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1953 CONVENTION PAPERS

**NATIONAL
SHELLFISHERIES
ASSOCIATION**



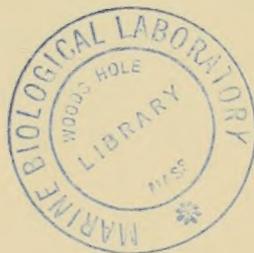
PAPERS DELIVERED
AT THE CONVENTION OF THE
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OBSERVATIONS ON THE RATE OF PROPULSION OF WATER AND RETENTION OF COLIFORM BACTERIA BY THE OYSTER

By Paul S. Galtsoff, Research Biologist, Shellfish Laboratory, Fish and Wildlife Service, Woods Hole, Massachusetts, and William Arcisz, Bacteriologist, Shellfish Sanitation Laboratory, U. S. Public Health Service, Woods Hole, Massachusetts

For respiration and feeding, oysters and other pelecypod mollusks depend on a continuous propulsion of water through their gills. The pumping mechanism consists of a very complex system of folds and tubes covered by rows of ciliated cells some of which beat inward and force the water through the small openings, so-called ostia, into the water tubes and cloaca. During this passage some of the particles suspended in water are retained by the gills. They are enmeshed in mucus secreted by the gill epithelium and are pushed toward the mouth and into the digestive tract. Part of this material may be discarded before it has reached the mouth. It is usually referred to as pseudofeces.

Two organs, independent of the pumping mechanism of the oyster, regulate the access of water to the gills. The adductor muscle controls the opening and closing of the oyster shell, while the mantle guards the gill chamber. The free edges of the mantle are beset with highly sensitive tentacles and the entire organ, abundantly supplied with muscle fibers and nerves, retracts or expands depending on circumstances. The two opposing edges of the mantle may remain far apart closely adhering to the shell and providing free access of water to the gills. They occupy this position when the oyster is actively feeding. On the other hand, they may bend toward each other, the tentacles of the opposite sides interlocking and completely closing the gill chamber. In this position no water has access to the gills in spite of the fact that the shell is open.

Bacteriological studies (Gorham, 1912; Round, 1914; Hunter and Harrison, 1928) and physiological experiments (Galtsoff, 1928; Hopkins, 1933; Th. Nelson, 1938) firmly established the fact that the rate of propulsion of water decreases or increases with the fall or rise in temperature. Water currents produced by the ciliated epithelium of the gill cease completely at 40°F.; they reach the maximum at about 80°F. The response of the isolated gill epithelium to temperature changes is so definite that the relationship may be expressed by a suitable mathematical equation and represented graphically by a curve. Feeding behavior of an intact oyster is, however, more complex. It is true that the pumping capacity of the gill epithelium is controlled by temperature, but within the limits attainable at a given temperature the oyster may change the rate of propulsion of water, apparently, in response to its needs. As an illustration, we may cite an experiment conducted in our laboratory in which the oyster, kept under stable conditions, propelled the water at three distinctly different rates. This is clearly shown in a diagram summarizing 90 hours of continuous recording made on a large adult oyster kept in running sea water at the temperature of 16.8°C. (Fig. 1). Dur-

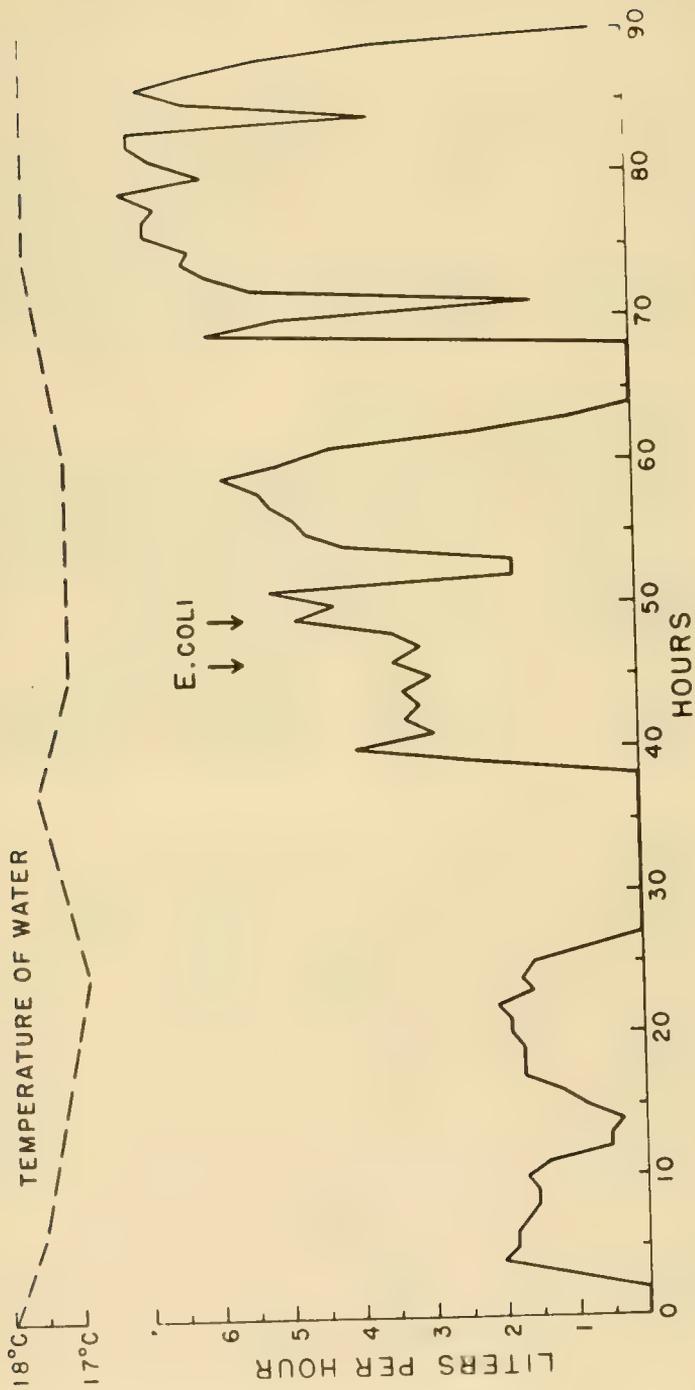


Figure 1. Ninety hours of continuous record of the rate of propulsion on water by the oyster. Note three distinct levels of activity of the oyster in spite of no significant changes in temperature of the water.

ing the first period of activity the rate of propulsion did not exceed 2 liters per hour; during the second period it varied between 1.7 and 5.8 liters per hour, and at last it increased to a higher level between 3.5 and 7.2 liters per hour. The recording was made by means of constant level tanks (Galtsoff, 1926; Galtsoff and Chipman, 1949) combined with the apron technique of Nelson (1935, 1936). The general arrangement of the apparatus used is shown in Figure 2. For the purpose of the present investigation it was found convenient to record the rate of propulsion of water not by a dumping vessel previously described by Galtsoff and Chipman (1949) but by means of a small water wheel. Each revolution of this wheel, recorded by the writing lever, corresponded to the discharge of 80 ml. of sea water. The wheel was made of plastic; it was calibrated by pouring measured volumes of sea water and recording the number of revolutions. Its accuracy was found to be \pm 5 percent.

Substances to be tested were delivered either through a mixing chamber (Fig. 2) or added directly to the gills through a capillary tubing placed in front of the gaping valves of the oyster. Each test was started by adding plain sea water. After 20 minutes of operation, the two-way stopcock was turned to deliver the material which was being tested. It was found that a continuous stream of water striking the mantle and gills has no effect on shell movement and on ciliary motion.

Oysters used in these experiments were large sized adults from Prince Edward Island. They were kept for at least 60 days in the laboratory tanks and were fully adjusted to their environment. Their dimensions were as follows: Size--14.6 by 8.0 and 11.7 by 7.1 cms.; total weights--305 and 227 grams; weight of meat--45.1 and 35.8 grams, wet; and 7.7 and 4.1 grams, dry. The rate of propulsion of water at the time of the experiments varied between 3 and 4 liters per hour. The temperature range was 13.0 to 13.2 in the first experiment and 16.8 and 18.0°C. in the second one.

The following bacteriological procedure was employed: A suspension of Escherichia coli was prepared by washing the growth from an agar slant containing approximately 25 billion organisms into 100 ml. of phosphate buffered dilution water. Appropriate dilutions were made of the initial suspension so that when a calculated amount was added to the sea water entering the mixing chamber and oyster tank, it contained between 20,000 and 30,000 E. coli per 100 ml.

The reaction of oyster to the presence of an irritating or harmful substance in the water usually consists in a series of partial or complete closures of the valves and in the reduction in the rate of propulsion of water. When the increase of its concentration is gradual and the disturbing agent acts slowly, the oyster responds by typical "stairway" contractions of the adductor muscle, each consecutive contraction starting from the level reached before. As can be seen from the records obtained during our experiments (Figs. 3, 3a and 4) the oyster remained completely indifferent to the presence of E. coli in the concentrations varying from 20,000 to 35,000 per 100 ml. Likewise, heavy suspensions of E. coli of about 500,000 organisms per 100 ml. supplied directly to the mantle and gills have no effect on shell movements and on rate of water propulsion.

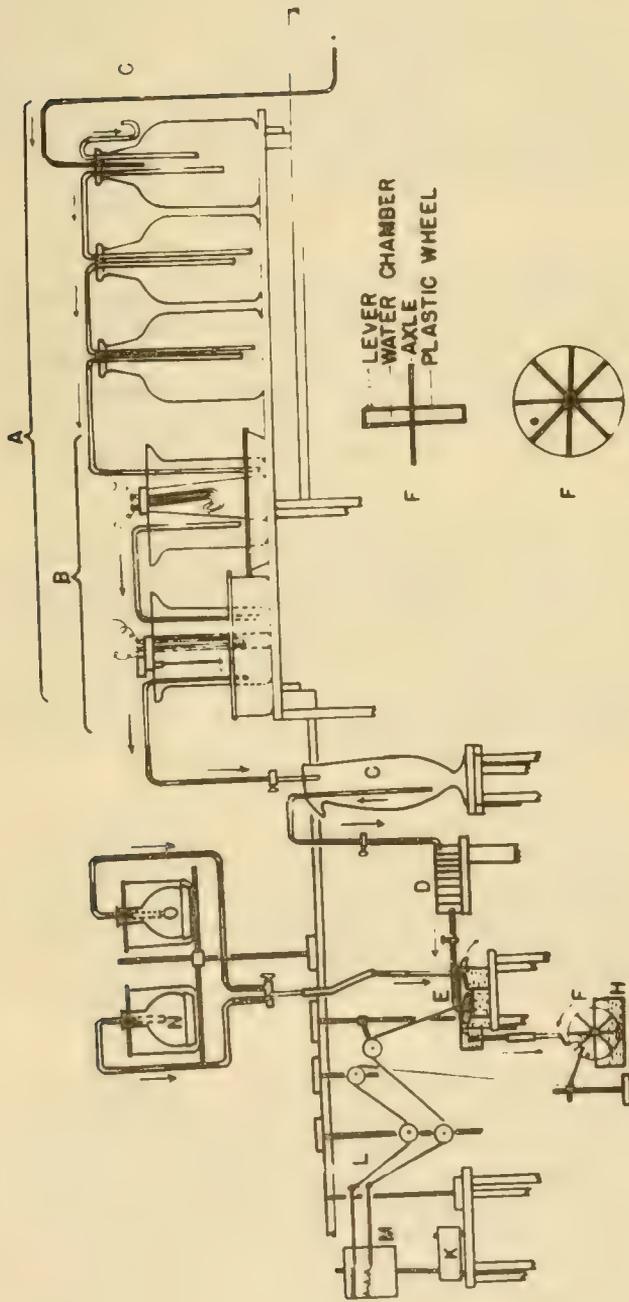


Figure 2. Diagram of the setup used in the experiments.

- A. Glass jars through which sea water is run from the supply.
- B. Jars in which water is being heated by electrically controlled heaters.
- C. Supply jar from which water is being fed through mixing chamber.
- D. Mixing chamber.
- E. Constant level tank in which the oyster is kept.
- F. Water wheel, on which the oyster effluent drops.
- K. Kymograph for recording the revolutions of water wheel.
- L. Lever which records revolutions of water wheel.
- M. Lever which records the shell movement of the oysters.
- N. Flasks used to supply the material either to the mixing chamber or directly to the constant level tank.

O and N.

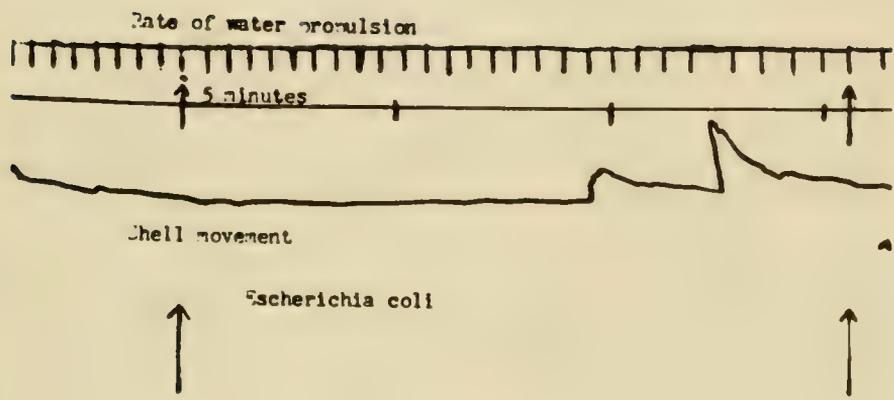


Figure 3. Shell movement and rate of water propulsion of oyster recorded by means of the water wheel. It shows no reaction of oyster where E. coli was added. (The space between the two arrows.)

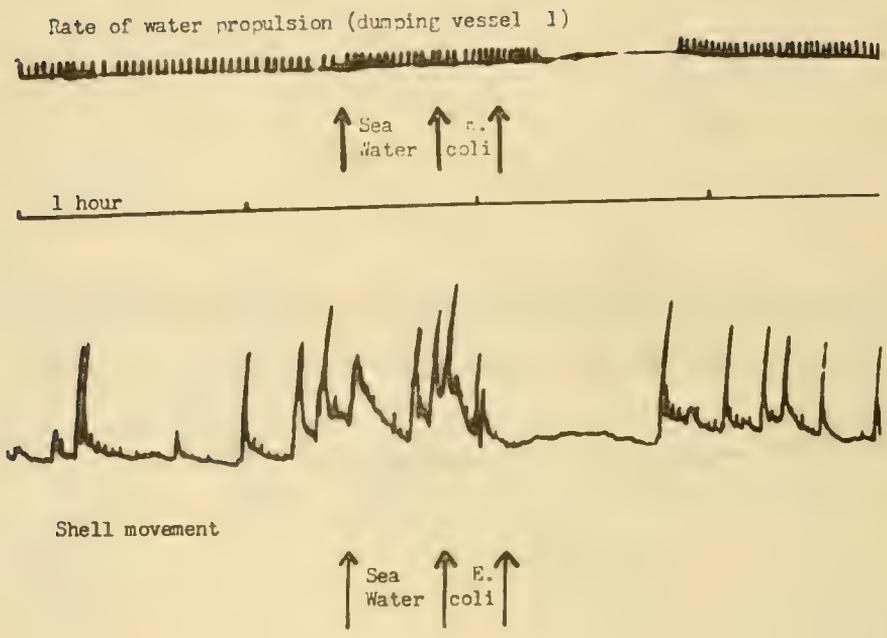


Figure 4. Rate of water propulsion and snell movement when E. coli was added directly to the gills of the oyster. At first, sea water was delivered directly to the gills from one of the flasks shown in Figure 2. Then by turning the two-way cock to the flask containing E. coli. No significant changes were noticed. A lack of vertical marks on upper line immediately after E. coli was cut off was due to the fact that oyster effluent was disconnected from the dumping vessel and the water was collected in a carboy for checking up the capacity of the vessel.

We deduce from these observations that within the limits of the concentrations of E. coli used in our experiments the oyster is indifferent to the presence of this microorganism in the water.

To study the retention of coliform bacteria by the oyster, the sea water in which the E. coli were suspended was examined for the presence or absence of these organisms. A period of 15 minutes was allowed for the passage of the E. coli from the pollution reservoir through the mixing chamber to the oyster tank.

Samples of water in the mixing chamber and of water which had passed through the oyster were collected simultaneously at the following intervals:

1. 0 hour or 15 minutes after pollution added to mixing chamber
2. 15 minutes
3. 1 hour
4. 2 hours
5. 30 minutes after pollution had been cut off

Samples of water from the mixing chamber were withdrawn by a pipette into sterile test tubes. The water discharged from the oyster was collected in a test tube from the discharge tube immediately above the water wheel. In all instances the E. coli densities were determined by using the membrane filter disc, and placing it on Albimi M. medium for a preliminary 2-hour incubation. The discs were transferred to E.H.C. modification of Endo medium and incubated for an additional 14 to 16 hours. The colonies were then counted. The results are shown in Table 1.

Examination of the table shows that during the first 15 minutes after the start of pollution the two oysters retained 49 and 21 percent, respectively, of the E. coli available in sea water. The accumulation of coliform bacteria in the body of the oysters soon reached the point at which no more microorganisms were retained and the effluent leaving their bodies contained more E. coli than the surrounding water. This may be considered as an indication of the elimination of accumulated pollution. In the second experiment (right column, Table 1), retention of E. coli was renewed two hours after the beginning of pollution. Thirty minutes after pollution was cut off, the effluent of both oysters contained fairly large numbers of E. coli, indicating that the elimination process was under way.

The number of the experiments is obviously too small to permit drawing final conclusions. The results are, however, in agreement with previous observations of Galtsoff (1928) and may be therefore interpreted as an indication of definite trends in the reaction of oysters to pollution; namely, that large percentages of coliform microorganisms pass freely through their gills and the discharge of the retained bacteria starts shortly after the pollution is cut off.

Retention and elimination of E. coli by the oyster are probably associated with the secretion and discharge of mucous by the gill epithelium. This tentative explanation is based on the findings of McGinnitie on the role of the mucous sheet in the feeding of pelecypods.

TABLE 1.--Passage of E. coli Through Oyster

Time	Source of Sample	Organisms Diff.	per 100	ml. Diff.
	Initial sea water	5	0	
	Pollution added			
0 hr	Mixing chamber	21,000	34,000	
	Oyster effluent	10,000	-11,000	28,000 -6,000
15 min	Mixing chamber	19,000	35,000	
	Oyster effluent	20,000	+ 1,000	28,000 -7,000
1 hr	Mixing chamber	11,000	29,000	
	Oyster effluent	14,000	+ 3,000	42,000 +13,000
2 hrs	Mixing chamber	-----	41,000	
	Oyster effluent	-----	27,000	-14,000
30 min	After pollution cut off			
	Mixing chamber	0	10	
	Oyster effluent	3,200	7,100	

Two New Methods of Research on Behavior of the Oyster

by

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The intact oyster presents a relatively limited range of behavior to observation. In part, its responses really are limited, in accordance with its sessile life; but in addition the shell offers a considerable obstacle to the observer. The methods here described do not extend the range of observations very far, but may be of service.

I. Observation through the transparent lower shell of young oysters.

For a short time after attachment to the cultch, the upper shell of the spat is transparent enough to permit limited visual examination of the soft parts, and use has been made of this possibility by Prytherch, A. E. Hopkins and others. However, as the oyster grows, it secretes a thicker horny layer (periostracum) at the edge of the upper shell, and although the calcareous layers secreted inside the horny layer remain glassy for a time, the pigmented, translucent horn soon obscures the view of the growing oyster completely.

In the course of work supported by the Freeport Sulphur Company in 1947-1949, it was noted that while the thin new growth at the edge of the adult oyster's upper shell is limber and horny, the corresponding shell-shoots of the lower valve are brittle and have a relatively low organic content. In agreement with this, a horny skin is conspicuous over the whole surface of the upper valve, but wherever the lower valve projects free of the surface to which it is attached, the limy layer is bare. This difference between the left and right valves occurs in both Crassostrea virginica and Ostrea equestris, so may be characteristic of the whole group. It may have been previously observed, but so far I have not been able to find an account of it. The difference would suggest that the lower valve of the young oyster, which is applied to the cultch, might, if exposed, prove to be more transparent, and transparent for a longer period, than the upper valve.

During studies undertaken at the Institute of Marine Science in 1950 for the Connecticut State Water Board, it was wished to observe the internal response of the undisturbed oyster to particles in the water. Young oysters, both C. virginica and O. equestris, which had set on clean glass, were examined through this transparent substratum. The lower valve proved to be as transparent as the glass to which it was attached, and this condition sometimes persisted until the spat were more than a centimeter long. At a varying size, opaque white patches developed in the shell, and gradually spread to block the entire view.

Under the dissecting microscope, the view of the oyster in action was fascinating. Particles whisked along the gills and were passed between the cupped palps, where they joined a stream of particles already received which circled rapidly in this sorting-chamber, clusters breaking into their components. At one side of the chamber, a selection of particles from the circulating stream was flicked into the mouth, while at the other side of the chamber was an area in which particles were sorted from the stream and passed to the mantle surface. At times, even though the water in which the oyster was placed remained unchanged and particles continued to enter and leave without a check, nothing was taken into the mouth. Since swallowing might later be resumed without obvious external cause for the change, it seems evident that feeding-rate may be in part independent of pumping-rate and of the nature of the materials suspended in the water.

The oyster in operation looks like a model of an automatic factory or power-plant, with an ordered confusion of express conveyor-belts continuously hurrying a mixture of raw materials past the entrance to a reactor, into which a selection of raw material is sorted as and when required. Feces, ejected at the other end of the gut, puff out like smoke from a busy chimney. At intervals, the conveyor belts fold together and the pump slows and stops as the whole operation is shut down.

The observations which I have made by the method are limited, and suffice chiefly to indicate that much can be learned by its use. Winston Menzel of Texas A. & M. College, working at the Institute of Marine Science, has however used the method for extensive investigations upon which his report is in preparation. A limitation of the method is, that the water-circulating system has not completely reached the adult state by the time opacity of the shell develops; and another is that a well-developed individual remains transparent for only a few days. On the other hand, the preparations are easily obtained, can be maintained without running sea-water, and lend themselves to many uses. The undisturbed living tissues can be examined under high powers of the compound microscope. The possibilities for cinematic recording are interesting, for purposes both of research and of educational exhibition.

II. Pattern of shell-opening under tension.

In the course of work supported by Freeport Sulphur Company in 1947-1949, need was felt for an index of vigor which could be repeatedly measured in an individual oyster without injuring it, and which would be simple and rapid enough for application to large numbers of individuals.

It is well-known that disease, injury or shock make it easier to insert and twist the shucking-knife; and it was noted that oysters in which windows had been inserted into the shell opened themselves sooner when undisturbed under water, and remained open more persistently, than intact controls. It therefore seemed possible that the response of an oyster to a short period of moderate pull on the shell might supply a useful routine test.

In order to disturb the oysters as little as possible in preparing them, it seemed best to apply the tension through rings cemented to the shell. A fairly satisfactory cement consists of fine sharp sand, 3 parts, to 1 part of U. S. Gypsum Co. Perf-a-Tape cement, mixed with the phenolic resin Cascophen to form as stiff a putty as can be stirred. A cleaned area of shell over the adductor insertion is painted with Cascophen, the putty applied, and the legs of a U of wire inserted. The gypsum cement sets quickly enough to permit attachment of a ring to the other side of the shell an hour or so later. The prepared oysters are then hung overboard in wire baskets while the resin cures (good circulation being desirable because of the formaldehyde catalyst used to polymerize the resin). Within twenty-four hours the rings are usually able to support a pull of at least twelve pounds. One worker could, without much practice, probably apply fifteen to twenty-five rings per hour. After the resin has cured, the cement appears to be inert in seawater. Oysters suspended overboard from their rings were in good condition after up to twelve months. The principal drawback is that if ringed oysters are at any time kept out of water for many hours, so that the shells dry out thoroughly, the cement becomes readily detached from the shell.

The ringed oyster's response to tension may be tested by means of a pivoted beam, the long arm of which bears a sliding weight like a beam balance. At the tip of the arm is a kymograph pen or pointer. The ring cemented to one side of the oyster is slipped through a hook suspended by a chain from a hook on the short arm of the beam. The sliding weight is moved back to the pivot in order not to place strain on the oyster while it is being mounted in the apparatus. The ring of the opposite side of the oyster is hooked to a vertical screw in the base supporting the column on which the beam is pivoted. By varying the length of chain and screw, the beam can readily be brought to horizontal with any oyster. A support is then swung under the beam to keep strain from the oyster while the weight is moved out to the position giving the desired tension for the test and while the kymograph drum is being set against the pen. A second pen fixed to the base of the beam balance gives a comparison line. The support used to stop the beam at horizontal is then replaced by one allowing a limited descent which will not strain the oyster's gape (in case the oyster opens with unexpected rapidity).

Although the above may sound complicated, the first apparatus used was constructed from scrap with household tools in a few hours, and an oyster can be attached for test in considerably less than a minute.

Placed under moderate tension in this way, even a strong oyster usually begins to open immediately, although at a slow rate (0.002 to 0.01 mm. per minute at the level of the rings on the shell, with a 9 pound pull). The rate of opening usually changes from time to time during the test, sometimes from slow to fast, sometimes the reverse; without clear relation to the degree of moderate opening of the shell (as long as tension is continuous. If the oyster is permitted to close and tension then reapplied, a marked change in rate of opening may result). The gradual opening is sometimes interrupted by an abrupt partial closure, presumably caused by contraction of striated muscle. The slow opening then begins again at the level of closure to

which the contracture attained. Repeated contractures tend to be of similar amplitude. The average rate of opening varies greatly between individual oysters, more than with the tension applied (within moderate limits). The individual variation obscures the expected relation to size, in the few dozen tests made. The pattern of opening displayed by a particular oyster seems characteristic, and may be repeated with little variation over a period of months. The fact that the pattern may be almost indistinguishable in tests of the same individual repeated at intervals of a day seems to indicate that gradual and limited forced opening is not injurious.

However, the pattern may also change sharply in a short time. For example, the vigorous clean-shelled oysters collected by hand from an up-river reef near Beaufort, N. C. customarily repeated their initial patterns unless subsequently subjected to shock or injury. Breaking the edge of the shell with pliers or drilling holes without touching the flesh was sometimes sufficient to cause a conspicuous immediate increase in rate of opening, traces of which might persist for a week. In contrast to these up-river oysters, sponge-riddled oysters from a down-river bed often showed a rapid and apparently spontaneous change from the initial pattern no matter how carefully handled. Oysters from this bed which at first responded at normal rates would after a few days begin to open so rapidly that tension had to be greatly reduced to obtain a detailed record. Sacrifice of these oysters revealed that boring-sponge under the cement had been killed and was rotting, that material from the sponge-chambers had been forced through the conchiolin caps covering recent sponge-perforations in the adductor scar, and that visible ulcers were developing in the adductor.

Records adequate for many routine purposes can be obtained at a rate of up to ten oysters per instrument per hour; although tests of longer duration are more revealing.

As in the case of the transparent lower shell described in earlier paragraphs, work with the pull-test just described was devoted chiefly to a preliminary examination of its possibilities. These appear promising for several purposes: for studies of the physiology of shell-closure; as a guide to uniformity of oysters selected for controlled experiments; and as an addition to the indices already available for following changes in individual oysters without sacrificing them.

Neither the pull-test method nor the method of looking through the lower shell is startling in principle, but there seem to be interesting possibilities for extension of knowledge of the oyster through their systematic use.

A STUDY OF THE RESPONSE OF OYSTERS TO TEMPERATURE, AND SOME
LONG RANGE ECOLOGICAL INTERPRETATIONS¹

by

Albert Collier

Introduction

PHYSIOLOGICAL EFFECTS OF TEMPERATURE²

The Pumping Rate of Oysters in Relation to Water Temperature

Much has been written on the response of oysters to temperature, but in respect to long term observations the experimental work on this subject has been limited. During the period from December, 1946, to July, 1950, the author had an unusual opportunity to study the detailed behavior of the oyster, Crassostrea virginica (Gmelin), in the laboratory as well as in the field. This paper will present the results of some of the studies on rates of filtration as related to temperature. These results will then be interpreted in the light of recent and paleoclimatology of the Gulf of Mexico area.

The discussion to be given here is based on the continuous recording of the shell movement and pumping rate of 66 oysters. The record for any given individual ranged from three weeks to six months, with minor interruptions. Some of the oysters made significant growth during the period of recording. Temperature observations were made with precision thermometers at intervals of four to eight hours. The sea water was pumped directly from the bay by a high pressure, high velocity system, without intermediate storage.

Involved in this analysis were 64,580 record hours, for each of which the effluent of the oysters has been computed, and the water temperature at that hour determined. Because of the comparatively long intervals (four to twelve hours) at which the temperature observations were made, we cannot indicate all of the hourly temperatures as observed, but only as interpolated values. Because of this, the data are presented in terms of temperature intervals of 5° C.

¹ The experimental work which forms the basis for this paper was performed by the author while he was engaged in investigations supported by private resources. The conclusions and interpretations do not necessarily represent the views of the present employer of the author.

² Acknowledgments: I wish to extend my appreciation to Messers. Sammy M. Ray, A. W. Magnitzky, and Joe O. Bell for their attentive and diligent assistance in the laborious field and laboratory exercises which produced the large body of data referred to in the following pages.

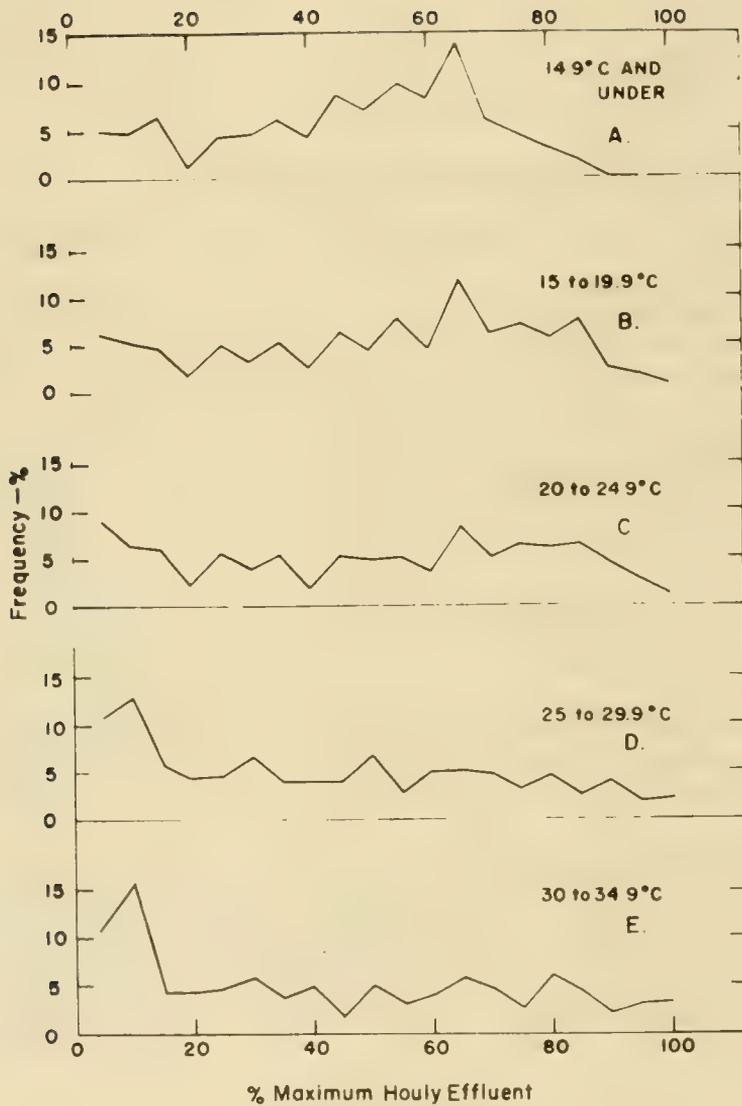


Figure 1. Frequency distribution of hourly effluents (in terms of percent of maximum hourly effluent for a given oyster) for the various temperature brackets. See text for full explanation.

TABLE I.

	Temperature intervals, °C.	Number of Oysters	Number of hours recorded
A.	14.9 and under	30	3,257
B.	15 to 19.9	37	23,964
C.	20 to 24.9	48	14,459
D.	25 to 29.9	39	19,138
E.	30 to 34.9	19	<u>3,762</u>
			<u>64,580</u>

Table I gives the temperature intervals into which the pumping rates were classified and the number of recorded observations in each. The number of oysters in the several intervals totals more than the 66 oysters used because of the overlapping of many of the specimens from interval to interval.

The hourly effluents for each oyster were converted to per cent maximum hourly effluents in order to permit a combination of all data for treatment. For instance, a small oyster pumping 10 liters per hour with a maximum of 12 liters per hour could not be compared with a large oyster pumping 10 liters per hour but with a possible maximum of 30 liters per hour. Frequency distributions of hourly effluents determined on this basis were then computed for the five temperature brackets shown in Table I, and the results are plotted in Figure 1.

In Figure 1 it is clear that as the intervals of higher temperatures were entered there was a tendency for the maximum pumping rate to increase up to interval C. In temperature interval A there is a definite mode at approximately the 60% level; in B, at about the 75% level with more spread towards higher rates. In interval C, the mode has advanced to about the 80% level with a tendency to build up the higher levels. In D and E the trend is reversed and the very low percentages are increased.

Figure 2 demonstrates the relationship between the hours of closure and temperature. As would be expected from the foregoing, the hours of closure increase with increasing temperatures.

Loosanoff (1950) noted the following relationships between pumping rates and temperatures: 8° to 16° C., increasing; 16° to 28° C., no fluctuations; 28.1° to 32° C., a further increase; 32.1° to 34° C., rapid; 34° and over, distress. He further stated that oysters which had been held at 3° to 5° C. and suddenly transferred to water of about 20° C. quickly increased their pumping rate to that of control oysters held at 20° C. No experimental data were given with these results and it is not known at what time of the year these experiments were performed, how many oysters were used, nor how long they were subjected to the experimental temperatures. In view of our results, the inference of Loosanoff's note is that his oysters responded as might be expected when temperature is the only variable. In fact, his results parallel those of Gray (1928) on the response of ciliary beat in mytilus to tempera-

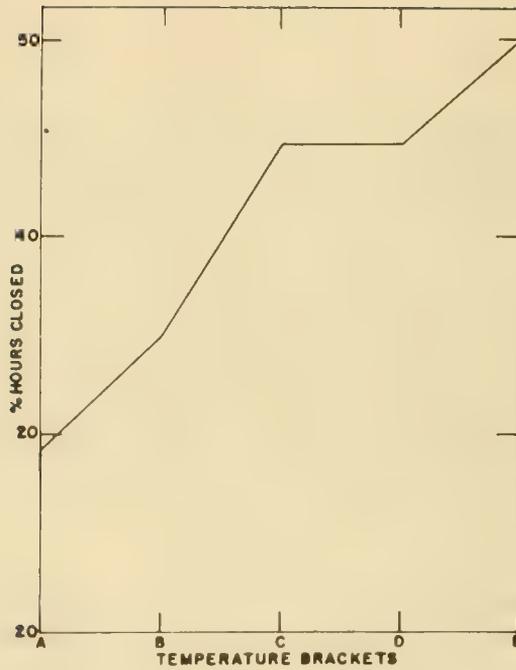


Figure 2. Closure time (percent of record hours) for all oysters at the various temperature levels. Total record hours involved at each level given in Table 1. Temperature brackets are also indicated in Table 1.

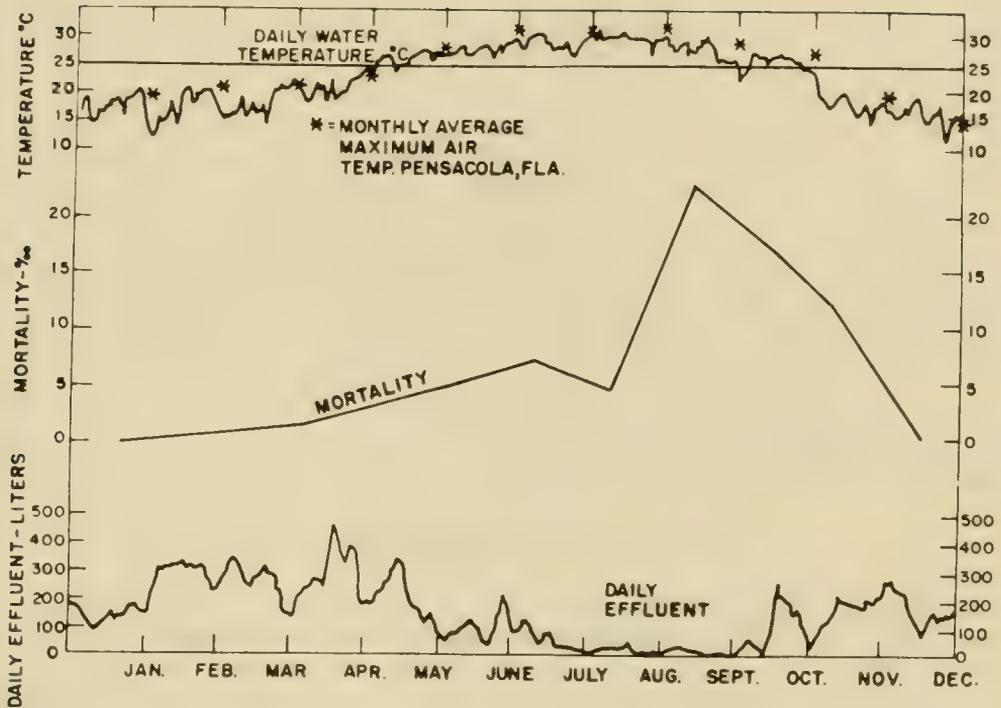


Figure 3. A simultaneous indication of daily water temperatures, mean monthly maximum air temperatures (Pensacola, Florida), monthly oyster mortality in percent, and mean daily effluent of experimental oysters in liters. The daily water temperatures were taken from a thermograph situated in Santa Rosa Sound near the laboratory, and the air temperatures were derived from the records of the Pensacola station of the U. S. Weather Bureau. The oyster mortality curve represents data from oysters held in rafts near the laboratory. The daily effluents of the oysters were computed from the same set of data summarized in Table 1.

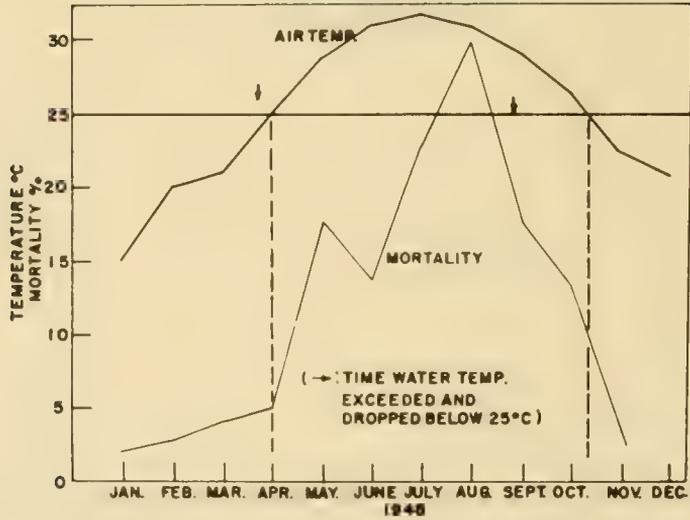


Figure 4. This figure summarized data collected in Grand Bay, Plaquemine Parish, Louisiana. The mortality curve was obtained from a set of oysters retained in tanks as explained in the text. The mean monthly maximum air temperature would show closer agreement with the water temperatures if the water temperatures were shown as monthly means as are the air temperatures.

ture. Gray shows that the cilia increase their activity with increasing temperature up to 28° C., and that beyond this the ciliated epithelium tends to disintegrate.

In an earlier report (Collier et al, 1950) it has been shown that there is a chemical factor which has an important influence on the pumping rates of oysters. This factor is what we call at present the "carbohydrates" or "N-ethyl-carbazol substances". It has been shown that these substances are required in increasing amounts as temperatures rise. Even at low temperatures the oyster seems to pump faster when these naturally occurring substances are present in greater quantity, but the absolute thresholds are lower. At higher temperatures the threshold value for minimum pumping is higher, and even greater quantities are required for maximum pumping. It would seem then, that the ciliated epithelium of the oysters' gills follows in general the equation of Arrhenius, provided there are sufficient quantities of some natural carbohydrate materials present in the water.

In view of the evidence given above, the optimum temperature range for the filtration of water by Gulf Coast oysters is approximately 15° to 25° C.

Temperature, Pumping and Mortality

In a series of raft experiments which were in progress near the laboratory at which the above temperature and pumping rate data were collected, mortality records were kept on a group of oysters during the same period of time. In Figure 3 the elements of daily effluent (mean daily effluent of all oysters), bi-weekly mortalities in percent, and temperature have been plotted. The mortality figures are based on a total of 1200 oysters. These were divided into sub groups held under a variety of conditions, but all groups followed the trend shown here. It is not difficult to see that high temperature, low pumping rates, and mortalities were coincident. During this period the growth rates were sharply depressed from June through September (unpublished data). This is in agreement with some temperature studies made in Long Island Sound (Loosanoff and Nomejko, 1949). In that case, a significant decline in rate of increase of shell length and shell width occurred in going from 20° to 25° C. In their discussion of the matter Loosanoff and Nomejko say, "Thus, under the conditions under which the experiment was run, the oysters grew most rapidly at temperatures of 15.0° and 20.0° C. Therefore the optimum range for their growth was either confined between these two temperatures or, what is more probable, extended a degree or two outside these two limits, giving a range from approximately 13.0° to 22.0° C." These authors did not give these values as final, but it is significant that we should be so nearly able to confirm them even in the waters of the Gulf of Mexico.

During 1948 another set of data were collected at Grand Bay, Louisiana, by the daily observation of 144 oysters held in tanks with water circulated over them. The water was pumped into the tanks constantly at a mean rate of 700 liters per hour in each tank with waste going overboard. Figure 4 shows the relation between this mortality and the average monthly maximum air temperatures at Burrwood, Louisiana. Here again, we find a close association

with the 25° C. temperature level. The arrows in the figure indicate where the water temperatures cross the 25° level in the spring and in the fall. These anticipate the maximum air temperatures by a few days, but the average maximum air temperature is still a good guide for classifying the temperature environment of the oyster in the shallow estuarial waters of the Gulf Coast.

Discussion

Evidence has been presented from several sources supporting the view that there is a point between 20 and 25 degrees centigrade above which conditions become unfavorable for the survival of the individual oyster. Stauber (1950) suggests the possibility of physiological species on the basis of minimal temperatures necessary for spawning, and it is suggested, on the basis of Hopkins' (1931) work, that the minimal spawning temperature for the Gulf Coast might be 25° C. It is possible to conclude, from the point of view of mortality studies alone, that spawning weakens the oyster to the point of death, and that the mortalities mentioned here resulted solely from spawning. This theory cannot easily be reconciled with the fact that pumping rates are also reduced by the advance of temperatures above 25° C. It would seem that the oyster should filter as much water as possible following spawning in order to reestablish its energy reserves.

It is possible that within the physiological optimum temperature range of the species (15° to 25° C.) there could be races defined according to the critical temperature thresholds for spawning. These have been reported as 16.4°, 20° and 25° C. (Stauber, loc. cit.). If this is so, and the optimum physiological range is 15° to 25° C., then the upper limit of this range coincides with the highest of the critical spawning temperatures. This means that spawning in water whose temperatures run over 25° C. exhausts the energy reserves of the oyster at a time when conditions are not favorable for recovery. Accordingly, the oysters will be favored or disfavored as the temperature fluctuates below and above the 25° C. level.

Another factor which must be considered insofar as the mortality picture is concerned is the attack of the oyster by various disease organisms and predators. If the oyster is forced to spend much of the time closed because of unfavorable chemical and physical environmental elements, particularly at temperatures above 25° C., then it uses its energy reserves very rapidly and becomes easy prey to the predators, and especially to Dermocystidium marinum, which is evidently endemic in the waters, and is always in position to attack from the inside of the organism (Mackin, Owen and Collier, 1950). This situation is made worse by spawning.

At this point it is clear that when water temperatures persist for prolonged periods above 25° C. the oyster is at a disadvantage in pumping, cannot recover from the exhaustion of spawning, and is open to attack by a pathogenic fungus and by predators. The temperature in itself is probably not the lethal agent, but serves to bring the oyster within reach of these enemies.

Since the oyster does pump at high temperatures when the "carbohydrate" concentration of the sea water is sufficient, one would expect that there should be times and places when temperatures above 25° C. might be tolerated with fair success. This is exactly what we find, but unfortunately, we do not yet know enough about these natural carbohydrates to define the environmental conditions required for their maximum production.

EFFECTS OF TEMPERATURE ON THE DISTRIBUTION OF CRASSOSTREA VIRGINICA IN SPACE AND TIME

In the preceding section we have established through physiological experiments and field observations that, at least on some points along the Gulf of Mexico, oysters do not do well when the water temperatures exceed 25° C. It should be profitable, then, to examine the seasonal fluctuations in water and air temperatures, and to review the climate of the northern Gulf of Mexico insofar as that is possible.

Since information on water temperatures is not as generally available, and the records are not of as long standing as those for air temperatures, we are forced to derive water temperatures from those of the air in order to survey the past. In comparing air and water temperatures for the estuaries of the Gulf Coast it is difficult to generalize. Water temperatures follow air temperatures closely when the body of water is comparatively shallow or presents a high surface-to-volume ratio. However, a great deal will depend on whether or not a given estuary receives a significant quantity of marsh drainage. This is particularly so in the summer months when the marshes are all exposed by low water during the day to a large amount of heat. Because of the black and gray mucks, the absorption of this heat is increased by the black-body effect. At night, when the marshes are covered by a relatively thin film of water, the temperature of the water will be raised by absorption of heat from the marsh muds, and then when it runs off with the ebb tide during the next day it will be exposed to additional radiation from the sun. Consequently, water temperatures in these shallow bays can run considerably higher than even the maximum air temperatures. Collier and Hedgpeth (1950, Fig. 6, p. 141) showed that the water temperatures of Copano Bay, Aransas Bay, and the Gulf of Mexico at Port Aransas, ran 3° and 4° higher than the average monthly air temperatures at Corpus Christi. This difference was quite consistent from April through September, but as would be expected, is a little more erratic during the winter months.

From the point of view of oyster biology it would be more useful to accept the temperature fluctuations of the estuarial waters as approximating the maximum air temperatures rather than the means.

Brownsville, Galveston, New Orleans, Mobile, Pensacola and Tampa were selected as land stations with sufficient length of record to permit a valid examination of air temperature trends for the northern shores of the Gulf of Mexico. This comparison is between the mean monthly maximum air temperatures for the period 1931 to 1952 (both inclusive) and the period 1930 back to beginning of record. All pertinent data are tabulated in Tables II through VII, and the differences in the means are plotted in Figure 5.

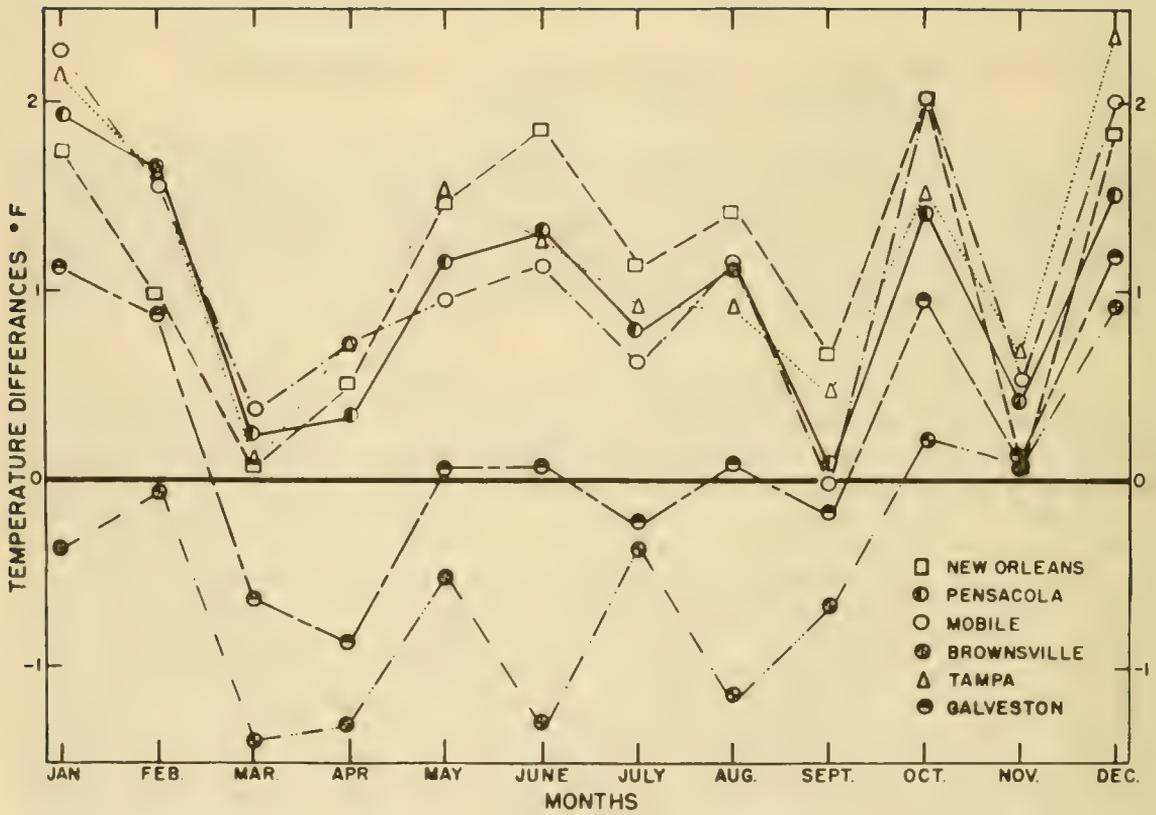


Figure 5. Differences in mean monthly maximum temperatures between the period of record preceding and including 1930, and the period 1931-1952. For actual values and statistical significance see Tables II through VII. See text for discussion.

The most outstanding characteristic is the negative difference shown for Brownsville (i. e., a tendency for the maximum temperatures to be lower), with little change for Galveston during the summer, and the large positive differences for the remainder of the points. This would make it seem that Galveston is a point at which the forces causing the changes are in balance and, in effect, provide a fulcrum for dropping maxima to the southwest and rising maxima to the east.

As shown in Figure 5 the month-to-month trend is similar at all points, and from this we can conclude the general trend for the northeastern Gulf is definitely upward for all months of the year except March, September and November. Even Brownsville, shows a tendency to increase in December. There are some points, Galveston for January, for instance, whose increase would not be significant according to the student t test (Table V) because of the erratic climate brought about by the northers so characteristic of the Gulf Coast. However, when these increases are in agreement with the trends at other points which are themselves significant it seems likely that the Galveston increases are significant, but with the element of mathematical probability obscured by the "northers".

Mitchell (1953) has correlated some portion of such secular climatic changes as we are pointing out with the growth of cities. His treatment is based on mean temperatures rather than mean maxima, and he has divided the United States into six rather broad regions. The one considered in this paper is split, in his work, into two regions (Zones V and VI), and Brownsville is omitted altogether from his considerations. The Gulf maritime province would have to be isolated for special study by a treatment such as Mitchell's before the effect of increasing population of Gulf Coast cities could be evaluated. For these reasons it is not possible to compare the results of Mitchell's investigation with those presented here.

Although it is possible that changes indicated here might be slightly aberrant because of industrial influences, it should be remembered that at the time that some of the largest changes are shown the winds are largely from over the Gulf of Mexico. In some cases the cities which have shown a significant increase in mean monthly maxima have had only moderate industrial or population growth - Pensacola, for example. For the purpose of this discussion we are justified in the examination of the climatic record on the basis of the average monthly maxima.

If the 77° F. line is taken as zero, and the years are divided into two 35 year periods (1882 to 1916, both inclusive, and 1917 to 1951, both inclusive) and the temperature deviations around the zero are summed algebraically, we will find that the results are as shown in Table VIII. It would certainly seem from this that summer temperatures are being extended into the fall months. This is probably to be expected if we follow Charlesworth (1953) in the following statement: "Today the glaciers of the world are in retreat, though not always with the same intensity or without sporadic spurts of short duration and small amount. This universal wasting is affecting the glaciers from the Arctic to the Antarctic, including those of the tropics. It set in

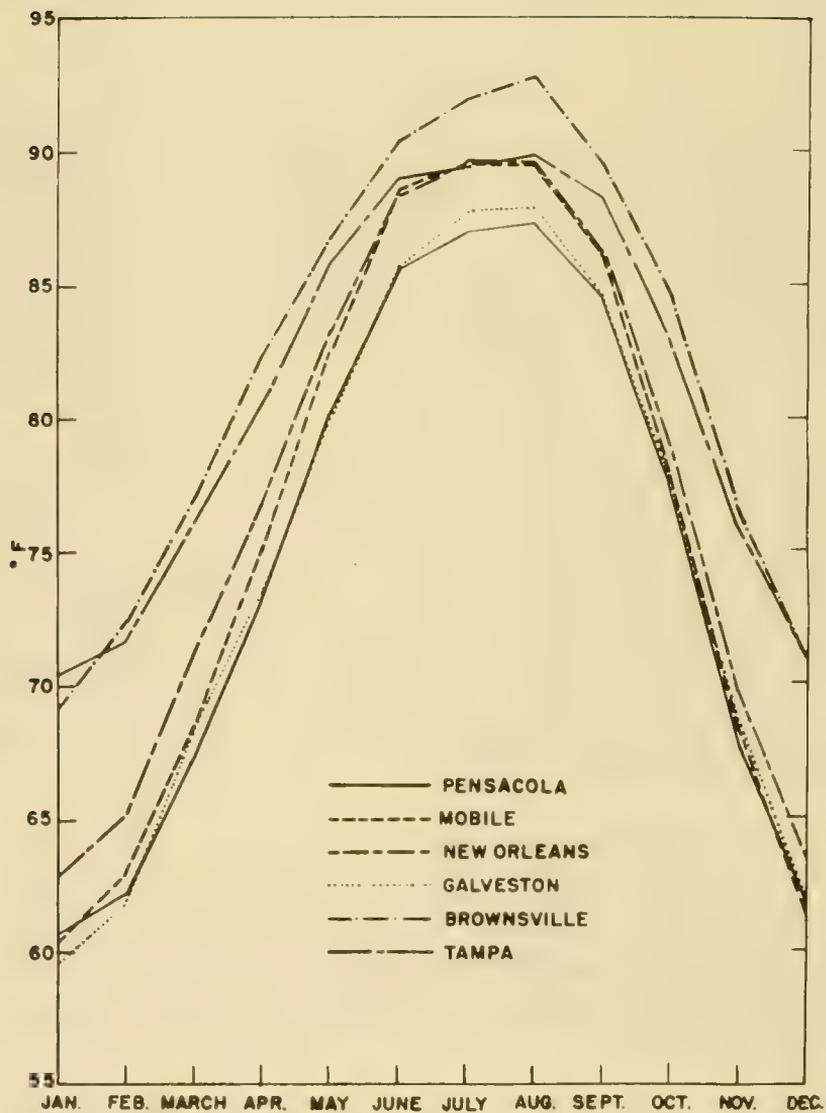


Figure 6. Mean monthly maximum air temperatures for all years of record for certain Gulf of Mexico points.

about 100 years ago and has accelerated in pace during the last thirty years or so." Again, quoting this time from Flint (1947): "Kinser demonstrated that mean annual temperatures have been rising at a rate falling between 0.5° and 2.2° C. per century. The increase is evident not only in the northern hemisphere, but in the southern as well."

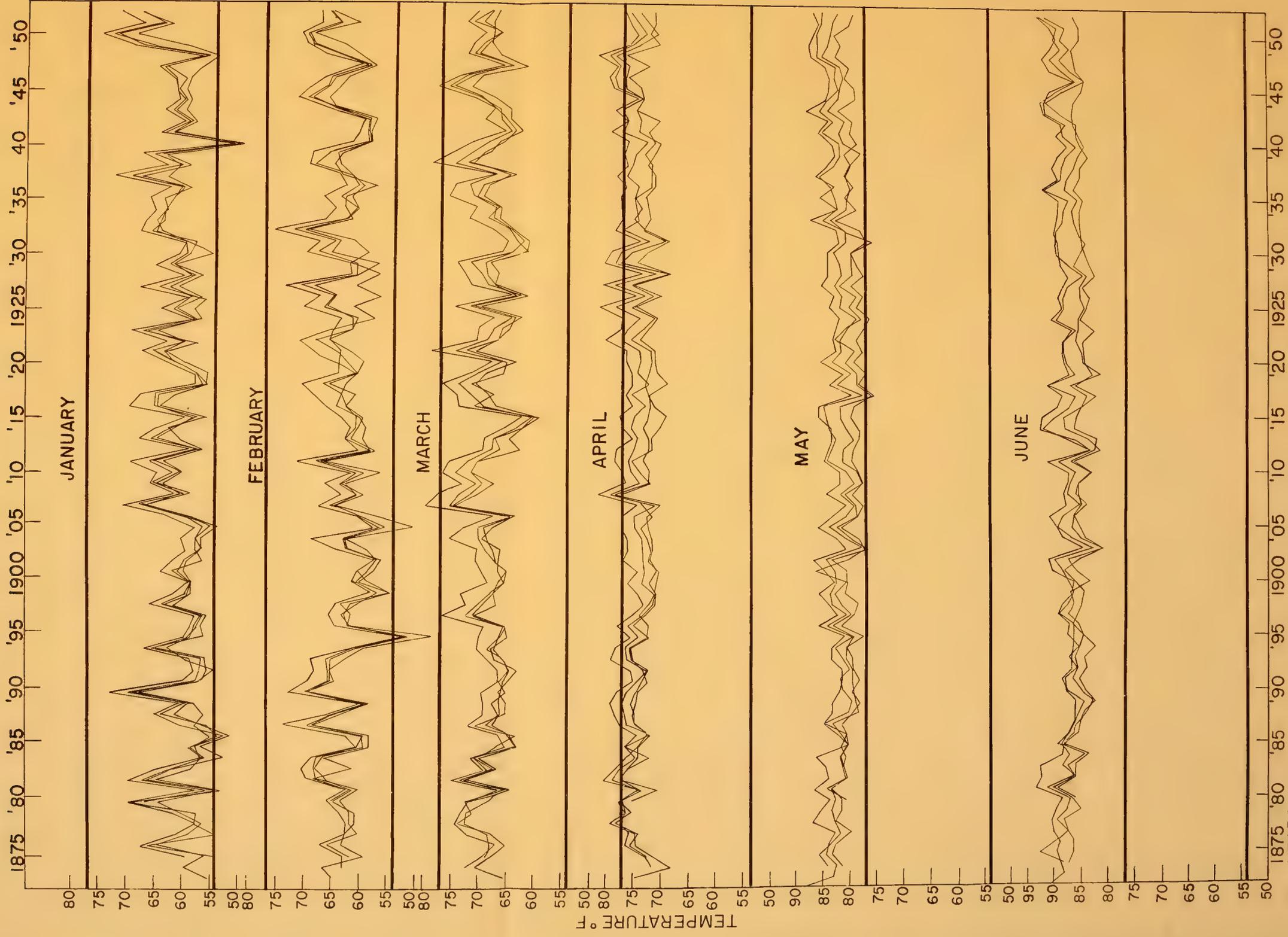
Collier and Hedgpeth (loc. cit.) point out some general similarities of the Galveston area of the Gulf of Mexico to the Beaufort region of the middle Atlantic Coast. Ekman (1953) considers the coastal waters of Texas as tropical in the summer and warm temperate in the winter with a corresponding faunal distribution. He also mentions Crassostrea virginica as being found further south, but says that it occurs as a community dominant only along the north coast of the Gulf of Mexico and along the eastern seaboard north of Florida.

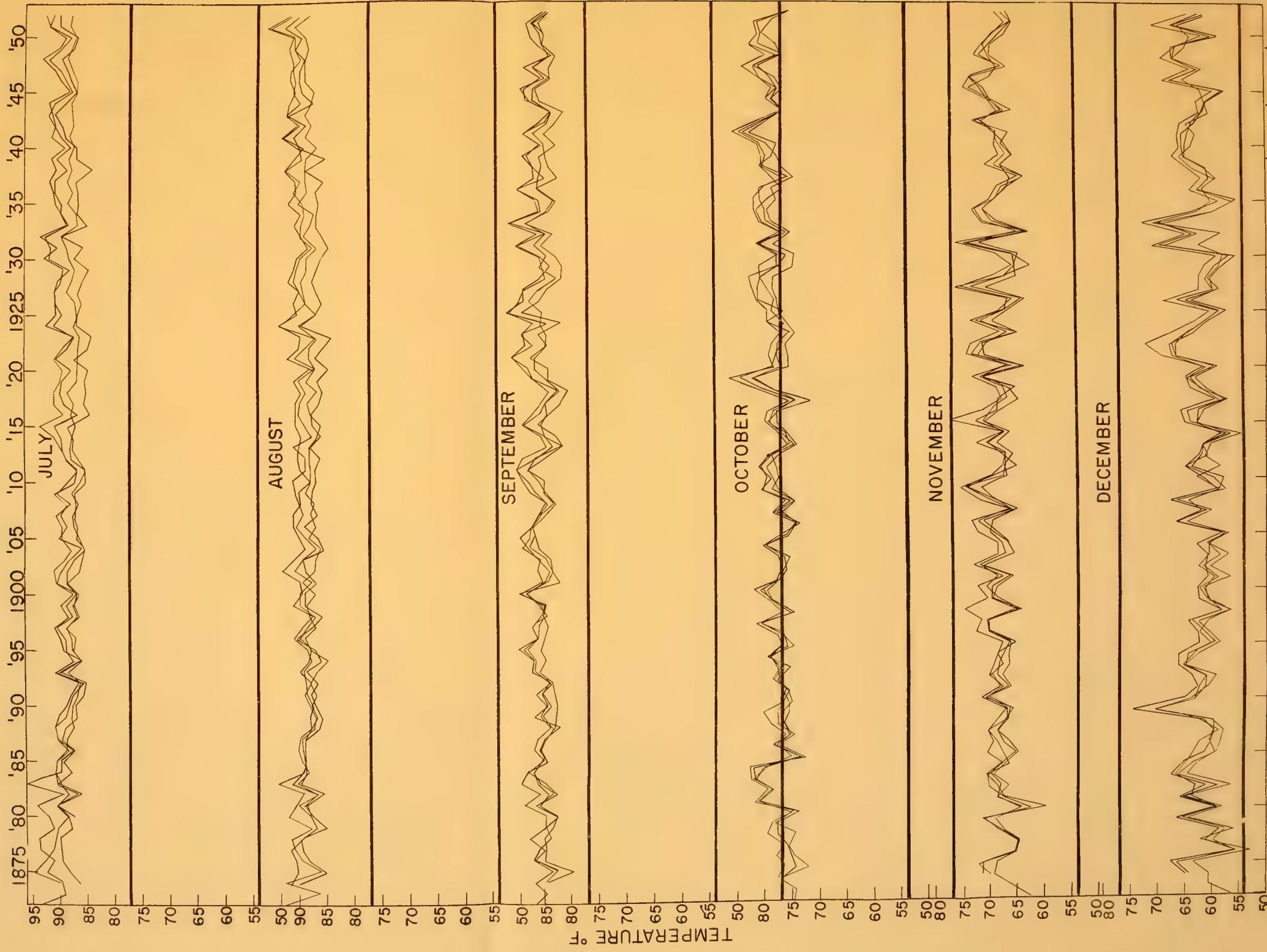
In Figure 6 we have plotted the mean monthly maximum air temperatures for certain points around the coast of the Gulf of Mexico. It will be noticed that Tampa and Brownsville have consistently higher maxima throughout the year with the exception that the summer maxima for Tampa are about the same as those of Mobile and New Orleans. With 25° C. (77° F.) as the maximum of the optimum range for Crassostrea virginica, this picture of seasonal maxima is consistent with Ekman's remarks concerning the distribution of this species in the Gulf of Mexico.

It is also to be observed that in the north Gulf (represented by Pensacola, Mobile, Galveston and New Orleans) there are five months of favorable temperature levels, while in the south Gulf there are only three months. These periods are subject to some variation from year to year as can be seen in Figures 7 and 8.

For the northern Gulf of Mexico, Pensacola, Mobile, New Orleans and Galveston have been selected as sample points. For all of these points, records go back to at least 1880. The average monthly maxima for these points have been plotted for each year of record in Figures 7 and 8. What we consider to be the optimum temperature range of the oyster, 53° to 77° F., has been indicated for each month by cross hatching. We note that it is only during the months of November, December, January, February and March that the temperature ranges are comfortably within these limits. In April and October, the range is on the upper border, and in these cases it is clear that there will be some years in which April and October will be favorable to oysters, and many years when conditions during these months will not be favorable. It will be particularly noticed that for October conditions have been becoming increasingly unfavorable since about 1916.

Crassostrea virginica was established in the Gulf of Mexico in or near the Pliocene period (Dall, 1889), and at this time air and water temperatures were lower than they are now. Phleger (1951) reported on a comprehensive study of the distribution of the Foraminifera in the Gulf of Mexico and the following interesting paragraph is quoted from his paper: "A cool water fauna is found in the lower part of most cores. This was deposited when surface water temperatures were lower than present surface temperatures in





MEAN MONTHLY MAXIMUM TEMPERATURE, GULF OF MEXICO . . . FIG. 8

the Gulf of Mexico. This cool water fauna is interpreted as late glacial and has features characteristic of the continental slope water between Cape Hatteras and Cape Cod in the western North Atlantic." This is evidence from another approach that lower water temperatures than those presently observed prevailed in the Gulf of Mexico at the time of the earliest known appearance of Crassostrea virginica. Hilgard (1878) reported the presence of specimens of Urosalpinx cinereus in sub-surface strata in the Mississippi River delta which are probably of the same age as the earliest formations in which Crassostrea virginica appears. As far as we know, Urosalpinx has long since disappeared from the Gulf of Mexico.

It is possible that if the climate continues its present trend Crassostrea virginica will decline as a community dominant. This will depend on whether or not there exists in this species the genetic potential to produce a race which can survive the high temperatures.

SUMMARY AND CONCLUSIONS

1. A combined study of pumping rates, growth rates, and mortality rates show that the optimum temperature range for Crassostrea virginica lies between 15° and 25° C., even on the Gulf Coast. The poleward limit for this species is determined by the minimum spawning temperature of approximately 16° C., and the equatorward limit is determined by the maximum temperature favoring survival, which we consider to be 25° C. This type of distribution fits Hutchin's (1947) group 3.
2. It is shown that in general, water temperatures for estuaries bordering the Gulf of Mexico can be inferred from average monthly maximum air temperatures.
3. The northern Gulf estuarial waters are within these temperature limits for approximately five months out of the year, but Weather Bureau records show a definite increase in the average monthly maxima of the last 22 years. September is already above the maximum, and since 1916 the average maxima for October have gone above 25° C.
4. Evidence that the Gulf waters were cool at the earliest known time Crassostrea virginica is recorded in the Gulf can be found in the works of the paleontologist and the paleoclimatologist. They were comparable to present day waters between Cape Hatteras and Cape Cod.
5. It is pointed out that although oysters can survive as individuals in the southern reaches of the U. S. Gulf Coast, it has been only on the north Gulf that they have survived as community dominants. This is in agreement with the above statement concerning the optimum temperature range and the temperature zones of the Gulf.
6. It is concluded that since Crassostrea virginica appears to be fundamentally a cool water animal, and since the waters have been becoming continuously warmer, particularly in the last thirty years, its position as a community dominant in the north Gulf is threatened if the warming continues.

7. There are other factors affecting the survival of oysters including Dermocystidium infestation, predators, changing salinities, silting, floods, river levees, and overfishing. Low water temperatures assist the animal to resist all of these except the last. Of these, the most important on the Gulf Coast at this time is probably Dermocystidium, but the attacks of this organism are predicated on the debilitation brought about by prolonged exposures to excessive heat. There is evidence that the presence of some natural carbohydrate substances in the sea water assist the oyster to filter water in spite of temperatures above 25° C.

8. The future of the Gulf oyster industry is not very bright. Cultch spreading activities and seed planting are probably helpful for brief periods, but are too expensive in view of the uncertain returns. The perfection of a heat resistant stock of oysters by husbandry offers some possibility, but the limited success of the species in maintaining itself in lower Gulf waters is not encouraging.

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TABLE II

Tampa

Comparison of mean monthly maximum air temperatures ($^{\circ}\text{F.}$) for periods from beginning of record through 1930 (period A), and 1931 through 1952 (period B) by "student" t test.

Month	Difference of means (B - A)	$\frac{t}{A:B}$	A			B		
			n	s	\bar{x}	\bar{x}	s	n
Jan.	† 2.153	2.1149*	40	3.287	69.688	71.841	4.686	22
Feb.	† 1.610	1.5203	40	3.633	71.135	72.745	4.579	22
March	† 0.081	0.0831	40	3.573	76.133	76.214	3.851	22
April	† 0.744	1.3025	41	2.205	80.415	81.159	2.076	22
May	† 1.514	3.6981**	41	1.532	85.422	86.936	1.580	22
June	† 1.273	3.4791**	41	1.286	88.559	89.832	1.555	22
July	† 0.917	2.9628**	41	1.010	89.183	90.100	1.427	22
Aug.	† 0.916	3.1019**	41	1.094	89.507	90.423	1.160	22
Sept.	† 0.475	1.4226	41	1.275	88.207	88.682	1.241	22
Oct.	† 1.523	2.9362**	41	2.027	82.415	83.938	1.730	22
Nov.	† 0.658	1.0322	41	2.232	75.861	76.519	2.640	22
Dec.	† 2.384	2.8220**	41	2.746	70.368	72.752	3.827	22

* Significant at .05 level

** Significant at .01 level

TABLE III

Pensacola

Comparison of mean monthly maximum air temperatures ($^{\circ}\text{F.}$) for periods from beginning of record through 1930 (period A), and 1931 through 1952 (period B) by "student" t test.

Month	Difference of means (B - A)	$\frac{t}{A:B}$	A			B		
			n	s	\bar{x}	n	s	\bar{x}
Jan.	+ 1.937	1.9415	51	3.5995	60.063	22	4.5692	62.000
Feb.	+ 1.667	1.7728	51	3.6355	61.710	22	3.8064	63.377
March	+ 0.247	0.3091	51	3.1149	67.180	22	3.1714	67.427
April	+ 0.343	0.6556	51	1.9760	73.125	22	2.2195	73.468
May	+ 1.151	2.2569*	51	1.9951	79.767	22	2.0095	80.918
June	+ 1.335	2.7146**	51	1.8355	85.233	22	2.1322	86.568
July	+ 0.7903	1.9706	51	1.5066	86.765	22	1.7163	87.555
Aug.	+ 1.113	2.6645**	51	1.3482	86.996	22	2.1769	88.109
Sept.	+ 0.064	0.0141	51	1.8658	84.641	22	1.5469	84.705
Oct.	+ 1.421	2.4856*	51	2.2436	76.984	22	2.2351	78.405
Nov.	+ 0.413	0.6688	51	2.2762	67.792	22	2.7346	68.205
Dec.	+ 1.502	1.8665	51	2.9570	61.425	22	3.5818	62.927

* Significant at .05 level

** Significant at .01 level

TABLE IV

Mobile

Comparison of mean monthly maximum air temperatures ($^{\circ}\text{F}$),
for periods from beginning of record through 1930 (period A),
and 1931 through 1952 (period B) by "student" t test.

Month	Difference of means (B - A)	t A:B	A			B		
			n	s	\bar{x}	\bar{x}	s	n
			Beginning of record through 1930			1931 - 1952 (both inclusive)		
Jan.	+ 2.280	2.1526*	58	4.016	59.743	62.023	4.763	22
Feb.	+ 1.522	1.6009	58	3.813	62.528	64.050	3.753	22
March	+ 0.374	0.4339	58	3.455	68.376	68.750	3.407	22
April	+ 0.745	1.3400	58	2.328	74.964	75.709	1.897	22
May	+ 0.964	1.8436	59	2.217	82.281	83.245	1.706	22
June	+ 1.142	2.2322*	59	2.145	88.081	89.223	1.751	22
July	+ 0.631	1.1640	59	2.364	89.369	90.000	1.511	22
Aug.	+ 1.169	2.8360**	59	1.672	89.217	90.386	1.587	22
Sept.	- 0.012	0.0241	59	1.961	86.207	86.195	2.092	22
Oct.	+ 2.026	3.2746**	59	2.511	77.469	79.495	2.380	22
Nov.	+ 0.543	0.8396	59	2.525	67.934	68.477	2.757	22
Dec.	+ 2.044	2.3699*	59	3.363	60.820	62.864	3.689	22

* Significant at .05 level

** Significant at .01 level

TABLE V

New Orleans

Comparison of mean monthly maximum air temperatures ($^{\circ}\text{F.}$) for periods from beginning of record through 1930 (period A), and 1931 through 1952 (period B) by "student" \underline{t} test.

Month	Difference of means (B - A)	\underline{t} A:B	A			B		
			n	s	\bar{x}	\bar{x}	s	n
Jan.	† 1.720	1.4700	56	4.384	62.325	64.045	5.283	22
Feb.	† 0.989	0.8966	57	4.387	64.856	65.845	4.412	22
March	† 0.059	0.0641	57	3.657	71.023	71.082	3.689	22
April	† 0.510	0.9942	57	2.136	76.726	77.236	1.776	22
May	† 1.489	3.6326**	57	1.577	82.856	84.345	1.775	22
June	† 1.857	4.0484**	57	1.931	88.016	89.873	1.517	22
July	† 1.120	3.0890**	57	1.501	89.335	90.455	1.283	22
Aug.	† 1.422	3.6935**	57	1.565	89.259	90.681	1.447	22
Sept.	† 0.682	1.3003	57	2.116	86.054	86.736	2.015	22
Oct.	† 2.037	3.3481**	57	2.455	78.181	80.218	2.338	22
Nov.	- 0.052	0.0687	57	3.030	69.747	69.695	2.978	22
Dec.	† 1.848	2.0591*	57	3.587	63.079	64.927	3.545	22

* Significant at .05 level

** Significant at .01 level

TABLE VI

Galveston

Comparison of mean monthly maximum air temperatures ($^{\circ}\text{F.}$) for periods from beginning of record through 1930 (period A), and 1931 through 1952 (period B) by "student" t test.

Month	Difference of means (B - A)	$\frac{t}{A:B}$	A			B		
			n	s	\bar{x}	\bar{x}	s	n
			Beginning of record through 1930			1931 - 1952 (both inclusive)		
Jan.	+ 1.130	1.1381	56	3.903	59.261	60.391	4.057	22
Feb.	+ 0.875	0.9015	56	4.024	61.675	62.550	3.383	22
March	- 0.643	0.8318	56	3.113	67.452	66.809	2.962	22
April	- 0.852	1.6306	56	2.138	73.729	72.877	1.905	22
May	+ 0.050	0.1090	57	1.936	80.000	80.050	1.502	22
June	+ 0.089	0.1886	57	2.125	85.702	85.791	0.953	22
July	- 0.232	0.5851	57	1.805	87.832	87.600	0.680	22
Aug.	+ 0.084	0.2589	57	1.406	87.875	87.959	0.927	22
Sept.	- 0.108	0.2725	57	1.594	84.681	84.573	1.541	22
Oct.	+ 0.972	1.9417	57	2.008	77.637	78.609	1.958	22
Nov.	+ 0.135	0.1833	57	2.979	68.510	68.645	2.808	22
Dec.	+ 1.108	1.4301	57	3.186	61.774	62.882	2.806	22

TABLE VII

Brownsville

Comparison of mean monthly maximum air temperatures ($^{\circ}\text{F.}$) for periods from beginning of record through 1930 (period A), and 1931 through 1952 (period B) by "student" t test.

Month	Difference of means (B - A)	$\frac{t}{A:B}$	A			B		
			n	s	\bar{x}	\bar{x}	s	n
			Beginning of record through 1930			1931 - 1952 (both inclusive)		
Jan.	- 0.375	0.3377	46	4.418	69.261	68.885	3.412	20
Feb.	- 0.087	0.9583	46	3.392	72.357	72.270	3.382	20
March	- 1.358	1.4435	46	3.553	77.428	76.070	3.413	20
April	- 1.304	2.2045*	46	2.205	82.874	81.570	2.216	20
May	- 0.526	0.9960	46	1.965	86.926	86.400	1.985	20
June	- 1.334	1.9727	46	2.454	90.839	89.505	2.683	20
July	- 0.368	0.7707	46	1.797	92.058	91.690	2.407	20
Aug.	- 1.175	2.0581*	46	2.346	93.120	91.945	1.507	20
Sept.	- 0.700	1.1084	46	2.591	89.730	89.030	1.679	20
Oct.	+ 0.205	0.3109	45	2.544	84.560	84.765	2.230	20
Nov.	+ 0.094	0.1040	46	3.824	76.711	76.805	2.795	20
Dec.	+ 0.931	0.8879	46	4.147	70.659	71.590	3.298	20

* Significant at .05 level

TABLE VIII

Comparison of deviations (in month-degrees, F.) during the month of October, from 77° F. (25° C.) for the two 35 year periods, 1882-1916 (both inclusive), and 1917-1951 (both inclusive) at each of the four sample stations on the north coast of the Gulf of Mexico.

	1882-1916	1917-1951
Pensacola	+ 6.9	+ 24.5
Mobile	+ 18.8	+ 79.1
New Orleans	+ 34.0	+ 109.6
Galveston	+ 14.3	+ 51.9

THE ANNUAL CYCLE OF REPRODUCTION, GROWTH, AND FATTENING IN LOUISIANA OYSTERS

By

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As this is the first meeting of the National Shellfisheries Association ever held on the Gulf Coast, we thought that the members from more northern states, as well as those from the South, might be interested in an account of some of the main points in the annual cycle of oyster life in Gulf waters, where conditions differ greatly from those of the better known regions such as Long Island Sound and Chesapeake Bay.

Since we are meeting in Louisiana, and since this state is the principal center of oyster production in the deep South, we shall discuss the oysters of Louisiana only. It should be understood that not all of our statements can be applied to other Gulf states such as Texas and Florida where some conditions are quite different.

Louisiana itself contains quite a variety of different oyster-growing waters. Each bay and each bayou differs to some extent from every other one. But, generally speaking, there are two main regions: the natural reef area east of the Mississippi River, which supplies most of the oysters used by canneries as well as most of the seed oysters used by growers, and the area west of the river, from the river to the western side of Terrebonne Parish, where most of the private leases are located. Our observations apply to this western area, and especially to the Barataria Bay and Terrebonne Bay regions.

The map shows the location of the waters mentioned. All of this area is old Mississippi River delta country. The marshy "land" shown on the map is practically all flooded at high tide. The bays are very shallow, usually only 5 to 10 feet deep, or even less. The tidal streams or "bayous" winding through the marshes are also important in oyster production.

Southern Louisiana is a region of very heavy rainfall. The bays just west of the river also receive some fresh water from the Mississippi when the river is high. Sometimes the water entering Barataria Bay from the Gulf is less salty than the bay water, because of a layer of fresh water spreading out over the surface of the Gulf from the mouth of the Mississippi, and only slowly mixing with the saltier water beneath it. The tide range averages only 1 to 2 feet, but the bays are so shallow that a few inches difference in tide level, or a few inches of rain, will cause a large and sudden change in the salinity.

The temperature of the water also has a wide range between extremes, although it does not usually change so radically from day to day or hour to hour as does the salinity. Although summer water temperatures are high,

often around 30°C. for long periods, the Gulf coast west of the Florida bight is not a subtropical region, but belongs to what Marston Bates calls the "Intemperate Zone". It gets hot, but it also gets cold, and water temperatures in Louisiana bays sometimes get down to within one or two degrees of freezing. However, low temperatures are not always maintained throughout the winter, and the water sometimes gets up above 20°C. in midwinter, though usually not for long periods.

Figure 1 is a graph of the thickness of the gonadal epithelium in Louisiana oysters. As the water begins to warm up in late winter and spring, the sperm and egg cells develop and the gonads become thicker. Spawning causes a sudden decrease in thickness, and then during the summer sperm and egg cells are thrown out about as fast as new ones develop.

Figure 2 shows the number of bivalve larvae found in plankton. Some of these may not be oyster larvae, but nearly all of them are. This graph agrees well with the one showing thickness of gonad, and with the graphs of setting which follow.

Figure 3 shows a record of setting each week during the 1949 season, in the lower and the upper parts of Barataria Bay. Note that setting was heavier in the lower bay where salinity was higher. The setting rate is shown as number per shell per day. This is the most convenient index figure to use on the Gulf coast. Figure 4 shows even heavier setting rates in the western Terrebonne Bay region. The two figures together show 3 seasons of setting records in 3 different localities, and all together they indicate that setting begins in late April or early May, that the May peak is the big one, and that successively smaller peaks of setting occur through the summer and into early autumn.

In northern waters, the idea is to put out culch in time to catch the peak setting, but this is not necessary or even desirable in Louisiana. The spat which set in May are subject to predation so much longer than those which set late in the season that they are reduced to about the same numbers by November, and the younger oysters grow so fast that the late set have almost caught up to the spring spat by the time they are a year old.

Figure 5 shows the growth of spat, from setting to market size, in trays at 5 localities in the Terrebonne Bay region. In this case length is used as an index of size.

Figure 6 shows growth curves for tray oysters, from setting to the age of 2 years, using average volume and average weight, as well as average length, as indices of size. Note that these curves show no indication of a slackening of growth during the warm months, as was reported from Florida.

Figure 7 shows even more clearly that growth does not slow down during the summer months, when the oysters are less than 2 years old. This does not mean that older oysters might not grow more slowly in warm weather, but we consider that a more or less academic point because Louisiana oysters should be marketed before they are 2 years old.

Figure 8 illustrates a rather interesting point. When oysters are reared in trays for the study of growth, it is very desirable to number each oyster and to keep separate records on the individuals. As the graph shows, the growth curve for those oysters which survived to the end of this experiment (when the oysters were 2 years old) forms almost a straight line, while each individual which died had reached a plateau and had stopped growing long before it died. As these oysters were all in one tray, this difference in growth rate is not caused by environment, but by something that happened to the individuals. If measurements of many of these sick individuals are included in the averages, as when a lot of unnumbered oysters are measured and the average of the entire lot is taken as an index of size, then you will get a curve whose slope decreases during the warm weather when the number of sick oysters is at its height. Also, when a number of these sick oysters die off between one measurement and the next, the average will show a sudden spurt upward due to the elimination of the stunted individuals, and not due to any actual increase in growth rate of the survivors.

Therefore, growth curves based on averages of entire lots of oysters, or averages of samples of a population, do not necessarily give a true picture of the growth of individuals. With this note of warning, we proceed with a series of graphs based on samples taken each month from 2 planted beds at locality "SE" and 5 planted beds at locality "BB". All beds were planted in September and October, 1947.

Figure 9 shows the increase in average length and width of the planted oysters at the two localities.

Figure 10 shows growth curves based on another index of size, the number of Louisiana oyster sacks (a sack equals $1\frac{1}{2}$ U. S. standard bushels) which 1000 oysters would fill.

Figure 11 shows the average shell capacity of the planted oysters. Note that this index of size seems to show seasonal variations, probably caused by differential mortality.

Figure 12 shows the volume of drained meat (dry pack) per sack and per oyster. Note that the volume of meat per sack does not increase in proportion as the oysters get larger, although the volume per oyster does.

Figure 13 shows the average wet weight of drained meat, per oyster, for the monthly samples from the planted beds.

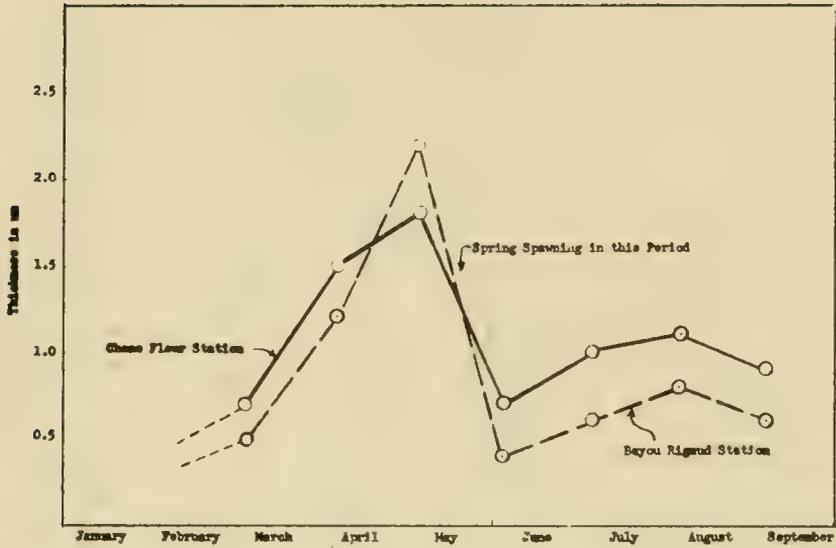
Figure 14 shows the so-called coefficient of fitness or index of condition, which is an index of the ratio between the amount of meat in an oyster and the size of the shell cavity.

Figure 15 shows the glycogen content of the planted oysters, each month.

In every index of quality, Louisiana oysters are at their best in late winter and spring, and are at their poorest in late summer and early fall. When they are at their best they are equal in quality, including flavor, to any oysters in northern waters. The trouble is that they keep this high quality for only two or three months. Possibly a really good and economical process of freezing oysters when they are at their peak may some day provide an answer to this problem of the Louisiana oyster industry.

Last year at the Atlantic City meeting, in a paper which was later published in the Atlantic Fisherman, we pointed out that planted oysters in Louisiana reach their peak yield within a few months after planting. After this peak, the yield decreases because growth is no longer fast enough to stay ahead of natural mortality. We now point out that this applies not only to the yield in sacks of oysters, but also to the yield in gallons or pounds of meat, and to the quality of the meats. Oysters planted in the fall will reach their peak in both quantity and quality the following spring, and should be marketed then. This is already a common practice in some parts of Louisiana. It is also possible to plant seed in April and harvest market oysters the following fall or winter, but this can be done profitably only if the seed are less than a year old, and only in places where predators, especially conchs (Thais) are not too numerous. In Dermocystidium territory, planted oysters should never be kept through more than one summer.

Louisiana oysters grow rapidly, and Louisiana oyster growers could greatly increase their production by taking full advantage of this rapid growth. To do so will require a better source of seed oysters than the natural reefs. This problem of seed production must be left for a future paper.



Thickness of the Gonadal Epithelium in Louisiana Oysters.

Figure 1

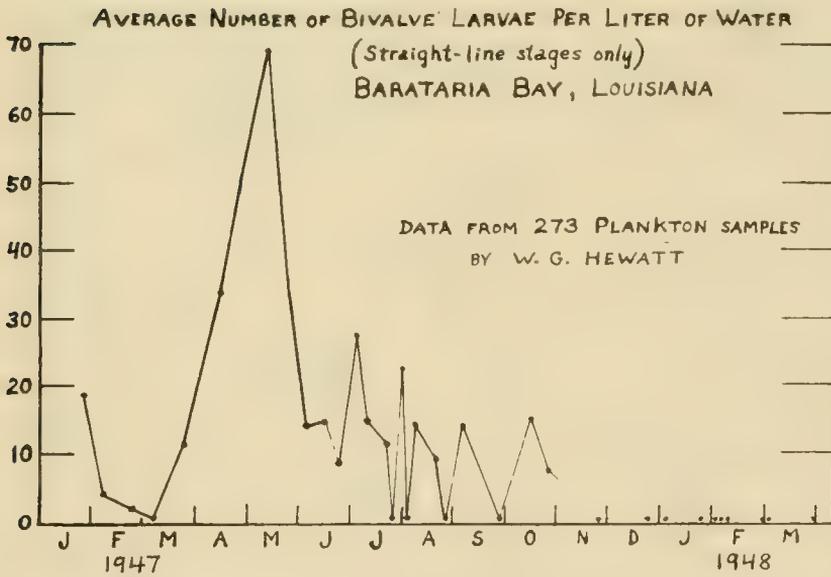


Figure 2

NUMBER OF SPAT SETTING PER SHELL PER DAY
BARATARIA BAY 1949

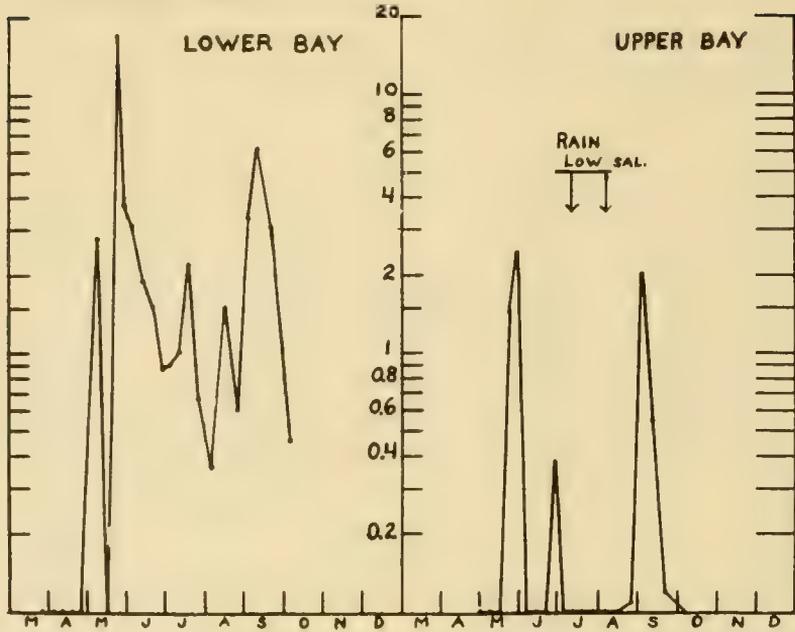


Figure 3

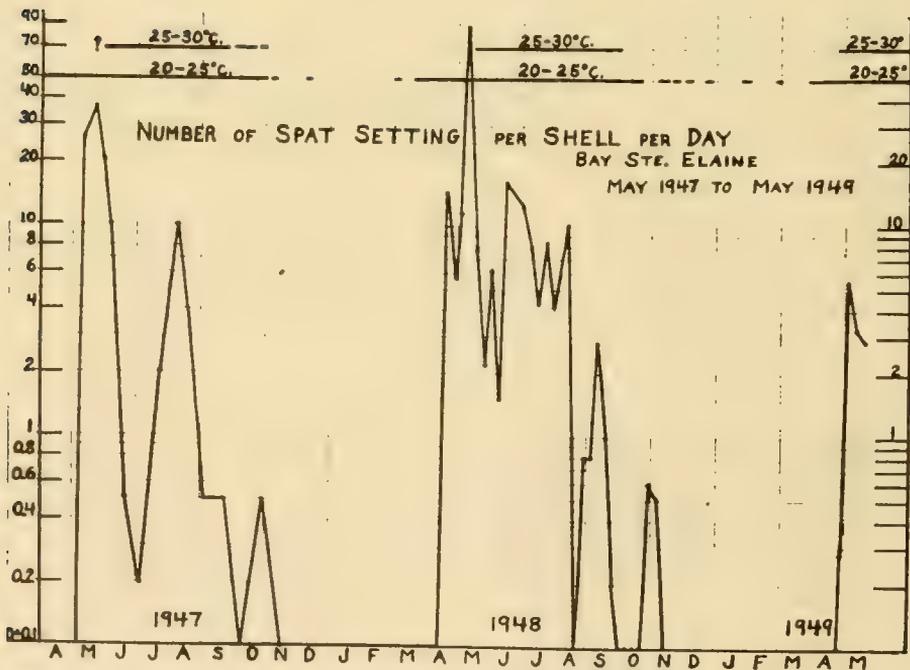


Figure 4

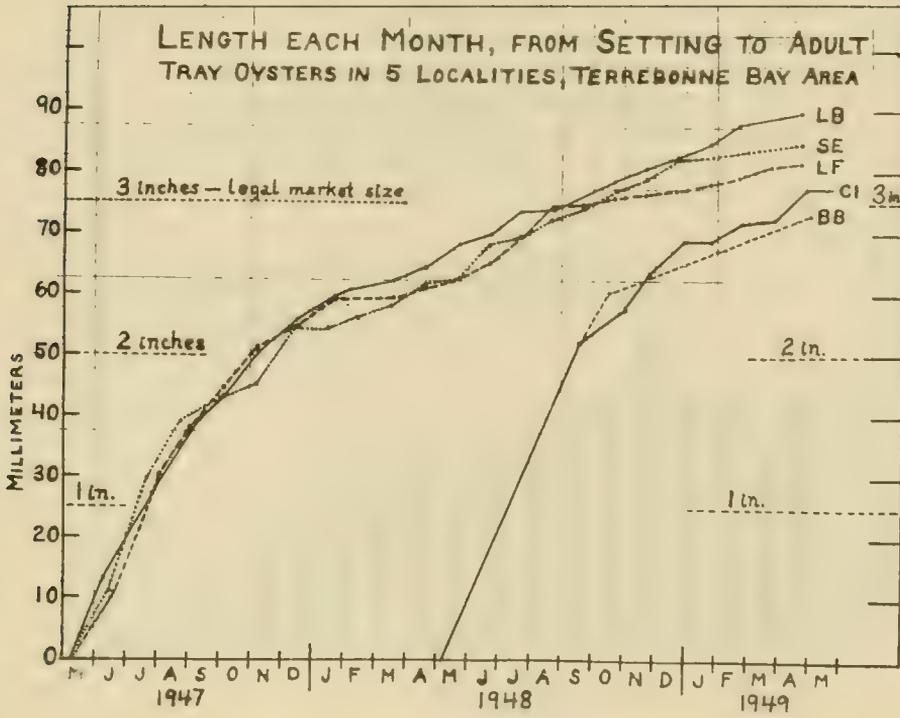


Figure 5

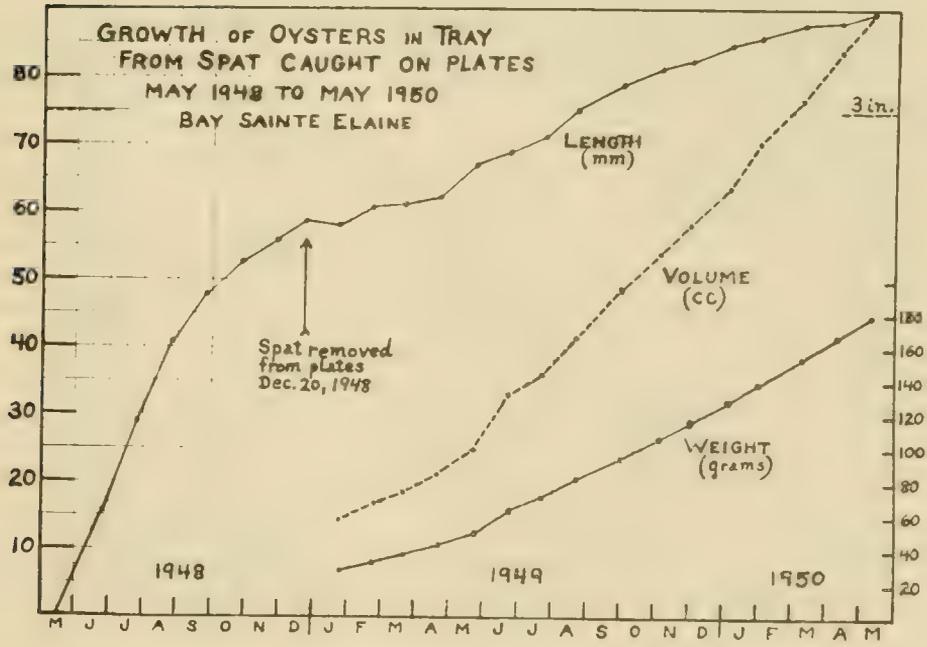


Figure 6

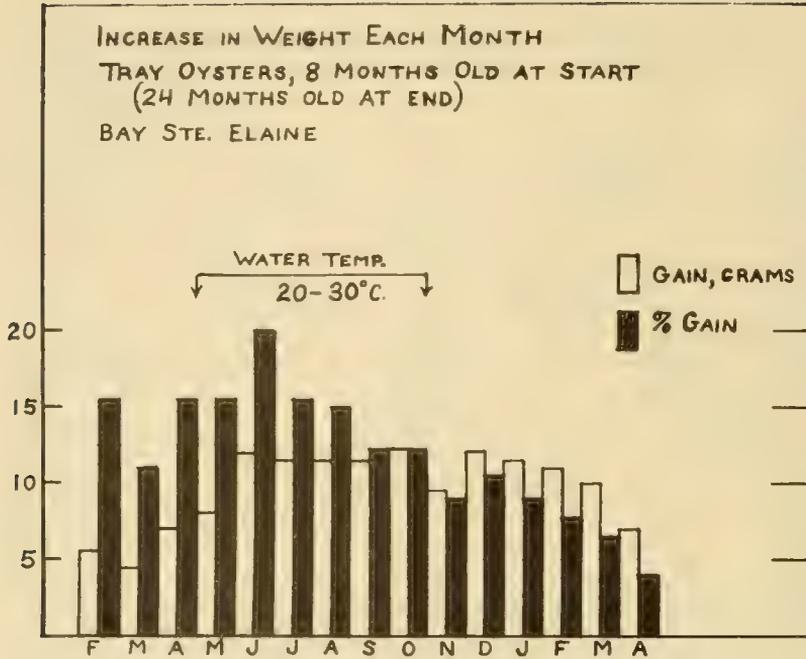


Figure 7

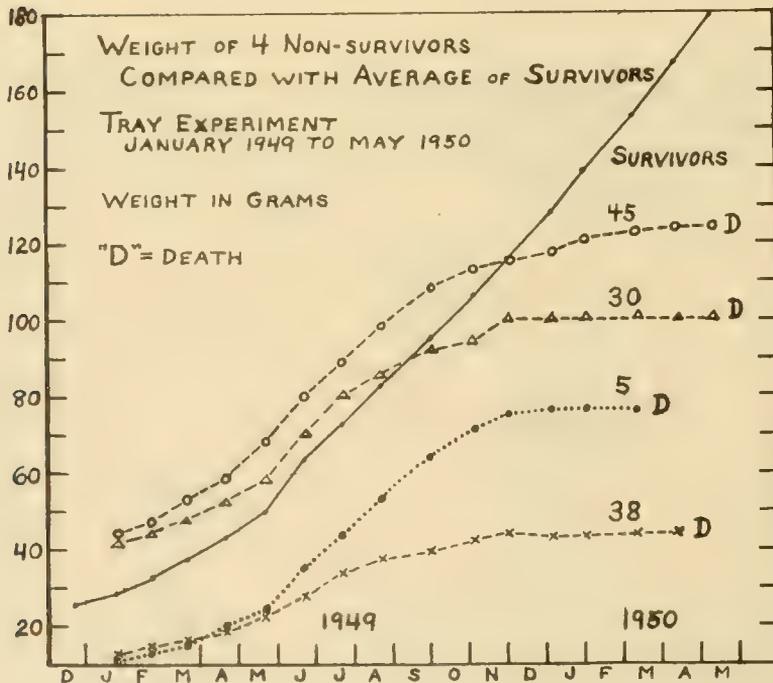


Figure 8

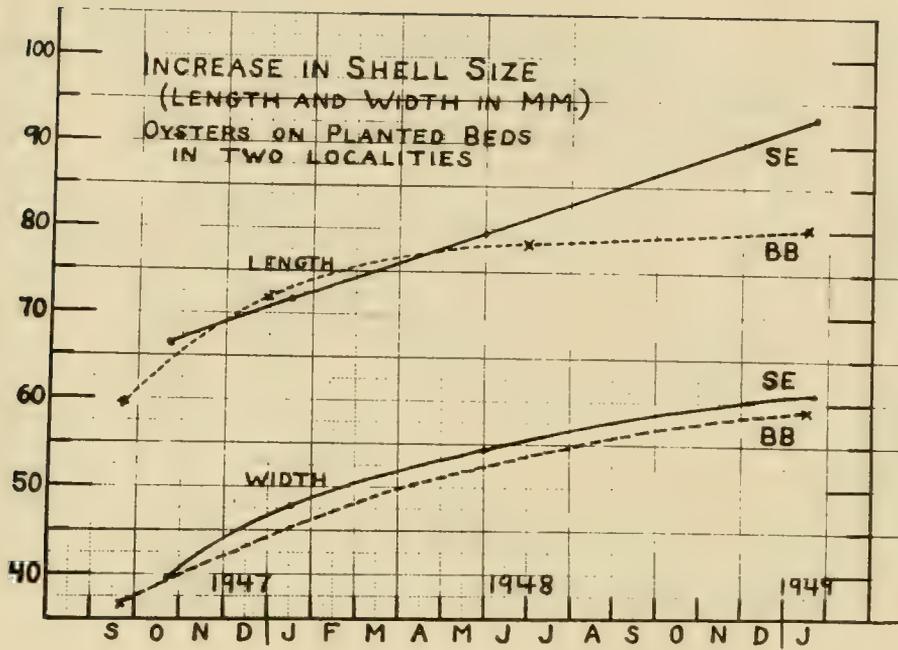


Figure 9

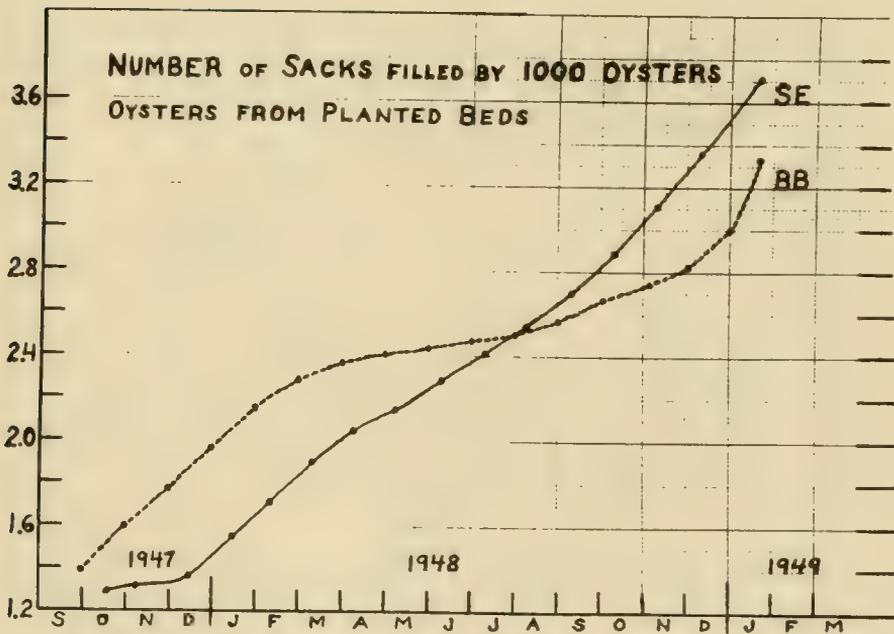


Figure 10

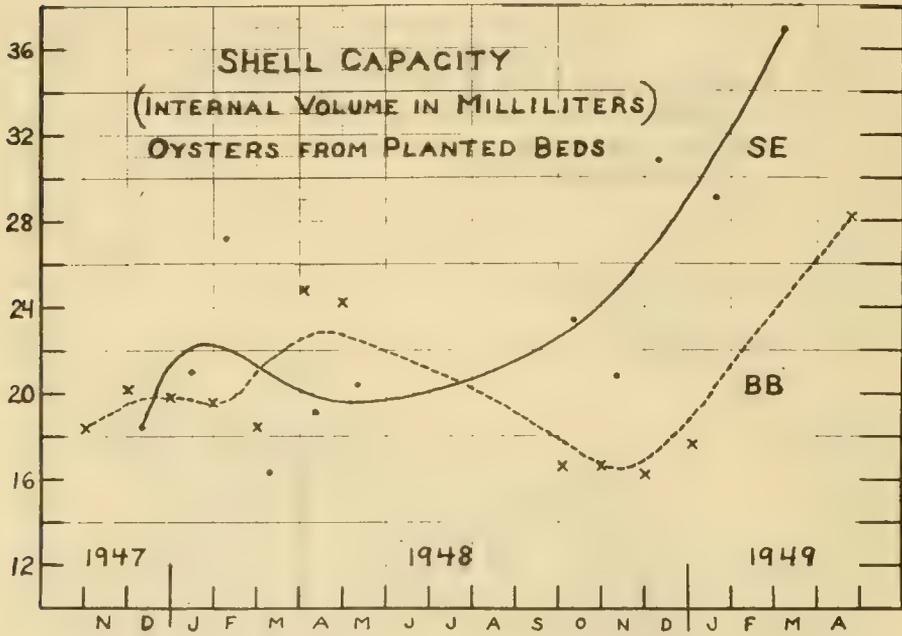


Figure 11

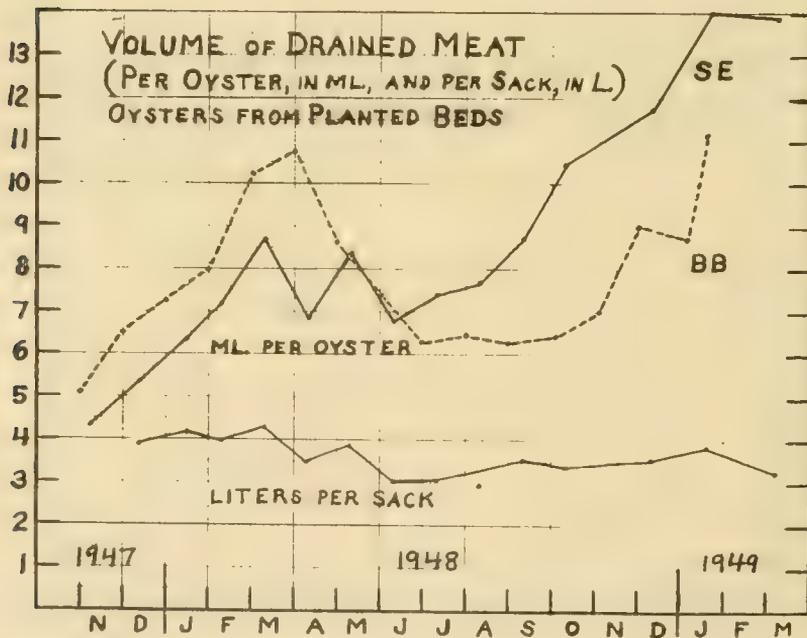


Figure 12

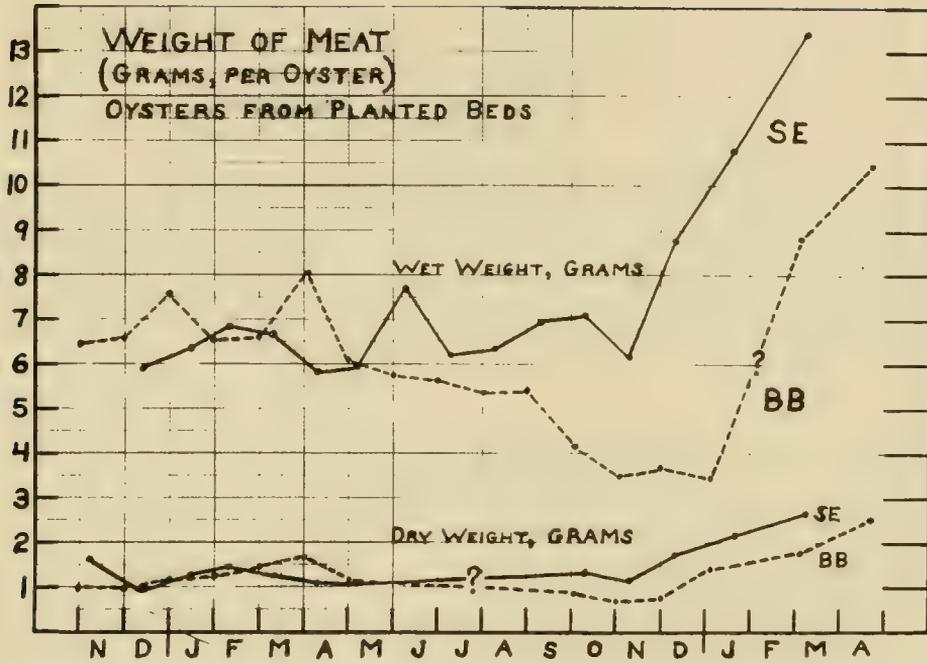


Figure 13

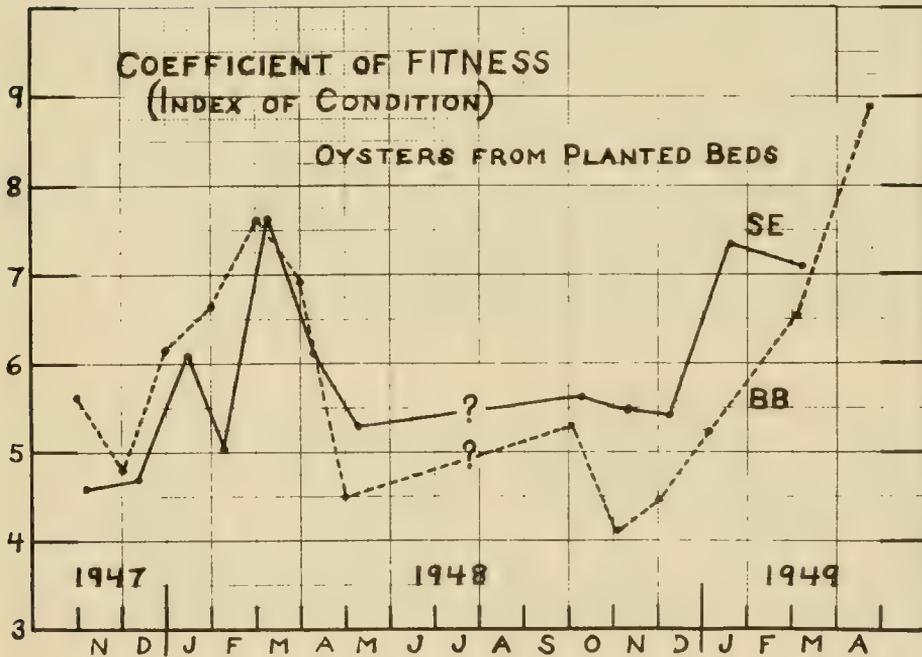


Figure 14

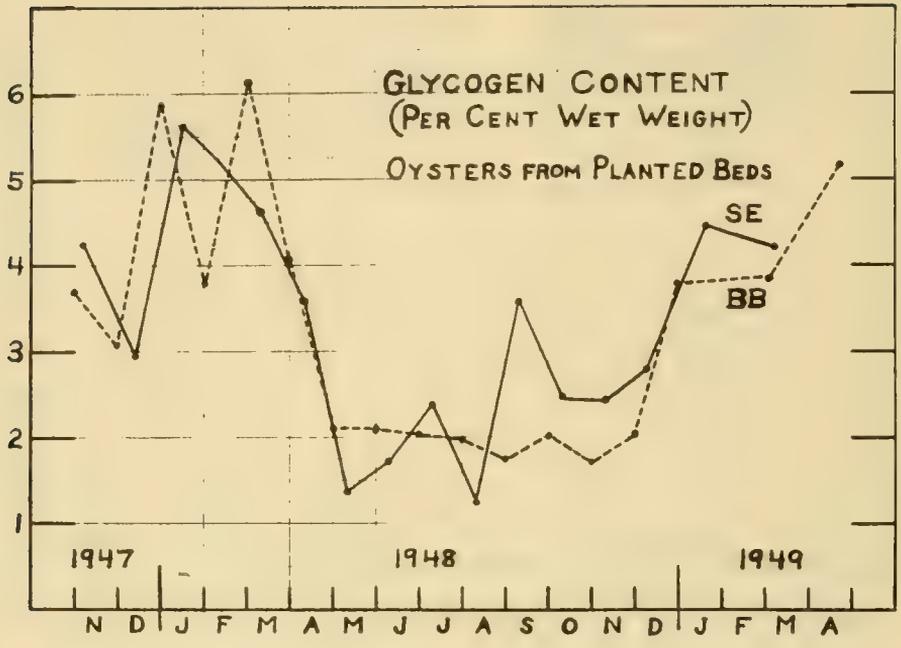


Figure 15

A REVIEW OF THOSE ASPECTS OF THE BIOLOGY OF THE OYSTER DRILL
UROSALPINX CINEREA (SAY) FUNDAMENTAL TO ITS CONTROL

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Relatively uncurbed, the oyster drill Urosalpinx cinerea (Say) continues to ravage the oyster beds of many parts of the north temperate world. The steady spread of its ranks from original areas of distribution and the increasingly evident destructiveness of the pest disclosed by critical observations of oystermen and marine biologists, have aroused genuine concern among those burdened with effecting control. The oyster drill is rightly recognized as one of the leading predators of all species of young oysters encountered in its range.

Thus it is appropriate at this time to re-examine the available knowledge on the biology of this small gastropod in order to provide a background for a re-evaluation of the kinds and intensity of drill control currently in progress. This report represents a partial condensation of a longer fully authenticated treatment of this subject which will appear elsewhere, and is in large part taken from a considerable number of technical reports - many unpublished - by J. R. Adams, H. A. Cole, J. B. Engle, H. Federighi, P. S. Galtsoff, H. H. Haskin, V. L. Loosanoff, J. R. Nelson, T. C. Nelson, H. F. Prytherch, I. W. Sizer, L. A. Stauber, and many others. Grateful acknowledgment is expressed to these investigators.

At present U. cinerea occurs in a number of localized areas on the east coast of Canada and along the Maine and New Hampshire coasts where it is not yet a serious pest. It abounds and is highly destructive in the coastal waters of Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Maryland, and Virginia; and is less abundant in North Carolina, South Carolina, Georgia, and northeastern Florida. It was unwittingly transported to the west coast of North America in the late eighteen hundreds and has become established in coastal embayments in western Canada, in Washington, and more successfully in California. By 1928 it had become firmly entrenched in British oyster beds, and now is numerous and a very serious predator in Essex and Kent. No evidence has been unearthed to confirm Federighi's statement that U. cinerea has been introduced in Bermuda.

It is probable that Urosalpinx has become a widespread uncontrolled pest since the advent of means for extensive transplantation, cultivation, and harvesting of oysters. Many of these methods inadvertently favor wide dissemination and augmented propagation of drills. Unless carefully checked, the transplantation of oysters of all ages from one region to another may introduce drills and their egg cases. Quite unintentionally live drills accompanying shell dredged for cultch may be shovelled overboard on other grounds. The washing which oysters receive when hauled aboard in standard dredges flushes most of the smaller drills through the dredge bags and returns them to the bottom. In this manner the extension of the range of this gastropod,

unknowingly transplanted from its original haunts on barnacle and black mussel bottoms and natural oyster reefs, has kept steady pace with increased human utilization of new grounds for the culture of oysters. Once established on oyster beds the drill soon attains high concentrations and persists there with phenomenal tenacity and success unless effectually controlled by man. The density of these stabilized drill populations fluctuates in large measure with the available food supply, drill breeding cycles, the abundance of enemies, and gradual or catastrophic changes in the physical and chemical environment.

Within their geographic range drills may be found from the intertidal areas of coastlines to depths of 90 feet; they have been collected on all types of hard and shelly bottom, and occur more plentifully on bottoms covered with oysters than on vacant ground. Although a muddy surface devoid of shell is least attractive, they do adapt themselves successfully to life on shell or oysters distributed on muddy bottom.

Water temperatures which may rise above 86°F for considerable periods of time, as in shallow waters along the New Jersey Cape May shore, Delaware Bay, do not prove a barrier to drill distribution. When subjected to such temperatures during low water they bury just beneath the surface of the bottom. Conversely *Urosalpinx* is able to withstand low temperatures of severe winters which in Great Britain apparently destroy the native English drills.

In the fall as the temperature of the water approaches 36°F in Delaware Bay, drills move onto the bottom. Some pass the winter attached to shell on the surface, or crawl into crevices among shell clusters, or may even retract within their own shells. Others dig into the bottom, crawling downward along the surface of buried shell or other hard surfaces. To date all evidence indicates that the latter remain just under the surface of the bottom, with siphon tip directed upward and in contact with the water. While at summer temperatures drills cling to hard surfaces with persistent tenacity, at low winter temperatures they may be readily dislodged because of the weak adhesion of the foot to the substratum. Further studies should disclose the proportion of drills that burrow preparatory to hibernation, the precise response of these snails to dropping temperatures before hibernation and to rising temperatures in the spring, the degree of dormancy exhibited by them during hibernation, and their behavior in the wide variety of winter conditions encountered over their latitudinal range.

Low salinity of the water does effect a barrier to the distribution of drills, particularly in estuaries at the mouths of rivers. The range of these lethal salinities appears to be associated with temperature and the salinity of the water in which drills have lived. *Urosalpinx* collected in Hampton Roads, Virginia, at prevailing summer temperatures, in salinities ranging from 15-20 o/oo were destroyed by a drop in salinity to 12 o/oo, whereas drills gathered in Beaufort, N. C., in salinities over 30 o/oo did not survive a drop to 16 o/oo. At lower temperatures drills are able to withstand correspondingly lower salinities. In Delaware Bay laboratory experiments conducted in outdoor winter temperatures demonstrated that drills

are able to attach to the sides of their containers in salinities as low as 7 o/oo, and drills in 10 o/oo were still alive and capable of movement after more than 19 months. Other experiments, run to duplicate conditions resulting from cold spring freshets followed by saltier periods, disclosed that 9 to 10 days exposure to salinities varying from 0 to 8 o/oo are required to kill 50% of the drills. At the other extreme, at least in some regions such as Beaufort, N. C., drills thrive in salinities which reach 37 o/oo for several days at a time.

The barrier against the distribution of *Urosalpinx* afforded by low salinities has stimulated the development of an extensive oyster industry in such areas, among others, as Delaware Bay, N. J., and the James River, Va. Natural oyster seed beds are located up bay in water of low salinity usually fatal to drills but favorable to oyster seed. By the time oysters are transplanted to lower more saline growing grounds, they have developed thicker shells less readily perforated by the drill. More detailed studies on the relation of salinity to the activities of the various stages of the life cycle of the drill in the whole temperature and geographic range of the species are highly desirable.

The foot of *U. cinerea* is quite small, scarcely extending beyond the broadcast outlines of the shell. A tough horny door, the operculum, is firmly attached to the posterior dorsal surface of the foot and when retracted, completely and effectively seals the fleshy organs of the snail within its shell. Just how long the mollusk can remain thus closeted is not known, though incomplete reports suggest a period of 5 days in the summer. The combination of the hard inconspicuously marked shell and the tough operculum constitutes a formidable armor against the intrusion of enemies.

These gastropods lack a free swimming larval stage and depend entirely on their own slow limited creeping or upon transportation by human or other agencies for dissemination. The economic implications of the possible movements of *Urosalpinx* onto drill-free oyster bottoms from surrounding infested grounds have promoted a number of preliminary investigations on migration. It is reported that under summer field conditions marked drills creep at an average rate of 0 to 24 feet per day. A maximum figure of 75 feet per day is recorded by one group of investigators, but this is probably an extreme figure. Since drills are known to hitch-hike on other animals such as crabs and on floating algae and debris, occasional extreme figures are not improbable.

Several factors modify the rate of creeping. Drills living among oysters and other food move very little while those on bottom devoid of food tend to crawl at an average rate of 15 to 24 feet per day in the direction of food which attracts them. It would seem that the impetus for drills to migrate away from bottoms covered with shell (which support food organisms) and/or oysters of all ages may not be as strong as has been supposed. Sand and mud bottoms devoid of shell considerably slow the locomotion of drills. Indeed, it has been suggested on some evidence that wide lanes of clean mud bottom may constitute relatively effective barriers to drill migration. Low salinities and temperatures also retard their progress. In a laboratory experiment

drills crept at an average rate of 31 feet per day at 77°F and only 1.4 feet per day at 40°F. Current velocities further influence their movements. A drill placed in flowing water will immediately and definitely turn into the current and move upstream. At much reduced current velocities this response is lost. Galtsoff has emphasized that the behavior of these snails is determined by the combined and antagonistic effects of the environmental factors. In general the rate of movement of these mollusks on native bottoms is probably quite low and extremely variable since the rate and direction may be influenced by so many factors.

The pronounced variability of drill densities observed by numerous investigators, even on adjacent oyster grounds differing markedly in the concentration of oysters, and the report that in Delaware Bay the proportion of drilled *Urosalpinx* shells increases with the degree of barrenness of bottoms, further imply a limited rate of migration. Federighi, Stauber, Cole and later Engle, conclude from the limited information available that it is unlikely that migration of drills is of more than local significance in the distribution of the species.

This discussion still leaves unanswered the important question of how *Urosalpinx* seems to appear suddenly and in devastating numbers on newly planted oyster bottom. Stauber has given a probable explanation which is supported by more recent information. There is a tendency for considerable numbers of drills, particularly the smaller stages, to be washed back onto the bottom during harvesting of oysters or cleaning of bottoms with standard dredges. Furthermore in drill-dredging operations these do not uniformly clear the ground of trash or drills. Consequently numerous *Urosalpinx*, most of which are small and thus generally overlooked, remain. When a commercial planting of oyster spat is made on these bottoms, destruction (its rate commensurate with the concentration of drills) occurs. The entire slaughter may be erroneously attributed to drills which have migrated from nearby grounds. If the majority of drills on these grounds are small and the newly planted seed oysters large, extensive drilling may not occur until later in the season or during the following year when small drills have attained a larger size, stimulated to rapid growth by the improved food conditions introduced with the new oysters.

This unfortunately is not the whole explanation. As the migration studies indicate, some limited localized migration of drills from surrounding areas may occur along the boundaries of the new planting. Further, a few young drills probably hitch-hike onto newly planted grounds on floating marine algae or on other debris as well as on widely roaming bottom dwelling animals. Much further research is urgently required on drill migration and on the role of floating materials and of roving animals in drill dissemination.

The head of *Urosalpinx* is small and bears a pair of long slender tentacles each of which in turn supports a small eye. These are sensitive to light. Laboratory experiments have demonstrated that drills tend to creep away from bright light, toward weak light, and are indifferent to very weak

illumination. These observations were confirmed in Delaware Bay. In the summer, on clear days, and in water 1 to 4 feet deep drills remain attached to the underside of oyster clusters; on overcast days they climb on top; and in deeper turbid water, they are more commonly collected on top of clusters than under them.

Over and behind the head and under the shell of this gastropod there is enclosed a spacious chamber, the mantle cavity, which houses a conspicuous double-comb-shaped gill. External respiration is accomplished by the passage of water over this organ. Water is drawn into the left side of the cavity by way of the siphon, a partly closed fleshy tube in the front part of the shell, and is ejected from the right side. Urosalpinx will remain alive only so long as the mantle cavity retains moisture. When the drill is forced to crawl out of water the loss of this vital fluid is accelerated and desiccation is thereby hastened. From the standpoint of control, it would be useful to learn the length of time that drills of various sizes can tolerate drying, particularly when exposed on the surface and when buried at various depths in bottom material drill-dredged from oyster grounds and heaped on shore. Similarly the capacity of drills to crawl out of different depths of such trash dumped on submerged vacant bottom after drill dredging operations would be valuable information.

Within the mantle cavity beside the gill there nestles a specialized sensory surface, the osphradium. This received the full flow of water drawn into the gill chamber and estimates the quantity of sediment suspended in the water. This function is of significant importance to the drill which often lives on muddy bottoms where the danger of fouling or blocking of the mantle cavity is a constant one. The response of Urosalpinx to the range of suspended sediments encountered in its normal habitats has not been investigated.

Dissolved substances in the water are probably perceived by the drill by means of sensory surfaces on the tentacles, the front part of the foot, the underside of the head, and the siphon. Preliminary precise research on the response of these mollusks to substances released by oysters was performed by Haskin, who demonstrated that the capacity of drills to detect minute traces of these substances in their native habitats plays an important role in food selection. He led bay water into 2 adjoining containers and placed oysters of different ages in them. The overflow water from these was led to opposite corners of a flat dish in the center of which drills were placed. The majority of the drills soon crept to the water coming from the youngest oysters. In these and in controlled field studies it was learned that oyster seed a few weeks old is approximately 4 times as attractive to drills as 3 year old oysters, and that the differences which enable the drill to distinguish between young and old oysters probably disappear after oysters have reached an age of 3 or 4 years. Four to 7 year old oysters imported from brackish water where growth was poor proved more attractive to drills than younger oysters. This apparent inconsistency is explained by the fact that oysters transplanted from inferior to superior growing conditions are stimulated to grow, and it is during this period of rapid growth, characteristic of young oysters, that the drill attracting substance is released. Watery suspensions

of oyster meats do not have the same appeal to drills as the water from young live oysters, suggesting that the drill attracting substance is produced principally by living oysters. While a decided preference for smaller oysters is usually expressed by *Urosalpinx*, larger oysters are not immune from their attacks even in the presence of oyster spat. The chemical nature of the attracting substance has not been investigated. The possible isolation and further detailed study of this substance could conceivably lead to a chemical produced synthetically and on a large scale which could be utilized in extensive trapping. Further research should also be performed on similar substances in other drill prey such as mussels and barnacles, which might prove to be stronger lures.

Urosalpinx is said to be entirely carnivorous although no tests have been performed to determine the possible role of minute encrusted plants in its diet. It is known to drill and feed upon a great diversity of animals: lower invertebrates such as encrusting moss animals, barnacles, small crabs, cnitons, periwinkles, slipper limpets, mud snails, its own kind, and smaller edible and ribbed mussels, soft and hard clams, scallops, oysters, and the carrion of fin fish. In drill traps baits of small living edible mussels, oysters, and barnacles have proved the most attractive. Contrary to popular belief drills will leave a bed of young oysters and seek out those in traps. This behavior is associated with the strong tendency of *Urosalpinx* to crawl upward at certain temperatures (above 41°F in Delaware Bay, and 50°F in Virginia and North Carolina). In addition young oysters collected in brackish water constitute more attractive bait than similar seed from more saline waters.

Ostensibly no one has induced *Urosalpinx* to destroy living jingle shells in the laboratory, even when starved for several weeks; yet starved drills readily consume the shucked meats of jingles. A report or two is available on the collection in the field of empty jingle shells perforated by drills. The apparent repelling qualities of living jingle shells merit further consideration.

The size of oysters destroyed by *Urosalpinx* varies from that of recently attached spat to those over 4½ inches long. By and large younger oysters suffer the highest rate of predation. This helps to explain the discrepancy often arising among the numbers of oyster larvae in the water, newly set oyster spat, and available commercial seed oysters.

The drilling operation of bivalves by *Urosalpinx* involves a mechanical and probably a chemical phase. Before drilling, *Urosalpinx* searches the surface of its prey with the sensitive tip of its long slender everted proboscis. Rasping at 77°F proceeds at the rate of approximately 60 strokes per minute, but is not particularly effective on the harder portions of the shell. A gland in the under surface of the foot is intermittently brought to rest over the drilling site and a "soaking" period follows during which the gland billows into, and the foot is held tightly about the drilling site. This alternate process of softening and rasping is continued until the shell is perforated. The duration of rasping varies from 2 to 15 minutes and the softening periods from 1 to 47 minutes. Some as yet unidenti-

fied substance appears to weaken the shell material, as suggested by the fact that after each softening period the radula effects noticeable headway in drilling. All rasped shell material is swallowed. The identification and possible utilization in control of the drill of this softening substance provides another worthwhile and provocative field for research.

The average rate of drilling through the shell of an adult oyster approximates 0.4 mm. per day, but this will vary with the hardness of the shell, size of the drill, temperature, and other conditions of the water. The average time required for perforation varies from 5 hours for a very young drill 3 mm. high to penetrate an oyster spat 2 mm. long, to 7 days for an adult *Urosalpinx* to drill through the shell of a $4\frac{1}{2}$ inch oyster. The quantity of oysters drilled per year is further altered by the presence in the habitat of other food organisms such as barnacles and mussels. These may be considered animal buffers in that they likewise attract drills and in consequence probably reduce predation of the oysters. The quantitative aspects of these relationships have not been explored to any degree. Moreover the season and the breeding cycle of the drill influence the rate of drilling. They feed voraciously after spawning, and thereafter the feeding rate declines gradually but irregularly as water temperatures drop.

Selection of the drilling site depends on factors not yet understood. *Urosalpinx* perforates either valve of an oyster, preferably the exposed side, and every portion of the shell, but the majority of the perforations occur over or near the center of the valve. It does not necessarily seek out and attack those portions of the shell which are softer or thinner and thus easiest to penetrate.

The diameter of the drilled hole is in general proportionate to the size of the drill. Snails under 15 mm. in height drill holes 0.5 mm. in diameter, and drills averaging 25 mm. produce holes 0.8 mm. in diameter.

Feeding is entirely mechanical. *Urosalpinx* extends its proboscis through the perforation in the shell of its prey and with the radula tears away the softer fleshy parts. Very little of the heavier muscle is removed. Drills are voracious feeders. In the laboratory one snail devoured about 0.4 cubic inch of oyster meat in 24 hours, and others have been observed to feed continuously for periods up to 20 hours. Federighi observed that oysters from which drills were removed after perforating them, with a few exceptions, eventually died; since oysters bored with a machinist's twist drill lived indefinitely, he ascribed the death of the oyster to some toxic substance injected into the oyster by the drill. These observations have not been confirmed and call for additional study.

The sexes are separate in *Urosalpinx* and no sex changes occur within an individual. Copulation has been described only once, and this between a male and female of the closely related drill, *Eupleura*. The male mounted the right side of the shell of the female and extended its long slender penis, protected between the inwardly folded lobes of the front lateral parts of the foot, into the genital opening of the female. Copulation persisted

over a period of 21 days even though the mating pair were separated several times. In New Jersey *Urosalpinx* reaches sexual maturity at about 15 to 25 months of age, and at a size of more than 15 mm. in height. In English waters full spawning is said to begin at 3 years of age and continues to an age of 10 to 13 years.

Spawning begins when the water warms in the spring. In Canada, Virginia, and North Carolina the spawning temperature is reported as 68°, in New Jersey as 57-59°, and in England as 54-55°F. Spawning terminates in the fall as water temperatures again fall below these temperatures. In New Jersey, Massachusetts, and England egg case deposition reaches a peak in early summer, almost ceases in mid-summer, and a second less intense wave appears in late summer and early fall which is believed to be oviposited by young drills maturing in the late summer. By contrast, in Chincoteague, Va., spawning is said to occur over a period of about 7½ months from June to January, with a climax in June and July. In shoal areas where water warms faster in the spring and cools rapidly in the fall, drills oviposit sooner and stop earlier than drills in deep water.

Urosalpinx can survive where it cannot reproduce. In estuaries of fluctuating salinities it may be able to oviposit but these eggs may not develop. In Delaware Bay minimum salinities close to 15 o/oo are thought to be necessary for spawning and development of the young.

Urosalpinx prefers living oysters for spawning sites but may deposit egg capsules on bags of shell, cement blocks, tin cans, snail shells, barnacle covered rocks, oyster stakes, or similar objects. Oviposition surfaces become much more attractive if projecting somewhat above the surface of the bottom. Before depositing her egg cases, the female carefully scrapes the egg laying site clean with her radula. Then at a rate of 3 or 4 capsules per day, she extrudes, carefully moulds, and cements each capsule to this surface. It is said that she does not feed during actual spawning, remaining attached to the spawning site, and unless disturbed, she continues until spawning for the season is completed. In Delaware Bay a female over 21 mm. in height can deposit an average of 72 and a maximum of 96 egg cases per season; it is questionable whether she will deposit this number of egg cases without feeding.

Drill egg cases range in height from 3.4 to 8.7 mm., the size being related to the size of the female. The tough leathery membrane of the case is composed of a double envelope permeable to sea water and to foreign chemicals, and protects the developing young from mechanical shock and from desiccation when exposed at low water in intertidal areas. The larger the egg case the greater the number of eggs per case, the number of eggs varying from 0 to 22, with an average of about 9 per capsule.

The larvae develop entirely within the egg case and emerge as fully shelled destructive snails. The rate of development is associated with environmental temperature, and in the field may occur in as short a time as 18 days or be prolonged to 78 days. All early prehatching stages may be killed

within the egg case by submersion in water of 5 o/oo, but it takes considerably longer at this salinity to destroy the unhatched shelled stages. All unhatched stages may be destroyed by exposing the egg case on shore for 3 or 4 days, or by dipping in boiling water for 10 to 15 seconds. They may also be destroyed by immersion in a 1 to 200 solution of copper sulfate for 1 minute, or in a formalin solution of 1 to 100 for 3 minutes; however, adult oysters with injured bills may also be destroyed by these chemicals. Insecticides such as amox and rotenone have also been tried as dips and sprays in the laboratory, but though non toxic to oysters they are useful only for the destruction of the earlier prehatching stages of *Urosalpinx*.

Some mortality of embryos occurs in the egg capsule, and is in part ascribed to the consumption of abnormal embryos by normal ones. The rate of this destruction as described by different investigators ranges from 13.9 to 58%. Fully developed shelled young drills, 0.8 to 1.5 mm. in height, emerge from the egg case by way of a small opening at the upper end of the case. Some may even drill their way out through the wall of the egg case. Four days to 5½ weeks may elapse between the time of emergence of the first snail and the last. Considerable cannibalism has been reported among newly hatched drills in captivity; it is questionable how wide spread this is in nature. Stauber from his extensive studies doubts that there is any appreciable survival through the winter of young drills hatching from the slight late summer spawning in Delaware Bay since so few of these young drills are recovered the following spring.

The average and maximum height of the shell of *Urosalpinx* varies in different regions. In most localities adult drills average over 26 mm. (1 inch), but in Seaside, Va., specimens as large as 56 mm. (2¼ inches) are common.

The most detailed research on the growth rate of *U. cinerea* to date has been performed by Cole in English waters. He collected large samples of all size groups of drills at monthly intervals and by analysis of growth curves and growth marks, concluded that in England the drill may live to an average of 10 and a maximum of 13 to 14 years of age, achieving the following sizes: for the female, average 39 mm., maximum 43 mm.; for the male, average 36 mm., maximum 39 mm. In America figures ranging from 9 to 19 mm. are reported for growth during the first year, but no comprehensive studies have been carried out, even though this aspect of drill biology seriously merits further exploration.

From available information on the life history of the drill it is possible to estimate grossly the average and maximum number of young drills produced by a female. Since this information is pieced together principally from preliminary, and in some cases unconfirmed, data on drills from scattered regions, it must be interpreted in this light. Assuming that a female drill deposits an average of 72 egg cases per season with an average of 9 eggs per case and a survival of young in each case of 50%, over an average productive period of 7 years, she may produce 2,268 young snails. On the other hand, assuming a maximum of 96 egg cases per drill per season, a maximum of 22 eggs per case, a mortality of 14%, and a maximum productive life of 11 years, a female could theoretically produce 19,980 young drills.

Although *Urosalpinx* is plagued by few enemies other than man, some unexplained mortalities occur. Stauber observed a sizable death rate in Delaware Bay during the winter and spring when temperatures were sufficiently low to inhibit migration. On one oyster bed which was drill-dredged periodically the percentage of dead ranged from 5% in December to 30% in March. A variable degree of cannibalism has also been reported among drills in the field.

The oyster drill possesses but few apparent weaknesses in its life cycle, and thus far has rather successfully resisted man's relatively uncoordinated and sporadic attempts to control it. Young drills pass their larval stages well protected in a tough egg case and emerge already covered by a strong shell and operculum and well equipped to plunder neighboring spat. The pest is highly adaptable to a wide range of environmental conditions. The firm attachment of egg capsules, the fixed adherence of all post hatching stages to living oysters and shell during the summer months, and their relatively small size and drably colored shells, increase the chances for dissemination of the species in the management of oysters.

To date drill control has been attempted by means of hand picking, flaming, forks, deck screens, oyster dredges, drill dredges, drill trapping, and hydraulic suction dredges. None of these methods or combinations of these has succeeded in completely eradicating the drill from oyster grounds. However it must be emphasized that when conscientiously applied, control has retarded drill destruction to a rewarding degree. By far the most modern and the most effective mechanical device yet used is the hydraulic suction dredge. This shows unusual promise and it is envisioned that continued experimentation on the design and operation of the dredge concurrent with further research on the life history, behavior, and ecology of the drill will in time bring about more thorough and lasting control of this gastropod pest.

The possibility of controlling drills by chemical means has been discussed by a number of workers. Loosanoff and his associates first attempted a study of chemical control on a large experimental scale. They began studies in 1947 in a search for substances which would prove inexpensive, affect oyster enemies injuriously, yet remain harmless to man and commercial marine organisms. They have screened over 1,000 chemicals, and the information so collected, though extensive, is based on preliminary laboratory experiments. Thus final conclusions are not yet available. Further screening experiments and actual field tests are required. Several compounds have been discovered which appear promising because their addition to the water even in small quantities causes the exposed parts of drills and other snail enemies to swell far out of the shell in a short time. During this distended stage, which lasts for several hours to several days, the drills are incapable of locomotion and are unable to contract, and thus are vulnerable to ready attack by such enemies as crabs which suffer no ill effects from consuming them.

From a purely practical point of view it is clearly evident in our present state of knowledge that far more effective control of this animal could be exercised if oyster farmers in each infested region would cooperate in a concerted continuing program of control. Because of the eminent success of the drill on commercial oyster grounds it would appear that long range effective control cannot be effected by a few.

OYSTER PRODUCTION AND OYSTER DRILL CONTROL

By Joseph B. Glancy

1. Need for Oyster Drill Control

By and large, the oyster drills or borers, *Urosalpinx cinerea* and *Eupleura caudata*, are the worst enemies of oysters. Oyster production in the U.S.A. over the last five decades has been gradually declining.

U. S. Oyster Production

1910	230,815,000 lb. of meat
1920	not available
1930	124,968,000 lb. of meat
1940	89,384,000 " " "
1950	76,636,200 " " "

A considerable part of this decline is due to the loss of productive areas because of pollution, but the main reason is the inability of oyster farmers to control drills. Most oyster growers are not aware of the seriousness of the drill problem. One reason for this is because so little has been done comparatively to control drills. Production of oysters in this country will never increase substantially until feasible methods of drill control are universally established. It is the purpose of this paper to review some of the work done on controlling drills and make suggestions for the development of better methods.

Several scientists have reported on drill damage. Dr. Thurlow C. Nelson and his associates have frequently given figures on oysters killed by drills in Delaware Bay. They estimated that an adult drill destroys 20 year-old oysters per season. The great and insidious damage done to oyster crops, however, is the destruction of newly set oysters by recently hatched drills. Engle described this from observations made at Milford. He found that a young drill could destroy 8 young spat per day. Practically all oyster bottoms, setting, growing and marketing, along the eastern seaboard where salinities are suitable are saturated with these young drills. As a consequence, seed oyster production is almost negligible on these grounds. I have observed frequently over the years excellent oyster setting on growing and marketing grounds which is wiped out in a few days by drills. This, curiously enough, is not without benefit. If much of this set survived, it would render the oysters practically unmarketable for shell stock. It occasionally does happen that set survives on adult oysters and when this occurs it is usually more profitable to handle the bed as seed. In some of the more southerly sections one of the drawbacks to oyster culture is the continual setting and survival of young oysters on older oysters resulting in a product that can only be handled in canneries, if used at all.

The reason that oyster growers are not aware of damage by young drills is because they do not know of the oyster set which occurs almost every year on their shellfish grounds, the set which is destroyed sometimes in a matter of hours by young drills. One of the best and most valuable series of observations on oyster setting ever made in this country is the recording made every year since 1937 by Dr. V. L. Loosanoff and his associates at Milford, Connecticut, and issued currently in bulletin form during the spawning and setting season by the Connecticut Shellfish Commission. The method used is to plant clean shell at about 10 stations and after 3 or 4 days make counts on the number of oysters attached. One of these stations, number 2, is adjacent to ground which the Connecticut Oyster Farms Company uses for growing ground and occasionally for marketing when meat yield is satisfactory. The count of spat per one side of 20 shells for Station 2 last summer (1952) which was considered a poor setting season was 135. This would be equivalent to approximately 5,400 spat per bushel. Practically no seed oysters survived at Station 2 for 1952. The spat was not all destroyed by drills, of course. There are normally present in this area other enemies and also smothering conditions which always take a toll of young oyster spat. Nevertheless, if this ground had been drill-free, a crop of seed would have been obtained in the vicinity of Station 2 for 1952.

Several of Dr. Loosanoff's stations are located in New Haven Harbor where annually about a half million bushels of shells are planted to obtain seed oysters. For many years I have made observations in New Haven Harbor on setting and survival of set. These may be summed up by stating that, if the number of larvae which attach is great enough to feed the drills, then seed will be obtained. In New Haven Harbor some drill control is secured, almost unwittingly, because oystermen, ostensibly to prevent fouling, dredge their grounds annually before planting clean, drill-free shells just prior to setting time. Comparatively few adult drills are removed by dredging, but considerable amounts of drill embryos attached to shells and oysters are taken from these areas.

The Bluepoints Division controls ground off Bridgeport in Long Island Sound on which they plant the bulk of shells. Of all the grounds owned by this company, these Bridgeport lots seem to have the lowest drill populations. Oyster seed is obtained here year after year. Comparatively light spatfalls survive fairly well. Unfortunately, the grounds in this vicinity are particularly prone to starfish invasions. It may be the low incidence of drills is due to the starfish. It has been reported that starfish kill drills. Our knowledge of other enemies of drills is scant and this matter should be a promising field of research on drills.

2. Drill Control Methods

(A) CONVENTIONAL OYSTER DREDGE

Some oystermen claim some degree of drill control with the use of the conventional oyster dredge. It is, of course, quite inefficient for adult drills because they readily escape through the large meshes of the dredge. The greatest advantage is perhaps secured by the removal of shells and oys-

ters bearing the drill embryos. Shells left on oyster bottoms enhance the drills' habitat by providing food and lodging.

(B) THE DRILL DREDGE, THE WIRE TRAP AND DRILL SCREENS

At our 1931 meetings at Sayville, Long Island, Mr. J. R. Nelson described the wire trap method which involves planting hundreds of poultry wire bags containing a peck of shells bearing oyster set which are pulled at weekly intervals so that the drills may be shaken out and at the same time drill egg cases removed.

Leslie Stauber at our Providence meetings in 1938 told of the use of the wire trap method as well as ingenious drill dredges and deck screens. I will not go into the detail of these, except to mention that all these methods are described together with the economics of their use in Bulletin 624 of the N. J. Agricultural Experimental Station by Leslie A. Stauber and George M. Lehmann as of Feb. 1937. Despite the fact that these methods appear feasible from a cost viewpoint, their use has not become universal throughout the industry. Some screening has been applied in the northern industry to effect drill separation as well as occasional use of special drill dredges and, in addition, in recent years several boats have been designed with mechanical drill screening. So far as I know, there has been no wide spread use of the wire trap method, despite the fact that it can be superior to the others in reducing the small drill population. It would be of interest to know just why oyster growers have not adopted these methods more widely.

(C) THE SUCTION DREDGE

The suction dredge is a hydraulic device for cleaning oyster bottoms. It appears to be a promising method of drill control. Drills, from egg to adult, can be captured at a rapid rate. Following the pioneering development by the Flower brothers, the inventors in the nineteen thirties, about 6 more suction dredges have been built, all with interesting innovations. One wonders, however, if the methods of operation have been based on the soundest biological principles as far as drill control is concerned.

If a symposium were to be held on the design and operation of suction dredges for drill control, here are a few questions which might well appear on the agenda:

1. Is it advisable to design a suction dredge for any other oyster cultural operation such as cultch procural, harvesting, or transplanting other than simple drill eradication?
2. Can the settling tanks be designed to insure that no small drills escape?
3. In relation to the topography of oyster bottoms, what is the best type of nozzle to use?

4. How much time is required for a suction dredge to clean drills off a piece of ground and what is the cost?
5. How large an area must be cleaned to prevent migrations from adjacent grounds?
6. How far away from oyster grounds is it necessary to dispose of tank settlings containing drills?
7. What does suction dredging do to oyster bottoms. Does it enhance it for oyster growing or does it make it inferior?
8. Does rile from a suction dredge cause sedimentation on adjoining beds?
9. How frequently must a ground be dredged to maintain it drill-free?
10. What is the best design in regard to nozzles, suction line arrangement, screening, and pumping with or without eductors?

Solutions to some of these questions may be aided by more complete biological knowledge of drills.

(D) CHEMICAL CONTROL

Land farmers have accrued great benefits from the use of insecticides to control pests. There is no doubt that drills would also be amenable to chemical control if we only knew what to use and how. Pacific coast oyster growers report that mercuric compounds can be used to destroy drills. Engle, some years ago, described a method of using copper sulphate to kill the embryos. More recently, Loosanoff has studied the effect of a great many chemicals on drill behavior in an effort to find repellants as well as specific poisons. At present, however, I know of no practical methods being used regularly for the chemical control of drills. It should be a very promising field of investigation.

(E) THE SALINITY BARRIER

This natural method of control relies on the fact that drills are killed or their activities inhibited in salinity zones where oysters can live, grow and reproduce. It has been and still is the most important factor in this country's oyster industry. It can almost be said that the history of the oyster industry is the history of the salinity barrier. In the northern industry of Massachusetts, Rhode Island, Connecticut and New York, many of the more brackish water oyster seed sanctuaries have been lost to pollution, industrial development, and depletion of spawning stocks. The Delaware Bay oyster industry has always placed almost sole dependence for its seed requirements on the so-called Natural Beds upstream of the Southwest Line in New Jersey and the East Line in Delaware. These areas are being endangered by pollution and depletion. In the Chesapeake and its tributaries the drill salinity barrier existing over large areas accounts for the bulk of the oyster production of

Maryland and Virginia. The James River seed beds are an outstanding example of oyster seed production as a result of lethal salinities for drills. Fortunately, this area has not as yet been seriously hurt by either pollution or depletion and for that reason Virginia is still the leading oyster producing state of the U. S.

What is the salinity barrier for drills? Federighi's work showed that it seems to vary somewhat according to the prevailing salinities in any one area. He found the death point salinity for the snails at Hampton, Va. was 12 p.p.th., while at Beaufort it was 14. At Milford, Connecticut, Engle's experiments showed a lethal salinity between 14 and 15 p.p.th. In this work, Engle pointed out the importance of temperature, high summer temperatures killing in much shorter time than during the winter. Stauber, in his paper before the National Shellfisheries Association at Atlantic City in 1941, described experiments in which Delaware Bay drills survived salinities below 12 p.p.th. for long periods. He mentioned that without any food two drills out of fifteen at the start were alive and capable of attachment and movement after being kept for more than 19 months in water of a salinity of 10 p.p.th. This amazing observation shows how difficult it would be to try to starve drills.

There is no doubt the salinity barrier offers a potent weapon for the control of drills in seed production. Unfortunately, most of the areas where these low salinities exist are state controlled, public grounds and unavailable for leasing to oyster growers. An exceedingly interesting experiment is now being carried out in Delaware Bay. Heavy oyster sets obtained in the more saline waters of the Bay are being transplanted to the Natural Beds before the drills can destroy them.

3. Conclusions

The number one problem in the oyster industry is the production of seed oysters. It will never be solved until better methods of drill control are developed by the oyster growers. Oystermen as a rule are unaware of the great damage done by drills particularly on newly set and small spat, and the scientists connected with the industry have never been urged enough or received adequate support for drill studies. The National Shellfisheries Association has had annual meetings consecutively since 1928, with the exception of the year 1945, at which many scientific papers on oyster culture have been presented. Only eleven papers have been devoted to drill investigations and almost all of these were given by Prof. Thurlow Nelson and his associates working in Delaware Bay. At least one section of the industry has realized the magnitude of the problem. Dr. Loosanoff in his bulletins out of Milford over the years has repeatedly called to the attention of the oystermen his observations on drills. Much of Engle's valuable work on drills has never been published.

In this paper I do not have the time to go into the scientific findings on drills, but fortunately Dr. Carriker will. This subject is in capable hands, I can assure you, judging by the fine paper he presented to us at our 1942 meetings at Atlantic City on the Boring Mechanism of the Oyster Drill

which was subsequently published in the Journal of Morphology for November 1943. We have learned much from the scientific work on drills, but in order to establish better control methods we need more laboratory and field studies on such matters as migrations, salinity tolerance, reproduction, food preferences, natural enemies, reaction to chemicals, growth rates, temperature effects, differences in behavior of the two species of drills, and their general life histories.

THE SOUTHERN OYSTER DRILL

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INTRODUCTION

The carnivorous gastropod, Thais, known locally as the oyster drill or conch, is probably the most destructive single agent affecting the oyster industry of the Gulf States. Incredible numbers of them exist in the estuaries and coastal bays. They are found primarily on the oyster reefs but are not restricted to any particular bottom type or water depth. They frequent the mean low tide line on rocky shores and are caught in shrimp trawls 15 miles at sea in sixty feet of water. Although sensitive to salinity levels of the water they occur naturally in the wide range from 15 to 35 ppt. and can withstand entirely fresh water for short periods. They show no reaction to changing pH levels within the normal range and they survive, experimentally at least, at oxygen levels too low to support most other marine life.

Thais is a very successful animal, biologically speaking, and there is evidence that it is continually extending its range. The occurrence of its shells mixed with oysters in the buried reefs shows that it has been here for thousands of years as a predator in the oyster community.

References to the drill appear in the literature almost exclusively in connection with oyster reef surveys. Investigators in the early 1900's, including Moore, Pope, Danglade, and Churchill, were interested primarily in the incidence of the snail and published few facts concerning its biology. Some of their comments were quite erroneous, as pointed out by Burkenroad in 1931, whose paper is one of the few concerned primarily with the biology of this snail. St. Amant's master's thesis on the biology of Thais is the most comprehensive study we have, but unfortunately this paper has been published only in abstract. Dr. St. Amant, who is now with the Department of Wildlife and Fisheries in Louisiana, made his field observations in Barataria Bay in the years 1936 and 1937. His very comprehensive study was hampered by the lack of facilities for maintaining snails under laboratory conditions.

The material I am presenting now is drawn from observations made at Pensacola and other Gulf areas in the past five years. Some of this information is new, and many of the details correct or extend our published knowledge of the snail.

SYSTEMATICS

Early papers discussed this snail under the name of Purpura haemostoma, although its correct name appears to be Thais floridana floridana Conrad 1837. Clench described the Louisiana snail as a sub-species in 1927 calling it Thais floridana haysae. Both he and Bartsch reviewed series of snails in 1937 and 1942 and concluded that the sub-species was valid. T. Haysae the

Louisiana conch is characterized by a more rugged shell, bearing a double series of blunt knobs around the body whorl. Thais floridana floridana is reported to range from Cape Hatteras to Texas and West Indies, while T. Haysae is found in the area from Mississippi Sound to Texas. There is much overlapping of range in the two forms along the Gulf Coast. I have recently found typical specimens of each type in bays near Pensacola, Florida. St. Amant found in Barataria Bay, Louisiana, that they occurred in the ratio of 6 to 9 haysae to one floridana.

It is reported that Clench later expressed doubt as to the validity of a subspecies. At Pensacola we have made reciprocal crosses between the two types and obtained normal oviposition and development of larvae. St. Amant reported he was unable to find anatomical differences in the two types. Certainly they are equally destructive to oysters and we may consider that we are dealing with a single animal in this discussion. I personally feel that we will be able eventually to associate the two shell types with different environments rather than with different hereditary patterns. Old snails measuring 3 or more inches usually have massive, chalky shells and are so different from younger snails that they might easily be considered a different species.

REPRODUCTIVE CYCLE

Thais is a dioecious snail, males and females occurring naturally in approximately equal numbers. External sexual organs are not sufficiently developed to indicate the sex until the snail reaches approximately 20 mm in length. In one of our surveys of a hundred mature snails collected at random, exactly half were female and half male. In older snails, i.e. over $2\frac{1}{2}$ " length, there is a slight preponderance of females.

The snails become sexually mature usually in their second summer, when they are one year old. In one instance, however, we observed normal capsule formation and egg deposition in a group of snails not over 8 weeks old. The maximum length of these snails was 25 mms. and the capsules were 4 mm long in contrast to the usual 10 mm capsule formed by the older snail.

Gonad activity is initiated when water temperatures reach 20° C although temperatures may be at this level or higher for a month before egg laying occurs. Breeding period reaches its height in April - May in the Gulf area but capsules are deposited regularly in the period March - August. The process is primarily a function of water temperature rather than a particular season. In the unusually warm winter of 1950, eggs were deposited both in the field and in laboratory aquaria during the middle of February, when water temperatures reached 22°. Breeding usually ends when temperature levels pass 30° C.

The snails deposit batches of eggs in capsules over a period of days or weeks. Fertilization is internal and copulation takes place as the first capsules are deposited. Copulation is not a necessary stimulus to egg laying, since we have had capsule formation in the absence of male snails. In all such cases the eggs were infertile and were soon destroyed by a fungus.

In most cases when egg laying is observed in early spring, the males will be found attached to the shell of the laying female. Later on, solitary females will be found laying fertile eggs and dissection shows masses of sperm in the female reproductive tract.

Development of the fertilized eggs, which lie embedded in a jelly-like matrix within the capsule, proceeds rapidly. Cleavage starts at the end of one hour, and the ciliated veliger larva, complete with shell, develops in about 8 days at a temperature of 25°. At this temperature level, total time for larvae development to the hatching state is 12 - 16 days. However, when cold weather follows egg deposition, development is slowed considerably. In 1950, for example, water temperatures dropped to 15 - 18° range after egg laying occurred, and larval development required 39 days. At the end of this period normal hatching took place and there was no apparent mortality or abnormality of the larvae.

The capsules vary in size depending on the size of the female, and are usually about one centimeter long. When new, they are a characteristic milky white color which rapidly becomes yellowish brown with age. Usually capsules are deposited at as high a level in the water as possible without having them exposed by the tide. The strong negative geotropism of the laying female at this time has been frequently observed and is the basis of one control method. However, it is by no means universal, and capsules are frequently placed on hard bottom materials and on under surfaces of submerged objects. When there is a scarcity of cultch material, the cases may be deposited in two and three tiers on top of each other. But in such instances, I have observed that succeeding tiers are always attached in such a manner as not to interfere with the escape of larvae from the underlying rows of capsules. Each capsule contains from several hundred to 4000 eggs, and in contrast to the eastern oyster drills, there is practically no mortality within the capsule. If not disturbed the female deposits from 50 - 150 capsules at the rate of 6 - 7 per hour and may repeat this several times in the breeding season. Seven females under observation deposited 500,000 eggs apiece in an eight week period under laboratory conditions. In one month 250 females, maintained for a growth experiment, deposited an estimated 100 million eggs. At hatching time, the operculum, a small closed pore at the apical end of the capsule opens by some unknown method and the young larvae pour out and take up their existence as plankters. The length of their free swimming life is unknown, but all of our field evidence points to a period of from 30-60 days. We have been able to maintain the free swimming stage for only 15 days in aquaria. By the end of this time they showed little growth and probably starved to death. Fortunately for the oyster industry, this long free swimming period greatly increases the chances for larval mortality, but unfortunately it also increases the dispersal range of the species. The fact that adult snails exist in such large numbers indicates that it is a remarkably successful animal as a plankter as well as in the adult crawling stage.

The escaped larvae have a strong negative geotropism which keeps them away from the bottom sediments after they emerge. Even violent agitation of the water does not cause them to sink to the bottom. They are also positively phototropic, which must help in directing them away from an early death in the mud.

We have recovered planktonic larvae in June, July, and August but have not found the first crawling stages until the first week of July. They are quite common from then until September. In 1951, the earliest observed egg depositions were mid May, and the first crawling larvae were found July 6th, indicating a possible minimum planktonic life of seven weeks.

When the larvae first settle to the bottom and assume the crawling habit, their shell consists of 3 whorls and is about 0.8 mm long. Within the first week they add two more whorls, increasing their length to approximately 1.5 mm, and at this time formation of the adult shell commences. The adult shell differs markedly, having a definite pigment pattern and having minute longitudinal ridges and granulations. The larval shell persists in the young snail as the terminal 5 whorls of the spire and may be identified until the snail reaches a length of 3/4 inch, when it is usually worn away.

GROWTH

With the establishment of the crawling habit, growth is extremely rapid. In the presence of an abundance of food, snails may reach a length of 25 mms in a month, 40 in 2 months, and 55 mms (that's over 2¹) in 5 months. Growth is necessarily a function of temperature and food availability. Some of our experimental snails have been larger at 6 months than others at 36 months of age. I might mention here that snails do not grow best on a diet restricted to oysters.

After the first year, growth rates become progressively slower, especially under laboratory conditions. The following chart shows average length increments of a series of 600 snails in different size groups held in large laboratory tanks. In these tanks, they were supplied with approximately 200 gallons of water per hour and a surplus of food was kept available.

Length Increases in 12 Months (1950)

Initial size in mms.	10-19	20-29	30-39	40-49	50-59
Average Increase	28	23	19	10	6
Maximum Increase	42	33	29	22	18
Minimum Increase	20	12	8	3	0

The effect of temperature on growth is demonstrated by conditions in 1949-50 when average winter temperature for December-February was 18°C and the average length increase for the group was 8.0 mm. The following year the average temperature level for the same months was 11°C and the snails failed to put on any new shell. Individual variations in growth are great. Under apparently optimal conditions, some snails may add 10-15 mms of shell while others in the same size and age groups do not grow at all.

We have held individually marked snails under observation for over 3 years. From our data, we can postulate a minimum normal life span of 5 years and the maximum may be twice as long. Mortality rates average 12% per year and are somewhat higher in younger rather than older animals under laboratory conditions.

In view of the extended life span of the adult snail, its low mortality rate, few natural enemies, and high reproductive potential, the numbers of larvae produced by a population of snails reaches astronomical proportions within a short time. We are fortunate indeed that larval mortality must be high or oysters might long since have disappeared from our coastal waters.

FEEDING HABITS

In Thais, as in other carnivorous snails, feeding is accomplished by means of a radula. This specialized structure is similar to a wood-working rasp and is moved back and forth over an object gradually wearing it away. There is no evidence that Thais uses a chemical as well as this mechanical method for drilling holes. The radula is well developed when the snail first takes up the crawling habit, and we have observed them drilling spat at this age and forming a hole .15 mm in diameter. A mature snail makes a hole ten or more times as large. The drilling of an oyster shell is a slow process and it may take two days for the snail to penetrate the shell of a mature oyster. It should not be thought that oysters are the food of preference, however. In repeated experiments we have found that snails attack almost all of the other sessile animals before attacking large oysters. Under laboratory conditions the order of preference is mussels, spat, barnacles, clams, hydroids, and finally mature oysters. As scavengers they go over the bottom somewhat like a vacuum cleaner, eating a great deal of the diatomaceous ooze which collects on objects. They also readily attack dead crabs and shrimps and other animals which they may find.

Feeding is by no means a continuous process with Thais. Scattered individuals enter into periods of inactivity lasting for days, even when other snails around them are actively feeding. In the laboratory some individuals may climb out of the water and rest for varying periods of time on the tank walls.

Snails may be kept at least three months in the absence of macroscopic food. They probably obtain a certain amount of nutrition from the slime on the walls of their container. Under starvation conditions, the snails will attack each other. There is a gradual killing off of the smaller snails until the entire population is approximately the same size.

The snail's sensory perception is well-developed. Currents of water are carried continuously down the ciliated siphon to the gill and pass over a specialized organ called the osphradium. This structure is well supplied with nerves and appears to be an important sensory organ. There is no doubt that the siphon has important sensory functions too, and it waves around in the water something like an elephant's trunk when food is placed in a tank. Starved snails react to the presence of food in less than a minute when it is placed at a distance of 4 feet from them. Trays of oysters placed on apparently barren sand bottoms near the laboratory are filled with drills at the end of 24 hours. When drills are removed from such trays, the tray will continue to attract drills on many successive days, indicating that snails from a very wide area can be attracted to a bait. When food is plentiful, however, snails move about very little. They will remain within a one foot circle indefinitely if the supply of food holds out.

The snail's activity, including feeding, is under the control of temperature as well as salinity levels. At temperatures of 12°C, about 55°F, snails stop feeding and come to rest; at lower temperatures they enter a state of hibernation and respond only slightly to mechanical stimuli. The length of such hibernation periods, of course, varies with the geographical area and the severity of the winter climate. Usually, when hibernating, they bury themselves in the sand or mud. We have observed on several occasions that one day the rocks around the island would be covered with snails and then after a cold spell, it would be almost impossible to find a snail.

Snails move fairly rapidly covering 10-12' per hour on a smooth hard bottom. They can move up to 30' per hour on glass surfaces. We have found no natural bottoms which serve as a barrier to their movements. Although they appear to prefer the hard shell reef or rocky bottom, it is probably because of food availability. They are able to cross without difficulty 15 yards of mud too soft to support an oyster in order to reach a baited trap.

SALINITY TOLERANCE

The only really effective barrier to snail migration is a chemical one-- lack of sufficient salt in the water. They are normally absent from those areas having a sustained salinity level of less than 15 ppt. Their tolerance to short exposures of fresher water is broad, however, and they can withstand entirely fresh water for several days without undue harm. They apparently do not have what might be termed a fixed minimal salinity death point. Like all estuarine animals they are capable of adjusting to rather wide salinity changes over a period of time. Animals that are acclimated to a salinity of 35 are seriously damaged if the salinity drops to 15 ppt. However, animals adjusted to a salinity of 20 ppt can endure a drop to 12 or even 10 ppt. without ill effects. The greater the salinity drop, providing it is for a short period, the less harm it is likely to do to the snails. They simply close their operculum and go into a condition resembling hibernation until the unfavorable conditions change.

In the past month, salinity levels at Pensacola have dropped to 8 for short periods during low tide stages and this has had no apparent effect on the snails, which were already adjusted to salinity levels between 14 - 19.

When fresh waters flood an estuary, significant segments of the snail population may be preserved because of their location in deep pockets where the salt water is retained. In Mississippi Sound, for example, in 1950 when fresh water conditions prevailed for about 6 weeks and many oysters as well as drills were killed on the reefs, large numbers of drills in the deep channel survived and repopulated the entire area by the following year.

ENEMIES

The natural enemies of the mature snail are few. Under experimental conditions we have observed hermit crabs attacking them in order to gain possession of their shells. Stone crabs, if sufficiently hungry, will crack them open and eat them. Probably these two situations do not occur in nature. There are at

least two trematodes which parasitize them and may cause considerable damage by invading the snail gonad. These parasites, common in Louisiana waters, are rare in the Pensacola area. It is doubtful that even in Louisiana they significantly decrease the numbers of snails.

Our chief allies in natural control methods are fresh water and cold temperatures. Both of these conditions are of short duration in the oyster producing areas of the south and such relief as they offer is temporary and likely to harm oysters almost as much as the snails. Those factors causing larval mortality during the free-swimming state are, of course, the primary limiting agents in the life cycle of this animal.

PREDATION

For many reasons it is difficult to assess the amount of damage caused by drills on the oyster reefs. Oystermen in the same area may vary from 0 to 50% in their estimate of loss due to the snail. St. Amant found in 1937 in Barataria Bay, for example, that much loss was being attributed to drills by some oystermen. In a dredged sample of almost 5000 oysters he found only 92 dead and of these he was able to observe drill holes on only two. Of course, all 92 may have been killed by drills, but as he states, this is still a mortality of less than 2%.

Snails kill mature oysters by drilling them, but they prefer to eat spat and the smaller oysters. It is possible for an oyster reef to support a large population of snails all summer and show no loss to the casual observer, since the larger oysters remain unharmed. We observed one large snail, for example, that was eating week old spat at the rate of 86 per day plus large numbers of tiny barnacles. It would have been difficult to observe this loss in the field.

A second difficulty in detecting drill damage in the field results from their habit of opening oysters primarily on the edge of the bill rather than drilling through the more solid portions of the shell. I have combined the data from a large number of controlled feeding experiments and find that approximately 10% of the oysters killed by drills have complete holes through the surface of the shell, while 90% were killed through incomplete marginal holes. Three quarters of those attacked on the margin gave no evidence of drilling to the casual observer. When complete holes are drilled through the surface of the shell, there is little preference shown in its position. They drill whatever surface of the oyster presents itself although there is some tendency for the holes to be in the posterior dorsal area, that is, near the oyster's exhalent current of water. We have found no preference for the muscle scar area as stated by some writers. Several drills may attack an oyster simultaneously, and we have seen them busily engaged drilling boxes. It is common to find oysters showing several incompleated holes.

The rate of kill as shown by laboratory experiments may be misleading, since usually a single type of food is presented and the snails have the chance to consume the oysters they kill. In the field there is a wide variety of food available and moreover when a snail kills an oyster, it is likely that secondary predators will consume most of the meat before the snail has a chance.

The following data give the consumption of oysters by semi-starved snails and probably represents the maximum rate of kill.

1" snails ate 0.3 spat (1") per day for 30 days.

2" snails ate 0.5 oysters (2") per day for 30 days.

2 $\frac{1}{2}$ " snails ate 3 spat (7 day old) per hour for 2 day period.

If these figures are expanded on a theoretical basis and assuming an average 5 year life span for the snail we may say that each mature conch represents during its lifetime the loss of a thousand market oysters plus an untold number of spat.

In experiments in which snails were presented with a choice of spat or mussels as food the results were quite striking. Within two hours 25% of the mussels were eaten and none of the spat were attacked. At the end of eighteen hours, all mussels had been eaten, 10 percent of the spat had been eaten and another 50 percent were being consumed. Forty percent of the spat were still untouched. Over a period of days there is a preferential selection of approximately 8 mussels to one spat.

We may abstract from this wealth of biological information certain items of possible interest to us in devising control methods. Some of these factors are utilized now by oystermen and have been used for a long time.

In the breeding season the snails are very gregarious and exhibit a marked negative geotropism. In other words, they gather in relatively large groups and climb up on any hard object sticking up from the bottom in order to deposit their eggs. It is possible to kill large numbers of adults and eggs by placing stakes on the reefs during this period and removing them when covered to dry out. This method is only partially effective because of the short period of development within the capsule and the extended period in which capsules are deposited.

The sensory keenness of the snail plus its fondness for young oysters makes it possible to place wire bags baited with spat on the reefs and attract large numbers of snails. By inspecting such traps regularly, it is possible to destroy a significant part of a snail population. This method is sufficiently satisfactory so that some private planters have in use over a thousand such traps on their leased beds. Their difficulty lies in the amount of labor required to handle and inspect them at regular intervals.

Little else has been done towards controlling the snail other than the hand-culling which is practiced when tonging or dredging oysters. The use of the suction dredge and screening of the material as done in New England is not possible in the south because of the generally soft nature of the reef bottoms.

What other factors should we consider in planning possible control methods? This question brings us pretty much up against a blank wall. There is the not very promising possibility of discovering some chemical cheap and selective

enough to be dumped in the water to kill just the snails. A somewhat more hopeful idea is that of developing a biological control, such as the trematode parasite which invades the snail gonad and destroys its ability to reproduce.

I offer two suggestions which may eventually prove helpful to the industry. The first of these, so far as I know, is an untouched field, i.e., the use of electrical currents - either in creating barriers to snail migrations or as a lure attracting them to traps. The second suggestion is the invention of a baited trap similar to those now in use but with the important addition that this trap should permit entry but not exit of the snails. As you know, the use of the word trap in connection with the present device is most misleading, since snails are able to wander in and out at will and only those are caught which happen to be feeding at the time the "trap" is examined. I believe that a trap permitting only entrance of the snails is entirely feasible. We have done some work on it, using a one-way swinging door in the entrances. Our early efforts along this line were only partially successful and we did not have sufficient assistance to continue the project. I feel certain that with the construction of such a trap on an economical basis we shall have gone a long way in solving our problem of snail control.

The Gulf Coast Conch, *Thais haemastoma*

James N. McConnell, Chief
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Louisiana Wild Life and Fisheries Commission

Probably the most destructive of all the natural enemies of the Louisiana oyster, *Crassostrea virginica*, is the oyster drill or borer, *Thais haemastoma*. This species feeds on oysters and other bivalves almost exclusively when available.

The borer, to obtain its food, drills a round hole through the shell of the oyster and extracts the meat by means of an extensible proboscis. Young oyster spat seem to be preferred when obtainable; however, any size oyster may be attacked. When larger oysters are used for food the ventral edge of the shell is most often the point of entrance, and in places where drills are plentiful and oysters scarce the writer has seen as many as eight drills attached and feeding on one large oyster, all of them entering through the shell edges. From my observations it seems that the softest parts of the oyster are eaten first and the tougher muscle area last. This serves the conch in good stead, for if the muscle were eaten first, the shell would partially open and crabs and fish would quickly devour the exposed oyster meats.

Breeding Habits

Breeding season in Louisiana seems to be governed by water temperature and usually starts in early April and runs into July, with a peak period reached in May. At this time *Thais* becomes negatively geotropic, for the breeding period only, and climbs on any elevated object to lay its egg capsules, being careful to deposit these capsules below the low water line. The egg capsules are fastened to this elevated object thereby becoming free from debris and silt. Martin D. Burkenroad, in his paper on *Thais haemastoma*, written while working in Louisiana, states that "each capsule contains about 1,000 eggs and a female produces 100 or more capsules."

Methods of Control

Permanent control is almost impossible, for the young larvae when hatched from the egg capsule are free-swimming, and although unable to stand water of low salinity will come back into areas again as soon as the salinities are favorable. It is my understanding we can use as a salinity tolerance the figure of approximately fifteen parts per thousand. Where many conchs invade privately leased grounds, fishermen take advantage of their natural climbing instinct during the breeding season and attach palmetto fronds to poles and stake off their leases with these traps which they run two or three times weekly, sometimes even daily. The stakes are pulled up and the climbing egg-laying conchs are shaken off into their boats. The conchs thus gathered are usually deposited on land in large piles where they dry up and become an olfactory nuisance for several months.

Although there has been some small demand for these animals to be used for food in the New Orleans area, it has only been in the last year or two that there seems to be a demand from the Eastern markets for processed canned conchs. The Golden Meadow Fisheries Company, one of our local canning plants, has been processing and canning these drills experimentally for the last two years. It is my understanding now that from one Eastern concern alone an order has been received for twenty to thirty thousand cases of these canned conchs.

When properly cooked and handled, these oyster enemies could become a tasty and nutritious addition to the nation's food supply.

As an incentive for the gathering of these animals in large quantities, the Golden Meadow Fisheries Company will pay much more per barrel for conchs than is now being paid for oysters, provided large enough quantities can be obtained for canning purposes. It seems probable that only during the breeding season when large concentrations of these animals occur can sufficient quantities be obtained for canning.

There is one other possibility that occurs to the writer of obtaining a large supply of conchs for canning. During the last several years, more and more of our oyster fishermen are turning to the transplanting, for steam canning purposes primarily, of seed oysters from the natural reefs east of the Mississippi River to the higher salinity growing grounds west of the River, principally in Plaquemines and Jefferson Parishes. This transplanting occurs during the months of September, October and November, and during the months of January, February, March and April and part of May are removed by "shovel back" dredging operations and brought to the steam canning plants for packing. In many places in this high salinity area large quantities of conchs have gathered to feast on the transplanted oysters, and since the fisherman ordinarily cleans his beds by dredging after the season so as to prepare his leased areas to receive the new planting in September, it seems quite feasible that at this time, for at least a two-week period, a collection system could be instituted that would make it financially profitable for a canning plant to continue to operate after the closing of the oyster season.

In summarizing, it seems to this writer that the best way in Louisiana to combat the oyster drill is:

First, to plant your seed oysters in areas where the water salinities remain normally below the tolerance figure of fifteen parts per thousand.

Second, to continue and increase the present method of trapping with poles and palmetto fronds during the months of April, May and June.

Third, to continue to increase the demand for canned conchs which seems to be a tasty and excellent food product.

As the demand increases, more fishermen will become interested in supplying the conchs not only for the financial consideration but primarily because they will be helping to eliminate (in high salinity areas) the Gulf oyster's most potent natural enemy.

MORTALITIES OF OYSTERS IN TRAYS AT GLOUCESTER
POINT, YORK RIVER, VIRGINIA

Willis G. Hewatt, Texas Christian University and Jay D. Andrews, Virginia
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ABSTRACT

Methods which have been used in the past for estimating oyster mortalities on natural or cultivated oyster beds have not only been unsatisfactory but have lead to confused and conflicting reports on the mortalities existing in oyster producing waters. The procedure of comparing the numbers of shells, boxes and live oysters has also created much speculation regarding the causes of reported high mortalities in different areas. Observations on oysters grown in trays appears to be the most satisfactory method of determining the rate and the time of occurrence of mortalities. If the trays are examined at frequent intervals gaping oysters can be recovered in sufficient numbers to permit a more accurate determination of the cause or causes of death.

In the summer of 1950 a few trays of oysters, from known sources, were suspended from the Virginia Fisheries Laboratory pier in the York River. The oysters in the trays were examined daily during the summer and at less frequent intervals during the remainder of the year. Other trays of oysters were added to the group in 1951 and 1952. Gaping oysters recovered from the trays were examined for evidence of the possible causes of mortality.

The annual mortalities recorded for the trays were relatively uniform, ranging from 20 to 34%. The combined mortality for all of the trays for the warmer months, June through October, was approximately six times as great as that recorded for the colder months, November through May. During the three months of July, August and September 18% of the mortalities occurred. Oysters transplanted from low-salinity waters revealed a distinct delay in the appearance of high mortalities.

The possible cause of the periodic high mortalities will be discussed in another report.

INCIDENCE OF DERMOCYSTIDIUM MARINUM, MACKIN, COLLIER, AND OWEN
A FUNGUS DISEASE OF OYSTERS, IN VIRGINIA

Jay D. Andrews and Willis Hewatt

ABSTRACT

Between July 1952 and March 1953, meats of 228 dying oysters were tested by the Ray method for Dermocystidium marinum, Mackin, Collier and Owen, a fungus disease of oysters. These gapers (dying oysters with meats) were obtained from trays of oysters suspended from the pier of the Virginia Fisheries Laboratory at Gloucester Point, Virginia. From July to October approximately 90% of all gapers had infections of the fungus. Heavy infections were by far the most common followed in decreasing order by moderate and light infections. Infections were less frequent during the winter months and almost disappeared entirely from February to May inclusive.

In August 1952, 398 live oysters from nine scattered locations were tested and showed only 25% infections. Heavy infections were few; the number of moderate and light infections was somewhat greater. The greatest number of infections of live oysters occurred in the lower bay, lower York River and Hampton Roads. The fungus was not found in the James River seed area.

Later tests have shown few infections of live oysters in December and scarcely any in April and May.

STUDIES ON THE OCCURRENCE OF DERMOCYSTIDIUM
MARINUM IN YOUNG OYSTERS¹

by

Sammy M. Ray²

INTRODUCTION

This investigation was conducted to determine the incidence of Dermocystidium marinum Mackin, Owen, and Collier, in young Crassostrea virginica (Gmelin) maintained in water in which this parasite is endemic. Data obtained by Dr. J. G. Mackin in some of his studies indicated that D. marinum occurred infrequently in oysters less than a year old. In the present studies the thioglycollate culture technique (Ray, 1952a and 1952b) was employed to examine oysters for the presence of this fungous parasite at various periods beginning shortly after setting, and extending approximately one year. Two groups of these young oysters were used. In addition, two other groups of oysters of more advanced age were studied in order to compare the intensities of infection in various age groups. One of these older groups was approximately one year old at the beginning of the study and the other group was composed of oysters four or more years old.

METHODS AND MATERIALS

On June 11, 1952, six bags of clean oyster shells were put out at each of two stations: Sugar House Station (extreme lower Barataria Bay, La.) and Station 51 (middle lower Barataria Bay, La.). These shells were taken up on June 25 and June 26, 1952. The shells bearing spat were separated, and each spat (usually one, rarely up to three per shell) was marked on the right valve with finger nail polish. The marked spat were placed in bags of galvanized chicken wire and suspended from the dock of the Texas A. & M. Research Foundation Laboratory located over Bayou Rigaud, Grand Isle, La. The bottoms of these bags were suspended approximately one foot above the water bottom. This group of spat was designated as Group A.

¹Grateful acknowledgement is made to the Texas A. & M. Research Foundation for the facilities made available for this investigation. Sincere appreciation is expressed to Dr. J. G. Mackin and Mr. J. L. Boswell for valuable assistance; to Mr. Fred Cauthron for the preparation of the lantern slides, and to Mr. J. L. Plock of Greenport, N. Y., for furnishing some of the oysters used in this investigation.

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On June 12, 1952, a large number of spat (estimated age, one to three weeks) were found on "boxes" in some of the storage trays in Bayou Rigaud. These spat were treated in the same manner as those described above. This second group of spat was designated as Group B. Spat that subsequently became attached to the shells in these bags were periodically removed to avoid confusion, and the marked spat were occasionally re-marked to insure proper identification. The bags containing both groups of spat were located within six to eight feet of several trays of oysters that were at least 75 percent infected with D. marinum during the summer months.

On June 25, 1952, Dr. J. G. Mackin removed a tray of approximately one year old oysters from the storage racks in Bay Chene Fleur, La., and placed it on the storage racks in Bayou Rigaud. These oysters were caught as spat in Sugar House Bayou between June 21 and July 23, 1951. They were maintained in Bay Chene Fleur, La., from July 24, 1951, until June 25, 1952. This group of oysters was designated as 11 to 12 month old oysters. In view of the fact that oysters approximately a year old were known to be susceptible to D. marinum, this group served as a control in this investigation. Twenty-five of these oysters were examined on June 25, 1952, and all were negative. This indicates that the level of infection was very low. However, a "gaper" which was heavily infected was recovered from this group on the same day, indicating that there were a few infected oysters.

The fourth group of oysters, estimated to be four years old, was obtained from Gardiner's Bay, Long Island, N. Y., on August 21, 1952. These oysters were used to determine the time required for oysters from a non-endemic area to become infected when placed in endemic waters. These oysters were maintained in trays on the tide gauge racks in Bayou Rigaud. Thirty of these oysters were examined on August 22, 1952, and all were found to be negative for D. marinum.

In most cases the diagnoses were based on the examination of rectal tissue after 48 to 72 hours of incubation. The Groups A and B spat were cultured in entirety through August 22, 1952. After this date rectal tissue and a part of the visceral mass were cultured for examination. The procedure followed in examining the cultured tissues was the same as previously described (Ray, 1952b). At the time of culturing the length of each of the spat in Groups A and B was measured to the nearest millimeter. The method used for estimating the intensity of infection was the same as previously outlined (Ray, Mackin, and Boswell, 1953) in which three intensities of infection (light, moderate, and heavy) were defined. In the present studies the intensities were further subdivided into very light, light to moderate, and moderate to heavy. While a heavy infection is sharply separable from a light infection, the limits of the intervening categories are not easily defined, for they are largely a matter of personal judgment based on more or less extensive experience. The criteria used for determining the six categories of infection intensities are briefly described below:

1. A heavy infection is one in which the parasites occur in such enormous numbers that the major part of the tissue macroscopically shows a dull

green-blue to blue-black color after treatment with iodine. With occasional exceptions, microscopic examination is not necessary for diagnosis of heavy infections. In a very few instances of heavy infection, macroscopic diagnosis is not possible because the organisms fail to enlarge or to stain normally after the usual treatment. However, in such cases microscopic examination reveals the heavy nature of the infection since the tissues are packed with the cells of the parasites.

2. A moderate to heavy infection is one in which the parasites are present in large numbers. This intensity differs from a heavy infection in that less than one-half of the tissue gives a macroscopic blue reaction with iodine. On the other hand, a moderate to heavy infection differs from a moderate infection in that some localized areas of macroscopic blue color appear after the iodine treatment.

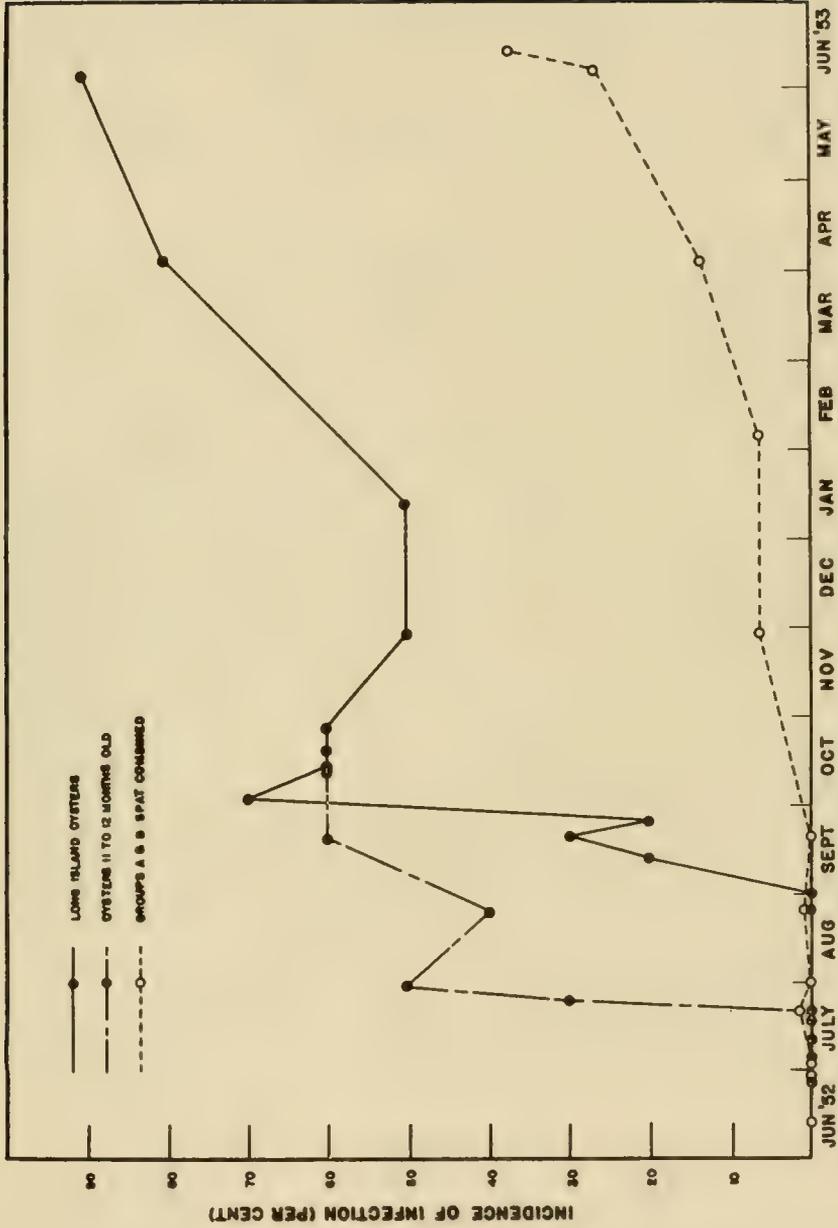
3. A moderate infection is one in which the organisms are so numerous in the tissues that one may expect to find some in every field at 100X magnification, but the masses of the cells of the parasites are more or less localized, i.e., there are concentrations of parasites in some areas while others have relatively few of them. A moderate infection does not show a macroscopic blue color when the tissue is treated with iodine, although sometimes in cultures more than 72 hours old the tissues may be slightly tinged with blue. This may also occur when the majority of the organisms enlarge to an unusual degree (100 to 150 μ in diameter).

4. A light to moderate infection is one in which some areas may be entirely free of the parasites and other areas may show localized concentrations containing something like 25 to 50 parasitic cells, or one in which the organisms may be scattered throughout the preparation more or less uniformly so that two or three parasites may be seen in each field at 100X magnification.

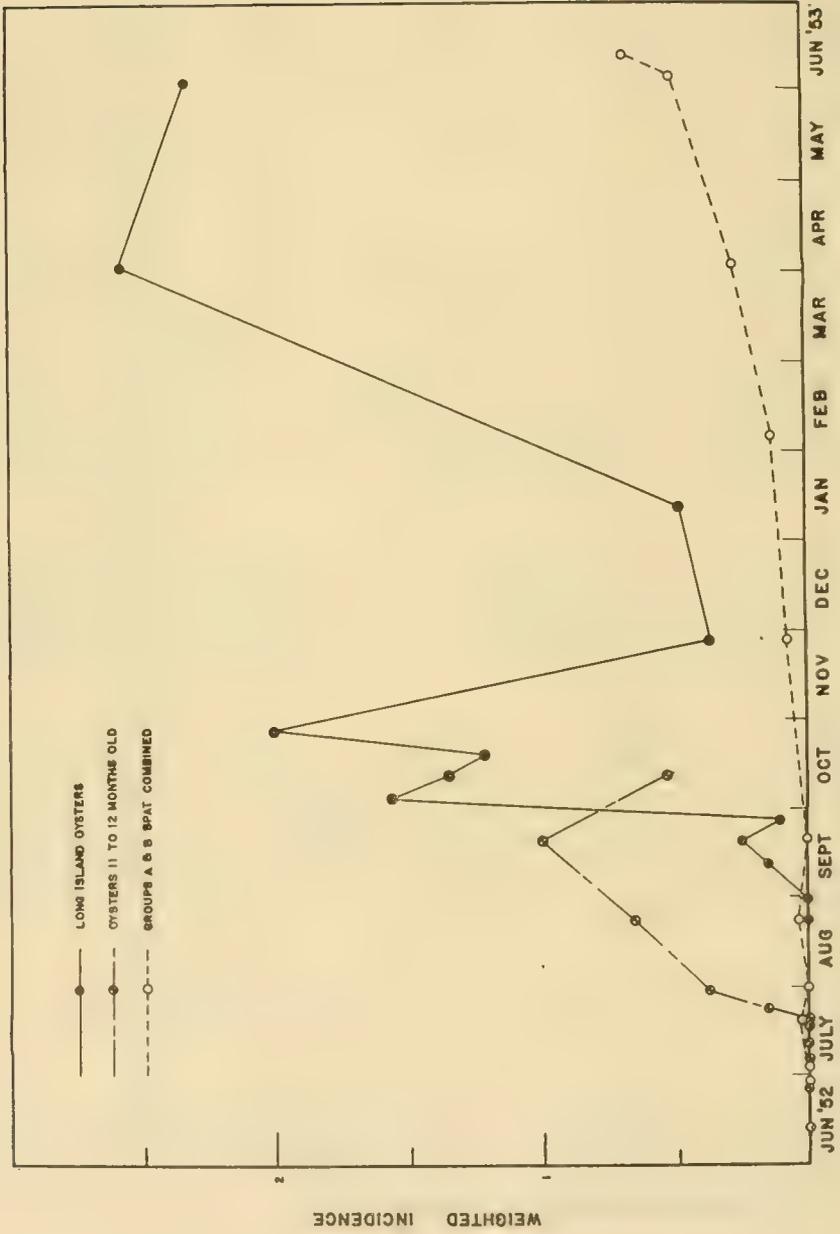
5. A light infection will show more than 10 organisms up to approximately 100 in the entire preparation. In such infections the parasites may be scattered singly in the tissues or they may occur in isolated clusters of up to 10 to 15 organisms. The total number of organisms per preparation is not used alone to define the upper limits of a light infection; consideration is also given to the concentration of the parasites with regard to the amount of tissue being examined.

6. A very light infection consists of finding one to ten organisms in an entire tissue preparation.

The estimated intensities were used to derive the "weighted incidence" for each sample checked. The system used herein for determining the weighted incidence is that devised by Dr. J. G. Mackin, in which a numerical value (weighted incidence) is obtained by arbitrarily assigning values of one-half, one, two, three, four, and five, to very light, light, light to moderate, moderate, moderate to heavy, and heavy infections respectively. The sum of these values when divided by the number of individuals in the sample gives the weighted incidence per oyster. The weighted incidence, which combines incidence and intensity of infection provides a much better index of the degree of infection than incidence alone. (See graphs 1 and 2).



GRAPH NO. 1



GRAPH NO. 2

Additional studies on the thioglycollate culture technique indicate that a slight modification of the method for rehydrating the medium results in a more uniform enlargement of D. marinum. This modification consists of using distilled water containing 20 grams of NaCl per liter for rehydrating the fluid thioglycollate medium instead of sea water or distilled water as previously advised (Ray, 1952a and 1952b).

RESULTS AND DISCUSSION

The results of the examinations for D. marinum, as well as other pertinent data, are recorded in Tables I, II, III and IV. Graph 1* was prepared by plotting the incidence of infection in percent in the three different age groups studied, against time in months. Graph 2** was prepared by plotting the weighted incidence in the three age groups against time in months.

An inspection of Tables I and II shows that the incidence and intensity of D. marinum in young oysters is extremely low (incidence less than one percent) during the first three to four months after setting. Only two individuals were found to be infected out of 275 spat that were checked between June 12 and September 19, 1952. One of these two cases of sporadic infection was found in a spat six to eight weeks old and the other was found in a spat nine to ten weeks old. Because of the paucity of data obtained during October and November, and the fact that lower water temperatures during the fall and winter months bring about a decrease in the infection rate of D. marinum, no conclusions can be drawn at the present time as to whether or not this low degree of susceptibility continues for a longer period. Perhaps the high degree of refractivity to this fungous parasite is due to an "age immunity". If this be true, however, it is the reverse of the usual situation, for in all other cases of "age immunity" to animal parasites resistance increases with advancing age after the brief period when, in mammalian hosts, the offspring are passively protected by anti-bodies acquired from the mother. Other possibilities are (1) that this low infection rate may be due to some factors connected with the feeding of the host and/or the life cycle of the parasite; (2) that the phagocytes, which constitute an important defense mechanism in invertebrates, may be more effective in resisting the invasion of D. marinum in young than in older hosts; (3) that the high metabolic activity of the rapidly growing spat may contribute considerably to this apparent "age immunity".

Further examination of Table I shows a more or less gradual increase in the incidence and intensity of infection from November 28, 1952, to June 13, 1953. One oyster was found to be infected out of 15 examined at 23 to 24 weeks, and another one out of the same number was found to be infected at 33 to 34 weeks. At 41 to 42 weeks, two of the oysters out of 15 examined proved

* Incidences of Group A spat (7-17-52) and Group B spat (7-18-52) combined and plotted at 7-18-52.

**Weighted incidences of Group A spat (7-17-52) and Group B spat (7-18-52) combined and plotted at 7-18-52.

to be infected. It is evident from data presented in Tables I and II that the susceptibility of these oysters was significantly increased approximately one year after setting. At this age, 30 of the 92 individuals checked (about 33 percent) were diagnosed as positive for D. marinum. The weighted incidence for this group of oysters at this time was 0.6.

The growth of the spat in Groups A and B as indicated by the total lengths was very good. At approximately one year of age the majority of these oysters were well shaped and rather deeply cupped. The oysters in these two groups averaged 81 millimeters in length about one year after setting.

An examination of Table III reveals that oysters 11 to 12 months old became positive for D. marinum after one month in Bayou Rigaud. An examination of Table IV reveals that the Long Island oysters were found to be positive between the first and third week. There was some mortality among the Long Island oysters within six weeks after initial exposure which was probably caused by D. marinum. Except for the initial mortality, which was certainly due to the effect of transportation, the Long Island oysters showed a relatively low mortality until the weighted incidence reached a relatively high level.

A study of Graph 2 shows clearly that there was a very significant age differential in the degree of susceptibility among the three age groups considered in this investigation. In the first place, the Groups A and B spat required a year to attain the weighted incidence (0.6) which was reached by the year old oysters within eight weeks. Similarly, one may note that Long Island oysters attained within six weeks a weighted incidence (1.55) which was 50 percent higher than the maximum weighted incidence (1.0) which occurred in the year old oysters within 12 weeks.

Although this investigation was not set up as a mortality study, some data were obtained on the mortality that occurred in each group except the group of 11 to 12 month old oysters. The mortalities in Groups A and B were not checked until the spat were about one to two inches in length. These spat did not appear to have an appreciable mortality until small conchs (Thais) entered the bags. The mortality from conchs greatly decreased as the oysters increased in size. It is assumed that the small conchs were unable to pierce the valves of the larger oysters, and also that the activity of the conchs probably diminished during the cool months. The percent of mortality per day recorded for the Group A spat in Table I represents the combined mortality of the spat collected at Sugar House Station and Station 51. Except for the mortality due to conchs, the mortality of the Groups A and B spat remained at a low level during the entire year. From April 3, to June 6, 1953, the Group B spat showed a mean mortality of 0.08 percent per day; the Group A spat showed a mean mortality of 0.14 percent per day from April 3, to June 13, 1953.

The mortality figures given in Table IV for the Long Island oysters are not exactly accurate in all cases because the mortalities were not determined at each interval that oysters were removed for Dermocystidium examinations.

The mortality figures recorded in Table IV were obtained by taking the average of the percent mortality derived in two different ways. One value represented the percent mortality based on all oysters on hand at the beginning of each period, including those that were removed for examination. The other value represented the percent mortality based on the number of live oysters on hand at the end of the period. The mortality figures for April 3, and June 4, 1953, are accurate. On April 3, 1953, 13 "boxes" and five "gapers" were recovered. This suggests that a large percentage of the mortalities that accumulated between February 5, and April 3, 1953, had occurred very recently, or this number of "gapers" would not have been recovered. Three of these "gapers" were heavily infected and two moderately infected. This sharp rise in incidence and intensity of D. marinum as well as mortality is undoubtedly associated with the rise of water temperatures. Between April 3, and June 4, 1953, several conchs entered the tray containing the Long Island oysters. It is not known how many of the mortalities that occurred in this tray were caused by the conchs. It is the opinion of the author that damage from the conchs was negligible. The oysters were encrusted with large numbers of small oysters which the conchs were observed attacking at the time the tray was examined. Of the 50 "boxes" recovered at this time, three bore evidence of crab damage. Therefore it is not possible to state definitely what part of the mortalities occurring at this time was due to Dermocystidium infections; nevertheless, in view of the extremely high weighted incidence (2.3) among the living oysters at this time, the author believes that the great majority of the mortalities were caused by D. marinum. It also should be noted that there was a marked decrease in weighted incidence along with a slight decrease in incidence in November, 1952, and January, 1953. This decrease suggests that oysters are able to rid themselves of some of the parasites during the colder months.

CONCLUSIONS

The data presented in this paper permits the following conclusions:

1. That oysters three to four months old are highly refractive to D. marinum infections.
2. That oysters from the third or fourth month to one year old are increasingly susceptible. The data suggest that the increasing susceptibility is carried into the second year.
3. That oysters approximately a year or more old may become infected with D. marinum within three to four weeks when maintained in highly endemic waters.

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TABLE I

Spat (Group A)

Date	No. Exam.	Approx. Age	Min. Lgth.	Max. Lgth.	Av. Lgth.	% Pos.	Wghtd. Incid.	% Mortality Per Day
6-26-52 (Sta. 51)	26	1-2 wks.	6	13	10	0	0	--- ¹
7-2-52 (S.H.)	25	2-3 wks.	11	20	14	0	0	----
7-17-52 (S.H.)	34	4-5 wks.	16	33	25	0	0	----
7-31-52 (Sta. 51)	27	6-7 wks.	25	45	34	0	0	0.08
8-22-52 (S.H.)	27	9-10 wks.	29	52	40	3.7	0.04	0.08
8-31-52	--	10-11 wks.	--	--	--	---	---	1.5 ²
9-19-52 (S.H.)	25	13-14 wks.	37	58	45	0	0	0.2 ²
10-11-52	--	16-17 wks.	--	--	--	---	---	0.25 ²
11-28-52 (S.H.)	15	23-24 wks.	44	70	55	6.6	0.07	0.27 ²
2-5-53 (S.H.)	15	33-34 wks.	42	71	56	6.6	0.13	0.1
4-3-53 (Sta. 51)	15	41-42 wks.	58	84	71	13.3	0.27	0.02
6-13-53 ³	54	51-52 wks.	48	108	81	37.0	0.66	0.14

¹Dashes indicate that no data were obtained.

²Mortality due mainly to small conchs.

³Sugar House Station (29 individuals) and Station 51 (25 individuals) combined.

TABLE II
Spat (Group B)

Date	No. Exam.	Approx. Age.	Min. Lgth.	Max. Lgth.	Av. Lgth.	% Pos.	Wghtd. Incid.	% Mortality Per Day
6-12-52	35	1-3 wks.	4	15	9	0	0	--- ¹
6-26-52	25	3-5 wks.	12	25	17	0	0	---
7-18-52	26	6-8 wks.	21	48	31	3.8	0.08	---
8-30-52	--	12-14 wks.	--	--	--	---	---	0.33 ²
9-19-52	25	15-17 wks.	27	59	47	0	0	0.14 ²
10-11-52	--	18-20 wks.	--	--	--	---	---	0.19 ²
2-5-53	--	35-37 wks.	--	--	--	---	---	0.08
4-3-53	--	43-45 wks.	--	--	--	---	---	0.04
6-6-53	38	52-54 wks.	50	99	81	26.3	0.5	0.08

¹Dashes indicate that no data were obtained.

²Mortality due mainly to small conchs.

TABLE III

Oysters 11 to 12 Months Old

Date	No. Exam.	Time in Highly Endemic Waters	Percent Positive	Weighted Incidence
6-25-52	25	None Since First Month After Setting	0	0
7-2-52	10	1 wk.	0	0
7-9-52	10	2 wks.	0	0
7-16-52	10	3 wks.	0	0
7-18-52	10	3 wks. & 2 days	0	0
7-23-52	10	4 wks.	30	0.15
7-30-52	10	5 wks.	50	0.35
8-22-52	10	8 wks.	40	0.65
9-19-52	10	12 wks.	60	1.00
10-11-52	20	15 wks.	60	0.53

TABLE IV

Long Island Oysters

Date	No. Exam.	Time in Highly Endemic Waters	Percent Positive	Weighted Incidence	Percent Mortality Per Day
8-22-52	30	1 day	0	0	(27 died out of 2 bushels)
8-30-52	10	8 days	0	0	(4 died since 8-22-52)
9-12-52	10	3 wks.	20	0.15	--- ¹
9-19-52	10	4 wks.	30	0.25	---
9-26-52	10	5 wks.	20	0.10	---
10-3-52 ²	10	6 wks.	70	1.55	---
10-11-52	10	7 wks.	60	1.35	0.20
10-17-52	10	8 wks.	60	1.20	----
10-25-52	10	9 wks.	60	2.00	---
11-28-52	10	14 wks.	50	0.35	0.08
1-12-53	10	16 wks.	50	0.50	---
2-5-53	--	19.5 wks.	--	---	0.14
4-3-53	10	27.5 wks.	80	2.55	0.24
6-4-53	10	36.5 wks.	90	2.30	0.77

¹Dashes indicate that no data were obtained.

²A heavily infected "gaper" recovered on this date.

EXPERIMENTS ON THE EFFECTS OF SEISMOGRAPHIC EXPLORATION ON OYSTERS

By

Fred W. Sieling

Introduction

To investigate the effects of seismic exploration for oil on oysters in the Barataria Bay, Louisiana, region, two experiments were conducted during 1949-1950. This work was initiated because claims were made by commercial oystermen that (a) the explosive charges used in this work killed oysters outright; (b) the gas which bubbles up from the shot holes is lethal to oysters; and (c) the bottom is softened or shifted by the explosions, thus causing the oysters to be buried and subsequently die. Therefore, the experiments using normal operating procedure for seismic exploration for oil were planned to provide answers to these questions.

In the marshlands along the Gulf coast large deposits of oil have been found under the bayous and marsh. To locate these deposits without the drilling of unnecessary holes involving large expenditures of money and time, the system of seismographic exploration is used. As this system is used in the shallow water and marsh along the Gulf of Mexico, the charges of explosive are placed in pipes which are in holes drilled into the bottom. These holes are drilled or jetted into the bottom by powerful pumps forcing water thru a nozzle set into the bottom mud. As the hole goes deeper extra lengths of pipe are screwed on and the drilling continued to the desired depth. These pipes are of light steel and may extend into the bottom for over a hundred feet. The explosive used, usually Nitramon, is packed in one pound cans which are equipped with screw threads at each end. By means of these threads the cans are put together in long sticks and electrical detonators are attached to the upper end and they are lowered into the pipes to the desired depth. The charge is set off when the seismographic recording machines are in the proper positions. Several charges may be set off in the same hole, the deepest charge always being first. The visual results of these explosions are small geysers of mud and water blown up to a height of 50 to 100 feet from the top of the pipe which is usually several feet above the water level. The sensation to a person standing in a boat nearby is that of a sharp slap on the bottom of the feet. After the explosion the top section of the pipe which is above ground is unscrewed and removed, water and mud filling the hole.

Review of the Literature

In reviewing the literature on the effect of underwater explosions on marine life it is found that research has been limited. However, when the field is narrowed to the effects of explosives on oysters it is found that the work of the Louisiana Department of Wildlife and Fisheries is almost the only work which has been done. In the Louisiana Department of Conservation report for 1933-1939 it is stated that some experimental work of a preliminary

and rather crude nature was done along this line. In this study, carried out by L. D. Kavanagh, the charges were laid on the bottom at different distances (varying from 175 to 750 feet) from oyster beds. After the explosions, the oysters from the beds were tonged up and examined. It was stated that some oysters were stunned and some had mud in them. Charges varying from 30 to 70 lbs. were used, but no particulars as to combinations of charges and distances were given. The report ends by saying that more work should be done.

In 1941 a series of regulations was established which set up restrictions which it was felt would permit satisfactory exploration for oil and still not injure either public or private oystering interests. These regulations are still in force and virtually unchanged since their promulgation.

J. N. McConnell, writing in the 16th Annual Report of the Department of Conservation, stated that further studies were carried out in 1943, but no details of methods, charges, or results have ever been reported.

In 1944, Gowanloch and McDougall conducted a series of experiments to determine the effect of heavier charges on oysters. This was at the request of certain oil exploration interests in the Gulf of Mexico and the proposed exploration was in the heart of the shrimp fishing grounds. In this work 800 pounds of dynamite failed to kill oysters suspended in cages at 50 feet distance from the charge, but it is stated that the authors felt that additional work was needed. Gowanloch and McDougall again in 1945 report on a series of experiments "in which great care was used to check the validity of the conclusions" as stated by the authors. In this work they found that oysters were not damaged by 400 pounds of dynamite which was exploded 25 feet under the surface of an oyster bed. It is also stated that oysters were not damaged by gases emitted as a result of the explosions, either at the time of the shot or at the time of a subsequent examination of the experimental animals. An anonymous report of the Chesapeake Biological Laboratory states that approximately 2% of the oysters exposed in bags on the bottom within 100 feet of a thirty pound suspended charge of TNT and within 200 feet of a 300 pound charge of TNT were killed at once. The report also states that somewhat incomplete data indicate that 5% of the oysters so exposed in the same radius suffered fatal injury. This report also states that oysters growing naturally probably are less damaged than those which are held in wire bags during the experiments. All charges in this work were suspended between the surface and the bottom. Most of this information is again summed up in a report of a 1947 conference on the effects of explosives on marine life (Naval Ordnance Laboratory Memorandum 9424), but no new information is added. Subsequent papers on the effects of explosions make no reference to their effects on oysters or other shellfish. Much work has been done in California in the last several years, but unfortunately no oysters or other shellfish have been used in any of the experiments which are reported.

Procedure

It was decided to plan two separate experiments and to vary the handling and holding of the experimental animals slightly. The oysters in the first experiment were placed in trays and then placed in racks which kept them

slightly above the bottom. In the second experiment the oysters were placed on trays and the trays placed directly on the bottom. In choosing the locations for the two series of explosions, four main considerations were taken into account. These were (a) to stay away from commercial oyster operations as far as possible; (b) to stay in water which does not have a salinity of less than five parts per thousand; (c) to stay out of waters which are normally inhabited by conchs (Thais sp.); and (d) to select areas where the bottom was not too soft to support oysters. After consulting salinity and temperature records and considering the general conditions of the bottom and protection from heavy wave action, two areas were selected. These were in Bay Batiste and Bay de Chene, two areas which were about ten miles apart. After the areas were selected three lines of chemical investigation were initiated. Samples of bottom mud were collected before any charges were fired, then collected again after the shots, and again at regular intervals after the charge was fired. These samples were analyzed to determine the amount of hydrocarbon present in the mud normally, and to detect any changes which may have occurred due to the effect of the explosion. Also samples of the ebullient gases from the explosions were collected at regular intervals after the charges were fired, and these were analyzed to detect any noxious gases which might pollute the water. Water samples were taken before and immediately after the explosions and analyzed for pH, salinity, oxygen and hydrogen sulphide. Samples were also taken and checked for these same factors at regular intervals after the shots were fired to determine any changes which might have occurred due to the experiment. This procedure was followed in both of the experiments.

After the areas for the experiments were selected the patterns for the shot points were laid out and the positions for the experimental animals were determined. The shot points formed a diamond with the points 1000 feet apart and one shot point in the middle. This distance simulated the worst operating conditions possible under the law as when two lines of seismographic explosions cross at right angles. These distances were surveyed so as to be accurate and the locations for the experimental animals were carefully measured from the center shot point. The oysters were placed at 20 feet, 60 feet, 130 feet and 250 feet from the point of explosions and in a staggered line so that each group would receive the full force of the shot. The distances were selected after studying published reports of the distances at which damage was done to oysters. The law of Louisiana states the minimum distance from a shot point to a natural oyster reef shall be 250 feet. In each experimental area, at a distance of 40 feet from the shot point, an area 18 x 18 was marked off and 10 bushels of rough-culled oysters were planted on the bottom. This was also done at the control stations which were located 750 feet from the nearest shot point. At these control stations oysters were also put in trays, which were placed on a rack in Experiment 1 and placed on the bottom in Experiment 2. At two other locations control facilities were established: one in a low salinity area, namely Bay Chene Fleur, and one in a high salinity area in the lower part of Barataria Bay at a location known as Station 51. The purpose of these will be explained later.

Two explosive charges were used in each shot hole, one of 50 pounds and one of 20 pounds of Nitramon, and these were exploded at a depth of 50 feet and 30 feet respectively. These charges were selected because 50 pounds is the maximum charge allowed in inland Louisiana waters and 20 pounds is a typical operating charge. The depths at which they were exploded were those set by law in that state, and standard operating procedure was followed. The foregoing procedure simulates the worst operating conditions for the survival of oysters in the vicinity.

Two weeks before the shots were made in the first experiment, trays of culled healthy oysters were placed on the racks erected in the experimental area. These oysters had already been under observation for several weeks to be sure that no mortality would ensue from handling. At the same time control trays were placed on the racks at the two control stations. These trays were in place several weeks before the explosions so that they would be acclimated to local conditions before they were exposed to the experimental conditions. All trays were covered on the inside with $\frac{1}{4}$ inch ratwire to protect the oysters from being damaged in handling and also to prevent the depredations by conchs and large crabs which can cause considerable damage to unprotected oysters. Each tray contained 175 oysters and the day before the explosions all the trays were examined, and dead ones were removed, and the trays were replaced in position. The same procedure was followed in both experiments.

Plans had been made to have pictures taken of all phases of the work and the oysters on the bottom were to have been photographed with an underwater camera to detect any sinking into the bottom due to the explosions. However, this last was not done due to breakdown of the camera at the last minute.

The first series of explosions was made at Bay de Chene on August 23, 1949 and the second series was made on August 31, 1949 at Bay Batiste. In each experiment the explosions were set off at intervals of approximately 25 minutes. The general procedure was to drill the hole from the drilling barge, using the procedure already described and then move the barge to the next location. Then the barge carrying the shooting equipment and the explosive would move in and load the first charge into the pipe and fire it, then as quickly as was safe load the second charge in the pipe and fire that. The two pieces of equipment would then move around to the other four shot holes and fire the charges at those holes in the same manner. Then the drilling barge would go back to the shot points and pull up the top piece of pipe or more sections where practical. This last was done so that no pipe would be left protruding above the bottom to menace boats or to damage fisherman's nets. This sequence is normal operating procedure for seismographic crews in the area. Biologists went to the shot point as soon as the shot was made and examined the water for the presence of dead or stunned animals around the pipe. All trays of oysters were examined as soon as the shots were made for the detection of gaping oysters.

As soon as possible after the day of the explosions in each experiment, five trays of oysters were taken from each control area and placed one at each measured distance from the shot. At the same time one tray was taken

from each position in the experimental area and taken to the control areas. The first part of this was to check any mortality caused by the handling prior to the explosions and by chemicals released in the water by the explosion. The second part of this was to determine if the explosions harmed the oysters; that is, these oysters were removed from the experimental area and kept at a location which was not exposed to the experimental conditions; and mortality was subsequently checked at regular intervals. Some oysters were left in the regular positions at the time of the explosions and subsequently checked at regular intervals to note the longtime effect of any products which may have been released by the shots. All trays were checked at regular intervals for a period of eight months after the explosions (or until as happened in some cases they were removed by persons unknown). The oysters placed on the bottom in the area were also checked for possible sinking into the bottom due to effects of the explosions. Other trays of oysters from the original source were kept in the two control areas for the duration of the work and checked for mortality at regular intervals.

Results of Experiments

The two experiments will be considered separately as a slightly different technique was used in exposing the oysters to the explosions. The work in Bay de Chene will be referred to as Experiment 1 and the Bay Batiste work will be called Experiment 2. In Experiment 1 the animals were put in trays and placed on racks above the bottom, and in Experiment 2 the animals were placed in trays directly on the bottom.

In Experiment 1 no dead or gaping oysters were found when examined immediately after the explosions. Observations were made on these, to count and remove dead oysters, at approximately two week intervals for four months at which time most of the oysters were stolen from the racks. However, it is thought that the four months during which they were checked would have shown up any mortality due to the effect of the explosions. It was planned to keep them under observation for at least eight months, and the trays which were held at Bay Chene Fleur and Station 51 were observed for 7½ months. From Table 1 it will be seen that the percent survival after four months of those oysters exposed 20 feet from the shot was 83.7%; those exposed 60 feet from the shot, 86.7%; those exposed 130 feet from the shot, 85.3%; and those exposed 250 feet from the shot, 84.4%; while the controls showed a survival of 76.0%. It can also be seen that those animals which were exposed to the explosions and then moved to the control areas showed no correlation in their survival rate and their distance from the shots. Also, it may be seen from Table 1 that those animals which were moved from the control areas into the experimental area showed no correlation between their percent survival and their position in reference to the shot point.

The only correlation found between the chemical and hydrographic data and the mortality data is that concerning salinity and temperature. It was found that the period of high mortality and the period of high salinity and temperature coincided, and the period of low mortality was the same as the period of low salinity and temperature. It will be seen from Table 1 also

that there is a great difference between the survival of the animals which were at the low salinity control area at Bay Chene Fleur and those at the high salinity control area at Station 51. The average percent survival of those oysters at Station 51 was 52.8% and the average percent survival of the controls at Chene Fleur was 78.6%. From Table 1 it will also be noticed that those animals which were exposed to the explosions and then moved to the low salinity control area had an average survival rate of 86-90% and those which were moved to high salinity area had an average survival rate of 78-85%. This is not a great numerical difference, but it follows the general trend of the percent survival falling as the animals are exposed to waters of higher salinity. To sum up the data from Experiment 1, we could find no correlation between the distance of the oysters from the explosions and the survival rate, and the control rack showed the lowest survival. Neither could there be found any significant correlation between those oysters left in the experimental area and those animals moved into a control area away from any possible effect of any gases arising from the shot holes. The only correlation was between mortality on one hand and salinity and temperature on the other.

The experimental animals in the second experiment remained in place for the total time planned; however the data are treated the same as in Experiment 1, that is, a cutoff is made after four months so that the data will be comparable. However, the observations were continued for the full 7½ months as originally planned for both experiments. It will be seen from Table 2 that the percent survival of the oysters at 20 feet from the shot point was 82.3% at four months, and it was 75.7% at 7½ months. Those animals which were exposed to the shots at 60 feet had a percent survival at the end of four months of 82.4% and at 7½ months it was 78.5%. At 130 feet from the shot point the percent survival at four months was 86.8% and at 7½ months it was 78.7%. The percent survival of those exposed at 250 feet was 85.1% at the end of four months, and at the end of 7½ months it was 73.5%. The percent survival of the controls was 86.2% at four months, and at 7½ months it was 77.4%. Table 2 also shows that there was no correlation between the distance at which the animals were exposed to the explosions and the survival rate shown by the oysters when moved to the control areas at Station 51 and at Bay Chene Fleur.

As in Experiment 1, the mortality data show no correlation with the chemical and physical data except that when the salinity and temperature were high the mortality was high and when the temperature and salinity dropped the percent of mortality also dropped. From both Table 1 and Table 2 it will be seen that animals which were exposed at any time to waters of higher salinity suffered a higher mortality rate than those which were never exposed to high salinity water. Oysters which were in the experimental area during the explosions and were then moved to the low salinity area after the shots showed a survival percentage of 87.7% after four months, and those which were moved to a high salinity control area after the shots showed a survival of 76.0% after four months. After 7½ months this percent survival was 83.1% and 67.9% respectively for the two areas. It will be noted that those animals which were moved from the control areas to the experimental area after the explosions showed the same trend but it was not as pronounced, as the oysters from the low salinity area showed a survival after four months of 88.7% and

after 7½ months of 78.6% while those from the high salinity control area showed a survival after four months of 85.4% and a percentage of 76.9% after 7½ months. It is interesting to note here that those oysters which were exposed to the explosions and which stayed in the experimental area showed a rate of survival of 84.5% after four months and a rate of 76.6% after 7½ months, thus showing that there was no significant difference between those which were exposed to the explosions and those which were not exposed. These data in Table 2 also show that there was no effect on the oysters from the ebullient gases from the shot hole as there was no significant difference in the survival rates of those left there and those oysters carried to the control areas.

In Experiment 1 the oysters which were placed directly on the bottom as a simulation of a commercial planting were stolen, so that no results were obtained from that experiment. However, the ones which were treated in a like manner in the second experiment remained in place and were subsequently taken up and examined for evidence of their sinking into the bottom. It was found, however, that there was no evidence of their sinking as would be indicated by the presence of blackened shells, dead oysters, or boxes.

A chemical analysis of the bottom mud run both before and after the shots showed no significant change nor did the chemical nature of the water show any change during the period of the shots. Sampling was continued for several months after the explosions and any change due to the bubbling of gases from the shot hole would have been detected. Oysters exposed very close to the gases for seven and a half months after the shots showed no noticeable effects.

Immediately after the explosions the area around each shot point was carefully observed to see if any evidence of dead or stunned animals such as fish, shrimp or crabs could be seen. However, none were to be seen at any time during the period the explosions took place, except those which were behaving in a normal manner.

Discussion of Results

These two experiments produced data which when used in connection with the chemical and physical observations made, and the personal observations of the author, make several conclusions obvious as to the effect of seismic exploration on oysters when carried out under the existing regulations of the State of Louisiana. In these experiments several possible effects of the explosions have been studied and the results of this study fall into three general categories.

The first of these is the effect of the explosions on the bottom. It can be said without any reservation that the oysters which were on the bottom near the shots showed no evidence of sinking which was different from that of oysters on the bottom in the control area. No silting of the bottom due to the shots was observed except for a distance on the average of about three feet from the shot hole. As the regulations do not permit seismic exploration on an oyster bed this would not damage oysters. The report of turbidity

measurements showed that less turbidity was caused by the explosions than by commonly encountered factors, such as wind.

The second effect to be studied was the presence of the ebullient gases which come from the shot hole after the explosion. The data obtained and shown in Table 1 show that the oysters which stayed in the vicinity of the bubbling gases during the course of Experiment 1 suffered no more mortality than those which were not near the source of the gases. The percent survival of those which were not exposed to the gases, in some instances, was less than that of those which were exposed. Animals which were exposed to the gases for from four to seven and a half months showed as high percent survival as those which were used as controls. There is no evidence that the gases caused by explosions are harmful to oysters which are in the area and which may stay in the area for some length of time.

The glycogen content of the experimental animals did not differ significantly from that of the control animals. In Experiment 1 the glycogen content of the experimental animals was slightly higher than that of the controls and in Experiment 2 the glycogen content of the control animals was slightly higher than that of the experimental animals. In neither case was the difference enough to be considered significant.

However, the primary purpose of both the experiments was to determine the effect of the shock wave from the explosions on the oysters. It will be recalled that the position of the oysters relative to the shot point was varied in several ways and that in the first experiment the oysters were on racks and in the second experiment the oysters were on the bottom. Table 1 gave a summary of the results of the first experiment and it was seen that there was no significant difference between the experimental animals and the controls as to the percent survival. In the second experiment the controls actually showed a lower percent survival than the experimental animals. Again, as was previously stated, those oysters which stayed for any length of time in water of high salinity showed a higher percent mortality than those oysters which were never in the high salinity water. It will be noticed that those trays of oysters which had as their origin Station 51 showed a higher percent mortality than those which were exposed to the explosions or which came from Bay Chene Fleur, and Station 51 has a high salinity at nearly all times of the year.

In Experiment 2 it was seen that the same general trend was followed as was followed in the first experiment, that is that there was no significant difference between those exposed to the explosions and those not exposed, and that the only difference was between those which had been in high salinity water and those which had not been in high salinity areas. Thus, no apparent effects were noted on the oysters due to the explosions.

Conclusions

The conclusions reached in connection with our original three questions concerning the effect of explosions on the bottom, the effect of gases from the shot holes of the oysters, and the direct effect of the shock wave on the

animals are fairly clear cut when operating procedure as outlined by law in Louisiana is followed. As to the first of these questions it can be said that the oysters did not sink into the bottom when explosions were detonated 40 feet from them, nor were they covered with silt or affected with turbidity caused by drilling, shooting, or pulling up of the pipes. Nor did oysters suffer any mortality due to gases from the shot hole during a period of eight months after the experiment (four months in the first experiment as the oysters were stolen at that time). The second of the questions can be answered by saying that the oysters which were exposed to the gases for as long as eight months showed no significant difference in the percent mortality from those which were used as controls and which were not exposed to the effect of the gases. Again the only difference was between those which were exposed to high salinity water and those which were not exposed to it at all.

As to the last and most important question, whether the shock wave of the explosions killed oysters, it can be said that those animals which were exposed at 20, 60, 130, and 250 feet from the shots and were kept for eight months in the control area did not differ significantly from each other in mortality, and did not show any higher mortality than those oysters which were in the control area all the time. Also, those oysters which were exposed to the explosion and left in the experimental area for eight months afterwards showed no significant difference among themselves, and did not show any higher mortality than those which were in a control area during the whole course of the experiment. Oysters which were brought into the experimental area after the explosions did not show any significant difference from those which were exposed to the shots and stayed in the area. The only oysters which showed any significant differences in mortality were those which were brought from Station 51 to the experimental area and they showed a higher percent mortality. So it can be said that the shock wave of the seismic work as carried out under operating procedure prescribed by law will not kill oysters even at a distance of 20 feet.

Glycogen analysis showed no consistent trend which would indicate that nutrition or storage of reserve food in the tissue of oysters was disturbed by the explosions or by the after-effects of the seismic work. There was greater difference in the glycogen content between trays which were in the control area all the time than there was between trays of oysters which were exposed to the shots and those which were not exposed to the explosions.

All tests which were conducted with regard to the possible adverse conditions arising from seismographic work gave negative results. Therefore, the only logical conclusion that can be drawn is that seismographic shooting as done according to Louisiana law has no adverse effects on oysters as close as 20 feet from the shot either immediately or over a period of eight months.

TABLE 1

BAY DE CHENE - EXPERIMENT 1

Distance from Shot	Tray Numbers	No. Oys- ters Started*	No. Oys- ters Sur- viving 4 Months**	% Survi- val 4 Months	No. Oys- ters Sur- viving 8 Months***	% Sur- vival 8 Mos.
<u>Rack #1 20 feet</u>						
Permanent Trays	503,504	345	289	83.7		
From Chene Fleur	95	172	157	91.2		
From Station 51	350	155	92	59.3		
<u>Rack #2 60 feet</u>						
Permanent Trays	507,508	348	302	86.7		
From Chene Fleur	93	172	152	88.3		
From Station 51	342	166	100	60.2		
<u>Rack #3 130 feet</u>						
Permanent Trays	511,512	336	287	85.3		
From Chene Fleur	98	170	147	86.5		
From Station 51	341	156	73	46.8		
<u>Rack #4 250 feet</u>						
Permanent Trays	515,516	333	281	84.4		
From Chene Fleur	99	170	156	91.7		
From Station 51	343	162	96	59.2		
<u>Controls in Bay de Chene</u>						
Permanent Trays	519,520	338	257	76.0		
From Chene Fleur	94	169	133	78.6		
From Station 51	344	163	86	52.8		
<u>Controls at Chene Fleur</u>						
Permanent Trays	90,91,92, 96,97	864	789	91.3	761	88.1
From Rack 1	501	172	150	87.2	136	79.1
From Rack 2	506	171	148	86.8	141	82.5
From Rack 3	509	168	152	90.4	144	85.7
From Rack 4	514	173	151	87.3	143	82.6
From Control Rack	518	171	146	85.4	140	81.9
<u>Controls at Station 51</u>						
Permanent Trays	345,346, 347,348, 349	825	625	75.8	567	68.7
From Rack 1	502	164	139	84.7	123	75.0
From Rack 2	505	169	143	84.6	132	78.1
From Rack 3	510	169	137	81.0	121	71.6
From Rack 4	513	169	132	78.1	116	68.7
From Control Rack	340	166	142	85.5	126	75.9

* Permanent trays at Station 51 started 8-20-49, trays at Chene Fleur started 8-19-49, all other trays started 8-23-49.

** Dates taken as end of 4 months period were 1-5-50 for controls at Station 51 and 1-9-50 for all other trays.

*** Dates for end of 8 months period were 4-22-50 for Station 51 and 4-23-50 for Chene Fleur.

TABLE 2

BAY BATISTE - EXPERIMENT 2

Distance from Shot	Tray Numbers	No. Oys- ters Started*	No. Oys- ters Sur- viving 4 Months**	% Survi- val 4 Months	No. Oys- ters Sur- viving 7½ Months***	% Sur- vival 7½ Mos.
<u>Rack #1 20 feet</u>						
Permanent Trays	593,597	334	275	82.3	253	75.7
From Chene Fleur	588	168	149	88.6	134	79.8
From Station 51	599	143	123	86.0	105	73.4
<u>Rack #2 60 feet</u>						
Permanent Trays	591,594	326	269	82.4	256	78.5
From Chene Fleur	584	164	152	92.7	131	79.9
From Station 51	560	127	118	92.9	111	87.4
<u>Rack #3 130 feet</u>						
Permanent Trays	572,598	324	281	86.8	255	78.7
From Chene Fleur	585	166	142	85.5	127	76.5
From Station 51	600	151	119	78.8	108	71.5
<u>Rack #4 250 feet</u>						
Permanent Trays	575,578	329	280	85.1	242	73.5
From Chene Fleur	586	164	148	90.2	129	78.6
From Station 51	565	148	126	85.1	111	75.0
<u>Control Bay Batiste</u>						
Permanent Trays	574,590	341	294	86.2	264	77.4
From Chene Fleur	582	170	147	86.5	133	78.2
From Station 51	571	150	128	85.3	118	78.6
<u>Controls at Chene Fleur</u>						
Permanent Trays	579,580, 581,583, 587	834	766	91.8	736	88.2
From Rack 1	592	159	146	91.8	139	87.4
From Rack 2	595	166	148	89.1	138	83.1
From Rack 3	596	128	104	81.2	97	75.7
From Rack 4	573	165	151	91.5	143	86.7
From Control Rack	576	171	143	83.6	139	81.3
<u>Controls at Station 51</u>						
Permanent Trays	561,562 563,564, 559	820	653	79.6	596	72.7
From Rack 1	569	164	131	80.0	121	73.7
From Rack 2	567	167	122	73.0	112	67.0
From Rack 3	570	167	120	71.8	104	62.2
From Rack 4	568	166	134	80.7	120	72.2
From Control Rack	566	170	127	74.7	109	64.1

* Permanent trays at Chene Fleur started 8-30-49, all other trays 8-31-49.

** 4 months ended 1-5-50 for controls at Station 51, 1-9-50 for controls at Chene Fleur, and 1-6-50 for trays at Bay Batiste.

*** 7½ months ended 4-22-50 for Station 51, 4-23-50 for Chene Fleur, and 4-21-50 for Bay Batiste.

THE PRODISSOCOONCHS AND THE SETTING BEHAVIOR OF THREE SPECIES OF OYSTERS

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Introduction

The obtaining of "strike" is one of the more important aspects of the oyster industry. This is true for all of the important species grown in the United States. The private planter and the oysterman who harvest oysters from state owned reefs both depend on an adequate spat-fall. On the Atlantic and Gulf Coasts, where Crassostrea virginica is the only commercial species, from North Carolina southward and even in parts of Virginia, oyster set is usually abundant and often the problem is one of getting too much. In some places the young spat attach so thickly that the seed must be broken apart or else many of the small oysters must die if the remainder are to reach market size. On one occasion in Terrebonne Parish, Louisiana, over two thousand newly attached spat were counted on the inside surface of an oyster shell that had been in the water for only one week.

In the saline waters along the Gulf Coast, one problem is to find the level where the commercial oyster attaches most readily. On the edge of the Gulf where the salinity approaches that of seawater, the species occurs naturally in reefs, and on pilings, jetties, and other structures, mainly in the intertidal zone. The limitation of this species to the intertidal zone is due partly to the destructive action of the oyster conch (Thais floridana), the stone crab (Menippe mercenaria) and other enemies below this zone. That it is also due in part to the setting behavior of the larvae is suggested by the following evidence. In certain parts of the northern Gulf two species of Ostrea (O. equestris and O. frons) occur together with Crassostrea virginica. These are found attached to surfaces at or near the bottom. On templates of an oil well platform off the Louisiana Coast, Gunter (1951) found that the zone of occurrence of two species of Ostrea was lower than but overlapping that of C. virginica.

In an effort to clarify the setting behavior of the different species, stratified culch experiments were conducted continuously at the Institute of Marine Science, University of Texas, at Port Aransas, Texas, from June 6, 1951 to November 8, 1952. Two species of oyster, C. virginica and O. equestris, occur in this area, where the salinity is about 30 o/oo. In addition, Dr. Philip Butler, Director of the Pensacola Laboratory of the Fish and Wildlife Service, kindly furnished two series of culch material from a somewhat similar experiment in Florida. In the latter material, a third species, O. frons, was also present.

Prodissoconch Shell

The majority of the spat that attached to the culch were less than 5 mm. in shell length and the characters of the adult shell that distinguish the

species had not developed. Ranson (1948) has separated the living oysters into two genera partly on the basis of the prodissoconch shell¹. He found that oysters belonging to the genus *Crassostrea* have a slight hook or beak at what would be the umbo of the larval shell, whereas the prodissoconch shell of the genus *Ostrea* has a more rounded shell.

Mounted slides of the prodissoconch shell of the three species were made. The prodissoconch shells were obtained from large oysters in which there was no difficulty in identification. These slides were compared, camera lucida drawings made, and sizes determined. From this comparison, it was then possible to identify the spat even though they had just recently attached.

Figure 1 shows camera lucida drawings of the right and left valves of the prodissoconch larvae of the three species *C. virginica*, *O. equestris* and *O. frons*. All three species have a shell under 500 micra and hence magnification must be used to distinguish the species. *C. virginica* and *O. equestris* prodissoconchs are approximately the same size, averaging a little over 350 micra; however, *C. virginica* can be distinguished by the characteristically beaked shell, and *O. frons* is readily distinguished by the fact that its shell is about 50 to 60 micra longer, measuring about 410 to 425 micra, and contains brown pigment.

Culch Experiments at Port Aransas, Texas

The experiments at Port Aransas, Texas, involved two series of observations. Clean weather-bleached shells of *C. virginica* were used as culch material and 3 mm. holes were bored in these shells. Two shells were wired at 0.5 meter intervals, starting at the bottom, to a chain that was suspended in water of three and a half meter depth. A weight was placed at the bottom so that the chain would remain taut and the shells retain their proper level.

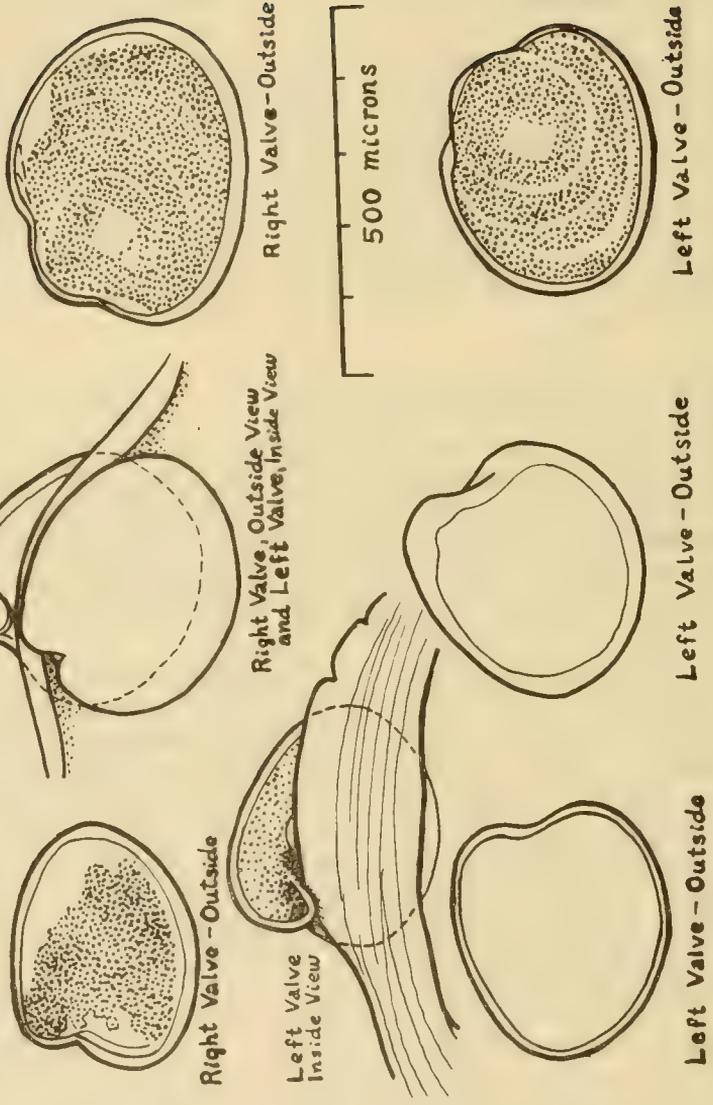
The culch chain was examined at approximately monthly intervals during the summer, and three times (October 21, 1951, December 30, 1951 and March 22, 1952) during the period from October of 1951 to May, 1952. At each examination one of the two shells at each level was removed and two additional shells were attached. Also at each examination (after the first) the remaining shell at each level (which had been down for two intervals) was examined. Therefore Series A was a series of examinations of culch that had been in the water one month (longer during the winter months) and Series B of culch that had been in the water for two months or longer. At first duplicate shells were put down in Series A and there were a total of fourteen examinations in this series and eleven for Series B.

¹ Ranson advocated calling the genus to which our common commercial east coast oyster belongs *Gryphaea*. Gunter (1950) called this genus *Crassostrea* and his nomenclature is followed here.

Figure 1

PRODISSOCONCHS OF THREE GULF OYSTERS

CRASSOSTREA VIRGINICA OSTREA EQUESTRIS OSTREA FRONS



All shells were examined under magnification of from 10 to 50 diameters, and the numbers of spat of each species were determined for each level. On several occasions certain of the shells were lost, especially in Series B. Table I shows the average number of individuals per shell of each species for each level for the duration of the experiment. There was an average of from 25 to over 50 spat per shell. On some individual shells the spat numbered several hundred. It is noted from the table that O. equestris was more abundant than C. virginica. Even though the total number of O. equestris was greater, C. virginica was more abundant at the higher levels.

Table II shows the percentage of the total number of individuals of each species which occurred at given levels. Series A has been plotted on a graph and is shown in Figure 2. It is seen that O. equestris became progressively scarcer near the surface, whereas the reverse was true of C. virginica. The percentage of O. equestris that attached at the surface, 3.5 meters from the bottom, was only one tenth of one percent of the total number of spat of this species in Series A, and none was found at the surface in Series B. In Series A, four tenths of one percent of the total number of C. virginica attached at the bottom level and there was none that survived at this level in Series B. C. virginica showed a marked decrease in numbers below one meter from the surface (2.5 meters from the bottom) in Series A and an even more striking decrease below 1.5 meters below the surface in Series B.

All the data gathered are really survival figures for the period the culch was in the water. Since all culch had been in the water at least one month when examined, many spat succumbed through competition among themselves and with other epizootites. The actual number of larvae which attached at the various levels would be difficult to determine because of the labor involved in very frequent observations. Shells examined at such long intervals do not reflect the maximum possible strike, since the shells soon become less suitable for spat-fall because of fouling.

Random plankton samples during the summer of 1951 revealed no "eyed" larvae of O. equestris at or near the surface, and none of C. virginica at or near the bottom. These observations agree with the stratification of the strike.

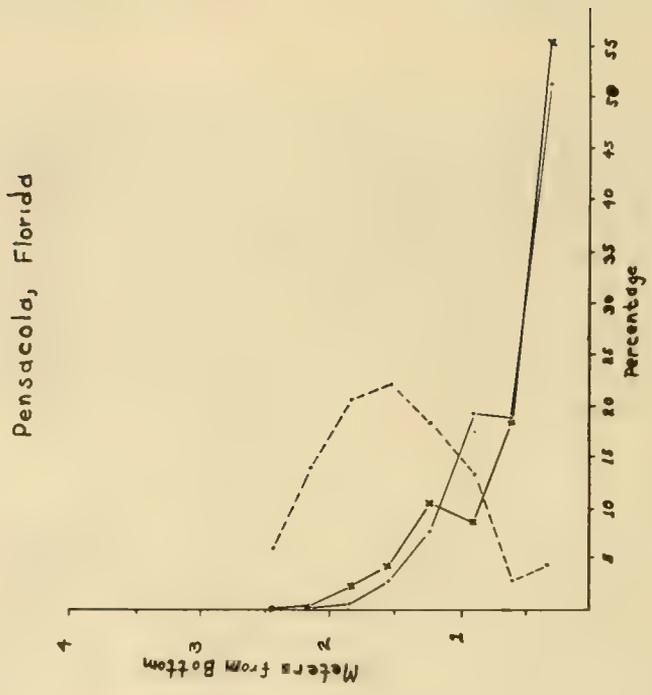
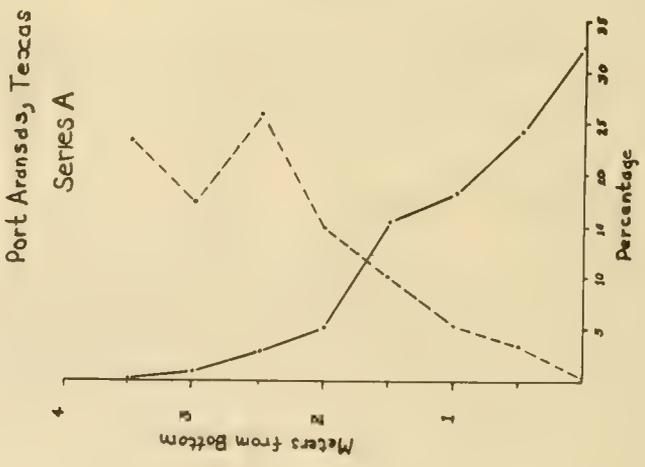
Culch Data from Pensacola, Florida

At Pensacola, the salinity range was from 19.5 to 26.0 o/oo during the period that the culch material was in the water. There were two series, the first from May 27, 1952 to June 6, 1952, and the second from June 6, 1952 to June 16, 1952. The culch consisted of asbestos plates, twelve inches long by four inches wide, placed in a ladder-like arrangement at one foot intervals. The bottom plate was one foot above the bottom and the surface plate nine feet above. However, the surface plate received no spat-fall and was not examined. The plate six feet above the bottom in the second series was lost.

For examination, the plates were marked off in ten blocks 5 cm. square, and two of these blocks on each surface were selected at random and examined. The oysters on the top surface of the plates of the first series were so

Fig 2
 Percentage of Attachment at Depths from Bottom

- *O. squamatus*
- - - *O. fross*
- · - · *C. virginica*



crushed that it was not possible to distinguish the species from the larval shell. Therefore the area examined was 150 square centimeters for each level (except for the level six feet above, where only 50 square cm. were examined, because this plate was lost in the second series.)

The percentage of individuals of O. equestris, O. frons and C. virginica attached at each level is shown in Table III. These are averages for the two series. These percentages have been plotted on the metric scale for depth in Figure 2. Both species of Ostrea attached more abundantly near the bottom. There was no culch on the bottom but from the information obtained at Port Aransas, it would be expected that had there been, this level would have received the majority of the Ostrea spat. At Pensacola, with salinity range of 19.5 to 26.0 o/oo, spat of C. virginica occurred most abundantly at about mid-depth. Dr. Butler tells me that in 1951, when no attempt was made to distinguish species, the heaviest set was at mid-depth. Adult Ostrea were virtually absent from the area at that time.²

The patterns of stratification for the two species of Ostrea are very similar, in these limited samples. At Pensacola as at Port Aransas, O. equestris was much more numerous than C. virginica. Spat of C. virginica made up only 20% of the total number of spat examined at Pensacola, and only 30% of the total number examined at Port Aransas.

Discussion and Conclusions

Some of the data from the published literature bear upon the stratification of spat in waters of high salinity. (No attempt has been made to include all the references, and no references to setting of the genus Ostrea are given.)

Chapman and Esveltdt (1942) found that the Pacific oyster, C. gigas, showed no stratification of set in Southern Willapa Bay (State of Washington), where the salinity range was from 15 to 26 o/oo. In Puget Sound, where it is expected that the salinity would be higher, this species has a narrow zone of setting from one to three feet deep and "almost always well above the mean low tide". Ranson (1943) states that C. angulata, the Portuguese oyster, forms reefs on the bottom in the less saline areas and intertidal reefs in more saline waters nearer the coast. In these respects its behavior is similar to that of C. virginica.

Thus stratification of set in the waters of higher salinity is reported for other species in the genus Crassostrea as well as for C. virginica.

According to Nelson (1938), the genus Ostrea occurs in the less turbid, more saline areas, whereas Crassostrea has been able to adapt itself to the more turbid, less saline areas such as river mouths and bays. He says that

² Letter from Dr. Philip Butler, Chief, Gulf Oyster Investigations.
30 January 1953.

this is because *Crassostrea* has a promyal chamber and a more efficient filtering system to handle the more turbid waters and still obtain abundant food. Other work, including unpublished data of the author, support the idea that *Crassostrea* has evolved a more selective feeding mechanism than has *Ostrea*, which would make it better adapted to turbid waters. No natural reefs of *C. virginica* are known to the writer in deep water of near ocean salinity; in such waters, the reefs are all intertidal.

However, if other factors, especially predators, are controlled, *C. virginica*, if transplanted, can be grown successfully in deep water of high salinity. It seems likely that factors other than feeding efficiency and predation affect the different zonations of *Crassostrea* and *Ostrea*. The present data suggest that *Crassostrea* is actually unable to strike effectively in deep water of high salinity.

In conclusion, it may be said that the commercial oyster, *C. virginica* sets abundantly in waters of high salinity, but in such waters the spat are abundant only in the intertidal zone. In highly saline waters of the Northern Gulf region, the oysterman and oyster biologist will see spat on culch planted on the bottom below low tide level, but most of these will be of the genus *Ostrea*, which is difficult to distinguish from *Crassostrea* except by the prodissoconch shell.

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TABLE I

AVERAGE NUMBER OF EACH SPECIES AT EACH LEVEL AT PORT ARANSAS, TEXAS

Level From Bottom	<u>Series A</u>		<u>Series B</u>	
	O. equestris	C. virginica	O. equestris	C. virginica
Bottom	61.1	0.3	86.0	0.0
0.5 M.	46.1	2.4	84.1	3.0
1.0 M.	34.1	4.7	56.7	4.4
1.5 M.	30.4	7.7	32.7	8.3
2.0 M.	10.5	12.8	13.3	18.9
2.5 M.	4.1	20.9	4.8	16.4
3.0 M.	1.6	15.0	2.5	18.9
3.5 M.	0.3	20.8	0.0	23.6

TABLE II

PERCENTAGE OF EACH SPECIES AT EACH LEVEL AT PORT ARANSAS, TEXAS

Level From Bottom	<u>Series A</u>		<u>Series B</u>	
	O. equestris	C. virginica	O. equestris	C. virginica
Bottom	32.6	0.4	30.9	0.0
0.5 M.	24.6	3.1	30.2	3.3
1.0 M.	18.2	5.9	20.4	4.9
1.5 M.	16.2	9.8	11.7	9.2
2.0 M.	5.2	15.2	3.8	16.1
2.5 M.	2.2	26.5	2.1	22.0
3.0 M.	0.7	16.3	0.8	18.5
3.5 M.	0.1	22.7	0.0	25.9

TABLE III

PERCENTAGE OF EACH SPECIES AT EACH LEVEL AT PENSACOLA, FLORIDA

<u>Level From Bottom</u>	<u>O. equestris</u>	<u>O. frons</u>	<u>C. virginica</u>
1 foot	51.7	55.4	4.6
2 feet	18.6	18.4	3.0
3 feet	19.5	9.2	13.0
4 feet	7.2	10.7	16.8
5 feet	2.3	4.6	22.9
6 feet*	0.7	1.5	20.6
7 feet	0.0	0.1	13.7
8 feet	0.0	0.0	5.3

* Only 50 square centimeters examined.

CHANGES IN THE PHYSIOGRAPHY OF OYSTER BARS IN THE JAMES RIVER, VIRGINIA

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INTRODUCTION

The intent of the Baylor Survey of 1892 was to define the naturally-producing oyster grounds in Virginia waters and to set them aside for public use. Actually, some natural ground was omitted and much barren ground was included within the bounds of the Survey; however, it stands as a definition of public grounds from which any citizen of the state may, for a small fee, obtain a license to take oysters. Only hand tongs are permitted in this public fishery.

These oyster bars within the Baylor Survey are about twice as extensive as the ground outside leased to private planters but they yield only about one-third as many oysters of market size (Marshall, 1951). On the other hand, certain of these public grounds provide the seed or small oysters that are transplanted to private grounds and are essential to the success of leased bottoms which are seldom self-sustaining. Based on this relationship an extensive area in the lower James River (above the boundary shown in Figure I), noted for its capacity to produce great numbers of small oysters, has been established by law as a seed area. This area is not subject to the usual regulation that oysters must remain on public grounds till they grow to marketable size. Tongs harvest and sell to private planters from one to two million bushels of seed oysters from this James River area annually. As a conservative estimate, each bushel contains 1500 small oysters, making a total approaching three billion oysters harvested annually as seed. Also removed from these grounds is a considerable amount of the shell to which the seed oysters are attached and an additional quantity of more mature oysters.

The removal of this mixture of live oysters and shell might reduce critically the amount of cultch available for the setting of larvae. This might also cause decline and other changes in the surface of the bars, modifying the hydrodynamics of the region and otherwise disturbing ecological conditions. The widely varied speculations concerning such effects make it especially imperative to search for data on this subject. A step that can be taken toward this end is to compare soundings on early hydrographic surveys with those taken more recently and thus to observe some of the physiographic changes that have actually occurred. The first complete depth surveys of the portion of the lower James River in question are those made by the U. S. Coast and Geodetic Survey in 1854-55. Additional comprehensive surveys, also by the U.S.C.G.S., were conducted in 1871-73 and 1943-48. All such soundings are plotted on what are known as hydrographic surveys which, of course, provide the primary reference data for drafting navigation charts, etc.

METHODS

From Moore's (1910) maps of the oyster grounds in the area, as his party observed them shortly before his publication, traverses were selected across

PUBLIC OYSTER BARS IN LOWER JAMES RIVER

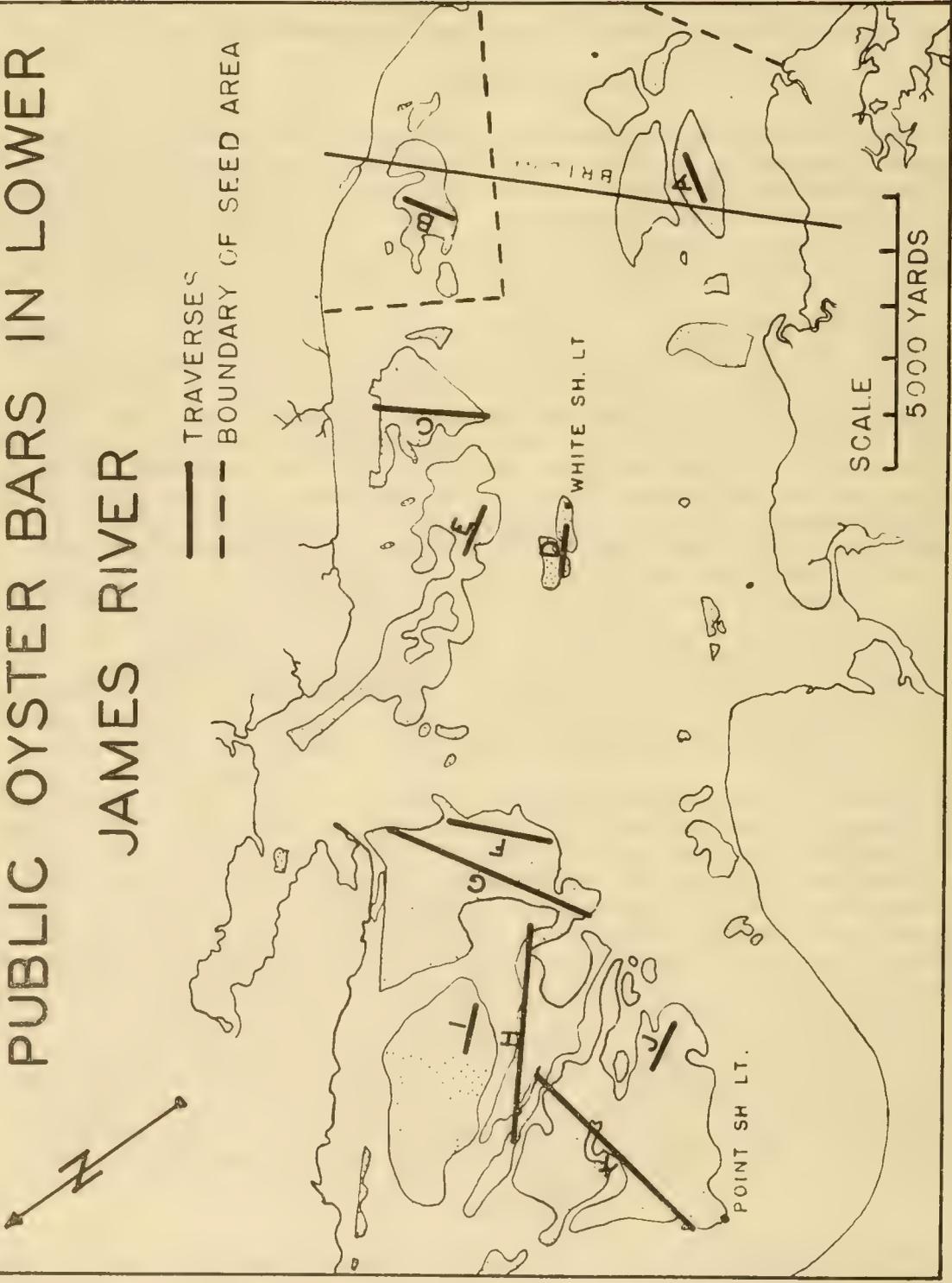


figure I

representative bars. Each such traverse (Figure I and Table I/1) is a straight line, crossing, for at least 75% of its length, grounds which Moore designated as supporting a dense growth of oysters. An exception is the straight line across Brown Shoal which, though not covering such a high percentage of dense growth, was studied to represent bars that were formerly exposed at low water.

These traverses were plotted on the hydrographic survey of each period mentioned above and, using the shortest interval practical on the less intensive older surveys, the depths were read every 100 yards. Thus every hundred yards a point was established at which depths could be compared on successive surveys. As reported by the U. S. Coast and Geodetic Survey (correspondence from the Acting Director) there has been an 0.6 foot rise in sea level in the region between 1855 and the late 1940s. To allow for this in the present study concerned with bottom changes, a one-half foot correction factor was used when comparing soundings of the 1940s with those of earlier years.

The points on these traverses that lie over slopes and channels are not well suited for this study. Though oystering is not limited to the crests of the bars, many such points are not over oyster bottom; their depths are likely to be altered primarily by hydrographic conditions, perhaps with little relation to oystering and oyster growth; and errors in surveying and draftsmanship are exaggerated in contrasts over sloping surfaces. For this reason only those points over relatively level stretches of the bottom, well up on the bars, were compared.

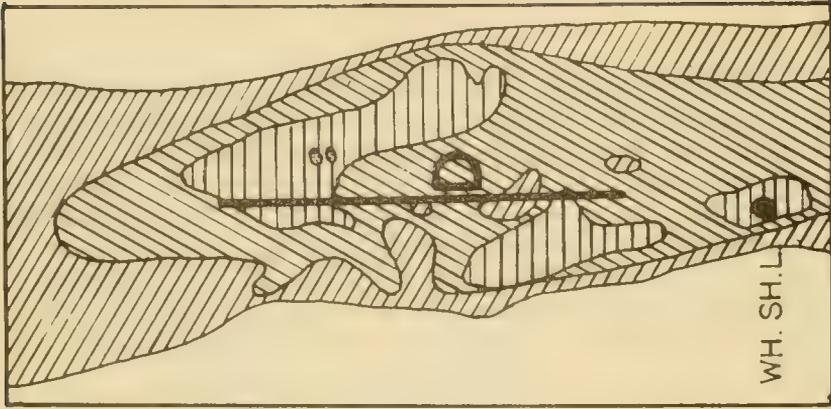
RESULTS AND DISCUSSION

At 121 of the points compared (Table II) the bottom was more elevated in the 1850s than it was in the 1940s, whereas the reverse was true at 38 points. An analysis of the comparisons for the entire ninety-year period (Column B of Table II) is as follows:

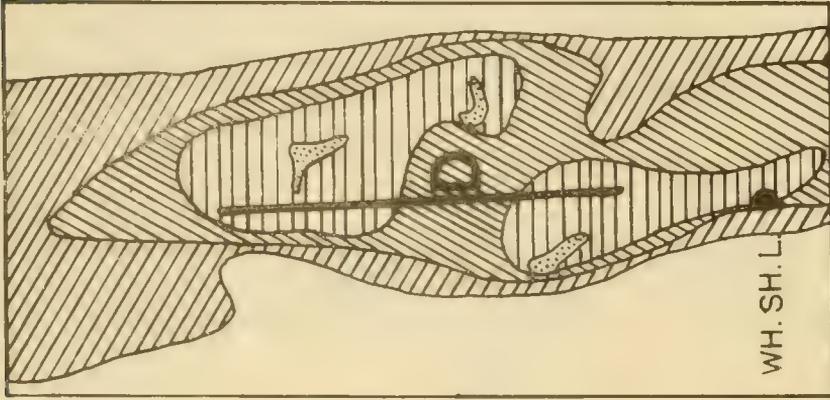
No. of points compared	=	173
Mean of depth differences	=	-1.1 ft.
Standard deviation of differences	=	1.9 ft.
t value	=	7.9
95% fiducial limits of differences	=	-0.82 to -1.38 ft.

/1 Point of Shoals Light and White Shoals Light, used as reference points in defining the traverses, are not operating but still stand. In some cases it was obvious that a traverse drafted on Moore's maps of the area crossed slightly different grounds than when plotted on the hydrographic surveys of the U. S. Coast and Geodetic Survey. It was assumed that this was due chiefly to errors in Moore's maps. This does not, of course, affect the accuracy of comparisons between hydrographic surveys.

1943-48



1871-72



1854-55

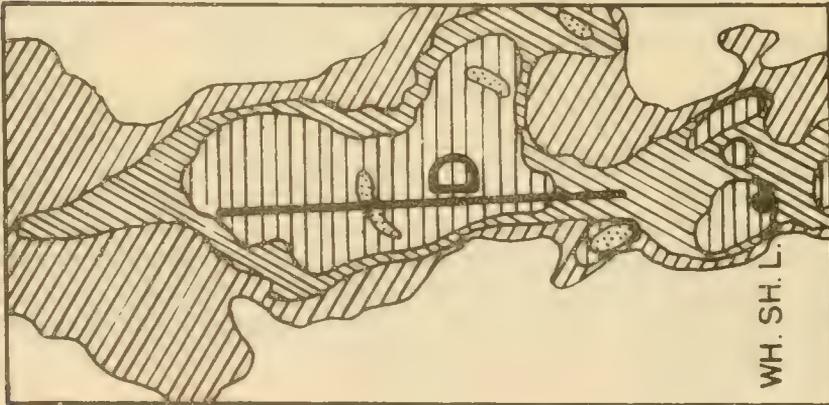


Figure II. Depth Contours over 200 Sq. Yd. Area at White Shoals Light.

An analysis of the comparisons for the first 17-18 years (Column A of Table II is as follows:

No. of points compared	=	173
Mean of depth differences	=	-0.3 ft.
Standard deviation of differences	=	2.2 ft.
t value	=	1.8
95% fiducial limits of differences	=	-0.04 to -0.64 ft.

Thus, to the extent that these data represent the general history of the crests of the bars examined, they indicate that over the ninety-year span there has been a mean depth change of about one foot. This figure is statistically significant, far better than the 1% probability level, and lies within relatively narrow fiducial limits that justify the approximation of one foot as to the magnitude of the change. The analysis for the first thirteen years leaves greater doubts as to the validity of the calculated depth change of 0.3 feet. The low t value indicates that the mean difference is not highly significant and the fiducial limits indicate the change could have been a slight increase rather than a loss from the surface of the bars.

Quite noticeable in survey comparisons is the almost complete disappearance of emergent or intertidal oyster shoals since the 1870s (see, for example, the history of Brown Shoals as portrayed by traverse B B¹ in Table II). Though the surveys do not always indicate it, it is probable that the typically elongate, exposed areas in middle of the oyster grounds are intertidal oyster reefs. Ignoring the shoals adjacent to and thus essentially a part of the shore, approximately 12,000 yards of intertidal reef were noted from the 1854-55 surveys, 17,000 yards in the 1871-73 surveys and less than 100 yards in recent surveys. On the original surveys there are so many indications of more thorough work in the 1870s than in the 1850s that the added reef measurements of the second period seem to result from more critical surveyings. On the other hand, the difference indicated between the 1870s and the 1940s seems real, as would be expected with a one-foot mean loss from the surfaces of the oyster bars and the half-foot rise in sea level.

DISCUSSION

The depth comparisons may be discussed under two interdependent headings: the net loss of surface over the ninety-year period and the variation from point to point. The existence of this variation is sometimes seen more vividly by observing depth contours, as illustrated in Figure II of the White Shoals area. That the variability should be so pronounced seems to stress the importance of natural environmental factors, cutting, building and depositing sediments to mold the shape of the grounds. Thus the bars are quite dynamic, everchanging in form. Though the physical and biological forces involved must be set aside in this discussion as a complex not thoroughly analyzed, it is important to recognize that final effects involve an interaction between such natural forces and the fishery influences. Such interactions are similar to those encountered in dealing with the many fishery populations that obviously vary in response to environmental forces.

Before considering the possible role of the fishery in the net decline of the bars, two forces should be mentioned that constantly tend to lessen the depth. Whatever oysters are on the bars continuously add to their shells and thus to the bottom development. Also sedimentation in the area tends to lessen water depths. Numerous data show that this is a region of steep salinity gradients, due to the mixing of river discharge and sea water. Thus it is an area of salting out of matter in suspension, accompanied by a marked downstream increase in the clarity of the water (see Chesapeake Bay Institute Data Report, No. 7, 1952, for tabulations of light transmission and salinity data). Whatever the sedimentation rate, it obviously has not fatally smothered the oyster grounds nor does it compare with the extreme deposition effects shown by Brown, Seavy and Rittenhouse (1939) for the York River and by Gottschalk (1945) for the upper Chesapeake Bay off the mouth of the Susquehanna River.

To consider the effect of oystering in the overall decline in the surfaces of the bars it is necessary to estimate the magnitude of shell removal by the fishery. Catch statistics applicable to the seed area, which coincides with the region of these depth comparisons except that the latter includes Brown Shoal (Traverse B B¹), are the only records from which we might estimate shell removal rates. The harvest of seed oysters in Virginia is given in various U. S. Fish and Wildlife Service (formerly U. S. Bureau of Fisheries) reports and is presented in what appear to be comparable summaries for the 1920-21 and the 1924-25 seasons and for the years 1929 through 1944. The average annual seed harvest in these years in terms of U. S. standard bushels was 1,675,000. Since only seed oysters are harvested from the James River seed area and since the recorded Virginia seed harvest is almost entirely that of the James, it seems reasonable to use a rounded figure of 1,500,000 bushels to represent the annual removal during the period covered by the data. The use of the bars in the lower James River has changed through the years, but as Wharton's (1948) historical review suggests, oystering in the state was probably as intensive in the 1850s as it is today and the James River has always been the center of this fishery. Thus the estimate of 1,500,000 bushels a year, taken from the region now defined as a seed area, may be used for the entire period since 1854. Of this harvest the actual volume in seed is small for the small oysters are generally attached to empty shells which may comprise about two-thirds of the total or 1,000,000 bushels annually. On the other hand, removal of empty shell from the surface of bars was undoubtedly less extreme in former years when the region was used more for market oysters. For an approximation of the average annual removal of empty shell incidental to the removal of live oysters from this area since the 1850s, I will use a figure of 700,000 bushels.

Tonging and shell removal obviously are concentrated in areas of dense growth and it is over such areas that most of the depth comparisons were made. Assuming that two-thirds of the estimated shell removal or 460,000 bushels of shell came from areas of dense growth and noting that Moore (1910) found, on the bars of the present seed area, about 3200 acres with such growth, it may be estimated that the fishery has been depleting these surfaces at the rate of 0.05 inches a year. At such a rate a little less than 5 inches of shell would have been removed in the ninety-year period whereas the net decline at the points compared was about 1 foot.

These removal estimates, being extremely crude, cannot be used as a basis for critical interpretations. On the other hand, if one makes allowance for factors tending to build the bars and then estimates the removal of shell involved in the net loss of a foot, the figure must greatly exceed my estimates from catch records. This raises many questions. The catch records may fall far short of the actual harvest. The removal of shell may initiate depletion processes which exceed the amounts tonged from the bars. Possibly there has been some settling of the river bottom in general, producing a lowering of oyster bars.

SUMMARY

This study compares original U. S. Coast and Geodetic Survey depth observations over the productive oyster bars of the lower James River for the period of 1854-55 through 1943-49. There was considerable variation in the physiographic changes in the surface of the oyster grounds during that period; however, at most points depth comparisons indicated decline and the net effect was a mean loss of about a foot in the elevation of the bars.

The variations in depth comparisons and changes in the courses of depth contours indicate that oyster bars are quite dynamic, changing form in response to environmental factors. The physiographic history of these bars is, therefore, the result of both natural and fishery influences just as fishery populations have complex histories of interwoven fishing and environmental effects.

ACKNOWLEDGEMENTS

Some of the expense and support for this study has been borne by the Virginia Fisheries Laboratory and the Chesapeake Bay Institute. My former associates at the Virginia Laboratory, Dr. Jay D. Andrews and Dr. J. L. McHugh, have been especially helpful in reviewing this manuscript.

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TABLE I. Location of Traverses of Oyster Bars in the Lower James River, Va.

	<u>Distance</u>	<u>Direction</u>	<u>Reference</u>	<u>Traverse Description</u>
A	5985 yds.	30.2°EofS	of Wh. Shoal Lt.	
A ¹	6795 yds.	35.6°EofS	of Wh. Shoal Lt.	1000 yds.across Fishing Point Rocks
B	6350 yds.	81.9°EofS	of Wh. Shoal Lt.	
B ¹	5539 yds.	76.0°EofS	of Wh. Shoal Lt.	1000 yds.across Brown Shoal
C	2168 yds.	87.9°EofN	of Wh. Shoal Lt.	
C ¹	3818 yds.	65.3°EofN	of Wh. Shoal Lt.	2000 yds.across Kettle Hole Rock
D	1328 yds.	45.6°WofN	of Wh. Shoal Lt.	
D ¹	315 yds.	42.5°WofN	of Wh. Shoal Lt.	1000 yds.across White Shoal
E	2025 yds.	12.3°EofN	of Wh. Shoal Lt.	
E ¹	1455 yds.	40.2°EofN	of Wh. Shoal Lt.	1000 yds.across Thomas Point Rock
F	9030 yds.	86.4°EofS	of Pt. Shoal Lt.	
F ¹	7680 yds.	75.9°EofS	of Pt. Shoal Lt.	2000 yds.across Wreck Shoal
G	6210 yds.	75.7°EofS	of Pt. Shoal Lt.	
G ¹	7585 yds.	87.8°EofN	of Pt. Shoal Lt.	4000 yds.across Wreck Shoal
H	4058 yds.	58.9°EofN	of Pt. Shoal Lt.	
H ¹	6488 yds.	84.3°EofS	of Pt. Shoal Lt.	4000 yds.across Mulberry Swash Rock
I	5640 yds.	70.8°EofN	of Pt. Shoal Lt.	
I ¹	6010 yds.	80.2°EofN	of Pt. Shoal Lt.	1000 yds.across Marshy Rock
J	3188 yds.	78.7°EofS	of Pt. Shoal Lt.	
J ¹	3855 yds.	66.4°EofS	of Pt. Shoal Lt.	1000 yds.across Point of Shoals Rock
K	615 yds.	25.8°EofN	of Pt. Shoal Lt.	
K ¹	4380 yds.	77.2°EofN	of Pt. Shoal Lt.	4000 yds.across Long Shoal & Swash Rock

TABLE II. Changes in Depths at 100-Yard Intervals over Oyster Bars in the James River, Virginia

Traverse	Points Compared	Mean Depth* <u>1854-55</u>	Change from 1854-55 to 1871-73		Change from 1854-55 to 1943-48, plus $\frac{1}{2}$ **	
			Average	Range	Average	Range
AA ¹	11	5	-0.6	-3 to $\frac{1}{2}$	-0.4	$-2\frac{1}{2}$ to $\frac{1}{2}$
BB ¹	9	$\frac{1}{2}$	-3.0	-4 to 0	-4.4	$-5\frac{1}{2}$ to -2
CC ¹	17	$7\frac{1}{2}$	$\frac{1}{2}$ 0.5	$-2\frac{1}{2}$ to $\frac{1}{2}$ 4	$\frac{1}{2}$ 0.3	$-2\frac{1}{2}$ to $\frac{1}{2}$ 4 $\frac{1}{2}$
DD ¹	8	$4\frac{1}{2}$	$\frac{1}{2}$ 0.8	$-5\frac{1}{2}$ to $\frac{1}{2}$ 4	-1.5	$-7\frac{1}{2}$ to $\frac{1}{2}$ 2 $\frac{1}{2}$
EE ¹	9	$9\frac{1}{2}$	0.0	-2 to $\frac{1}{2}$ 2 $\frac{1}{2}$	-0.6	$-2\frac{1}{2}$ to $\frac{1}{2}$ $\frac{1}{2}$
FF ¹	18	$5\frac{1}{2}$	-2.3	-6 to $\frac{1}{2}$ 1 $\frac{1}{2}$	-2.5	$-6\frac{1}{2}$ to 0
GG ¹	36	6	-0.3	-3 to $\frac{1}{2}$ 6 $\frac{1}{2}$	-1.0	$-3\frac{1}{2}$ to $\frac{1}{2}$ 3
HH ¹	19	10	$\frac{1}{2}$ 0.3	$-4\frac{1}{2}$ to $\frac{1}{2}$ 5	0.0	$-3\frac{1}{2}$ to $\frac{1}{2}$ 2 $\frac{1}{2}$
II ¹	11	10	-0.7	-2 to 0	-0.5	$-1\frac{1}{4}$ to $\frac{1}{2}$ 1
JJ ¹	7	5	-0.7	-2 to 0	-1.3	$-2\frac{1}{2}$ to $-\frac{1}{2}$
KK ¹	26	5	$\frac{1}{2}$ 0.9	$-4\frac{1}{2}$ to $\frac{1}{2}$ 6	-0.7	$-4\frac{1}{2}$ to $\frac{1}{2}$ 5

* These are mean depths, to the nearest $\frac{1}{2}$ foot, below mean low water at the point compared.

** This offsets an approximate 0.6 foot rise in sea level in the region since the 1850s.

COMPARISON OF THE M. F. TECHNIQUE AND THE M.P.N. TECHNIQUE IN
EXAMINATION OF SEA WATER

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Results of a study comparing coliform density values of sea water obtained by the membrane filter (M.F.) and the most probable number (M.P.N.)⁽¹⁾ procedures established the former method as a reliable technique, if due regard is given to turbidities and bacterial densities in determining the volume of sample for filtration. The two techniques gave results 87.1 percent in agreement.

The time and material saving features of the M.F. method make it particularly desirable for examining the frequently large number of samples required for determining sanitary quality of waters in shellfish-producing areas. The Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry ⁽²⁾ stipulates that, in making bacteriological examinations of shellfish-producing areas, the number of samples from each station should be sufficient to give a true picture of the number of coliform organisms present in the water under the various tide and weather conditions occurring during the shellfish harvesting period. The minimum number of samples required varies, but where examinations are made to define the line between approved and restricted areas, at least 15 samples per station, during the period of survey, appear necessary.

The advantages of the membrane filter in the bacteriological examination of fresh water sources have been indicated ^(3,4). It has also been suggested that the membrane filter (M.F.) method gives coliform densities comparable to those obtained by the M.P.N. procedure, as applied to fresh water ⁽⁵⁾. Goetz ⁽⁶⁾ reports that, in an ocean water survey, results obtained by the M.F. method were more consistent and accurate than data gathered by standard M.P.N. procedures.

Considering the advantages of the M.F. method in the bacteriological examination of fresh water, it was deemed advisable to initiate a study designed to demonstrate the comparative performance of the membrane filter in the examination of sea water. 1/ Consequently, the present study was undertaken to determine:

1. The comparison of coliform density values obtained by the membrane filter and the standard M.P.N. procedure.

1/ This study was conducted in the United States Public Health Service Shellfish Sanitation Laboratory located in Woods Hole, Massachusetts.

2. The effect of bacterial densities on the agreement of results by the two methods.

3. The influence of turbidity on the agreement of results by the M.F. and M.P.N. procedures.

Materials and Methods

Water samples were collected from three stations predetermined on the basis of degree of pollution and turbidity. An effort was made to choose stations approximately representative of water qualities defining the three areas of pollution pertaining to shellfish production. Station A represents an approved area (median coliform M.P.N. per 100 ml., 70 or less); Station B, a restricted area (median coliform M.P.N. per 100 ml., 70 to 700); and Station C, a closed area (median coliform M.P.N. per 100 ml., more than 700).

Samples were collected twice daily from the three stations at approximately 8:30 a.m. and 1:30 p.m. over a period of time extending from November 1952 through February 1953. During this period, tide stages and weather conditions varied widely.

All sample collections were made according to standard procedures⁽⁷⁾. Sterile, brown glass bottles with bakelite screw caps, having a capacity of 625 milliliters, were used for collecting and containing samples.

The elapsed time between the collection and the bacteriological examination of a sample never exceeded two hours. Samples were stored at a temperature between 6°C. and 10°C. until examined.

Quantities of a sample used to determine coliform densities by the M.P.N. and the M.F. methods were from the same bottle. Mixing of the sample was obtained by shaking the sample bottle vigorously, 25 times.

Turbidity readings were determined by procedures outlined in Standard Methods⁽⁷⁾ and were made on that portion of a sample remaining after appropriate quantities for bacteriological examination had been withdrawn.

The M.P.N. method for determining the bacterial density of a sample consisted of planting five replicate quantities in at least four decimal dilutions in lactose broth. Gas producing tubes were confirmed in brilliant green bile broth⁽⁷⁾.

Filtration techniques, culture media and incubation conditions employed were those developed and recommended by the Environmental Health Center⁽⁵⁾. Sterilization of the filtration apparatus, membrane filter and nutrient pads was accomplished by autoclaving at 121°C. for 15 minutes.

It was found that satisfactory sterilization of membrane filters by autoclaving was obtained by the following method:

Approximately 10 filters as shipped, i.e., between nutrient pads and paper discs, were placed in covered petri dishes and autoclaved. Care was taken to limit the height of the stack of filters per petri dish to a point sufficiently low to prevent the top of the dish from contacting the stack of filters. This precaution was necessary since it was found that the weight of the petri dish cover exerted sufficient pressure at sterilization temperatures to cause adherence of the membrane filter to its protective coverings. Filters and pads were stacked vertically in petri dishes. Edges of filters were not allowed to project from between their protective covers. Complete coverage of each filter during autoclaving prevented filter edges from curling and becoming brittle.

To increase the accuracy of colony counts and to minimize the suppressive effects of excessive bacterial density on coliform recovery rates, an attempt was made to filter sample volumes yielding not more than 350 total colonies per M.F. In the present study, the choice of sample volumes filtered per M.F. from the three sampling stations warrants discussion.

On the basis of previous coliform densities obtained by the M.P.N. method, filtration of 100 ml. of sample from Station A was indicated. Though it was possible to pass as much as 600 ml. of water (average turbidity 8 P.P.M.) from Station A through a filter without its being clogged, volumes in excess of 50 ml. produced a mat-like overgrowth. By using five membranes and filtering five 20 ml. portions of sample instead of one 100 ml. portion, no overgrowth was noted and the total colony count of the five membranes was never in excess of 350 colonies.

It is postulated that certain types of extraneous matter on the M.F. surface serve as a "blotter" to hold fluid which encourages diffuse growth of organisms, thus preventing colony formation. It was noted that overgrowth by non-coliform organisms rarely occurred on filters having moderate to light depositions of extraneous matter. Granular extraneous matter, when unaccompanied by fine suspended silt like material, did not interfere with colony formation or serve as a bridge for coalescence.

Experiments employing various turbidity concentrations and various particle sizes and shapes would have to be performed before any definite relationship between turbidities and colony growth and formation could be established.

Bacterial densities in waters from Stations B and C showed such wide morning to afternoon and day to day fluctuations, that it was impossible to obtain desired results by employing only one volume of sample for filtration. Satisfactory membrane counts were obtained by filtering 20 ml., 10 ml., and 1 ml. portions from Station B and 1.0 ml. and 0.1 ml. portions from Station C. For increased accuracy, duplicate membranes were examined for each sample volume.

Though average turbidities of waters from Stations B and C were relatively high, 20 and 14 P.P.M. respectively, no clogged and few overgrown filters resulted even from the maximum volume of water filtered, i.e., 20 ml.

Results

Tables 1, 2 and 3 present data obtained from Stations A, B and C respectively.

Table 1 is self-explanatory. In Tables 2 and 3, the method of calculating M.F. counts per 100 ml. needs description. Valid M.F. counts from all volumes of a sample filtered were included in calculating M.F. coliform counts per 100 ml. Membrane counts were deemed valid if filters were free from evidence of overcrowding, overgrowth and excessive non-coliform organisms.

Calculations were made by the following method of computation:

$$\frac{\text{Total number coliform organisms}}{\text{Total volume sample filtered}} \times 100 = \text{number organisms per 100 ml.}$$

For example, in sample 9, Table 2, M.F. counts from all sample volumes filtered (duplicate 20, 10 and 1 ml. quantities) were valid. Totaling coliform counts and volumes of water filtered and applying the above equation we have:

$$\frac{75 \text{ organisms}}{62 \text{ ml. of sample}} \times 100 = 120 \text{ organisms per 100 ml. of sample.}$$

Table 4 gives the percentage of agreement between bacterial densities obtained from samples by the M.F. and M.P.N. methods. The 95 percent confidence range of the recorded M.P.N. (Tables 1,2,3) was used as a base line for comparison. (For a 5-tube three dilution test, the 95 percent confidence range is the difference between the lower and upper limits; namely, 0.3 to 2.9 times the calculated M.P.N.)⁽⁸⁾. The 95 percent confidence limits were obtained by multiplying the recorded M.P.N.'s by the factors 0.3 and 2.9. Those M.F. counts falling within these limits were considered in agreement.

The percentage agreement of results by the two methods was higher for waters of high coliform density than for waters of low and moderate coliform content.

Summary and conclusions

Coliform densities were obtained by simultaneous examination of sea water samples by the M.F. and M.P.N. methods. A comparison of the data so obtained revealed that the two techniques gave results 87.1 percent in agreement.

Results obtained by the M.F. and M.P.N. methods from waters having large coliform counts gave greater percentage agreement than results obtained from waters having a low coliform count.

Water turbidity can greatly influence the coliform recovery rate and should be considered in conjunction with the bacterial density in determining the volume of sample to be filtered.

The use of small replicate volumes of water is indicated where a single large volume from sources having low bacterial numbers produces overgrowth.

On the basis of results from the present study, it is concluded that the M.F. method is a reliable technique for determining the coliform densities of sea water if due regard is given water turbidities and bacterial densities in determining the volume of sample for filtration.

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TABLE 1.-- Coliform Densities Obtained by M.F. and M.P.N. Methods

Station A*			
Sample No.	Turbidity P.P.M.	M.F. Count per 100 ml. **	M.P.N. per 100 ml.
1	5	2	4.5
2	5	170	920
3	5	5	2
4	5	2	2
5	5	2	2
6	20	17	33
7	20	3	7.8
8	20	1	13
9	10	4	4
10	10	5	2
11	10	59	79
12	10	4	6.8
13	10	71	79
14	10	2	17
15	5	3	2
16	5	18	46
17	5	11	2
18	10	5	13
19	10	8	7.8
20	5	5	13
21	5	5	4.5
22	10	5	17
23	10	2	2
24	5	3	17
25	5	20	22
26	5	2	1.8
27	10	1	1.8
28	10	9	2
29	5	7	4.5
30	20	2	2
31	5	12	23
32	10	2	4.5
33	10	6	7.8
34	10	4	4.5
35	10	32	46
36	10	11	17
37	5	1	6.8
38	5	1	1.8
39	10	3	33
40	10	6	7.8
41	10	29	17
42	10	4	2
43	10	22	13
44	5	110	33
45	5	35	33
46	5	3	4.5

* 20 ml. filtered per membrane filter.

** Total count of 5 membrane filters.

TABLE 2.-- Coliform Densities Obtained by M.F. and M.P.N. Methods

Sample No.	Station B		
	Turbidity P.P.M.	Calculated M.F. Count per 100 ml.	M.P.N. per 100 ml.
1	5	50	49
2	5	180	490
3	10	290	700
4	10	270	170
5	10	150	220
6	10	480	790
7	10	110	490
8	10	40	220
9	30	120	230
10	30	80	170
11	30	890	2,400
12	20	470	700
13	30	840	3,500
14	50	910	2,400
15	20	360	790
16	50	1,200	5,400
17	10	280	1,100
18	20	340	170
19	5	730	1,700
20	20	160	490
21	20	2,800	7,900
22	20	2,100	6,400
23	20	2,000	4,900
24	20	12,000	11,000
25	5	310	490
26	20	120	230
27	20	500	490
28	30	650	640
29	30	2,000	2,300
30	30	600	1,100
31	50	3,100	4,900
32	30	1,100	790
33	50	4,000	4,900
34	10	560	790
35	10	250	460
36	20	2,500	1,400
37	20	1,000	3,500
38	10	650	4,300
39	20	2,500	4,900
40	20	1,100	2,300
41	20	2,700	11,000
42	10	2,000	1,300
43	20	440	950
44	10	1,500	1,300
45	5	500	330

TABLE 3. -- Coliform Densities Obtained by M.F. and M.P.N. Methods

Station C			
Sample No.	Turbidity P.P.M.	Calculated M.F. Count per 100 ml.	M.P.N. per 100 ml.
1	5	2,500	7,900
2	5	9,500	7,900
3	5	860	1,300
4	5	500	460
5	5	9,900	11,000
6	5	1,200	3,300
7	30	5,300	4,900
8	30	12,000	35,000
9	20	4,800	3,300
10	20	7,600	17,000
11	10	16,000	11,000
12	10	4,700	3,300
13	20	54,000	24,000
14	20	5,000	4,900
15	20	2,000	4,900
16	20	19,000	35,000
17	20	7,700	7,900
18	20	3,500	11,000
19	20	5,300	4,900
20	20	76,000	54,000
21	20	14,000	13,000
22	20	140,000	110,000
23	5	84,000	110,000
24	5	150	78
25	10	2,900	7,900
26	20	1,800	2,300
27	10	3,800	11,000
28	20	23,000	23,000
29	20	140,000	130,000
30	20	7,000	13,000
31	20	4,200	4,600
32	20	7,400	13,000
33	10	3,300	3,300
34	20	6,800	3,300
35	10	16,000	33,000
36	20	2,800	1,300
37	20	6,000	11,000
38	10	11,000	4,900
39	10	4,800	7,900
40	20	23,000	46,000
41	10	5,100	3,300
42	10	5,600	4,900
43	10	3,800	3,300
44	20	1,000	2,200
45	10	240,000	130,000
46	5	14,000	23,000
47	10	13,000	13,000
48	5	91,000	220,000

TABLE 4. -- Comparison of Coliform Densities Obtained by M.F. and M.P.N. Methods Using 95 percent Confidence Limit of Recorded M.P.N. as a Base Line

Stations A, B and C					
Station	Average Turbidity PPM	Number of Samples	Number of Samples Agreeing	Number of Samples Disagreeing	Percent Agreement
A	8	46	36	10	78.3
B	20	45	37	8	82.3
C	14	48	48	0	100
Totals	14	139	121	18	87.1

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