5 lb. containers; the rest is sold fresh in local markets and restaurants. At one time some of the product was canned and some was dried but this never amounted to much and is now prohibited by law.

Divers are paid according to the amount of saleable meat that can be recovered from the abalone. Red abalone is the most desirable since it grows the largest and the meat is of better quality. Consequently it brings a higher price. At present market prices, red abalone bring from $7 to $9 per dozen to the diver. Pink abalone, on the other hand, are generally smaller and the meat is not of such high quality. As a result present prices are between $4 and $6 per dozen. In the fresh market abalone steaks sell from $1.95 per lb. for
large red abalone to 75¢ per lb. for the better grade pink abalone. Divers can make from $5,000 to $30,000 per year depending on weather and ability in that order.

Abalone may not be shipped from California in either the fresh or frozen state. It is felt that present stocks are sufficient for local market demands and that any large additional increase in production would tend to deplete severely the available supply. It is reported, however, that California abalone may be found on menus of restaurants in several large eastern cities.

Transplanting

Transplanting of abalone in California had not been attempted prior to the Department's first experiment in 1956. At that time over 800 red abalone were successfully transported over a distance of several hundred miles to a new area where there were none. These abalone were carried in bait tanks (approximately 4' x 5' x 6') aboard the research vessel YELLOWFIN. Because of abnormal spacing of the holes in the shells, injuries to soft parts and a general weakened condition, only 660 of the original 800 were tagged and replaced by divers on the bottom. A later check revealed that only 19 of the 660 had failed to survive transplanting. The remainder have apparently adapted themselves to the new environment.

Since this first large scale transplant, other experiments have been conducted by the Department in an attempt to improve the methods and techniques. It has been determined that abalone will survive transport but the length of time they can remain out of water depends primarily on the species. Survival and general condition are improved if during transport abalone are not confined to a bait box with circulating sea water, but placed in nets or sacks, kept out of the direct sunlight, and a constant flow of water maintained over them. If kept submerged in freely circulating sea water red abalone may be stored in live boxes in which they will live without food for at least 70 days. The pink abalone of southern California require more careful handling than the reds; they cannot remain out of water for such long periods of time and must be kept constantly covered by streams of sea water and out of the sun while in transit.

Further transplanting experiments are planned and it is hoped that it will be possible (1) to establish abalone in favorable areas in which they do not occur, (2) to establish abalone where they are in such limited concentration that the addition of new stocks might help build up the population, and (3) to use abalone as biological indicators, since they appear to be sensitive to certain chemical changes in the water. This fact has possibilities as an aid in pollution studies.

It should be kept in mind that transplanting is far from an established procedure. From the little we have done, however, we
know that, if certain precautions are observed, it is possible to transplant abalone from one area to another. An adequate supply of food, i.e., the seaweeds, Nereocystus or Macrocystus, and an area more or less exposed to the open sea are required.

The Japanese have done a limited amount of experimental transplanting. The abalones transplanted from northern Japan transformed themselves into what was formerly thought to be a distinct species confined to southern Japan.

Movement and Migration

Abalone inhabit rocky shores in certain areas along the coast. They are found from high tide mark out to depths of over 300 feet, with the maximum concentration between 25 and 40 feet.

Tagging experiments have shown that there is no migration and very little movement among abalone. Those tagged and released in shallow water (5 to 8 feet deep and 10 feet offshore) were later collected by shore fishermen within a few feet of original sites. Those released in deeper water 20 to 25 feet offshore never appeared on shore; when checked by divers a year later these abalone were still in the same general area. Abalone tagged and released in still deeper water 40 to 50 feet deep and up to 1/4 mile offshore are still in the area of release after two years. This would indicate that although individual abalone may move around considerably they never move any great distance. In the tidal zone they have been observed to move over 100 yards parallel to the shore, but none has ever been observed to move from shallow to deep or deep to shallow water.

Life History

There has been no attempt to cultivate abalone artificially in California. All the abalone taken have been the result of natural propagation. Until recently too little was known of the early life history to attempt culture experiments. It is now known that spawning takes place in spring and summer; it was formerly thought that spawning took place during winter. There is a free-floating stage which probably lasts from eight to ten days. The small abalone then sinks and becomes attached to the bottom and starts to grow into an adult.

Although we now have a better knowledge of the early life history, other considerations enter into attempts to cultivate abalone. One of the principal difficulties is in collecting spat. The fishing grounds face the open ocean and weather conditions being what they are along the Pacific Coast, it is a matter of conjecture whether collecting equipment could be set and maintained during the spawning season. Spat collecting nets, prepared according to methods suggested by Cedric Lindsay, Supervisor Shellfisheries Investigation, Washington
Department of Fisheries, were exposed but abalone had not matured at the expected time. At this time in previous years in this area the majority of abalone were in spawning condition.

It is planned to continue to attempt to collect abalone spat; experiments will have to be conducted to determine the best methods.

Growth rate is not accurately known but it is strongly suspected that young abalone grow to about one inch in diameter the first year, and when they reach 4 to 5 inches in diameter they are probably 3 to 4 years old. From then on the growth rate varies. In some areas growth is fairly uniform with the majority reaching a size of 8 inches or more. Most of the growth takes place during winter months with some abalone adding as much as 1-1/2 inches in shell diameter during this period. Some abalone will reach a certain size and grow no more; others apparently keep on growing. Abalone up to 11 inches in diameter are known. In some locations most abalone are under 7-1/2 inches, while in other areas, often times close by, specimens of the same species are almost all 8 to 9 inches in diameter. The causes of this difference in growth are not known. Previously it was thought that growth was constant and that the size of an abalone was directly proportional to its age. It is only recently that the Department's investigation has disclosed that growth rate is not constant but varies from area to area for the same species, also from species to species in the same location. In areas where abalone are being harvested by divers growth rate is more rapid than in those areas where they are left undisturbed. Whether harvesting surplus animals is the deciding factor, or whether growing conditions are superior in these areas, are moot questions.
THE MARYLAND SOFT SHELL CLAM INDUSTRY: ITS POTENTIALS AND PROBLEMS

J. H. Manning and H. T. Pfitzenmeyer

Maryland Department of Research and Education, Solomons, Maryland

Abstract

Four of Maryland's tidewater counties are now producing about half the total U. S. catch of soft shell clams. Maryland's production in 1957 is estimated at about 250,000 bushels, worth $1,000,000 to the dredgers. Some of the problems confronting the industry are briefly discussed.

A preliminary report on the Maryland soft shell clam fishery was presented at the Baltimore meeting of the National Shellfisheries Association in 1955. At that time 60 hydraulic clam dredges were licensed in the State, and annual production of soft shell clams was estimated at 110,000 - 120,000 bushels, worth about $500,000 to the dredgers. Figure 1 shows the growth of the fishery since the beginning of dredging on a commercial scale--from seven dredges in 1952 to 132 in July 1957. The accelerated rates of expansion between 1954 and 1955 and between 1956 and 1957 are attributable, at least in part, to the enactment of legislation recognizing and regulating the industry.

Value of the fishery to primary producers during the past three years is indicated in Figure 2. These estimates, which are considerably in excess of estimates based solely on collection of the production tax, are believed to be conservative. The soft shell clam resource should be worth about $1,000,000 in gross income to dredgers during the current year.

Operation of the clam dredge fishery is limited by law to waters of four counties, and within three of these counties it is further limited by local restrictions. Effective operation of the gear is limited to depths not exceeding about ten feet by a legal restriction on the length of the conveyor system (19 feet, axle to axle). As shown in Figure 3, less than 25 per cent of the "barren bottom" within the ecological range of the soft shell clam and the operational range of the hydraulic clam dredge is open to dredging. If the clam resource were distributed proportionally throughout Maryland tidewater and a ready market existed for increased production, full territorial expansion of the fishery should result in a four-fold increase in dockside value--to about $4,000,000 annually. Whether the full potential of the Maryland clam fishery will be reached is a question only time can answer. The clam fishery must co-exist with other commercial fisheries of long-established value
Fig. 1. Growth of the soft shell clam fishery in Maryland, 1952-1957.

Fig. 2. Estimated value of Maryland's soft shell clam catch to primary producers.

within an estuary rapidly growing in importance as a residential and recreational center. Many of the problems confronting the clam industry involve economic and social rather than biological relationships.

In general, Maryland production of clams is controlled by demand. The dockside price has remained steady at about $4 a bushel for more than two years. Any development that depressed the price would be most unfortunate for the fishery, since costs of operating a clam dredge are high. Figure 4 indicates some reason for optimism, concerning capacity of the market to absorb increased production. The New England catch has declined from more than 600,000 bushels in 1950 to less than 250,000 bushels in 1956. The Maryland catch has taken up some of the slack in recent years, but Atlantic Coast production is still far short of the 1950 level. Currently, Maryland's catch accounts for about one half of the total.

Maryland is not getting the ultimate value per unit catch from the clam resource. A very high percentage of the catch is shipped out of the State, and the price differential on the New England market favors New England clams by about 75 per cent. This is not justified by the difference in shucking yield, which is not more than about 25 per cent, often less. Maryland clams, taken from brackish waters, may be inferior to Maine clams as steamers, but for other purposes they need no apology. It seems likely that at least part of the price differential can be erased by quality control. Shell stock which is exported must be in first-class condition when shipped, and substantial, sanitary containers are needed to replace the fruit baskets now used. A most encouraging recent development in the industry is the trend to local processing. Before 1956 practically the entire catch was shipped as shell stock. Twelve shucking houses are now operating in Maryland, with resultant gains to tidewater economy and simplification of transportation and sanitation problems.

Creation of local demand is a virtually unexplored approach to market development. A few dealers have taken steps in this direction, but there has been no concerted effort by the industry. Fried clams, when placed on the menus of local restaurants, have received enthusiastic acceptance.

Perhaps the most serious internal problem of Maryland's clam industry is the occurrence, from early autumn to early spring, of red coloration in clams. Pink yeast has been isolated from a few shipments of Maryland clams, but there is no clear evidence that contamination originated in Maryland or that pink yeast is the sole or primary cause of the pigmentation. At least one Federal and two State agencies are preparing for a concerted attack on this problem in the coming months.
Fig. 3. Proportion of actual and potential clam-producing bottoms in Maryland.

Fig. 4. Production of clams in New England (1950-56) and Maryland (1955-56).
We do not feel that any of the problems of Maryland's clam industry, internal or external, are unsolvable. The industry is now recognized as a major factor in Maryland's tidewater economy, and as such deserves full support and attention from State and Federal agencies concerned with the harvesting, processing, and marketing of seafood resources. If the industry will make known its needs to these agencies and displays a willingness to cooperate, it will find them more than willing to help. With adequate organization, representation, and management, the Maryland clam industry can look forward to a promising future.
FILTERING EFfICIENCY OF HARD CLAMS IN MIXED SUSPENSIONS OF RADIOACTIVE PHYTOPLANKTON

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Abstract

Simultaneous removal of two species of phytoplankton by Mercenaria (= Venus) mercenaria L. was measured by labeling one species with P32 and the other with Ca45. Since both species are removed from the same volume of water pumped, a faster removal of one indicates a greater efficiency of the clam in filtering that species. A species of Carteria was always one of two species in suspension, since it alone of 15 species tested had sufficient uptake and retention of Ca45 for use in these experiments. In general, algal cells 4μ or larger in diameter were filtered with uniform efficiency. Cells 2 to 3μ usually were not filtered as efficiently as larger cells in the same suspension, even though clams secreted much mucus, probably in an effort to retain these smaller cells. A dinoflagellate, Gymnodinium sp., caused most clams to close soon after filtering activities began. When clams remained open, they efficiently filtered Gymnodinium from suspension, but ejected chains of compact cells, which were 94% Gymnodinium, through their incumbent siphons. The population density, varying from 2 to 20 million cells per liter in most suspensions, did not affect the efficiency with which the species were removed.

Although extensive studies of the feeding behavior of filter-feeding lamellibranchs have been made, there remain some basic problems relative to filtering efficiency and factors affecting it. Variations in filtering rates of the hard clam, Mercenaria (= Venus) mercenaria L., result from a change in efficiency of particle removal or in volume of water pumped. In recent investigations Rice and Smith (in press) found that filtering rates of hard clams varied with different species of phytoplankton. Since these filtering rates were based on radiological measurements of P32 incorporated into the cells of only one species, it was not possible to determine whether these variations involved filtering efficiency of gills or volume of water pumped. A comparison of the simultaneous removal of two species from the same suspension would eliminate changes in rate of water propulsion as a
factor. With both species in the same water, a faster removal of one would indicate greater efficiency in filtering that species. Removal of two species of algae from the same suspension can readily be followed when each species is labeled with a different radioisotope. The purpose of this investigation was to study the filtering efficiency of hard clams for different species of phytoplankton.

The radioisotopes employed for labeling algae were P$^{32}$ and Ca$^{45}$. Their radioactive characteristics differ sufficiently to allow separate determination of each isotope when both are present in a mixture. Calcium, like phosphorus, is essential in metabolism of algae. Only a small per cent of the calcium which occurs naturally in sea water is necessary for algal growth. Since the uptake of Ca$^{45}$ is the same as that for non-active calcium, the dilution which occurs when this radioisotope is added to natural sea water prevents much Ca$^{45}$ from being taken up by the algae. To increase uptake of Ca$^{45}$, artificial sea water was prepared without the addition of calcium (Rice 1956). Fifteen species of algae were tested for rate of cell division and uptake of Ca$^{45}$ when grown in this artificial sea water containing Ca$^{45}$. In all experiments Carteria sp. was labeled with Ca$^{45}$, since it was the only species that had sufficient uptake and retention for use in these experiments. In suspension with Carteria, other species were labeled with P$^{32}$ following the methods of Rice (1953) for obtaining highly radioactive cells.

The phytoplankton used may be grouped according to their length as follows:

<table>
<thead>
<tr>
<th>Algae</th>
<th>Classification</th>
<th>Size (μm)</th>
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<tbody>
<tr>
<td>Chromulina pleiades</td>
<td>Chrysophyceae</td>
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</tr>
<tr>
<td>Nanochloris atomus</td>
<td>Chlorophyceae</td>
<td>3</td>
</tr>
<tr>
<td>Chlamydomonas sp.</td>
<td>Chlorophyceae</td>
<td>8</td>
</tr>
<tr>
<td>Gymnodinium sp.</td>
<td>Dinophyceae</td>
<td>9x12</td>
</tr>
<tr>
<td>Platymonas sp.</td>
<td>Chlorophyceae</td>
<td>9x14</td>
</tr>
<tr>
<td>Carteria sp.</td>
<td>Chlorophyceae</td>
<td>10x14</td>
</tr>
<tr>
<td>Amphydinium klebsi</td>
<td>Dinophyceae</td>
<td>12x17</td>
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<td>Nitzschia sp.</td>
<td>Bacillariophyceae</td>
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<td>Nitzschia closterium</td>
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</tbody>
</table>

The twelve clams used in this study were collected in the vicinity of the Fishery Radiobiological Laboratory, Beaufort, North
Table 1. Comparison of rates of filtration by the clam, *Mercenaria mercenaria*, of different species of algae with that of *Carteria* sp. when both species were present in the same suspension. (Half hour observations during an experimental time of 3 hours.)

<table>
<thead>
<tr>
<th>Species mixed with <em>Carteria</em> sp.</th>
<th>Total</th>
<th>Both species removed at same rate&lt;sup&gt;1&lt;/sup&gt;</th>
<th><em>Carteria</em> sp. removed faster</th>
<th><em>Carteria</em> sp. removed slower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitzschia closterium</td>
<td>37</td>
<td>32</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Nitzschia sp.</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amphidinium klebsi</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chlamydomonas sp.</td>
<td>13</td>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Platymonas sp.</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nannochloris atomus</td>
<td>17</td>
<td>6</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Chromulina pleiades</td>
<td>17</td>
<td>8</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Gymnodinium sp.</td>
<td>13</td>
<td>9</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>1</sup>Variance of less than 10 per cent between species
Fig. 1. Similarity of filtering efficiency of clams for two species of planktonic algae present in the same suspension in two tests in which the rate of filtration was different.
Carolina and were kept in running sea water when not being used in experiments. They ranged from 80 to 88 mm in length and from 66 to 77 mm in height. Each of three clams was placed in ten liters of filtered sea water to which had been added measured numbers of Carteria cells containing Ca\textsuperscript{45} and cells of a second species of algae labeled with P\textsuperscript{32}. The suspensions were adequately stirred to prevent any settling of cells. In control jars containing no clams, the numbers of cells in suspension at the beginning and the end of experiments were the same. When clams were placed in suspensions of some algal species, considerable numbers of cells quickly adhered to shell surfaces. Further tests showed that coating the shells with thin layers of paraffin prevented significant losses of cells from suspension.

Samples were taken of the suspension before adding a clam and at 30-minute intervals for 3 hours after it had opened. After filtration of cells from samples with millipore filters, measurements of their radioactivity due to Ca\textsuperscript{45} and P\textsuperscript{32} were made. Rates of removal of both algal species in suspension were calculated from these measurements.

It is believed that the decrease in cell numbers in suspension represents filtration by the clam, although it is not definitely known whether filtered cells which may be rejected are sufficiently free of mucus to go back into suspension. There is reason to believe, however, that rejected cells are bound in mucus and do not break up and re-enter the suspension.

With the exception of tests using Nannochloris atomus, Chromulina pleiades and Gymnodinium sp., the decrease in cell numbers of any species during any half hour of a 3-hour observation period never varied over 10 per cent, and usually not over 5 per cent, from that of Carteria. In only 6 out of 80 experiments was the rate of removal of one species different from that of the other (Table 1). Even in these instances the difference in filtering efficiency was only significant during the first hour of filtering activity.

Whether cells of these algal species were completely filtered from the water pumped was not determined. When algae of different sizes such as Nitzschia and Carteria were filtered with the same efficiency, it is believed that all cells were removed from water passing through the gills. It is improbable that algae of such unlike size and shape could escape the gills at the same rate. If the species in suspension are similar in size, a uniform percentage of escapement is possible.

The rate of filtration apparently did not affect the efficiency with which clams removed either species. Figure 1 shows results of two representative experiments. During the observational time the clam in Test 1 removed cells of Nitzschia sp. and Carteria sp. as efficiently as the clam in Test 2, yet filtered them at a much slower rate, due to less water being pumped through its gills. Figure 4
Fig. 2. Differences in filtering efficiency of clams for two species of planktonic algae in the same suspension.
shows that all three clams removed both algal species with the same relative efficiency during the 3 hour period, yet filtered the cells at a faster rate during the first 1 1/2 hours. When both species were removed at a faster rate during one time interval than another, this indicated a change in volume of water pumped, since the filtering efficiency for each species had not changed.

In 20 of 34 experiments when Chromulina pleiades or Nannochloris atomus were in suspensions with Carteria, the clams removed Carteria at a faster rate than either of the other species (Table 1). Figure 2 shows the results of one of these experiments in which many small cells escaped the gills. A more abundant formation of mucus was observed in tests containing N. atomus and C. pleiades, but this was ineffectual in preventing considerable escapement of cells.

In 14 of 34 experiments the percentage decrease in cell numbers of N. atomus and C. pleiades during each hour did not differ more than 10 per cent from that of Carteria cells. Rice and Smith (in press) have shown that filtering rates of hard clams in certain unialgal suspensions were altered considerably when other algae were added. A more efficient retention of N. atomus was observed when Nitzschia closterium was present; this may have resulted from a clogging of gills by the large cells of Nitzschia. In other experiments yet unpublished, the percentage escapement of N. atomus through the gills of oysters averaged 88 per cent; this was reduced to 51 per cent by addition of Carteria to the suspension. It seems possible, therefore, that the presence of relatively large Carteria cells increased the efficiency of retention of small cells of N. atomus and C. pleiades by the hard clam.

In 9 of 13 experiments Gymnodinium and Carteria were removed with similar efficiency (Curve B in Figure 3). In the other 4 tests the presence of Gymnodinium resulted in variations in rates of removal of Carteria. Curve A shows these fluctuations in the filtering rate of Carteria, but a steady rate of removal of Gymnodinium.

Apparently the use of different cell densities of each species did not change the efficiency of removal. The total number of cells in each suspension was kept low, never exceeding 20,000,000 cells per liter, except in 6 tests containing 73,000,000 Nitzschia closterium cells per liter and 26,000,000 Carteria cells per liter. Previous experiments (Rice and Smith, in press) have shown that dense suspensions stimulate secretion of mucus with a resultant increase in production of pseudofeces. A comparison of the results of tests using suspensions of different population densities (Figure 4) shows that cells of Nitzschia closterium and Carteria sp. are removed with similar efficiency at the various time intervals.

The presence of Gymnodinium sp. seemed to have a definite effect on the feeding behavior of clams. In many tests it was impossible to obtain data since the clams closed soon after filtering.
Fig. 3. In nine experiments Gymnodinium and Carteria in mixed suspensions were removed with similar efficiency (Curve B) but in the remaining four experiments the presence of Gymnodinium affected the rate of filtering of Carteria (Curve A).
Fig. 4. Effects of densities of two species of planktonic algae in mixed suspensions on efficiency of removal by clams.
activities began. When they did remain open, chains of compact cells were ejected through the incumbent siphons. Some of these rejected cells were collected, washed several times in sea water and dissolved in acid so that thin samples could be prepared to minimize self absorption of beta particles of Ca$^{45}$. These samples were measured for their P$^{32}$ and Ca$^{45}$ content. Ninety-four per cent of the cells in these chains were Gymnodium, which demonstrated that the clams were selectively rejecting this dinoflagellate after filtering it from the water. This ejection of chains of cells was frequently observed only in tests using Gymnodinium.

Identification of the species of Gymnodinium used in this investigation needs to be made, since different results have been obtained with other species of this dinoflagellate. Ballantine and Morton (1956) found that Gymnodinium veneficum caused the small clam, Lasaea rubra, to cease filtering and to close its shell. It is known that Gymnodinium brevis produces a substance that is highly toxic to marine animals. Gymnodinium splendens, however, has been reported to be an excellent food organism for oysters.

The results of these studies demonstrate that the hard clam, Mercenaria mercenaria, does not filter algal cells of different sizes with the same efficiency. Cells of 2 to 3$\varpi$ are not removed as efficiently as those of larger size in the same suspension, even though these larger cells aid in retention by gills. In general, cells which are 4$\varpi$ or larger in diameter are filtered with uniform efficiency.

Thanks is given to Dr. W. A. Chipman and Dr. T. R. Rice of this laboratory. Dr. Chipman offered helpful suggestions and assisted in evaluating and reporting results. Dr. Rice advised in investigational procedures and made available unialgal cultures of phytoplankton.

Literature Cited


HISTOPHYSIOLOGY OF THE OYSTER KIDNEY

Milton Fingerman and Laurence D. Fairbanks

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Abstract

Histophysiology of the oyster kidney, Milton Fingerman and Laurence D. Fairbanks, Newcomb College, Tulane University, New Orleans 18, Louisiana

The morphology and physiology of the kidney (organ of Bojanus) in the oyster Crassostrea virginica are described. The morphology was determined by injection of Evans Blue and by histological section. Two bladders are present, one on each side of the animal with a secretory portion in between and posteriorly. A funnel-shaped duct runs from the floor of the pericardium to each bladder and opens close to the urinary pore. The latter is immediately posterior to the genital pore. Both openings are covered by a common flap. Injection of dye revealed that the tubules of the organ of Bojanus are bathed in blood from the adductor muscle which is supplied directly by the posterior artery from the ventricle. Blood then collects and flows into a large contractile vessel adjacent to the visceral ganglion. This vessel proceeds directly to the portion of the gills posterior to the intestinal loop and bifurcates anteroposteriorly as the afferent interbranchial vessel. In extremely dilute sea water the blood was hypertonic to the environment. The situation was reversed when oysters were placed in concentrated sea water. Some osmoregulation must have taken place. Only in the most dilute medium does the possibility exist that the organ of Bojanus serves in an osmoregulatory capacity. The wall of the ventricle appears to be the primary organ of filtration. In the lowest salinity tested the pericardial fluid was hypotonic to the blood whereas at higher concentrations the

1This investigation was conducted under a contract between Tulane University and the United States Fish and Wildlife Service. Funds were made available under provisions of P. L. 466, 83rd Congress, approved July 1, 1954, commonly called the Saltonstall-Kennedy Act.
Fig. 1. An oyster whose pericardium was injected with Evans Blue to reveal the location of the bladder of the organ of Bojanus. A, adductor muscle; P, pericardium; B, bladder. The line indicates where the section shown in Figure 2 was taken.
pericardial fluid was more concentrated, which indicates salt elimination to keep the oyster's tissue fluids more dilute than the surrounding waters.

Introduction

Several aspects of the morphology of oysters have been studied by a number of previous investigators (Leenhardt, 1926; Hopkins, 1934; Nelson, 1938; Shaw and Battle, 1957). However, very little is known of the urinary system of bivalve mollusks except for the physiological investigations of Picken (1937) and Florkin and Duchateau (1948) on the freshwater genus Anodonta and the morphological study of Awati and Rai (1931) on the Bombay oyster, Crassostrea cucullata.

Fingerman and Fairbanks (1956a, b) demonstrated that the commercial oyster, Crassostrea virginica, has some ability to osmoregulate. After 12 hours the salinity of body fluids had not changed to the same extent as the environment. Fingerman, Fairbanks, and Plauche (1957) determined chloride concentrations of fluids taken from several portions of the body. These investigators found that the urine of oysters kept in diluted sea water was hypotonic to blood. They postulated, therefore, that the organ of Bojanus functions in osmoregulation by conserving salt when oysters are in a dilute environment. The investigation described below was undertaken to obtain more information on the morphology and physiology of the organ of Bojanus in Louisiana specimens of Crassostrea virginica.

Morphology

Differences of opinion concerning the orientation of non-cephalized animals are found in the literature. We shall use the descriptive terms defined below in reference to the oyster.

Anterior, oral or in the direction of the hinge.

Posterior, anal.

Ventral, toward the gills.

Dorsal, opposite the gills.

Right, toward the side of the flat valve and promyal chamber.

Left, toward the side of the cupped valve.

The location of the organ of Bojanus and related excretory structures is best demonstrated in the living animal by injection of a dye such as Evan Blue into the pericardial cavity (Figure 1). Four to five ml of dye solution are usually required when a large
animal is used. The dye enters each bladder of the organ of Bojanus from the pericardial cavity. A bladder and separate duct from the pericardium are present on each side of the animal. Urinary orifices open into the right and left epibranchial chambers. The bladders lie ventral and adjacent to the pericardial cavity. As the bladder fills, dye is forced out through the urinary orifice located in the mid-ventral edge of each bladder. The genital orifice is immediately anterior to the urinary orifice (Figure 2). Both openings are covered by a common flap. A large nerve, visible without magnification extends anteriorly from the visceral ganglion along the mid-lateral wall of each bladder. The lateral wall of each bladder is capable of contracting to such a degree that the greater part of the fluid is forced out of the bladder. This has been observed in several oysters, apparently in response to distension of the bladder caused by fluid flowing in from the pericardial cavity. If an incision is made in the lateral wall of the bladder dorsal to the nerve and perfusion into the pericardial cavity is continued, dye can be seen flowing from the pericardial cavity through a rather large duct that empties into the bladder near the external urinary orifice. The proximal openings of the ducts leading from the pericardial cavity are in the ventral wall of the pericardium at the antero-lateral base of each of the two auricles. The ducts run along the medial wall of each bladder. They are flared at their origins in the pericardial wall and taper distally. The diameter of the outlet in the wall of the bladder is about one-third the size of the opening in the pericardial wall. The ducts are slightly curved with their distal ends directed posteriorly and are lined with cilia that beat distally from the pericardial cavity.

Histological sections reveal that both bladders of the organ of Bojanus are bordered medially and posteriorly with blind tubules that diminish in size with distance from the bladders (Figure 2). The line in Figure 1 shows where the section in Figure 2 was taken. The majority of the tubules lies ventrally adjacent to the adductor muscle. The most anterior tubules are at the level of the anterior end of the bladder, next to the antero-ventral portion of the pericardium, and lateral to the gonadal ducts. In this region the tubules are relatively large, rounded and few in number. Considerable space surrounding the tubules indicates a liberal supply of blood. The tubules are thick-walled structures composed of tightly-packed well-differentiated columnar cells having bulbous or vacuolated ends exposed to the lumen.

When both adductor muscle insertions are left intact and ties are placed around the anterior artery and the auricles, injection of dye into the ventricle reveals that the posterior artery sends blood directly to the adductor muscle. This blood next bathes the tubules, collects in the region of the visceral ganglion and posterior extremities of the tubules, and then flows out into a large contractile vessel adjacent to this ganglion. The vessel next proceeds directly to the portion of the gills posterior to the intestinal loop and bifurcates antero-posteriorly as the afferent interbranchial vessel.
Fig. 2. Histological section through the organ of Bojanus. B, bladder; T, tubules of organ of Bojanus; U, urinary pore; G, genital pore; D, digestive tract; M, mantle; C, ctenidia; E, epibranchial chamber; H, heart; P, pericardial cavity; L, lower end of duct from pericardial cavity to bladder.
The excretory system of *Crassostrea virginica* differs in two major respects from that of *Crassostrea cucullata* as described by Awati and Rai (1931). In the latter species the urinary and reproductive openings are not separate as in *Crassostrea virginica* but unite to form a single reno-gonidal aperture on each side of the body. Secondly, *Crassostrea cucullata* has an internephridial canal, connecting the two bladders, that does not appear to be present in *Crassostrea virginica*.

**Physiology**

In an attempt to understand the physiology of the organ of Bojanus, determinations of the chloride concentration in fluids taken from several portions of the body were performed according to the method described by Schales and Schales (1941) on oysters maintained at three different salinities. Body fluids were sampled from 30 oysters kept at each salinity for periods up to 14 days. These samples were taken from the bladder of the organ of Bojanus, the ventricle, the mantle, the pericardial cavity, and the blood vessel running from the secretory portion of the organ of Bojanus to the gills and mantle. The chloride concentration of the water in which the oysters were maintained was also determined. The results are presented in Figure 3 as differences in chloride concentration between the blood from the ventricle and the other body fluids. The concentration of the sea water and the difference in concentration between the sea water and blood in each of the three instances is also shown. Data obtained with oysters in the most concentrated sea water are at the top of the figure and data from oysters in the most dilute sea water at the bottom. At the right of the figure are probability coefficients, determined by t test, based on the difference between the heart blood and each fluid. At low salinities (161.7 milliequivalents of chloride per liter) all body fluids were hypertonic to the environment. However, only the mantle fluid was hypertonic to blood from the ventricle. At the intermediate concentration (227.0 meq/l), mantle and pericardial fluids were slightly more concentrated than the environment whereas the remaining fluids were less concentrated. At the highest concentration (515.6 meq/l) all fluids were hypertonic to the environment; the fluid from the organ of Bojanus was least concentrated followed by blood from the ventricle.

Evidently some sort of osmoregulation occurred to keep the oysters hypertonic in diluted sea water and hypotonic in concentrated sea water. Only in the most dilute medium does the possibility exist that the organ of Bojanus serves in an osmoregulatory capacity, tending to eliminate water from the blood to keep the latter hypertonic to the environment. Prosser, Green, and Chow (1955) studied the role of the antennary glands of the shore crab, *Pachygrapsus crassipes*, in osmoregulation and arrived at the same conclusion concerning these organs as that found for the organ of Bojanus. The antennary organs may function in hyper-osmotic regulation but not at all in hypo-osmotic regulation.
### Differences in Concentration of Cl (MEQ/L) in Oyster Body Fluids From Blood Taken From the Heart

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<tr>
<th></th>
<th>SW 515.6</th>
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<th>MF</th>
<th>PC</th>
<th>P &lt; 0.20</th>
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</table>

**H** - Blood from Heart  
**MF** - Mantle Fluid  
**PC** - Pericardial Fluid  
**SW** - Sea Water  
**KR** - Blood from Kidney Region  
**B** - Fluid from Bladder of Organ of Bojanus

**Fig. 3.** Concentration of chlorides (MEQ/L) in various body fluids compared with blood from heart of oyster.
In the oyster, as in *Anodonta*, the wall of the ventricle appears to serve as the major filtration structure. In the lowest salinity tested, pericardial filtrate was hypotonic to the blood in the ventricle showing that salt was being conserved to keep the oyster hypertonic to its environment. At the two higher salinities, the pericardial filtrate was hypertonic to ventricular blood showing that salt was being eliminated in order to keep the oyster hypotonic to its environment. The opening of the duct from the pericardium to the bladder of the organ of Bojanus lies close to the urinary pore. Presumably, contraction of the pericardial wall could result in elimination of the contents of the pericardial cavity with little mixing of this fluid and the urine in the bladder.

**Literature Cited**


SOME FACTORS IN THE USE OF NANNOPLANKTON CULTURES AS FOOD FOR LARVAL AND JUVENILE BIVALVES

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Abstract

To guide in estimating food quantity required, densities of larvae and phytoplankton in our cultures are compared with natural densities of grazers and food cells. Orders of magnitude are similar, which supports use of our present feeding schedule, 0.01 ml of wet packed algal cells per day per liter of larval culture containing 3-15 larvae per ml.

Selection of food species is critical. Thirty-seven isolates of 26 genera in 6 algal classes were tested as food for larval or juvenile oysters (Crassostrea virginica) or clams (Venus mercenaria). Small naked flagellates are necessary for oyster larvae, best for clam larvae, and adequate for juveniles of both species, though the latter grow best on cryptomonads or small diatoms. The dinoflagellates Amphidinium carteri and Gymnodinium sp. are useless as food. Species such as Chlorella "A" or Stichococcus sp. are moderately toxic, particularly to oyster larvae; the chrysophyte Prymnesium parvum is extremely toxic to all stages of both bivalves.

Mass unialgal cultures of good foods such as Isochrysis galbana and Monochrysis lutheri (chrysophytes) have become toxic. This correlates with bacterial contaminants and high growing temperatures of 23 - 26°C. The toxin appears to be heat-labile and adsorbable by paper or membrane filters. Certain bacterial cultures isolated from toxic algae have similar properties. Algal food cultures should be started from pure stocks and grown at the lowest temperatures the algae tolerate.

A major problem involved in raising bivalves, whether for experimental purposes or in a commercial hatchery, is to supply adequate food. Because no synthetic diet has been devised, the only available foods are cultures of various nannoplankton organisms.
This discussion is offered in the hope that it will be of help to those responsible for selecting and growing food organisms. It is based largely upon work carried out at the Milford Laboratory during 1956-57. Feeding experiments with larvae and juveniles of the oyster (Crassostrea virginica) and hard clam (Venus mercenaria) were carried out in cooperation with Harry C. Davis and Paul Chanley of the Milford Laboratory. Davis and Guillard (in press) have presented results on the use of ten genera of micro-organisms by larvae. Ecological data are drawn in part from studies of the micro-biota of two brackish ponds on Martha's Vineyard. Bioassays of toxins produced by micro-organisms were made in cooperation with Mr. Davis.

The comparatively simple question of food quantity is considered first. Food organisms must not only be present in adequate total quantity, but also in suitable concentration. Loosanoff, Davis, and Chanley (1954) have shown that larvae must not be too crowded, but whether there is an optimum concentration, due perhaps to some social phenomenon, is still unknown. We are concerned first with whether or not conditions in our larval cultures differ markedly from natural conditions in respect to the relative abundance of phytoplankton and grazing organisms.

In culture methods commonly used at Milford, also by Walne (1956), the larvae are maintained at concentrations of 3 to 15 per ml, while phytoplankton concentrations vary from 10,000 to 100,000 cells per ml, depending upon sizes of the cells.

Data on zooplankton concentrations in nature are frequently deficient because small organisms are not quantitatively retained by collecting devices. Deevey (1948) recorded from 10 to 500 zooplankters per liter, exclusive of ciliates, in samples collected with a #10 net in West Tisbury Great Pond. She noted (1956) a maximum of about 200 animals per liter, again exclusive of ciliates, in Long Island Sound in late summer. The writer's observations of preserved whole samples from W. Tisbury and Oyster Ponds indicate that small ciliates (probably of the suborder Oligotrichina) often outnumber all other zooplankters. Lackey et al (1952) reported that ciliate concentrations in the Woods Hole region were generally of the order of a few per ml, but that exceptional concentrations of the order of 100 per ml occurred.

It is seen that total animal population densities in nature approach our culture densities at times. The volume of animals per unit volume of water, however, is estimated to be somewhat higher in the cultures. A culture having ten 200μ oyster larvae per ml has about 20 cc of larvae per cubic meter. Deevey (1956) found the maximum plankton displacement volume (collected with a #10 net) to be 6 cc per cubic meter in Long Island Sound. Note that bivalve larvae are often but a small proportion of the animal population. Rees (1954) cites maximum larval concentrations of the order of 10 per liter. However, a recent report by the State of Washington Department of Fisheries (1957) describes concentrations of 2 larvae per ml
in the region of Willapa Harbor. Perhaps high concentrations in confined waters are not unusual.

To compare the concentrations of food organisms in culture with those in nature one must refer to studies made by methods allowing small and fragile organisms to be counted. Lackey et al. (1952), by examining the entire contents of small water samples concentrated by sedimentation or centrifugation, determined that the concentrations of micro-organisms over oyster grounds (except for places like Great South Bay, Long Island) varied from about 300 to 300,000 per ml. Undesirable blooms of Stichococcus and Nannochloris in Great South Bay exceeded 2 million cells per ml at times. Hubert (1956) made a careful study of the living phytoplankton of Great Pond, Falmouth, Mass. Summer maxima were of the order of 100,000 cells per ml; more general levels were about 10,000 cells per ml. Conover (1956), using formalin preserved samples, found that summer maxima in Long Island Sound were of the order of 10,000 cells per ml. Our samples taken in the summer of 1956 from West Tisbury Great Pond and Oyster Pond, Martha's Vineyard, were preserved with acid I2-KI, allowed to settle, and examined with a water immersion lens. Holmes (1956) discusses the accuracy of this method. Total counts varied from 4,000 to 40,000 cells per ml in these samples.

The range of summer maxima found in nearby waters (excluding tidal pools and local blooms) is similar to the range of food concentrations in the larval cultures. It is suggested that the feeding schedule described by Davis and Guillard (in press) be used as a guide in estimating the quantity of algal cells needed to feed given numbers of larvae. This schedule provides 0.01 ml of wet-packed algal cells per day per liter of larval culture (ca. 10 larvae per ml). The wet-packed cell volume of an aliquot of the algal culture is found by centrifuging it at 1000 g. for 25 minutes in Hopkins or Goetz tubes. The number of cells per feeding will vary, depending upon the average cell size of each food species.

There are fewer data to serve as a guide in estimating juvenile food requirements. Some data from experiments designed to compare growth of oyster spat on different foods are of interest. For example, 50 spat averaging 0.7 mm in maximum dimension were kept 30 days in 3 liters of water (changed every other day) and fed an unidentified cryptomonad (Rhodomonas ?) at three times the rate used in larval cultures. The average increase in size was 4.5 mm. Unfed controls grew 0.6 mm in the same time. Martin (1928) and Prytherch (1924) observed faster growth of spat under favorable circumstances in nature, suggesting that more food may be required, perhaps supplied continuously, rather than batchwise. These data may serve as a starting point for estimating food requirements for juveniles of this size.

It is more important and difficult to supply the right kind of food than the right amount. Growth of the animals is the only criterion
by which values of different food organisms can be measured at present. Larvae fed equal volumes of different foods (according to the schedule mentioned above) are compared with unfed controls and with others given known good foods. Methods are given by Davis (1953), and Davis and Guillard (in press). Assay is most indicative of true value of food organisms themselves if cultures are bacteria-free and harvested just before the end of the logarithmic phase of growth. This yields maximum cell density and minimum accumulation of debris and soluble metabolites. The assay should be repeated using unialgal but not bacteria-free cultures if the organism to be used routinely as a food. Impure cultures may become toxic, especially if growth conditions are sub-optimal.

From such experiments one cannot make an unqualified evaluation of the worth of a given species of micro-organism in nature, partly because growing conditions there are not known, and also because the interactions with other micro-organisms and with physical factors of the environment are more numerous. For example, Shilo and Aschner (1953) found that the toxicity due to Prymnesium parvum was decreased by bacteria, by adsorption of the toxin on materials such as mud, and by oxidizing agents.

During the past 20 months we have assayed the food value of 37 isolates of 26 different genera of micro-organisms. These were classified as follows: 18 green algae, 7 diatoms, 6 chrysophytes, 3 cryptomonads, 2 dinoflagellates, and one unicellular blue-green alga. In some experiments only larvae were fed, in others only juveniles, and in some, both larvae and juveniles were fed from the same algal cultures. In some experiments the cultures were bacteria-free, in others only unialgal. Over 100 different assays have been made using hard clam or oyster larvae, and roughly half that number with juveniles.

Of the organisms we have tested, only certain small naked flagellates (no larger than 12 μ) have benefited oyster larvae in their earliest stages (48 hrs. after fertilization). The chrysophytes Isochrysis galbana and Monochrysis lutheri were best. As the larvae grew, after about the sixth day, they became capable of using certain other forms, such as Platymonas sp., Phaeodactylum tricornutum and Chlorella #580. Clam larvae have been able to use more different types of foods than oysters of the same age. Juveniles of both species utilized a still wider range of organisms. Chrysophytes and diatoms have been notably successful. Those naked flagellates which were best for larvae were relatively good also for juveniles, but those foods best for juveniles (cryptomonads, Skeletonema costatum, Actinocyclops sp.) were useless to larvae. Factors besides nutritive composition of the foods are obviously involved in their acceptability to larvae of different sizes and species. Davis and Guillard (in press) discuss the influence of presence and thickness of algal cell walls (external to the plasma membrane) and of production by the algae of toxic substances. It is significant that certain organisms, in particular the
dinoflagellates _Amphidinium carteri_ and _Gymnodinium_ sp., were useless to larvae and juveniles alike although they did not kill larvae. The chrysophyte _Prymnesium parvum_, on the other hand, was extremely toxic to both larval and juvenile clams and oysters. This organism, which unfortunately is widely distributed, produces a toxin of high molecular weight. Exposure to normal feeding concentrations of _Prymnesium_ prevented the development of oyster eggs and caused heavy mortality in cultures of larval clams and oysters. In a 17-day feeding experiment with 3 mm oyster spat and 3.8 mm juvenile clams (average sizes) 100% of the oysters and 75% of the clams died. During this period the control oysters fed a mixture of good foods grew 28% in length, while the clams grew 44% in length. Unfed controls grew less than 5%.

The fact that a micro-organism may be toxic when fed to larvae or juveniles does not necessarily imply that it can prevent the development of eggs to the straight hinge stage. _Chlorella_ isolate 'A' is an example. It is of interest that two different _Stichococcus_-like organisms, one from Great South Bay and one from the Martha's Vineyard ponds, were also relatively toxic. Gibor (1956) considered the _Stichococcus_ sp. which he fed to _Artemia_ not toxic but only indigestible.

Again, it is instructive to compare ideas derived from laboratory experiments with those derived from observations in nature. Lackey et al (1952), from analysis of the plankton of such places as St. Mary's River and Peconic Bay, supported the idea that small flagellates are good larval food and that diatoms and dinoflagellates are better food for adult oysters. The idea generally agrees with out findings, although we have not yet found any dinoflagellates which are useful as food to larvae or juveniles. The samples of phytoplankton taken from the Martha's Vineyard ponds, had, as far as we know now, a favorable composition for both larvae and juveniles. The fraction of cells over 12 microns in greatest dimension was less than 20% in all samples except one in which about 50% were large pennate diatoms. This came from a shallow inlet of low salinity. Very small chrysophyte-colored cells were usually most numerous. Some of these were obviously flagellated; others were minute diatoms. The frustules of these small diatoms can be demonstrated only with difficulty by the methods normally employed. At some times cryptomonads were dominant; often small green flagellates were numerous, some of them possibly zoospores. Hulbert's findings (1956) in Falmouth Great Pond were generally similar, although at times the plankton was dominated by diatoms brought in from Vineyard Sound.

To summarize, our experience suggests that for best results the algologist should provide a mixture of cultures of two or three small naked flagellates - especially chrysophytes - for larvae, and should supplement this with diatoms and cryptomonads for juveniles. Note that this is for best results -- larval clams grow fairly well on such forms as _Phaeodactylum_ or _Chlorella_ #580, and very well on _Chlorococcus_ isolate 'C'.
Still another problem besets the use of cultures as foods. Presumably assay of a healthy bacteria-free culture measures the effects of such relatively constant properties as cell size, wall thickness, and average chemical composition. However, one cannot always wait to get a culture bacteria-free; moreover, large cultures for routine feeding can seldom be kept pure, and may not always be in the log phase of growth. At times this may make little difference, but there is clear evidence that impure cultures may be toxic under some circumstances.

Toxicity of good food organisms was first recognized in carboy cultures of Isochrysis galbana and Monochrysis lutheri grown at room temperature, exceeding 23°C at times. These cultures had been used successfully through the fall and early winter; erratic growth of the algae and toxicity to larvae became apparent in late winter.

Assays were made with various preparations from a toxic Isochrysis culture and with bacteria derived from this culture. Newly fertilized oyster eggs give clear results in 48 hours or less. Highly toxic preparations cause the eggs to disintegrate. Dilutions of such preparations usually allow eggs to develop into ciliated stages or deformed shelled larvae. On occasion larvae develop apparently normally to straight hinge stage, then die.

Certain properties of toxic culture medium resemble those of toxins derived from pure cultures of other organisms. The following brief summary will make this clear. The toxic principle from the Isochrysis cultures is destroyed by boiling and is not carried over in the distillate. It is not destroyed by heating to 60°C. An aliquot of toxic culture is made non-toxic by Millipore filtration, which removes the algae and bacteria and adsorbs certain molecules. Filtration through Whatman #5 filter paper, which removes few algae or bacteria, also removes most of the toxicity. Rapid centrifugation, which removes most of the algae and some of the bacteria, leaves the toxicity. Exposure to low pressure which occurs during filtration does not remove toxin. The material therefore has many of the properties of the toxin found in pure cultures of Prymnesium parvum, as described by Shilo (Shelubsky) and Aschner (1953). It differs from the Gymnodinium brevis toxin described by Ray and Wilson (1957) in that it is adsorbed on both Millipore and paper filters, while Gymnodinium toxin is adsorbed only on paper. The Gymnodinium veneficum toxin studied by Abbott and Ballantine (1957) has a molecular weight greater than 1000 and is dialyzable and adsorbable. The toxin described by Shelubsky (1951) from the blue-green alga Microcystis is a compound of small molecular weight with quite different properties. For the sake of completeness we mention that the toxicity of Chlorella, Chlamydomonas, Stichococcus, and certain other algae may well be due to the fatty acids they liberate. This is well reviewed by Proctor (1957).
Pure and mixed cultures of bacteria were obtained by streaking toxic algal cultures and by inoculating aliquots into various enrichments of sea water. Preliminary studies on toxicity, adsorption, and heat lability showed that the preparations made from bacterial and from toxic algal cultures had similar properties, but definitive experiments have yet to be made.

Efforts to induce toxicity in pure algal cultures by the addition of bacteria or aliquots of toxic cultures were not uniformly successful, but succeeded more often when the algal cultures were grown at 23 - 26°C, than when they were grown in the 20°C room. Cultures grown at 20°C sometimes became toxic when transferred to 23 - 26°C, and once altered, remained toxic when returned to 20°C. Isochrysis is less tolerant of high temperatures than Monochrysis, and becomes toxic more often. We have never observed toxicity in pure cultures of either Isochrysis or Monochrysis. Impure cultures may become toxic when grown in artificial sea water (medium ASP 2 of Provasoli et al 1957), which eliminates the possibility that toxicity is due to some interaction between the micro-organisms and organic substances in sea water.

The hypothesis is offered, therefore, that toxicity is due to bacteria that get into cultures in the course of handling. Toxin production, presumably by bacteria rather than by algae, takes place when algae provide the necessary substrate. This is influenced by the condition of the algal culture. We noticed, for example, that a dense culture in a carboy sealed to an aerating system became toxic in a day when the supply of air and CO₂ was accidentally shut off. The fact that larvae too young to feed can be killed or injured suggests that some toxin is liberated into the medium.

In our experience, erratic results in feeding experiments have tended to coincide with the use of impure cultures, especially those grown at room temperature. Possibly some of the irregularities mentioned by Walne (1956) were caused by bacteria. Lest bacteria be blamed for all ills, let us record that they can also detoxify cultures. The report that bacteria destroy Prymnesium toxin led us to incubate an aliquot of toxic Isochrysis culture with a mixed culture of marine bacteria. This treatment removed the toxicity. In another trial, toxicity was only reduced.

It is recommended that whenever possible large algal cultures be started from bacteria-free stocks and grown at the lowest temperatures that provide rapid growth. Simpler methods of control may be devised when the nature of the toxicity is determined and factors responsible for its production are better understood.


DISPOSAL BY THE OYSTER OF INTRACARDIALLY INJECTED RED BLOOD CELLS OF VERTEBRATES

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Abstract

Various vertebrate erythrocytes were injected intracardially into the oyster, Crassostrea virginica, and their fates were determined histologically. In many vessels and sinuses the particles are phagocytized within a few hours. Some phagocytes then migrate through tissue and surface epithelium to be voided. Those erythrocytes not removed in this fashion are digested intracellularly. It is suggested that the ability of the oyster to defend itself against foreign particles is dependent upon both the migratory and the digestive capabilities of its leukocytes.

Introduction

The present study is one of a series of steps to be taken in elucidating the defense mechanisms of the oyster, Crassostrea virginica Gmelin. The first step was taken by Stauber (1950) when he injected India ink intracardially and traced its fate. The series of experiments to be reported here is concerned with the fate of a metabolizable particle—the vertebrate red blood cell—placed in the circulatory system of the oyster. This work confirms the findings of Stauber (1950) and extends the concept of the phagocyte as an active participant in the defense of the oyster body.

Methods

The experiments were designed to reveal phagocytic, dispersal and digestive activities at several times following intracardial injection of red blood cells. Rabbit, weakfish, and normal duck erythrocytes, as well as cells from a duck infected with the avian malaria parasite, Plasmodium lophurae, were used as inocula. The red cells were washed thoroughly and resuspended in sea water. The heart was carefully exposed by filing an opening through the left valve of the shell directly over the pericardium. Then, by means of

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a tuberculin syringe and a 30 gauge needle, 0.2 ml of the inoculum was introduced into the ventricle. The oysters were replaced in tanks of sea water and one was sacrificed at each of the following times: 10 minutes, 30 minutes, 6 hours, 1 day and 2 days, and then every other day up to about three weeks. Each oyster sampled was relaxed in cold magnesium sulfate solution overnight, then fixed in Bouin's solution, washed, dehydrated in a series of alcohols, cleared in xylol, and mounted in paraffin. Twelve transverse sections were obtained from each animal. Two sections were cut from each of the following levels: palps, stomach, visceral mass posterior to the stomach, heart, adductor muscle and the most posterior portion of the gills. The sections were stained with hematoxylin and eosin.

In each animal certain vessels, sinuses and tissues were examined under high dry and oil immersion objectives. The presence or absence of phagocytosed red blood cells in epithelium was ascertained by oil immersion examination.

The following blood channels were sampled: heart, anterior and posterior aortae, subepithelial spaces of the gut at all levels, large arteries of the visceral mass, sinuses between the digestive diverticula, dorsal and ventral circumpallial arteries, proximal mantle vessels, medial and lateral gill axis sinuses, vertical gill vessels, palp vessels and the blood spaces of the adductor muscle and kidney.

The movement of phagocytes containing red cells through epithelium was determined by examination of the following sites: walls of esophagus, stomach and rectum, walls of about twenty digestive diverticula, inner and outer aspects of the palps, outer aspect of the mantle in several areas, nephridial tubules, gonaducts, external lining of the heart, and internal lining of the pericardium.

In addition to the sections, smears of heart blood were made before the oyster was relaxed. These preparations were fixed with methyl alcohol and stained with Giemsa stain. Also, in vitro experiments with mixtures of oyster heart blood and chicken red cells have confirmed the phagocytic abilities of oyster leukocytes.

**Results**

Following intracardial injection of red blood cells, five phases of oyster response are discernible: 1) large masses of red cells virtually occlude many arteries; 2) these particles are phagocytized; 3) phagocytes migrate into tissue; 4) phagocytes migrate through epithelium resulting in elimination of the particles from the body; and 5) those red blood cells retained in the body within phagocytes for twelve days are digested.

Phagocytosis of red blood cells begins immediately after injection. Within ten minutes large numbers of red cells are engulfed...
by phagocytes. This is confirmed by sections and blood smears. From one to eight red cells per phagocyte are usually seen and not uncommonly up to fifteen or twenty are found within a single cell. Six hours after injection approximately 95% of the red blood cells in oyster blood vessels are within phagocytes.

Masses of phagocytized red cells in arteries are removed in two ways: 1) blood pressure forces the particles through smaller arteries into sinuses, and 2) phagocytes containing red blood cells begin to migrate from the vessels and sinuses to tissue. As the emboli are resolved, blood flows more freely.

As early as 48 hours post-injection, the migration of red cell-laden phagocytes across epithelial layers can be detected. This movement to the exterior reaches a peak at about eight days. The surfaces traversed are: gut wall at all levels, gonaducts, mantle, digestive diverticula, pericardium and inner and outer aspects of the palps. After emerging into lumina, red cell-laden phagocytes are easily voided.

In all experiments, evidence of intracellular red blood cell destruction was obtained. Experiments using avian red blood cells containing malaria parasites were particularly clear in this respect. The pigment associated with malaria is the product of hemoglobin destruction wherein the parasite splits off globin, leaving the acid hematin residue behind. This acid hematin is extremely resistant to destruction. In the oyster, when a parasitized red blood cell is destroyed, the readily recognizable pigment remains as a marker. Twelve days after injection, malaria pigment in oyster phagocytes was distributed in the tissues while intact red cells were difficult to find. Destruction by osmotic effects can be ruled out since an occasional free red cell could be found which was still intact. The amount of free pigment due to rupture of cells during inoculation was negligible and virtually undetectable in early samples. These observations, coupled with the evidence for intracellular proteases and lipases (Yonge 1926), make inescapable the conclusion that vertebrate red blood cells injected intravascularly are digested by oyster phagocytes. Further evidence for this process is supplied by the pioneer work of Yonge (1926), who has clearly shown that when shark corpuscles are fed to Ostrea edulis they are phagocytized and digested to a mass of fat globules in 12 hours.

Discussion

As Stauber (1950) has noted, it is to be expected that in the oyster, a poikilothermic animal, the degree of activity of phagocytes would be affected by temperature changes. In his experiments, at temperatures from 12° to 21° C., ink-laden phagocytes were seen migrating across epithelial layers after eight days. With temperatures at 21° C. movement of phagocytized red cells across
epithelium was first recorded at two days. This early movement may have occurred in the india ink work also, but it was not discovered because no samples were taken between 22 hours and eight days. More recent data show the period between one and eight days post-injection to be a very significant time in the oyster's attempt to rid itself of foreign particles.

The response of an oyster phagocyte to particulate matter is to engulf the particle and then migrate. If the particle is not metabolizable, it may be eliminated by phagocytic migration. When the particle is metabolizable, such as a red blood cell, this migratory response may be supplemented by intracellular digestion. In the experiment discussed here, some red cells were quickly removed by phagocytic transport before they could be digested. After twelve days, most of the red cells in phagocytes had been digested. Degradation of red cells seems to be the more important means of elimination. Although migration may be able to remove a large number of particles over a long period of time, digestion of these particles would greatly enhance the efficiency of the defense mechanism. In this manner perhaps some potentially pathogenic microorganisms are destroyed by digestion while such intracellular parasites as Nematopsis and Dermocystidium, which appear to be resistant to the intracellular enzymes of oyster leukocytes, may be eliminated by migration of host cells to the exterior.

No matter what the defense mechanism, it depends on the oyster maintaining a physiological optimum. Any added stress might reduce the efficiency of the phagocytes, allow parasites to accumulate, and lead to the death of the oyster.

Summary

When vertebrate red blood cells are injected intracardially in the oyster:

1) Phagocytosis begins immediately and is over 90% complete within six hours.

2) Phagocytes carry these particles from blood channels through tissues and epithelia to various lumina from which some are voided.

3) Those erythrocytes not removed in twelve days are digested within phagocytes.

4) Digestion seems to be more important than migration, as a means of eliminating vertebrate red blood cells from the oyster.
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SOME OBSERVATIONS ON BLOOD CIRCULATION IN THE OYSTER, CRASSOSTREA VIRGINICA

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Abstract

It was found that the American oyster has three veins that return blood to the heart, the branchial, the pallio-branchial and the pallio-cloacal.

Each accessory heart was found to possess a branch which follows closely the adductor muscle dorsad. Each branch empties its blood into the shell side of the mantle.

A study of the gill circulation reveals an afferent and an efferent system. The medial gill axis sinus is the major afferent branchial vein and the pallio-branchial vein is the major efferent vessel. In the demibranchs, afferent and efferent systems are connected by spaces in the interfilamentar junctions via the vessels associated with the principal filaments.

The present study deals with the venous return and gill circulation of the American oyster, Crassostrea virginica (Gmelin). Other investigators (Kellogg 1890, Leenhardt 1926, Hopkins, A. E. 1934) have shed much light on the arterial system of the oyster but details of venous circulation and its relation to the gills have never been adequately explained.

There are three pairs of major vessels which return blood to the heart:

1) the branchial vein, which drains blood from the anterior portion of the lateral gill axis sinus and the lateral portions of the visceral mass,
2) the pallio-cloacal vein, which drains blood from the cloacal chamber and the dorsal portions of the mantle and visceral mass and,

3) the pallio-branchial vein, which drains blood from the ventral regions of the mantle and the entire posterior portion of the gills.

The branchial vein is a dorsal extension of the lateral gill axis sinus. It ascends in a dorso-posterior direction to join the atrium. During its course toward the atrium it lies embedded in the epibranchial septum, which divides the anterior epibranchial chamber on each side into two separate channels. The pallio-cloacal vein originates in the mantle of the cloacal chamber and runs close to the adductor muscle as it proceeds dorsally over the muscle then bends sharply ventrad to join the atrium. The pallio-branchial vein originates in the posterior mantle just where the mantle joins with the most lateral gill lamella. It proceeds anteriorly and, at the level of the end of the oral process, turns dorso-anteriad and runs up to the atrium.

Blood which drains from the sinuses of the organ of Bojanus (kidney) empties into the accessory hearts first described by Hopkins (1934). The accessory hearts beat in a proximo-distal direction and propel blood into the circumpallial artery and, via numerous short connectives, to the shell side of the mantle all along its course. In addition, there is a branch of the accessory heart which, instead of crossing the cloacal chamber, follows the adductor muscle dorso-posteriad for approximately half its length and empties, via numerous terminal sprouts, into the shell side of the mantle. Blood after filtering through the organ of Bojanus has probably lost what little pressure it had hence the accessory heart takes on the important function of increasing the pressure in this critical region. The accessory heart sends blood out the circumpallial artery into the pallial curtain and, through numerous small branches, to the shell side of the mantle. This blood, then, filters through the numerous tissue spaces of the mantle where it collects either in the pallio-branchial vein, which receives blood from the ventral side of the mantle, or the pallio-cloacal vein, which receives blood from the general area of the cloacal chamber.

One of the major veins that drains the visceral mass as well as the medial palps runs along the medial axis that separates the two gills, and is known as the medial gill axis sinus. All the blood that enters and circulates through the gill filaments comes from this major vein in which the blood flows in a posterior direction. This vessel then, marks the beginning of the afferent branchial system (Figure 1-top water tubes). As it proceeds, it gives off branches to every other principal filament in the adjacent gill lamellae. It also gives off in every interlamellalar junction a vessel which runs across to the outermost lamella of each side and empties into branch
Fig. 1. Dorsal view of the demibranchs and water tubes on the left side in the region of the adductor muscle. The afferent branchial system is indicated by the vessels associated with the middle water tubes and the combined circulation is indicated in the lower water tubes.
vessels serving every other principal filament in these lamellae. From here the blood can go across the sub-interlamellar junctions, which connect opposing principal filaments of the demibranch at different levels across the water tube, to the corresponding principal filament of the adjacent lamellae (see upper two water tubes, Figure 1). Finally, at all levels, blood moves in the spaces of the interfilamentar junctions to the vessels of the interdigitating adjacent principal filaments which constitute the beginnings of the efferent system. This efferent branchial system can be broken down into three separate parts which all eventually empty into the pallio-branchial vein (see middle water tubes, Figure 1).

1) The blood vessels of the principal filaments of the medial lamellae of the inner demibranchs: these efferent vessels collect into a common vessel at the top of the water tube then proceed to the anterior end of the water tube, then turn laterally across the interlamellar junction toward the pallio-branchial vein.

2) The vessels of the principal filaments of the lateral lamellae of the outer demibranchs empty separately into the pallio-branchial vein.

3) The efferent vessels in the principal filaments on either side of the lateral gill axis: these vessels drain into a common vessel known as the lateral gill axis sinus; previously this vessel was thought to be continuous the whole length of the gill, but there it only proceeds to the anterior end of the water tube which it drains, turns sharply laterad, and thence proceeds across the interlamellar junction to join the pallio-branchial vein. As it turns laterad to join the pallio-branchial vein it receives the vessel that drains the medial lamellae of the inner demibranchs (part "1" of efferent branchial system) and the two empty into the pallio-branchial vein as a common vessel.

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AN ABNORMAL VIRGINIA OYSTER WITH A BIFURCATED MUSCLE

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In October, 1955 Mrs. Jack Ross brought in an oyster which he had taken in Davis Bay, on which this Laboratory is situated. He had shucked this oyster and noted that it was abnormal.

The muscle was split into two parts where it attached to the left valve. The more forward and dorsal part was oblong in shape, being 2 cm long and about 1.3 cm wide, with the long axis parallel to the axis of the body. The lower part of this muscle was somewhat irregular in shape and it was approximately 1.4 by 1.2 cm. The posterior margin of the dorsal part of the muscle extended almost 2 centimeters posterior to the margin of the ventral smaller portion. Those two parts were separated by epithelial tissues of the body wall about 1.2 cm across. The left valve itself showed three dark areas. Two of them corresponded to the muscle attachment but one was ventral and posterior. Apparently there was no muscle attachment along this smaller area. The muscle scars on the shell were approximately 2.5 cm apart and, apparently, the measurements of the muscles and their separation, made on the alcoholic specimen, were somewhat modified by shrinkage. The left valve showed an irregularly ridged, white area splotched with irregular purple markings, apparently a former muscle attachment, between the real attachments. The shell at this point was approximately 2.5 cm thick. The remainder of the shell was less than a cm thick. The writer did not cut the shell or break it open.

The left valve of the oyster was 14.0 cm long. The muscle attachment to the right valve was somewhat hourglass-shaped with the lower lobe being much the larger and spread posteriorly. The "waist" of this muscle was slightly less than 1 cm across. The smaller lobe was a cm across and the other one was slightly over 2 cm across. This hourglass figure extended with the long way at right angles to the anterior-posterior axis of the oyster and with the small lobe towards the promyial chamber. It is probable that the abnormal pull of the double muscle of the left valve caused this abnormal shape of the attachment on the right valve.

The three attachment portions of this muscle all showed the closing and locking muscle, although the areas were somewhat irregular. The attachment area of the upper valve showed in general the hourglass shape described for the muscle of that side.

The shell itself was not particularly heavily infested with associates and boring organisms. There were a few small barnacles and signs of some worm holes. There were no signs of organisms within the shell which would have caused this abnormality.
The oyster was in excellent condition and was a creamy white in color. It had a considerable amount of glycogen. Apparently the muscle abnormality interfered very little with its life processes. The cause of this aberration is unknown.

I have presented this specimen to Dr. Paul S. Galtsoff as an addition to his collection of abnormal oysters.
OBSERVATIONS ON MUSCLE ATTACHEMENTS, CILIARY MOTION, AND THE PALLIAL ORGAN OF OYSTERS

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A detailed study of the anatomy and functions of the oyster which I have conducted during past years has revealed several facts which heretofore have remained unnoticed. The present discussion deals with the adductor muscle, the skeleton and muscular apparatus of the gills, the ciliary tracts of the mantle and gills, and the structure of the so-called pallial organ.

Attachment of Adductor Muscle

The adductor muscle of all pelecypods is a very conspicuous and powerful organ. Experiments which I described at the 1952 meeting of the Association show that the muscle of a fully grown Crassostrea virginica withstands a pulling force up to 22 pounds (10 kilograms). Under this force the muscle fibers usually break in the middle and the remaining parts still adhere to the valves. Only rarely is the muscle separated at the place of attachment. On the other hand, by applying heat to the outside of the shell over the area of the muscle scar the connection between the muscle and the valve may be severed or at least greatly weakened.

The muscle scar is always smooth and glossy. Examination of sections made across the decalcified shell with the muscle attached to it show that the fibers end in a layer of epithelial cells next to the inner surface of each valve (Figure 1). The boundaries of these cells are not visible and the entire layer has the appearance of a syncytium. The nuclei are very large. Fibrillar protoplasmic strands extend across this layer and end at the inner surface of the shell. In the areas adjacent to the adductor muscle the mantle is covered with a typical epithelium which gradually changes into a specialized layer as the mantle approaches the adductor muscle. Brück (1913) who described this epithelium in Anodonta and Cyclas called it "adhesive" epithelium and considered that it provides a mechanism for attachment of muscle fibers to the shell. He did not notice, however, an organic film, no more than 2 microns in thickness, by which the muscle fibers are cemented to the shell of Crassostrea virginica. This film is always present in the oyster but is usually destroyed when muscles are cut from the shell. The cement by which muscles are attached to the shell probably consists of collagen, a protein generally found in connective tissue. To verify this assumption various connective tissue stains were used. The staining reactions were not too reliable, however, and better results were obtained by observing effects of proteolytic enzymes.
Fig. 1. Attachment of adductor muscle to shell. A thin layer of cement, in one place detached from the muscle, lies over a row of epithelial cells through which pass muscular fibrillae. Drawing from decalcified preparation of oyster shell with muscle attached. Hematoxylin and eosin--X 650.
It is known that under proper conditions collagen may be digested by an enzyme called collagenase. A series of experiments conducted last month at Woods Hole indicates that this protein plays a major role in the attachment of muscles in oysters. The experiments consisted of injecting small amounts of phosphate buffer solution containing 1 mg of collagenase per ml into the adductor muscles through small holes drilled in the valves. In another set of experiments the muscles of small oysters with the shells attached to them were immersed in the solution of collagenase and were kept at a temperature of 24-25°C. for 24-48 hours. Solutions of trypsin and of phosphate buffer, without enzyme were used for control experiments. In all cases muscles treated with collagenase became detached within 36 hours, but controls remained attached to shells. There is no doubt that the oyster uses collagen as a cement for the attachment of its adductor muscle.

Gill Apparatus and Ciliary Motion

The muscle fibers of the gills present a different picture. They end in root-like processes which are deeply embedded in chitin material. There is no sign of any cement by which they may be glued to the skeletal bars. The framework of an oyster gill is an inverted V-shaped structure with two sets of muscles. One set is located between the two converging arms of the frame; the contraction of these muscles brings the arms together and closes the gills. The other set of muscles located on the outside of the frame surrounds the sharp point where the arms are joined together. The contraction of these muscles pulls the arms apart and opens the gill. In this way access of water to the gill lamellae is facilitated. The opening and closing of the gill lamellae is one of the essential mechanisms by which the oyster regulates the rate of pumping of water through the gills.

Control of size of ostia is another mechanism which the oyster uses to regulate passage of water through the gills. The importance of ostia in feeding and respiration was correctly emphasized by T. C. Nelson (1940). Observing live oysters with the gills exposed on one side, I found that muscular contraction of the gills closes the ostia and that this method of regulating the dimensions of the ostia is much more important than filling and emptying of blood vessels of the gill filaments. The latter view has been maintained by Elsey (1935) but was not confirmed by my observations.

The lateral cilia which surround the ostia force the water into the water tubes and at the same time sort the particles suspended in water by throwing the large ones back to the surface of the filaments and by permitting the smaller ones to be drawn inside the gills. It is generally considered that the removal of particles from water is the function of the latero-frontal cilia. My observations show that this removal is incomplete and that the lateral cilia constitute another barrier which prevents the larger particles from passing through the ostia. In order to study ciliary currents the gills were
Fig. 2. Small portion of exposed surface of gill with expanded ostia. Horizontal arrows indicate direction of metachronic waves of the two rows of lateral cilia. Small particles of carbon (right) are sucked in while large particles (left) are thrown off by the recovery strokes of the cilia. Drawn from life--X 250.
exposed by removing parts of the shells, and phosphorescent powder (observed in ultraviolet light) or a suspension of colloidal carbon was added to the water. The behavior of the lateral cilia was recorded by the artist. It was clearly seen that while minute particles were sucked into the water tubes through the ostia, the larger ones were thrown back by the recovery strokes of the lateral cilia. In several instances the larger lumps of powder were tossed back and forth for a long time until they finally were discarded (Figure 2).

The control of ciliary motion is a problem of great physiological interest. Since the ciliary motion continues after the severance of the tissue from nerve ganglia, it is generally considered that ciliary activity is not under control of the nervous system although the possibility of such control was suggested by Lucas (1929, 1935) and others. I found that in the oyster with exposed gills, but otherwise intact, the motion of the frontal cilia and of the cilia of the terminal grooves of the gills ceases with the spontaneous contraction of the adductor muscle and is restored when the muscle relaxes. There is undoubtedly some sort of connection between muscular contraction and ciliary motion, but whether this control is exercised through the nervous system or by stimulation of the protoplasm of ciliated cells cannot be answered without a detailed physiological study. Electric shock applied directly to the ciliated epithelium produced no effect on ciliary rhythm.

The Pallial Organ

The pallial or abdominal organ of *Crassostrea virginica* is an inconspicuous little protuberance on the side of the adductor muscle in the right suprabranchial chamber. The surface of the organ is covered by long cells with still cilia (Figure 3). Numerous sensory cells with long processes are found compressed between the epithelial cells. Typical connective tissue underlies the epithelium. The large nerve with numerous branches ending at the periphery of the organ suggests that the pallial organ is of great importance to the oyster. The location of the organ inside the superbranchial chamber makes study of its physiology difficult (Figure 4). The organ probably serves for detection of mechanical disturbances in the water--a view which was advanced by several investigators who described this structure in various lamellibranchs, (Dahmen 1923, Awati and Rao 1931). It does not seem reasonable that the organ is concerned with chemical sense. The location of the organ in the suprabranchial chamber speaks against this possibility. Furthermore, it is a well established fact (Hopkins 1932) that the edge of the mantle, with tentacles which come in contact with water before it enters the mantle cavity, is very sensitive to irritating or poisonous substances and gives the oyster a warning signal to close the shell. The long stiff cilia for the pallial organ seem to be more suitable for detecting mechanical disturbances than for responding to chemical stimulation.
Fig. 3. Cross section of pallial organ showing long epithelial cells, narrow sensory cells on right and left sides of protuberance, and very large nerve trunk in connective tissues under the organ--X 100.
Fig. 4. Location of pallial organ (pal or) on wall of adductor muscle (ad m) in suprabranchial chamber which was cut and the opposing sides of mantle pulled apart. Accessory heart (ac h), mantle (m), and rectum (r). Drawn from life.
The observed details are of minor nature but they provide better understanding of the operation of the feeding and respiratory organs. Such an understanding is essential for the solution of practical problems of self-purification, feeding, and fattening of shellfish. The aim of these investigations is to learn more about the life of the mollusk in order to find ways of producing better oysters.

References


OBSERVATIONS ON DISTRIBUTION AND ELIMINATION OF SPORES OF NEMATOPSIS OSTREARUM IN OYSTERS*

Sung Yen Feng**

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Abstract

A non-random distribution of cysts of Nematopsis along the mantle margin of oysters was discovered. Sampling sites must be carefully chosen for comparative studies. When oysters with high and low initial infections of Nematopsis were transplanted into areas of low and high infestation respectively, the transplants attained the characteristic level of infections of native oysters in that area. Presumably a dynamic equilibrium of elimination and reinfection of the parasite was reached. Spores discharged from living oysters may infect crabs. -- S. Y. Feng.

INTRODUCTION

Nematopsis ostrearum, a sporozoan parasite of oysters, was first described by Prytherch (1938). Sprague (1950) emended the description. The known definitive hosts for N. ostrearum are three species of xanthid crabs, Panopeus herbstii, Eurypanopeus depressus, and Eurytium limosum, according to Prytherch (1940) and Sprague (1950). The most commonly recognized stages in crabs are mobile sporadins or gregarines and spherical gametocysts which produce gymnospores. Only vegetative spores or sporozites are found in oysters. These spores occur most abundantly in mantle tissues, but also in adductor muscle, heart, gills, labial palps, and perhaps other organs (Prytherch 1940, Sprague and Orr 1955). In this paper the peculiar distribution of cysts in the mantle margin and evidence of discharge of spores by oysters are investigated.

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Fig. 1. To determine distribution of cysts, the edge of the mantle was excised, measured, and subdivisions designated by letters. In three oysters, cysts were counted in a continuous band on the entire excised strip (Fig. 2) but in later groups carefully spaced samples were taken. In subsequent discussions and figures, data are arranged in sequence A to C (ventral mantle) and C to E (dorsal mantle). The shaded area beneath the adductor muscle was selected as a sampling site for other studies.
RESULTS

Distribution of *Nematopsis ostrearum* Cysts in Mantles of Oysters

Sprague discovered that in mantles of oysters, *N. ostrearum* cysts occurred predominantly in a band two mm wide, near and parallel to the margin. He also reported uniform distribution of cysts within the band, but this conclusion presumably was based upon examination of short sections of the mantle margin. In June and August, 1956, the author counted cysts in the band along the entire mantle margins of three oysters. A strip of tissue five mm wide was excised from the margin of the mantle, and lengths of ventral (A to C) and dorsal (C to E) mantles were measured as shown in Figure 1. The counts, from 305 to 393 microscopic fields of one mm$^2$ each, were grouped by zones, each representing 10 per cent of the mantle margin (Figure 2).

Great variations in counts were noted but typically there were many cysts opposite the adductor muscle and few cysts at the anterior and posterior ends of the mantles.

The burden of counting the entire mantle margin in heavily-infected oysters led to refinements of technique and the choice of oysters from an area of relatively light infections for further study of the pattern of distribution of cysts. On October 4, 1956, 20 oysters were collected from Hoghouse Bar in the Rappahannock River and opened by removing their cupped valves. In each oyster, incisions were made in the mantle of the flat valve at B and D to mark the position of the adductor muscle (Figure 1); the strip of tissue was excised, and the subdivisions were measured. Instead of counting cysts in the whole margin, the strips were divided into 10 equal sections. An 8 by 5 mm sample of tissue was taken from the center of each section and treated with 10 per cent KOH solution for one minute. The partially-digested tissue was placed between two slides and compressed until it attained a length of 10 to 12 mm. The preparation was examined at 100 X magnification under a microscope equipped with an ocular Whipple cell. The intensity of infections in each section was determined from the mean number of cysts per mm$^2$ calculated from the count in 10 fields. Each point in Figure 3 represents the mean value for 20 oysters.

Counts of 20 oysters confirmed the pattern observed in the first three oysters (Figure 3). The lowest numbers of cysts were observed at the anterior (oral) ends of the dorsal and ventral mantles. The number increased rapidly from the anterior ends of both mantles towards the adductor muscle. The greatest concentration of cysts occurred immediately above the adductor muscle in the dorsal mantle and slightly posterior to it in the ventral mantle. A decrease in the number of cysts was found posteriorly in the region of the pallio-branchial fusion.

This general pattern is usually shown by individual oysters including those with low counts but seems to be accentuated in those
Fig. 2. Variation in individual oysters in distribution of *Nematopsis ostrearum* along margin of flat-valve mantle. Each line represents counts made along the entire mantle margin of a single oyster. Areas enclosed by the two sets of broken lines mark position of adductor muscle.
Fig. 3. Mean distribution of Nematopsis ostrearum in margin of flat-valve mantle of oysters, October 1956. The mean count of 20 oysters is plotted. The area enclosed by the two sets of broken lines marks position of the adductor muscle.
with high counts. In any one oyster the highest concentration of
spores may occur in either the dorsal or ventral mantles.

Elimination or Disappearance of *N. ostrearum* Cysts 
from Mantle Margins of Oysters

Controversial opinions concerning the nature of *Nematopsis*
infections in oysters have been found in the literature. Prytherch
(1940), Lanadau and Galtsoff (1951), and Sprague and Orr (1955) note
that spores were more abundant in older oysters and, therefore,
believe that *Nematopsis* was cumulative in oysters. The occurrence
of spores in walled cysts probably encouraged this viewpoint. Owen
et al. (1951) were of the opinion that oysters eliminate spores be-
cause some old oysters comparatively free of spores were encountered.
Prytherch (1940) estimated that the sporozoites suffered a better than
50 per cent mortality from phagocytosis before they reached the final
stage in oysters. Stauber (1950) noted, "It is possible that in
addition to the mortality mentioned above, there is an important
sporozite loss due not to intracellular digestion by phagocytes but
to emigration and elimination of the sporozoite-laden phagocytes"
(p. 239). In the present studies, field experiments were carried
out to determine whether oysters did retain spores once they were
encysted in the tissues.

Experiment I

On May 2, 1956, three stations, representing the full range
of salinities in the oyster-growing areas of the James River-Hampton
Roads system, were selected for holding oysters in trays. The lower
station was Darling's Watchhouse (J4) in the relatively high-salinity
(20 parts per thousand) waters of Hampton Roads; the intermediate
station was Miles' Watchhouse (J12) with salinities of 16 ppt; and
the upper station at Deep Water Shoal (J24) had mean salinities of
about 7 ppt. Two groups of oysters were collected for the experiment
-- one from J4 where infections of *Nematopsis* were moderately heavy
(21 cysts per mm²) and the other from J24 where infections averaged
0.1 cysts per mm². At each of the three stations, oysters from the
two sources were placed in separate compartments of a tray which was
set on the bottom. On September 4, 1956, a sample of 25 oysters was
examined from each lot at the three stations.

After a period of four months, oysters with high initial in-
fec tions showed slight increases in number of cysts at J4 and J12,
and a significant decrease at J24 (Figure 4). Oysters with low initial
infections had a marked increase in number of cysts at the lower
stations, but no increase at J24.

*J4 indicates distance in nautical miles above the mouth of the
James River.
Fig. 4. Changes in level of *Nematopsis* infections when oysters were moved to areas characterized by lower and higher intensities of parasites. At each station (D.W., M.W., and D.W.S. representing Darling's Watchhouse, Miles Watchhouse, and Deep Water Shoal Light-house respectively), oysters with low and high initial infections (represented by horizontal lines) were placed in opposite ends of a tray on the bottom. The levels of infections four months later (September 4) are denoted by vertical bars. N is the mean number of cysts per mm².
At each station it appears that a characteristic level of infection was reached in a relatively short time. If cysts do accumulate in tissues, the numbers in oysters at J4 and J12 should have increased as much in heavily-infected individuals as in lightly-infected ones. At J24, oysters with heavy initial infections showed a decline in number of cysts from 21 to 6 cysts per mm$^2$, and this can be explained only by removal of cysts from the mantle. This decrease occurred during the warm summer season when spores are probably most abundant. Little is known of seasonal variations in infections and there is a possibility that spores may be redistributed in the oyster. However, this experiment strongly suggests that oysters can destroy spores or remove them from their tissues. Unfortunately the trays were lost after September and subsequent changes could not be followed.

Experiment II

The hypothesis that oysters eliminate or discharge cysts of *Nematopsis* is further supported by observations made on oysters in suspended trays. On June 1, 1955, oysters collected from Hoghouse Bar in the Rappahannock River, an area of relatively low level of infection, were placed in Trays 56, 57 and 59 suspended at Gloucester Point in the York River. These oysters had been fully acclimated during an 8- to 12-month period at Gloucester Point where both salinities and level of *Nematopsis* infections in oysters on natural grounds are high. On January 6, and June 5, 1956, respectively, Trays 56 and 59 were moved to the Fleet Pier near J24 in the James River. In this low-salinity area the level of *Nematopsis* infections in oysters is low. Tray 57 was retained at Gloucester Point and Tray 67 containing native Deep Water Shoal (J24) oysters was suspended at the Fleet Pier in the James; these two trays served as control groups for high- and low-incidence areas. On September 23 and October 27, 1956, samples of 25 oysters from each tray were examined and the results are given in Table 1.

The mean number of cysts in oysters decreased significantly in all trays with the exception of Tray 57 which was retained in a high-intensity area (Table 1 $\chi^2$ values). Since all trays were suspended off the bottom, most mud crabs were kept out and this may explain the relatively low intensity of *Nematopsis* infections at Gloucester Point. It must be remembered also that the experimental groups were moved to the low-salinity area several months before these examinations were made. The experiment was designed for purposes other than *Nematopsis* studies.

**Level of Nematopsis Infections and Distribution of Mud Crabs**

Surveys of James, York and Rappahannock Rivers revealed that species of crabs known to be hosts for *Nematopsis* were scarce in low-salinity (under 15 ppt) waters. Low incidence of *Nematopsis* at J24
Table 1. Decrease in number of spores of *N. ostrearum* in oysters suspended in trays between September 23 and October 27, 1956.

<table>
<thead>
<tr>
<th>Source of oysters</th>
<th>Tray no.</th>
<th>New location</th>
<th>No. of cysts per mm²</th>
<th>$\chi^2$</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-intensity area (Gloucester Point)</td>
<td>Experimental</td>
<td>56</td>
<td>James River Fleet Pier</td>
<td>5.7</td>
<td>2.9</td>
<td>24.109</td>
</tr>
<tr>
<td></td>
<td></td>
<td>59</td>
<td></td>
<td>10.7</td>
<td>2.1</td>
<td>19.474</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>57</td>
<td>Remained at Gloucester Point</td>
<td>5.2</td>
<td>4.6</td>
<td>9.384</td>
</tr>
<tr>
<td>Low-intensity area (Deep Water Shoals)</td>
<td>Control</td>
<td>67</td>
<td>Remained at James River Fleet Pier near Deep Water Shoal</td>
<td>0.14</td>
<td>0.07</td>
<td>5.997</td>
</tr>
</tbody>
</table>

1. Average number of cysts in samples of 25 oysters.

2. Based upon 24 oysters.
can probably be attributed to paucity of mud crabs and low death rate among oysters. The high-level of infection at J4 may have been accentuated by abundance of mud crabs around the watchhouse which had unusually favorable habitat niches. Furthermore, the death rate of oysters—-from causes other than Nematopsis—-is high at J4.

DISCUSSION

The non-random distribution of cysts in the oyster mantle reveals that standard counting techniques and sampling methods are essential; it also suggests that samples must be taken from various parts of the mantle margin in order to estimate accurately the total number of cysts in a particular oyster. For most purposes, however, it should not be necessary to make counts from numerous parts of the mantle, for by sampling carefully in a chosen site, comparisons between oysters and locations can be made. The shaded area of the left ventral mantle (Figure 1) where infections approach a maximum, is suggested as a sampling site.

The question arises: What are the causes of this peculiar distribution? Hopkins (1934, 1936) first discovered the accessory heart in oysters, observed much surging back and forth of blood in the circumpallial arteries, and advanced the theory that the circumpallial arteries received both arterial and venous blood. Stauber (unpublished data, 1957) has clearly demonstrated the pattern of blood circulation in the mantle margin by injection of green vegetable dye into the ventricle, which supplies the anterior circumpallial network, and red dye into the accessory heart which feeds the posterior circumpallial arteries at the fusion of left and right mantles; the two colors were found to meet in dorsal and ventral mantles in areas where the highest counts of cysts were observed. This suggests that distribution of N. ostrearam cysts in the mantle is probably associated with the pattern of circulation. It is possible that cysts tend to accumulate where blood flow is slowest. There are indications, however, that localization of cysts can not be explained, solely on the basis of mechanical transport; the special affinity of cysts for gill tissue in N. prytherchi and for mantle tissue in N. ostrearam suggests specific physiological requirements.

Seasonal patterns of infection, about which much more information is needed, are now of great importance. For example, if oysters are continually discharging spores which reinfect crabs, there is no need for an oyster to die to complete the cycle, and the incidence and intensities of infections may not be as closely related to the pattern of mortalities of oysters as previously suspected. Furthermore, the whole cycle may be speeded up considerably. It is already evident that Nematopsis infections are more common and the number of spores produced by crabs is much greater than had been assumed under the theory of accumulation of spores in the oysters. Stauber (1950, p. 239) conjectured that, "unless the hypertrophy of the sporozoite-
infected phagocyte restricts amoeboid activity, it is possible that elimination of developed spores in this fashion may constitute the more normal route of infection for crab hosts". The finding of substantial Nematopsis infections in James River, where deaths of oysters are few, tends to support this supposition. Viability of discharged spores could be easily tested by placing starved mud crabs (free of gregarines) in closed aquaria with live oysters heavily-infected with Nematopsis. If crabs become infected, it would prove that some spores are discharged; if not, the problem of disposition of spores would remain unsettled.

The exact mechanisms by which oysters remove spores is still a mystery; however, Stauber (1950) and Tripp (1957) have demonstrated the rates and methods by which India ink and metabolizable vertebrate red blood cells are digested or eliminated by phagocytes. Presumably Nematopsis is eliminated by similar activities. Probably temperature plays an important role in regulating the rate.

Many phases of the ecology of this parasite are still unsolved, though it now appears that cysts, which have been reported to be non-toxic, are even less harmful to oysters than was previously supposed—as indicated by the ability to discharge them and thereby prevent massive accumulations over long periods of time.

SUMMARY AND CONCLUSIONS

Distribution of Nematopsis ostrearum along mantle margins of oysters was non-random. Lowest numbers of cysts were observed at the anterior ends of dorsal and ventral mantles. Numbers increased posteriorly toward the adductor muscle. The greatest concentration of cysts occurred immediately above the adductor muscle in the dorsal mantle and slightly posterior to it in the ventral mantle. A decrease in number of cysts was found posteriorly in the region of pallio-branchial fusion. Techniques for estimating number of cysts in the mantle margin of oysters and the possible significance of this unusual distribution of cysts are discussed. A standard sampling site on the mantle is suggested for estimating levels of infection.

Evidence for the hypothesis that oysters eliminate or discharge encysted spores of Nematopsis ostrearum has been presented. It is possible that spores discharged from living oysters may infect crabs. Seasonal patterns of infection, mechanisms of discharge of spores, and toxicity of this parasite to oysters have not yet been adequately studied.
Literature Cited


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INCIDENCE AND LIFE HISTORY OF PARORCHIS ACANTHUS, A DIGENETIC TREMATODE, IN THE SOUTHERN OYSTER DRILL, THAIS HAEMASTOMA

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Abstract

This paper is the initial report of a program to develop biological control of the Southern Oyster Drill, Thais haemastoma, cause of considerable economic loss in some areas on the Gulf of Mexico.

Information on the life cycle, known hosts, reported localities, synonymy, and morphological descriptions of developmental stages of the digenetic trematode Parorchis acanthus has been compiled from various sources.

The pathology of Parorchis infection in T. haemastoma is briefly described and figured.

Preliminary data indicate that incidence of natural infection of P. acanthus in T. haemastoma is about 2% in Northwest Florida, 3.4% in Mississippi and less than 1% in Texas. All natural infections were massive, parasite larvae constituting 70 to 90% of the liver.

Parorchis infections experimentally induced in herring gulls (Larus argentatus), in ring-billed gulls (L. delawarensis), and in oyster drills are reported. Sexually mature Parorchis adults were first recovered from the cloacas of four out of four gulls 30 days after feeding each gull approximately 200 encysted metacercariae suspended in sea water. In oyster drills continuously exposed to droppings of Parorchis-infected gulls, the first infection was found on the 21st day of exposure and on the 33rd day of exposure, four of 33 examined were infected. The elapsed time and the recovery of only first generation rediae indicate that these were true experimental infections.

Possible use of P. acanthus as a biological control agent for T. haemastoma is discussed.
Introduction

Although very few data have been published, the Southern Oyster Drill, *Thais haemastoma*, is probably responsible for extensive economic loss to the Gulf oyster fishery. In an attempt to develop a method of biological control, the natural enemies of this snail are being investigated. Scattered observations on the parasites of *Thais* have been reported in the literature. Few of the papers containing these observations were concerned directly with the parasites. Schechter (1943) reported the emergence of *Parorchis acanthus* cercariae from occasional *Thais* from Barataria Bay, Louisiana. Butler (1953) noted that at least two trematodes parasitizing *Thais* in Louisiana and in the Pensacola, Florida, area may cause considerable damage to the gonad. During a study of the reproductive cycle of *Thais*, Butler (1956) found some drills heavily infected with larvae of parasitic worms which damaged the gonad so badly as to render it unfit for cytological study. The latter two observations suggested the possibility of biological control of *Thais* populations. Accordingly, in the summer of 1956, a survey of the parasites of this drill was begun in hope of finding a parasite which could be used for this purpose.

In September, 1956, heavy infections of a larval digenetic trematode, later identified as *Parorchis acanthus* Nicoll, 1907, were found in the livers of drills from Gulfport, Mississippi, and a study of this worm was begun. Since then, the parasite has been found in a number of drills from Biloxi, Mississippi, from Pensacola, Florida, and from Galveston, Texas. This paper is the first report of these investigations.

Life Cycle of *Parorchis Acanthus*

The life cycle, based on the descriptions of Stunkard and Cable (1932) and Rees (1940), is shown in Figure 1. The sexually mature worm, living in the cloaca and bursa Fabricii of the herring gull, releases eggs, each of which contains a completely developed miracidium. The miracidium contains a single well-developed redia. The egg hatches immediately after release into the cloaca of the gull, and the miracidium is discharged in the feces of the gull into the water. If the free-swimming miracidium does not enter a snail host, it dies in less than a day. Upon finding a snail, the miracidium apparently enters the aperture of the shell, passes up between the shell and body of the animal, penetrates the tunica propria of the liver at the spire and disintegrates, releasing redia (Redia I). The germ balls of Redia I give rise to a second generation of rediae (Rediae II) which penetrate deeper into the liver, ultimately lodging in the connective tissue between the hepatic tubules. In massive infections, Rediae II also invade the gonad and, according to Stunkard and Shaw (1931), may cause parasitic castration in *Urosalpinx cinerea*. The germ balls of Rediae II develop into cercariae only, no additional
Fig. 1. Life cycle of *Parorchis acanthus* (based upon Stunkard and Cable 1932, Rees 1940).
generations of rediae being produced. The mature cercaria, released through the birth pore of Redia II, leaves the snail, swims about briefly in the water, then settles down on any available submerged object and rapidly secretes a double-walled cyst membrane about itself while detaching its tail. The larva is now an encysted metacercaria. The metacercaria, when attached to food, is ingested by the gull, passes through the digestive tract and excysts in the lower intestine. The juvenile worm attaches itself, by means of a powerful ventral sucker, to the wall of the cloaca, bursa Fabricii and, less commonly, the lowermost part of the intestine and develops into a sexually mature adult.

A list of hosts of P. acanthus, sites of infection in drills, localities from which infections were reported and authorities are given in Table I.

Morphology of Parorchis Acanthus

The morphology of the different developmental stages had been described by a number of authors (Table 2) under the names of Zeugorchis acanthus, Parorchis acanthus, P. avitus, Cercaria sensifera, and C. purpurea. As far as I can determine from the literature, all are generally regarded to be synonyms of P. acanthus. I have seen all developmental stages, and although there are minor differences, my material is in general agreement with the published descriptions. A complete description of my material will be published elsewhere at a later date.

Effect of Larval Stages of Parorchis Upon Thais

Invasion of the snail host by any sizeable number of miracidia results in production of a very large number of second generation rediae (Figure 1). In all natural infections examined by dissection, rediae were easily seen with the naked eye through the transparent tunica propria covering the liver. In cross sections of unfixed livers they appeared as enormous numbers of tiny white wormlike objects interspersed among irregularly distributed small masses of liver tissue. All natural infections were rated massive, since 70 to 90% of the liver had been replaced by rediae. Any slight tear in the tunica propria permitted the escape of a flood of rediae and cercariae into the surrounding medium.

The gross appearance of cross sections of livers of infected and uninfected snails may be compared in Figure 2. The liver of the uninfected drill is variable in color, usually being light gray or beige, but may be very dark, almost black. Its consistency is like that of soft cheese. The surface is covered by a fairly tough tunica propria. The swollen liver of the infected drill is less variable in color, usually being lighter gray or light yellow. Its consistency is softer and its tunica propria more easily torn.
Table 1. Known hosts of Parorchis acanthus

<table>
<thead>
<tr>
<th>HOST</th>
<th>SITE OF INFECTION</th>
<th>LOCALITY</th>
<th>AUTHORITY</th>
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<tr>
<td><strong>ADULT STAGE</strong></td>
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<td>Gulls</td>
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<tr>
<td>Herring</td>
<td>Cloaca, bursa</td>
<td>Scotland</td>
<td>Nicoll 1906</td>
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<tr>
<td>Fabricii</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Mass</td>
<td>Wales</td>
<td>Linton 1914, Cable 1937</td>
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<tr>
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<tr>
<td>Western</td>
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<td></td>
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<tr>
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<td>Mass</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
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<td>Cloaca</td>
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<td>Willett</td>
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<td>Penner 1957, pers. com.</td>
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<td>Dowitcher</td>
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<td><strong>LARVAL STAGE</strong></td>
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<tr>
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<td>Mass</td>
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<td>Littorina planaxis</td>
<td>Liver</td>
<td>Calif</td>
<td>Penner 1957, pers. comm.</td>
</tr>
<tr>
<td>Melogena sp.</td>
<td></td>
<td>Florida</td>
<td>Penner 1957, pers. comm.</td>
</tr>
<tr>
<td>Batillaria sp.</td>
<td></td>
<td>Florida</td>
<td>Penner 1957, pers. comm.</td>
</tr>
<tr>
<td>Cerithium sp.</td>
<td></td>
<td>Florida</td>
<td>Penner 1957, pers. comm.</td>
</tr>
</tbody>
</table>

1See Clench (1947: 86-87) for synonymy.
Table 2. Developmental stages, synonymy, and sources of morphological descriptions of *Parorchis acanthus*.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Name given</th>
<th>Authority</th>
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<tbody>
<tr>
<td>Adult</td>
<td>Zeugorchis acanthus</td>
<td>Nicoll 1906</td>
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<tr>
<td></td>
<td><em>Parorchis acanthus</em></td>
<td>Nicoll 1907</td>
</tr>
<tr>
<td></td>
<td><em>Parorchis avitus</em></td>
<td>Linton 1914, 1928</td>
</tr>
<tr>
<td></td>
<td>P. avitus (immature)</td>
<td>Stunkard &amp; Cable 1932</td>
</tr>
<tr>
<td>Egg</td>
<td>Z. acanthus</td>
<td>Nicoll 1906</td>
</tr>
<tr>
<td></td>
<td>P. acanthus</td>
<td>Nicoll 1907</td>
</tr>
<tr>
<td></td>
<td>P. avitus</td>
<td>Linton 1914</td>
</tr>
<tr>
<td>Miracidium</td>
<td>Z. acanthus</td>
<td>Nicoll 1906</td>
</tr>
<tr>
<td></td>
<td>P. acanthus</td>
<td>Nicoll 1907, Rees 1940</td>
</tr>
<tr>
<td></td>
<td>P. avitus</td>
<td>Linton 1914</td>
</tr>
<tr>
<td>Redia</td>
<td>Cercaria purpureae</td>
<td>Lebour 1914, Rees 1937</td>
</tr>
<tr>
<td></td>
<td>C. sensifera</td>
<td>Stunkard &amp; Shaw 1931</td>
</tr>
<tr>
<td></td>
<td>P. acanthus</td>
<td>Rees 1940</td>
</tr>
<tr>
<td>Cercaria</td>
<td>C. purpureae</td>
<td>Lebour 1907, 1914</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rees 1937</td>
</tr>
<tr>
<td></td>
<td>C. sensifera</td>
<td>Stunkard &amp; Shaw 1931</td>
</tr>
<tr>
<td></td>
<td>P. acanthus</td>
<td>Lebour 1914, Rees 1937 &amp; 1940</td>
</tr>
<tr>
<td>Metacercaria</td>
<td>C. purpureae</td>
<td>Rees 1937</td>
</tr>
<tr>
<td></td>
<td>C. sensifera</td>
<td>Stunkard &amp; Shaw 1931</td>
</tr>
<tr>
<td></td>
<td>P. avitus</td>
<td>Stunkard &amp; Cable 1932</td>
</tr>
<tr>
<td></td>
<td>P. acanthus</td>
<td>Lebour &amp; Elmhirst 1922</td>
</tr>
</tbody>
</table>
Fig. 2. A. Liver of uninfected drill, transverse section, X 7.5. Note homogeneous appearance of normal tissue. B. Liver of drill with massive Parorchis infection, transverse section X 7.5. Whitish objects occupying most of liver are parasite larvae, gray patches are remaining liver tissue.
A comparison of stained cross sections of the livers of infected and uninfected drills (Figure 3) reveals the extensive displacement of the liver tissue produced by developing rediae. The histopathology of liver infections is characterized by:

(1) extensive displacement and reduction of liver tissue to 10 to 30% of the surface area of the cross section;

(2) compression of liver tubules resulting in obliteration of most if not all of their lumina;

(3) presence of basophilic inclusion granules of uncertain significance in the cytoplasm of the large triangular cells of the hepatic tubules, revealed by hematoxylin-azure B-eosin staining;

(4) presence of certain cells, thought to be phagocytic blood cells, in groups next to some of the parasite larvae; and

(5) apparent absence of direct destruction of liver tubules by invading parasites.

Invasion of the gonad was observed in a few specimens. The gonad of the uninfected drill is cream-colored or dark beige to brown, its size and thickness varying according to seasonal changes in the reproductive cycle; that of infected animals has been thin or patchy and dark yellow or orange in color. Whether the differences observed are an effect of infection or constitute normal seasonal changes is uncertain at this time.

Some Results of Experimentally-Induced Infections with *Parorchis Acanthus*

Gulls:

One herring gull (*Larus argentatus*) and three ring-billed gulls (*L. delawarensis*), known by repeated examinations over a one-month period to be *Parorchis*-free, were used in this experiment. Large numbers of cercariae obtained by dissection of naturally infected drills were permitted to encyst on the bottom of a Petri dish of sea water. The encysted metacercariae were scraped free with a scalpel, suspended in sea water, and several hundred pipetted into the esophagus of each gull before feeding the daily meal of chopped fish (usually mullet). This treatment was repeated a week later in order to insure infection of all birds. The fish, obtained locally, were quick-frozen, cut up into small pieces, and stored at 0° F for several weeks to prevent possible infection of the gulls with fish parasites. The fish were thawed just before feeding time. At intervals of seven to ten days, the cloaca of each bird was aspirated carefully with a medicine dropper pipette and the aspirate examined for worms.
Fig. 3. Effect of Parorchis infection upon Thais liver. Bouin, Weigert's iron acid hematoxylin and eosin, transverse section, 7 u, ca. X 50. A. Uninfected liver. B. Infected liver. Note extensive displacement and compression of liver tissue by parasites, occluded lumina of liver tubules.
No worms were found until the 30th day after first feeding encysted metacercariae, when sexually mature worms were recovered from each of the birds. For three months after being fed encysted metacercariae, none of the birds exhibited any symptoms of illness. However, three months and eight days after being fed cysts, the herring gull died suddenly. Three days later, one of the ring-billed gulls died, apparently in the same manner. Post-mortem examination failed to reveal the cause of death in either case, but mature Parorchis adults were recovered from the cloaca of each bird. The other two infected ring-billed gulls have shown no symptoms of illness during the slightly more than four-months period of their infections, although they are now (July) somewhat less greedy at feeding time than they were when captured last January.

Drills:

This experiment was designed to determine whether (1) Parorchis infections could be induced in large numbers of Thais in outdoor tanks and (2) whether there is any relationship between the size of the drill and its susceptibility to infection.

The experiment was performed in two 10 by 20 foot concrete tanks. Running sea water, at a constant depth of about 4 inches (roughly 2 cubic meters of water), was supplied to each tank at the rate of approximately 2 gallons per minute, permitting a complete change of water in about 4½ hours.

Into each tank a lot of 653 snails was introduced from the rocky shore of our laboratory island in Santa Rosa Sound. Each lot consisted of 408 small drills (15 to 35 mm long) and 245 large drills (45 to 60 mm long). These size ranges were chosen arbitrarily to facilitate recognition of each size class. During the 33 days of the experiment, no food was supplied to the drills.

Three ring-billed gulls and one herring gull, known to be infected with sexually mature Parorchis adults and discharging miracidia in their feces, were hung in hardware cloth cages from the walls of the experimental tank; a single ring-billed gull, known to be free of Parorchis infection, was placed similarly in the control tank. The droppings of the gulls fell freely and directly into the water in each tank. The birds were fed daily either frozen small pinfish (Lagodon rhomboides) or frozen chopped mullet (Mugil cephalus).

Repeated sampling of the drill population about our laboratory island over a period of several months has never revealed infected snails, suggesting that incidence of Parorchis infection in the population is very low, if not absent. The incidence in nearby Pensacola Bay, on the other hand, is slightly above 2%. Thus, it was decided that a sample of 5% of the total number of drills in each group (roughly 2.5 times the natural incidence in the bay) should be adequate
to reveal any induced infections. Accordingly, 33 drills (about equal numbers of large and small individuals) were removed from each group initially and on the 13th, 21st, and 33rd days of exposure to gull droppings. All snails without visible infections when the liver was examined under a dissecting microscope were fixed in Bouin's fixative (Allen's modification) and stained sections were examined with a compound microscope to detect light infections.

No infections were observed in the samples prior to the 21st day, when one small infected snail was found. On the 33rd day, 2 large and 2 small drills (about 12% of the sample) proved to be infected. All infections were light and stained sections contained only first generation rediae, indicating that these were bona fide experimental infections. Although the time required for the development of each larval stage is unknown, these observations indicate that an extended period is required.

It is difficult to explain satisfactorily the low incidence of infection in this experiment in which it appeared certain the experimental snails would be exposed to a high concentration of miracidia. One might speculate that these results were due to (a) resistant snails (see Hyman 1951, pp. 307-308), or death of most of the miracidia before they could penetrate snails (See Linton 1941, pp. 379-381), or a combination of these causes. However, this experiment must be repeated under various conditions before a precise explanation can be given.

On the basis of this study we may conclude that (1) Parorchis infections were experimentally induced in Thais; (2) For some unexplained reason the rate of infection was low; and (3) There seemed to be no important difference in the susceptibility of large and small drills to infection with Parorchis.

Incidence of Natural Infections of Parorchis Acanthus in Thais

Although the survey of the natural incidence of infection in drills from oyster-producing areas of the Gulf Coast region begun in the fall of 1956 has not been completed, some preliminary data from 2050 drills can be reported at this time (Table 3). These drills were collected in Galveston Bay, Texas, from Mississippi Sound, at Biloxi and Gulfport, Mississippi, and in Pensacola and Apalachicola Bays, Florida. Only massive infections detected by dissection are reported.

During repeated sampling of the drill population of the Pensacola Bay area, some samples of a hundred or more drills contained no infected snails, while in other samples of comparable size from different locations up to 7% were infected. Samples from one point on the north shore of the bay, where large numbers of herring and ring-billed gulls roosted during the fall and winter, almost invariably
Table 3. Incidence of Parorchis acanthus infections in Thais from three oyster-producing areas.

<table>
<thead>
<tr>
<th>Area</th>
<th>Number examined</th>
<th>Snails</th>
<th>Infected</th>
<th>Number</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northwest Florida</td>
<td>1123</td>
<td>23</td>
<td></td>
<td>2.05</td>
<td></td>
</tr>
<tr>
<td>Mississippi</td>
<td>475</td>
<td>16</td>
<td></td>
<td>3.37</td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>452</td>
<td>3</td>
<td></td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>2050</td>
<td>42</td>
<td></td>
<td>2.05</td>
<td></td>
</tr>
</tbody>
</table>
contained infected drills, while another area on the south shore where few gulls were noted, sampled on four occasions from November 1956, to February 1957, yielded only one infected snail out of 196 examined.

Of several hundred drills collected from the rocky shore of our laboratory island in Santa Rosa Sound, none was infected. Only a comparatively few gulls frequented the laboratory area during the past fall and winter.

The large gull populations which build up during the fall and winter, although important, are not the only source of Parorchis infection in the Pensacola area, for willets, dowitchers, and water turkeys, reported by Penner (1957) to harbor the adult worm elsewhere in Florida, also occur here to some extent, and could constitute an additional source of infection.

Possible Use of P. Acanthus in Biological Control of the Oyster Drill

The serious drill predation existing in certain areas of the Gulf oyster fishery requires development and institution of effective drill-control measures.

Although the natural incidence of Parorchis infections in Thais is low, it is evident that it causes considerable damage to the individual drill. This experimental work demonstrates that Thais can be experimentally infected with P. acanthus. The percentage experimentally infected was low, but still higher than natural incidence. Further study to determine how the rate of infection may be increased to the extent necessary to utilize this parasite as a biological control agent is warranted by this initial success.

Acknowledgments

The personnel of the United States Fish and Wildlife Service Shellfishery Laboratory, Gulf Breeze, Florida, furnished varied assistance: field collections of drills were made; Figure 1 was drawn by Charles R. Chapman and photographed by Jack I. Lowe; and Dr. P. A. Butler and Charles R. Chapman contributed certain unpublished data. Other field collections of drills were made by Dr. Abraham Fleminger, United States Fish and Wildlife Service, Gulf Fishery Investigations, Galveston, Texas, and William Demoran, Gulf Coast Research Laboratory, Ocean Springs, Mississippi. Dr. Lawrence R. Penner, Zoology and Entomology Department, University of Connecticut, kindly permitted the use of unpublished data. The aid of these individuals is gratefully acknowledged.
Literature Cited


Penner, L. R. 1957. Personal communication: letters dated 8 April and 11 May 1957.


THE CROWN CONCH Melongena CORONA Gmelin; ITS HABITS, SEX RATIOS, AND POSSIBLE RELATIONS TO THE OYSTER.1

Ralph R. Hathaway
Oceanographic Institute
Florida State University

Abstract

Studies on the crown conch in northwest Florida from St. Marks to Panama City indicate large populations living on soft, protected intertidal bottoms, often associated with oysters, and usually displaying sex ratios in favor of females. Animals in natural habitats, tagged and observed for up to 193 days, confined their movements to small areas. Tagged animals removed up to 150 m from their habitat quickly moved back into it. Attempts to observe captive crown conchs feeding on oysters were unsuccessful, although this was often seen among free animals on oyster reefs. Sample populations from oyster reefs were made up of large, predominantly female crown conchs. Adjacent soft bottoms and high intertidal areas of protected beaches yielded smaller animals and greater numbers of males. The ability of an intertidal oyster to hold its valves closed upon the proboscis of a crown conch for hours during low tide is cited as an argument against the idea that Melongena kill their prey by secreting a toxin from the proboscis. It is concluded that crown conchs are not serious predators of oysters.

Introduction

In recent years biologists and fishermen alike have become more aware of the importance of fundamental biological information in solving problems faced by commercial fisheries. This is a well recognized fact in the oyster industry, as is shown by its interest in the National Shellfisheries Association. Here, and in many other places, the biological and physical milieu of the oyster Crassostrea virginica Gmelin is explored in great detail. Animals associated with oysters account for a large part of this effort, and

1 Contribution No. 86 Oceanographic Institute, Florida State University. This work was supported by contract 14-19-008-2307, U. S. Fish and Wildlife Service. The data are taken from a thesis in partial fulfillment of the requirements for a Master of Science degree, Florida State University, under the direction of Dr. R. W. Menzel.
of these, other mollusks are a prominent element. Gastropods known to prey on *Crassostrea* are subjects of active research, while other snails, whose relation to oysters is uncertain, remain to be investigated. The crown conch, *Melongena corona* Gmelin, is one of this latter group.

Previous studies on the crown conch are reported by Clench and Turner (1956). This report indicates there is no planktonic larval form, that the snails feed on a variety of mollusks and detrital material, and that there is a remarkable extension of the proboscis in the act of feeding. These authors define two subspecies, *M. corona corona* and *M. corona johnstonei*, blending in a "cline" in the region of Apalachee Bay, Wakulla County, Florida.

The present study was undertaken on the crown conch because of its abundance and frequent association with oysters on the west coast of Florida. Sex ratios, habitats, migrations, and possible relations to the commercial oyster were examined.

Methods and Areas Studied

Methods consisted of field observations of undisturbed animals, sex and length measurements, and techniques devised to study feeding and migration. These animals are sexed easily and accurately upon extraction from their shells after immersion in boiling water for five minutes. It has been necessary to define four sex classes: definitive male, definitive female, presumptive female, and male-with-small-penis. A large penis or capsule gland characterizes the definitive male and female classes, respectively. Absence of any kind of penis or capsule gland defines presumptive females, and male-with-small-penis is defined as it is named. These sex classes cannot be differentiated by any live-sexing technique. Hargis (1957) emphasizes the advisability of finding either a penis or a capsule gland in order to determined positively sex in *Urosalpinx cinerea*. Similar emphasis must be given to sexing in *Melongena*. Length was measured to the nearest mm from the apex of the spine to the end of the siphonal canal. Crown conchs were kept in laboratory aquaria and in wire cages in natural habitats with the intention of getting numerical information on oyster predation. Migration was checked by marking or tagging several dozen animals in the field and attempting to follow their movements by repeated trips to the habitats. The subspecific differences noted by Clench and Turner (1956) were not studied and both groups are treated together in this paper.

Areas covered by this study consist of the Northwest coast of Florida from Apalachee Bay to St. Andrew Bay. Specific areas were: (1) St. Marks estuary; (2) Alligator Harbor; (3) Indiana Lagoon in Apalachicola Bay; (4) St. Joseph Sound; and (5) St. Andrew Bay near Panama City, Florida. Apalachee Bay has a poorly defined shoreline consisting of great salt marshes and expanses of grass flats of the
species Syringodium filiforme, Diplanthera wrightii, Halophila engelmannii, and Thalassia testudinum. The bay is shallow, and influx of fresh water from numerous rivers, especially the St. Marks and the Ochlockonee, give much of it an estuarine character. The sounds to the west, including Alligator Harbor and the complex called Apalachicola Bay, are shallow and muddy, and protected by offshore barrier beaches. The oyster industry of Apalachicola Bay is centered where fresh water discharge from the Apalachicola River maintains lower salinities favored by Crassostrea virginica. West of Cape San Blas, where the harbors of Fort St. Joe, Panama City, and Pensacola open to deep oceanic waters a few miles offshore, influence of fresh water is less obvious. Oyster beds occur only in the upper reaches of these bays.

Results

Results may be considered under three headings: habitats, populations, and relations to oysters. Habitats along the varied coastline from Apalachee Bay to Panama City abound with Melongena. They occur on intertidal bottoms that are protected and soft, and in a high salinity range. Very young animals occur in high intertidal zones, especially in salt marshes. Older animals are found on intertidal oyster reefs and nearby subtidal flats, as well as in areas where there are no oysters. Nevertheless, crown conchs are always found in or near an intertidal zone. Those on oyster reefs will bury in winter, or if the reef is too hard for this, will move to adjacent soft bottoms.

Populations have been analyzed in regard to sex ratios, length distribution, and migration.

Sex ratios vary significantly from unity. Those found in this study are tabulated below. Samples from the reef in Indian Lagoon indicate more females than males in a population that lacks small animals. Snails from Indian Lagoon bottom are mainly small male animals. Snails from St. Marks were collected both on and off oyster reefs. These samples range in size from very small to large, and display variable sex ratios. Crown conchs from St. Andrew Bay and St. Joseph Bay were not associated with oysters. These samples were of great size range and variable sex ratios.

Migration studies indicate fast and direct movements by Melongena towards its original position after it has been removed up to 150 meters from its habitat. Undisturbed crown conchs remain within small areas of their preferred habitat for many months. These results are based on observations of 329 animals in four locations for up to 193 days. Evidence for large scale migratory activity was not seen, however, absence of small individuals suggests that populations on oyster reefs are immigrants. Crown conchs associated with oysters appear to be older, predominantly female animals that have moved in from other areas.
<table>
<thead>
<tr>
<th>Place and time of Sampling</th>
<th>No. of females per 100 snails</th>
<th>Size of Sample</th>
<th>Average Length mm</th>
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<tr>
<td><strong>Indian Lagoon Reef</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11 Sept. 1956</td>
<td>69</td>
<td>79</td>
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</tr>
<tr>
<td>17 Nov. 1956</td>
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<tr>
<td>30 Jan. 1957</td>
<td>60</td>
<td>137</td>
<td>114</td>
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<tr>
<td><strong>Indian Lagoon Bottom</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>30 Jan. 1957</td>
<td>28</td>
<td>46</td>
<td>77</td>
</tr>
<tr>
<td><strong>St. Marks</strong></td>
<td></td>
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<tr>
<td>9 Sept. 1956</td>
<td>64</td>
<td>146</td>
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<td>23 Sept. 1956</td>
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<tr>
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<tr>
<td>16 Jan. 1957</td>
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<td>29</td>
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<tr>
<td>14 Feb. 1957</td>
<td>43</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td>14 Feb. 1957</td>
<td>58(^2)</td>
<td>29</td>
<td>55</td>
</tr>
<tr>
<td>30 Mar. 1957</td>
<td>48(^2)</td>
<td>144</td>
<td>74</td>
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<tr>
<td><strong>St. Andrew Bay</strong></td>
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<tr>
<td>13 June 1957</td>
<td>39</td>
<td>96</td>
<td>84</td>
</tr>
<tr>
<td>18 June 1957</td>
<td>57</td>
<td>90</td>
<td>81</td>
</tr>
<tr>
<td><strong>St. Joseph Bay</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 June 1957</td>
<td>62</td>
<td>144</td>
<td>65</td>
</tr>
</tbody>
</table>

1. Include definitive female and presumptive female sex classes.

2. Not significantly different from 50 by Student's t test.
Relations to oysters are obscured by conflicting observations. It is common to find a Melongena feeding on an oyster whose valves are firmly closed upon the snail’s proboscis. Feeding of crown conchs on oysters has been studied in basket experiments and in aquaria. In none of these experiments was a crown conch seen to successfully attack and consume an oyster, although an unsuccessful attack was observed. This was seen among six Melongena kept in aquaria for 2 months. It occurred in the manner described by Gunter and Menzel (1957), the snail making a prolonged investigation of the oyster, and finally ejecting its proboscis in an attempt, presumably, to insert it between the oyster’s valves. This mode of attack appears to be clumsy when directed towards oysters since the opening between the valves is relatively small in this species.

Crown conchs confined in wire baskets did not increase the mortality of oysters kept in the same baskets. Six Melongena were observed under these conditions for up to 38 days. Snails in captivity were seen to continue such activities as mating and laying, but it is difficult to know if this behavior is representative of that which occurs in natural habitats. Feeding activity may be especially sensitive to unnatural conditions. A snail with the carnivorous habits of M. corona would be expected to be attracted to an oyster reef and there to make numerous attacks on oysters. If only a small percentage of attacks are successful, the limited number of observations on captive conchs might not include any successful attacks, while one might be observed occasionally among the much larger number of snails on a reef. Any reduction in feeding activity due to captivity would make the observation of successful attacks by captive animals even more unlikely. Also, there is the consideration that high summer mortality among Florida oysters may induce crown conchs to feed on dead and weakened animals, producing an exaggerated picture of Melongena predation on healthy oysters.

On an intertidal oyster reef exposed at low tide the sight of an oyster tightly closed upon the proboscis of a crown conch raises questions regarding the manner in which these snails kill their prey. If a toxin were being released from the proboscis the oyster would not be expected to remain closed long after the proboscis was inserted. It would appear more likely that Melongena kills oysters by mechanical action of the proboscis, since it is possible to find oysters that have held tightly to the proboscis for many hours, or at least since the receding tide exposed them. Upon exposure to air the conch attacking an oyster reduces its body activity, including that of the proboscis. Predator and prey then maintain a status quo until the tide comes in and covers them, at which time action of the proboscis begins again.
Conclusions

M. corona are abundant along the miles of shoreline in Northwest Florida where soft, protected, and intertidal bottoms provide ideal habitats for this animal. They usually remain within a small area throughout the year, and probably throughout their lives. Small individuals are not found on oyster reefs, indicating that Melongena populations associated with oysters are immigrants. Sex ratios vary from unity, most often in favor of females. This is most definite among crown conchs on oyster reefs. In addition, Melongena on oyster reefs are larger than those found elsewhere. Predation by crown conchs on oysters occurs under natural conditions, but experiments indicate Melongena is not a serious predator on Crassostrea.

Literature Cited


The 1957 Convention of the National Shellfisheries Association and the OysterGrowers and Dealers Association of North America was held July 21 to 25 at the Belmont Plaza Hotel, New York City. Through the courtesy of Frank M. Flower and Sons of Bayville, Long Island, oystermen and scientists were able to see a suction dredge and other equipment in operation. A new feature of this convention was the reports of laboratory directors on research in progress and plans for the future. This half-day session was sponsored by the Research Committee which was composed of industry and scientific members. Presentation of papers and attendance by oyster planters and biologists from the West Coast were encouraging signs of progress.

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A BRIEF HISTORY OF THE NATIONAL SHELLFISHERIES ASSOCIATION

During the early years of the present century, a need was felt for a national organization wherein those state and federal officials charged with conserving and administering the extensive shellfisheries of our nation might meet, exchange ideas, and gain information from scientists who were studying biological and sanitary problems related to the industry. The first organizational meeting was held in New York City on January 15, 1909 and on May 5 of that year a constitution and by-laws for permanent organization of a National Association of Shell Fish Commissioners was presented.

Successive meetings attracted increased attention from state officials, scientists and industry representatives. As scientists in fishery biology, sanitation, and nutrition became active in the Association, a revised constitution and by-laws was adopted on August 19, 1930, and a more descriptive name, The National Shellfisheries Association, was chosen.

The first joint meeting with the Oyster Growers and Dealers Association was held in 1929. In 1937 the Oyster Institute of North America became a part of the Annual Oyster Convention. Annual joint meetings of the three organizations, held in major cities along the Atlantic Coast, have been centers of attraction for all people concerned with shellfish.

The National Shellfisheries Association is a unique organization in that fishery administrators, business men, and fishery biologists meet to discuss their problems with mutual benefits. Reports of the latest research on shellfish are brought to the attention of administrators and members of the industry. During recent years, these reports and addresses have been reproduced and distributed annually to members as the Proceedings.
Candidates for membership in the Association should submit completed application forms to the Secretary-Treasurer for review by the Membership Committee.

NATIONAL SHELLFISHERIES ASSOCIATION
Application for Membership

Date____________________

Name__________________________________________________________

Mailing Address__________________________________________________

Official Title or Position__________________________________________

Special Interests__________________________________________________

Mail completed blanks to Secretary-Treasurer, Dr. Philip A. Butler,
U. S. Shellfishery Laboratory, Gulf Breeze, Florida.

Dues are $2.00 per year and include the Annual Proceedings. Make check payable to the National Shellfisheries Association.
EDITORS' NOTES

Volume 48 has been reproduced by the Xerox-offset process by the Duplicating Department of the University of North Carolina at Chapel Hill. Part of the cost of duplication was borne by the Oyster Institute of North America, whose help is greatly appreciated.

The Secretary-Treasurer reports that 419 copies of Volume 47 were distributed which included 208 to industry members, 135 to Association members, 62 to libraries in the United States, and 14 to foreign libraries. Copies are sent to libraries without charge. Author abstracts are collected and sent by the Editor to Biological Abstracts.

At best, editorial work is burdensome to the Editorial Committee and often irritating to the author. Much time and effort can be saved for both parties if authors will examine carefully for style the most recent volume of the PROCEEDINGS and use this as a model. Please note that all figures and tables must fit on an 8 x 10½ inch page with ample margins. Style, spacing, and general format should follow closely the examples in Volume 48.

Considerable confusion has arisen about submitting papers written in a style best fitted for oral presentation. For publication in the PROCEEDINGS, papers written in the best scientific style are desired. The content does not need to be limited to material presented at the Convention. Other papers will be considered for publication.

INFORMATION FOR CONTRIBUTORS

Original papers given at the Annual Association Convention, and other papers on shellfish biology or related subjects submitted by members of the Association will be considered for publication. Manuscripts will be judged by the Editorial Committee or by other competent reviewers on the basis of originality, contents, clarity of presentation and interpretations. Each paper should be carefully prepared in the style followed in previous PROCEEDINGS before submission to the Editorial Committee. Papers published or to be published in other journals are not acceptable.

Manuscripts should be typewritten and double-spaced; original sheets are required but carbon copies, if available, will facilitate reviews. Tables, numbered in Arabic, should be on separate pages with the title at the top. Scientific names should be underlined.

Illustrations should be reduced to a size which fits on 8 x 10½ inch pages with ample margins. Glossy photographs are preferred to originals. Illustrations smaller than a page should be carefully oriented and loosely attached to plain white paper with
rubber cement. Legends should be typed on separate sheets and numbered in arabic.


Each paper should be accompanied by an abstract which is concise yet understandable without reference to the original article. It is now our policy to publish the abstract at the head of the paper and to dispense with a summary. A copy of the abstract for submission to Biological Abstracts will be requested at a later date.

Reprints and covers are available at cost to authors. Master sheets will be retained for one year after publication. When proof sheets are returned to authors, information about ordering reprints will be given. The present agency from which authors may obtain reprints is the Duplicating Department, Bingham Y, University of North Carolina, Chapel Hill, N. C., Mr. J. Nelson Callahan, Head.

For inclusion in the PROCEEDINGS of the current year, all manuscripts should reach the Editor prior to October 1 following the summer Convention. Send manuscripts and address all correspondence to the Editor, Jay D. Andrews, Virginia Fisheries Laboratory, Gloucester Point, Virginia.
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