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CONVENTION SYMPOSIUM

on

PRODUCTION AND UTILIZATION OF SEED OYSTERS

The symposium was introduced by Dr. L. Eugene Cronin who also presided. Five biologists representing different major oyster-producing areas along the eastern and gulf coasts of the United States presented the following topics: Mr. Joseph B. Glancy, "The Supply of Seed Oysters in the New England-New York Area"; Dr. Harold H. Haskin, "The Seed Supply in Delaware Bay"; Dr. J. D. Andrews and Dr. J. L. McHugh, "A Critique on the Use of South Carolina Seed Oysters in Virginia Waters"; Dr. A. F. Chestnut, "Seed Problems in North Carolina"; and Dr. Philip Butler, "The Production and Utilization of Seed Oysters in the Gulf Area". Dr. Thurlow C. Nelson summarized the reports, including the papers by Mr. Glancy and Dr. Haskin which were not available for publication. The papers by Dr. Andrews and Dr. McHugh, Dr. Chestnut, and Dr. Butler, and the summary by Dr. Nelson are reproduced in the following section.

SYMPOSIUM ON THE PRODUCTION AND UTILIZATION OF SEED OYSTERS

L. Eugene Cronin

Chesapeake Biological Laboratory
Maryland Department of Research and Education
Solomons, Maryland

The production and utilization of seed oysters offer greater problems and greater challenges than any other aspect of oyster biology and the oyster industry. We have brought together speakers representing the oyster industry and the oyster biologists so that these problems and present opinions can be effectively summarized in one session. The panelists also represent every major oyster-producing area of the Atlantic and Gulf Coasts.

No aspect of oyster culture offers greater variety than the seed oyster problem. Some regions are faced with a grave and serious economic problem because of the shortage of seed oysters. In other regions seed oysters are so abundant as to interfere with growth and reduce the market quality of the crops. In between lie areas where seed production and utilization are more nearly in balance. All of these areas have grave and important problems which merit our concerted attention.

As a biologist, I am interested in the fundamental problems presented in the production and utilization of small oysters. We have begun to appreciate the differences between and similarities among seed produced at different sites and transplanted to new sites. There is an exciting opportunity for increased yield through improved use of various kinds of seed in different waters. We must understand the genetic differences between seed and the effects of various environmental situations on seed from different sources. This is one of the avenues by which we might achieve greater production, faster growth, and improved quality of oysters for specific uses.

The economic problems involved in various coastal areas are also varied and complex. They range from the relatively simple protection of natural sets on good growing areas to the possibilities of transplanting seed for many hundreds of miles along the coast.

Our panel members present reports from different coastal areas and these will be summarized and commented upon by Thurlow C. Nelson, Dean of American oyster biologists.

THE SURVIVAL AND GROWTH OF SOUTH CAROLINA SEED OYSTERS IN VIRGINIA WATERS[1]

Jay D. Andrews and J. L. McHugh

Virginia Fisheries Laboratory, Gloucester Point, Virginia

Introduction

Most of the seed oysters planted on private grounds along the Atlantic Coast of the United States are obtained from public seed beds. The supply depends largely upon a wild crop over which there is little control. It is to be expected, perhaps, that the quantity of seed available at various localities along the coast is in proportion to the duration of the warm season. It follows that oystermen are usually searching southward for their supply of seed and the ramifications of this hunt are complex and ever changing.

Between 1825 and 1880 millions of bushels of Virginia oysters were shipped north to oyster-growing areas from Delaware to New Hampshire (Goode 1887). In 1879, for example, two million bushels were exported from Maryland waters alone at a price of seven cents per bushel. Some 200 sail-powered "run boats" were engaged in the transfer of oysters from Chesapeake Bay to northern waters. The cost at the point of delivery was 25 to 35 cents a bushel. Most of these Chesapeake oysters were marketed immediately, but some were planted for use the following summer and fall. Evidently most were of marketable size when shipped north; the primary purpose of relaying was to hold them for sale in the succeeding summer and early fall when native oysters were spawning and poor in quality.

By 1880 northern dealers had established shucking plants in Norfolk and Baltimore, and thereafter shipments of oysters in the shell to northern ports declined. The search for southern oysters has never ceased, but now small seed oysters may be held in northern waters for several years before marketing, and few are taken north of New Jersey. Growing southern oysters for several years in northern waters is a far different task than holding large oysters through one summer season before marketing, for survival and growth become important as well as the ability to fatten.

As production of market oysters on private grounds increased in Virginia, the home market absorbed most of the supply of seed, and as recently as ten years ago less than 10 per cent of James River seed was sold out of State. Today the sale of seed oysters from public grounds of the James River for direct transport out of State is forbidden, and northern growers have turned to private grounds and the seaside of Eastern Shore for their supply.

[1] Contributions from the Virginia Fisheries Laboratory No. 73.

These limitations on the export of seed were necessary under the present organization of the industry in Virginia, for the amount of ground under lease has been increasing, the demand within the State has been great, and in the last few years the price has steadily increased. Despite the ban on direct shipments out of State, the annual catch of seed oysters from the James River has increased. Potential seed areas on public grounds in other rivers have not been utilized and seed production on private grounds has been slow to develop. Prior to 1947 considerable quantities of Pamlico Sound seed oysters were used in Chesapeake Bay and particularly on the seaside of Eastern Shore. This practice ceased when the state of North Carolina placed on oysters an export tax of 50 cents per bushel (Chestnut 1949). Until recently shipment of seed from South Carolina has been virtually barred by various laws of that state, but now that regulations have been revised and South Carolina is ready to encourage production of seed for northern planters (Wallace 1956).

In South Carolina most oysters are grown in the intertidal zone and the beds are characterized by heavy sets. Planters are intrigued by the high count per bushel but they recognize that consequent crowding may produce inferior shucking stock. It is not clear, moreover, whether oysters from the high-salinity waters of South Carolina can be transplanted successfully to the much less saline waters of upper Chesapeake Bay. In addition to these problems, scientists have been concerned about the growth and survival characteristics of southern oysters. Little attention was paid to quality and fitness of stock in the early days of extensive transplantation along the coast, and control of pests and diseases was given no consideration. It might be surmised that whatever damage could be done by mixing stocks and transplanting pests has already occurred, but recent troubles with the fungus Dermocystidium in Delaware Bay, and the possibility that the fungus may have been introduced in Chesapeake Bay some years earlier, suggest that unrestricted transplanting may yet be unwise.

The Chesapeake Biological Laboratory at Solomons, Maryland, began studying the characteristics of out-of-state seed grown in Chesapeake Bay a number of years ago (Beaven 1949); in 1951, in cooperation with the Bears Bluff Laboratory and the Maryland laboratory, studies of South Carolina seed oysters were begun in Virginia. Small numbers of these oysters have been held in trays for growth and mortality observations and upon these experiments is based a preliminary estimate of the usefulness of South Carolina seed in Chesapeake Bay.

We have attempted to compare the growth, survival, and fattening qualities of native and South Carolina oysters. We have assumed that the intensity and duration of setting in South Carolina waters will necessitate the removal of seed oysters at an early age--probably less than nine months. To hold stock longer in South Carolina produces a very dense cluster of oysters which can scarcely be separated a year later. In our experiments South Carolina and native spat of the same age were placed in trays when one to three months old and grown side by side. Data were obtained on oysters of three different year-classes

from the two sources. The history of each group is given in Table 1.

Patterns of Mortality

The pattern of mortality of native Chesapeake Bay oysters has been described by Hewatt and Andrews (1954). The death rate is high during warm periods (June to October) and extremely low during the winter and spring. Sporadic departures from this usual pattern, caused by mortalities from unknown causes, occur in some areas (Beaven 1946). In Figure 1 the pattern for native oysters is depicted over a period of three years (Trays 11 & 12). Figure 1 and Table 2 reveal also that in the warm period the mortality of South Carolina oysters (Trays 4 & 38) often is little more than half as great as that of natives. Andrews and Hewatt (1957) have shown that South Carolina oysters are more resistant to the fungus, Dermocystidium marinum, which is the cause of most summer deaths in trays. During winter and spring, however, the death rate in South Carolina oysters is appreciably higher than that of natives. In the warm winters of 1952-53 and 1953-54, these losses were relatively inconspicuous, but when winters were cold, as in 1954-55 and 1955-56, deaths were frequent in February and March and again in May and June (Fig. 1). The causes of these deaths in later winter and again in late spring are unknown. When organisms are transplanted to colder climates, minimal temperatures are often limiting, but oysters grown intertidally in South Carolina usually are exposed to lower temperatures and greater extremes than those held subtidally in trays at Gloucester Point. It appears that susceptibility to winter mortalities involves other factors in addition to low temperatures--perhaps diseases, favored by cold waters, to which South Carolina oysters are more susceptible than natives. The winter survival of South Carolina oysters in their native waters is unknown.

For convenience in computing biomass, it is best to express mortality in terms of survivors, as in Figure 2. Mortality and growth records were not collected in the first year because weights and counts of spat were difficult to obtain. For convenience, also, survivorship was computed on the basis of an original stock of 1000 oysters in each lot. Death rates for each period between observations were applied to the number of survivors at the beginning of the period. From Figure 2 the number or percentage of survivors at any age in months can be determined.

South Carolina oysters (closed circles) had less seasonal variation in death rate, hence the survivorship curve declines rather steadily, but the curves for native oysters (open circles) show steep declines in summer and almost no drop in winter. These curves include the unusual year of 1954 when over half the native oysters, but only one-fourth of the South Carolina oysters died. The South Carolina oysters had a distinct advantage in survival during this warm year.

Table 1. History of Virginia and South Carolina oysters grown in trays at Gloucester Point

Year of birth	Tray number	Origin	Date transplanted
1951	4	South Carolina	July 1951
	11	James River	Nov. 1951
	12	Corrotoman River	Nov. 1951
1952	28	South Carolina	Nov. 1952
	27	York River	Aug. 1952
1953	38	South Carolina	Nov. 1953
	39	Chincoteague	Nov. 1953
	40	York River	Aug. 1953

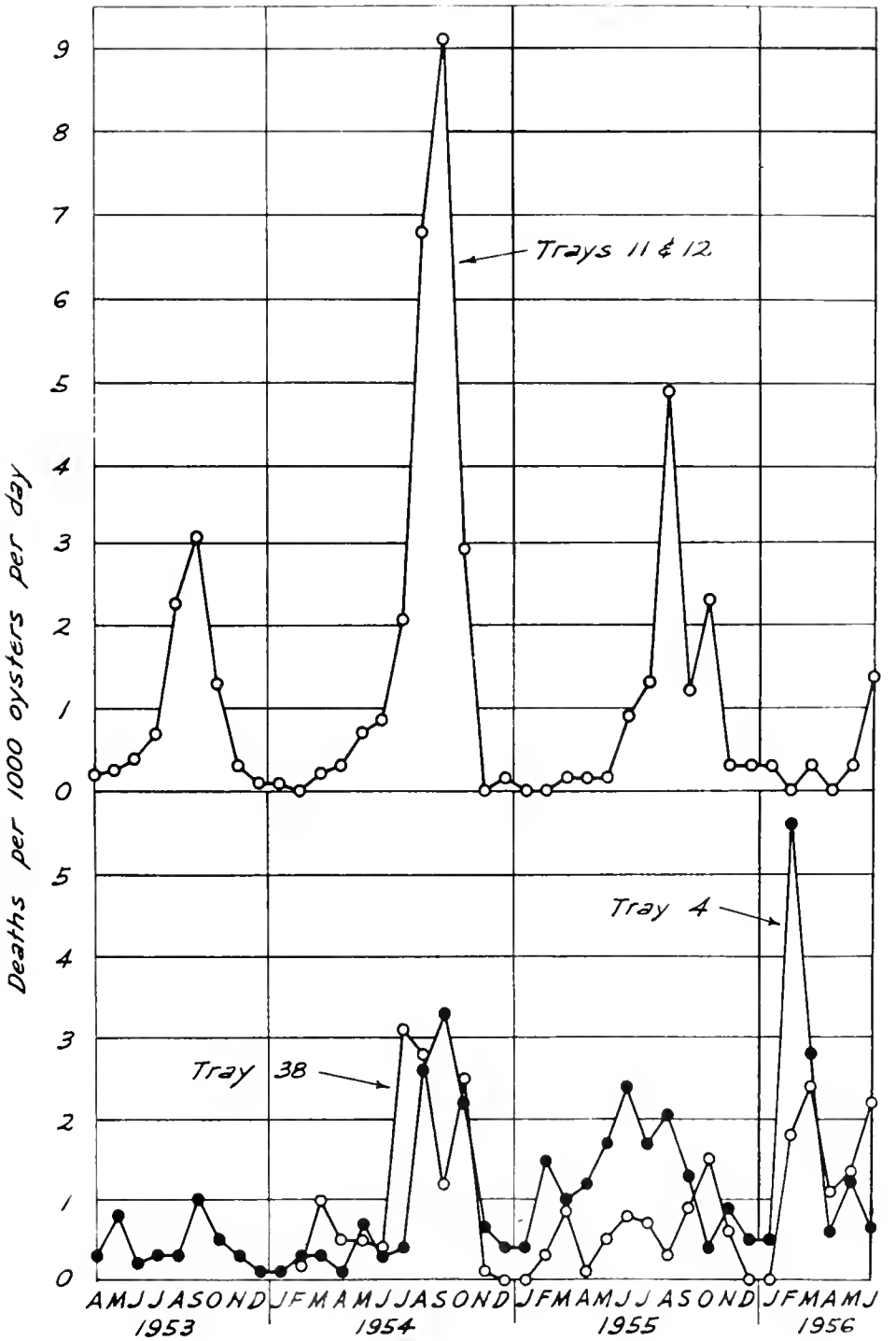

Fig. 1. Patterns of mortality in oysters from Virginia (Trays 11 and 12) and South Carolina (Trays 4 and 38). Mortality for each month is expressed as the average number of deaths per 1000 oysters per day.

Table 2. Mortalities of oysters in trays in the warm and cold seasons at Gloucester Point, Virginia

Year	Tray number	Source	Mortality in per cent		
			Warm months (June to Oct.)	Cold months (Nov. to May)	Annual (June to May)
1951	4	South Carolina	9	16	24
1952	4	South Carolina	7	6	13
	11	James River	4	4	8
	12	Corrotoman River	3	0	3
1953	4	South Carolina	10	6	15
	11	James River	24	5	28
	12	Corrotoman River	17	6	22
1954	4	South Carolina	24	19	39
	11	James River	57	1	57
	12	Corrotoman River	51	4	53
1955	4	South Carolina	22	31	46
	11	James River	26	8	32
	12	Corrotoman River	30	2	32
1956	4	South Carolina	25	..	..
	11	James River	16	..	..
	12	Corrotoman River	25	..	..

In years in which average winter and summer temperatures are nearly normal, it appears that losses in South Carolina and native oysters may be about equal. Although summer losses are less in South Carolina oysters, winter deaths are more serious than in natives. The designation of "warm" and "cold" winters is difficult, but after 1948 Virginia had six consecutive warm winters during which the three winter months rarely had average temperatures below normal. In each of the past two winters (1954-55 & 1955-56), two of the three winter months had average temperatures well below normal and these were by far the coldest winters since 1948. During this experiment (1952 to 1956), two quite warm and two rather cold winters were experienced. It appears that warm winters and warm summers (1952-53 & 1953-54) favor the survival of South Carolina oysters, but cold winters (1954-55 & 1955-56) and cool summers (1956 permit greater survival of natives (Table 2).

Apparently South Carolina oysters are not immune to winter mortalities at any age, whereas all oysters reach two years of age before summer losses from Dermocystidium become heavy. In low-salinity waters, where no deaths occur from the fungus at any age, South Carolina oysters may suffer high winter losses (Beaven 1953). In the lower bay, therefore, South Carolina oysters appear to have no advantage over natives in ability to survive and in the upper bay they may be quite inferior.

Growth

The growth of oysters, expressed as weight in the shell after cleaning, shows small differences between Virginia and South Carolina oysters of the same year-class but large variations among year-classes (Fig. 3). In other words, environmental differences apparently caused greater variation in growth than genetic differences between native and South Carolina oysters. The oysters of the 1951 year-class (Trays 4, 11, & 12) grew faster than those of the two succeeding year-classes. At the end of 24, 36, and 48 months of age they were 40 to 45 per cent heavier than the 1952 year class at the same age (Trays 27 & 28). In two of the three year-classes, South Carolina oysters were heavier than natives at the beginning of the experiment, but soon the natives exceeded them in weight. There is some indication that South Carolina oysters may never reach a size as large as natives. Marketable oysters of three to three and one-half inches weigh from 60 to 90 grams.

Yields

In these experiments the yield of oysters is the resultant of losses from deaths and gains from growth. In the computation, average weight is multiplied by number of survivors; this is less complex than the method used by McHugh and Andrews (1955). To facilitate comparison of groups, the biomass or total weight has been converted to relative biomass or yield based upon an initial weight of 19 grams per oyster.

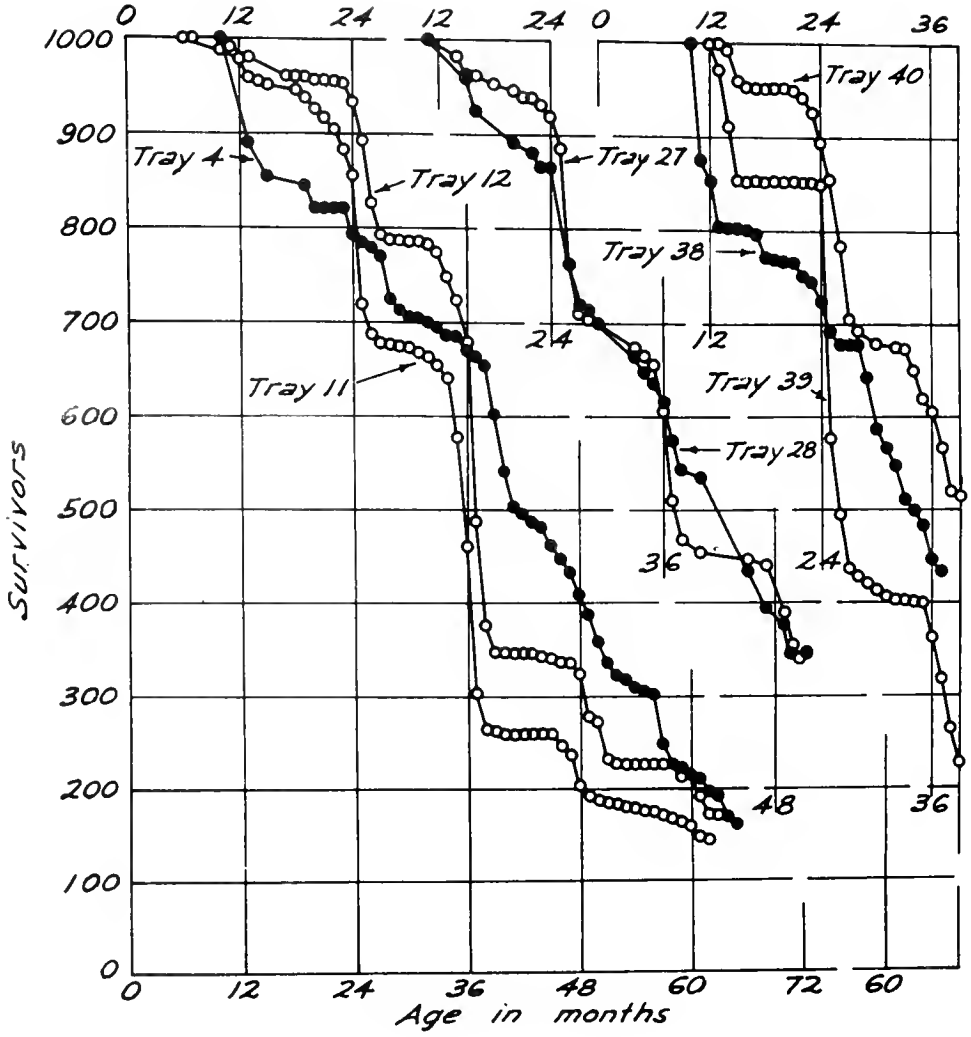

Fig. 2. Numbers of survivors from initial lots of 1000 oysters; calculations were based upon the death rates of oysters suspended in trays from the Virginia Fisheries Laboratory pier. The 1951 year-class is represented by Trays 11 and 12 from Virginia and Tray 4 from South Carolina; the 1952 year-class by Trays 27 (Virginia) and 28 (South Carolina). Tray 39 contained oysters from the seaside of the Eastern Shore of Virginia. Native oysters are represented by open circles and South Carolina by closed circles.

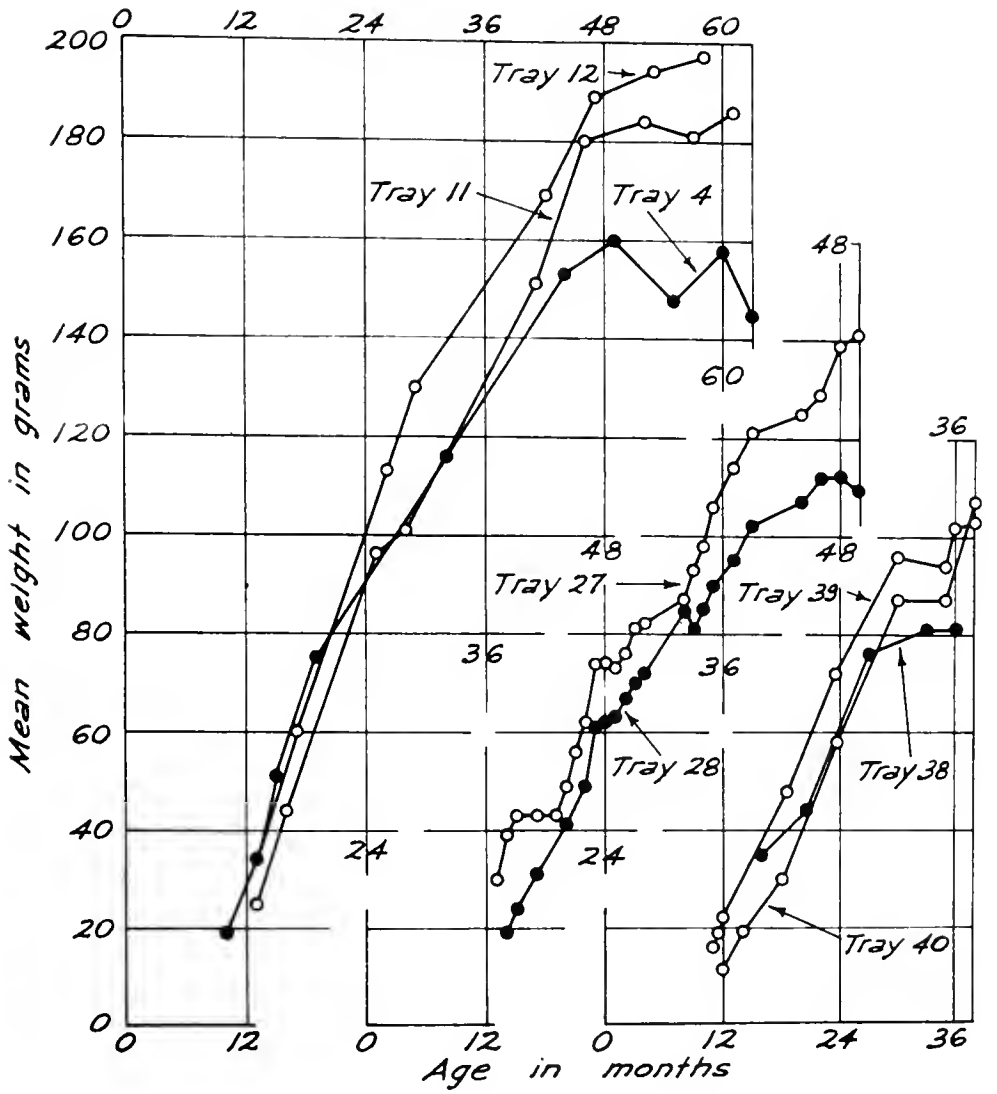

Fig. 3. Mean growth rate in total weight, including shell, of oysters from Virginia and South Carolina. The 1951 year-class is represented by Trays 11 and 12 from Virginia and Tray 4 from South Carolina; the 1952 year-class by Trays 27 (Virginia) and 28 (South Carolina); and the 1953 year-class by Trays 40 (Virginia) and 38 (South Carolina). Tray 39 contained oysters from the seaside of the Eastern Shore of Virginia. Open and closed circles represent native and South Carolina oysters respectively.

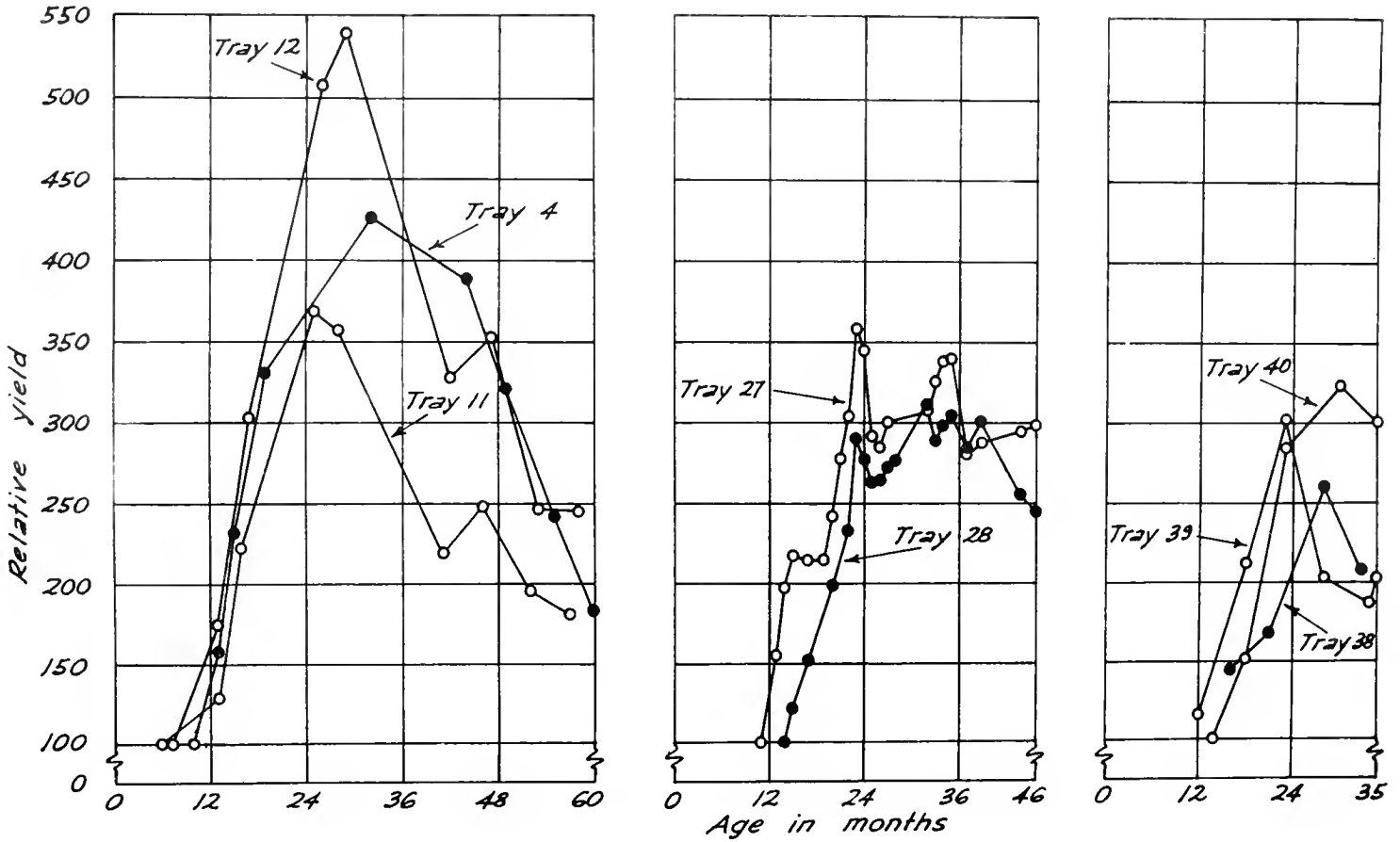

Fig. 4. Relative yield (biomass) of oysters from Virginia and South Carolina. The 1951 year-class is represented by Trays 11 and 12 from Virginia and Tray 4 from South Carolina; the 1952 year-class by Trays 27 (Virginia) and 28 (South Carolina); the 1953 year-class by Trays 40 (Virginia) and 38 (South Carolina). Tray 39 contained oysters from the seaside of the Eastern Shore of Virginia. Native oysters are represented by open circles and South Carolina by closed circles.

This was the approximate size at which each group of spat was separated from the cultch and weighed. Although actual weights varied, the month when each group reached an average weight of 19 grams was determined from the known weight-length relationship (McHugh and Andrews 1955). All points in Figure 2, however, are based on actual weights. A value of 100 was assigned to the initial biomass of 19,000 grams (1,000 oysters at 19 grams each). Yields are expressed as a percentage of the initial biomass, and at any age they can be read from the graph in any unit of weight or volume desired.

In all groups relative biomass increased rapidly during the first two years when growth was rapid and death rates low, and maximum yield was obtained in 24 to 30 months after setting (Fig. 4). In Trays 11 and 12 biomass declined rapidly thereafter for this was the period of excessive death rate in the summer and fall of 1954. Although there were rather wide differences in relative biomass of the two groups of native oysters of the 1951 year-class, the pattern was very similar. The decline in biomass was precipitous in the late summer and fall but tended to rise in spring when few deaths were occurring. If there had been a measurement at 34 months (late spring of 1954), biomass would undoubtedly have increased as it did in the spring of 1955 (41 to 46 months). In the spring of 1956 (53 to 58 months), these oysters were nearly five years old and growth had declined. The curve for South Carolina oysters (Tray 4) exhibited a distinctive pattern in which the inflections were less abrupt because the rate of survival was less variable. The sharpest declines in these oysters came in winter and spring when growth was slow and mortalities fairly high.

In Trays 27 and 28 (Fig. 4) the patterns were similar to those in the 1951 year-class but biomass was maintained near maximum levels longer because survival in 1955 was comparatively high. These groups never attained the maximum biomass of the 1951 groups because excessive mortalities in 1954 depleted the ranks early. It will be noted again that seasonal fluctuations in biomass are not as drastic in South Carolina oysters (Tray 28) as in natives (Tray 27).

Again, in oysters of the 1953 year-class (Trays 38, 39, and 40) biomass did not reach the level achieved by the 1951 groups (Fig. 4). In this latest year-class native oysters (Tray 40) had a distinct advantage over imported oysters; susceptibility to the fungus D. marinum caused high losses (48 per cent) in Chincoteague oysters (Tray 39) in the summer and fall of 1955 and many deaths occurred in the South Carolina oysters (Tray 38) in the winter and spring of 1956. Figure 4 clearly illustrates that these losses altered the biomass curve in Trays 38 and 39, and these oysters produced much lower yields at marketable sizes.

Yields of three, four, or five to one may not seem realistic to oystermen. It must be remembered that oysters grown in trays are protected from injury, smothering, drill predation, and other agents of attrition which operate on natural grounds; these are factors which

Table 3. The condition index in South Carolina and native oysters held in trays at Gloucester Point, Virginia[1]

Date	Source	Tray number	Mean length mm	Condition index
1 June 1955	York River	27	91	11.0
	South Carolina	28	84	12.5
10 Sept. 1955	James River	11	100	9.0
	South Carolina	4	106	7.1
4 May 1956	York River	27	93	7.8
	South Carolina	28	81	6.5
25 June 1956	York River	27	97	11.7
	South Carolina	28	96	9.2

[1] These determinations were made by Dexter S. Haven.

cause early losses in planted oysters when tray losses are negligible. The yield on natural grounds, consequently, never attains the level found in tray oysters; to achieve high yields, gains from growth must greatly exceed losses from deaths.

In yields, as in growth and mortality, South Carolina oysters appear to be at a disadvantage when compared with natives, although they may retain their peak biomass for a slightly longer time. In years of low temperatures South Carolina oysters do not attain the biomass of natives.

Condition

A preliminary attempt has been made to compare the condition index (Higgins 1937) or "fatness" of South Carolina and native oysters. In three of four samples natives had higher indices of condition than South Carolina oysters (Table 3). Samples have not been taken in the fall and winter when most oysters are marketed. Seasonal and annual fluctuations in condition factor have been so great from river to river that data must be collected for several years before any firm conclusions on condition index can be reached.

Discussion of Other Factors

The importance of several other characteristics of South Carolina oysters, when grown in Chesapeake Bay, has not been determined. These oysters are relatively more elongate than natives and the shell appears to be thinner. We have encountered more difficulty with breakage of shells in shucking South Carolina oysters, although it is not clear whether this is caused by a heavier infection of boring sponge or by thinner shells. The cupped valves have a deeper cavity in South Carolina oysters than in natives, and they are usually cucullated, that is, the cavity extends under the hinge. A few measurements indicate that the capacity of the shell cavity is greater than in natives for a given weight or size of oyster. The upper valve in South Carolina oysters lies on the cupped valve like a flat lid whereas in natives it contributes to the shell cavity.

Summary

Most oystermen and biologists recognize that native oysters are the most satisfactory seed for planting in a given area. Although the demand for seed in Virginia presently exceeds the supply, there is no reason why this situation should continue to exist, for the proper utilization of suitable public grounds such as the Corrotoman and Piankatank Rivers, and greater attention to the production of seed oysters on private grounds, should be adequate to supply all planters within the state.

If these obvious sources of local seed are not exploited, however, planters will continue to look elsewhere for a supply. The recent relaxation of laws in South Carolina already has aroused interest among Chesapeake planters. In comparison with native Chesapeake Bay oysters, South Carolina seed is definitely superior in resistance to the fungus, almost equal in growth, but usually inferior in rate of survival during the cold season. Planters who desire to experiment further with these seed oysters should consider the interaction of the various biological factors with the economic and fiscal problems associated with their import from South Carolina.

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THE SEED OYSTER PROBLEMS OF NORTH CAROLINA

A. F. Chestnut

Institute of Fisheries Research
University of North Carolina
Morehead City, North Carolina

Abstract

The greatest problem regarding seed oysters in North Carolina is probably the lack of utilization of available seed. Twenty-five years ago considerable quantities of seed were exported to the Chesapeake Bay area. An export tax of 50 cents per bushel on all oysters shipped out of the state in the shell was imposed in 1947 and curtailed further shipments.

Small quantities of seed oysters are transplanted from natural areas to privately-leased beds. The total leased acreage is less than 4,500 acres so the amount of seed oysters utilized is small. During the past three seasons plantings by the Division of Commercial Fisheries has increased almost five fold. In recent years the first plantings of any substantial quantity were made in 1954 when 42,550 bushels of oysters were transplanted from natural rocks to selected public areas. The total plantings in 1956 were 205,804 bushels. A program is under consideration to continue plantings each year.

The seed-oyster areas in the coastal waters are located in two ecologically different environments. Heavy concentrations of clustered oysters are found near the inlets growing in waters with salinities above 30 °/oo and are generally limited to the intertidal zone. Some of these oysters are transplanted successfully to subtidal grounds in areas of lower salinity where they grow to marketable size. In the Pamlico Sound area most seed oysters are located in waters with salinities below 10 °/oo and oysters are growing at various depths up to 20 feet. During years of excessive rainfall these oysters suffer heavy mortalities.

Oyster sets of high intensity frequently occur in many natural areas resulting in a high production of seed oysters. In some years continuous setting occurs from May through September producing large clusters of oysters in areas which are not worked for commercial harvesting.

PRODUCTION AND UTILIZATION OF SEED OYSTERS IN THE GULF AREA

Philip A. Butler

U. S. Fish and Wildlife Service Laboratory
Pensacola, Florida

The variety of environmental conditions along the extensive Gulf Coast imposes a great diversity of techniques in oyster culture, making it difficult for one person to have available current information on the entire industry. For this reason, I have called upon marine biologists in each of the states for assistance in assembling some of the data essential to our discussion. I should like to acknowledge their helpfulness and cooperation in providing information; the interpretation placed upon these data and any errors in statement are my own.[1]

In order to simplify organization of the information, I submitted a questionnaire to these gentlemen. This material can be covered most efficiently by itemizing some of the questions and replies. It is essential that we have a clear understanding of the term "seed oyster." While most biologists attach a definite meaning to the term, among oystermen, especially from different geographic areas, you will find that the concept of a "seed oyster" undergoes considerable change.

The first question, then, was "What does the term 'seed oyster' mean to oystermen in your state?" The replies were briefly as follows: no precise meaning, oysters to be replanted, any oyster under legal size, and any oyster not legally subject to harvest. Perhaps some of these definitions are broader than what your own local industry understands by the term. For the purposes of this discussion I am using the term "seed oyster" to include any oysters of less than legal size which would be transplanted primarily for the purpose of growing to a larger size. We are not concerned here with market-size oysters that frequently are replanted for conditioning, to await a more favorable market, or to cleanse them of pollution.

The next question asked was "Does your state provide for restricted or closed areas for the production of seed oysters?" We learn that in the approximately 3,500 miles of Gulf coast line there are about 350,000 acres of water bottom set aside for seed culture, all in the state of Louisiana. This does not imply that all, or even the majority, of this area is in production at any one time. We asked also if there are any public reefs or polluted areas where larger oysters are harvested primarily for transplanting to growing grounds. Two states answered in the affirmative; in the past biennium Florida had a special situation in which oysters

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[1] Robert M. Ingle, Assistant Director, Florida State Board of Conservation; Harold Loesch, Marine Biologist, Alabama Conservation Department; Gordon Gunter, Director, Gulf Coast Research Laboratory; Lyle St. Amant, Chief Biologist, Louisiana Wildlife Fisheries Commission; Robert Hofstetter, Marine Biologist, Texas Game and Fish Commission.

from a polluted reef were transplanted to a new growing area; and in Alabama, there was some production from deep-water reefs of older mixed oysters which were replanted on tonging reefs or on private growing grounds.

The question must arise in your minds that if this is the extent of seed oyster culture in the Gulf, is there no demand for additional seed. In answer to this question, the reply from three of the Gulf states was they have no significant demand. In Alabama, a few private planters could utilize additional amounts of seed oysters, and in Louisiana there is some demand, but it is almost satisfied by the state seed oyster program. In none of the states is there any import or export of seed oysters for transplanting purposes and none of the biologists felt that the oyster industry in his state provides a potential market for out-of-state seed.

The question must also arise in your mind then, as to just what the Gulf oyster industry does rely upon for its source of oysters ---certainly not exclusively on the natural reef community? The answer is found in the replies to our question "Has your state planted any cultch?" I will not give any of the figures, but all of the states do plant shell; although in Texas it has recently been only on an experimental basis. It is significant that the size of the oyster harvest in the various states corresponds roughly to the extent of the shell planting program.

This brief survey of the situation points out fundamental differences in the oyster industries of the Gulf and the North Atlantic states. Although a shell planting program is undertaken in both areas, transplanted seed oysters are the mainstay of the business in the northern waters.

Oyster communities in the Gulf of Mexico are still on a more nearly self-perpetuating basis than those in the depleted areas along the Atlantic Coast. But this is perhaps merely an illusion and we are actually witnessing the slow and progressive deterioration of an overharvested resource which eventually will require drastic help if it is to remain productive. It is remarkable that after a hundred or more years of commercial harvesting, the Gulf oyster community can still be productive with only the moderate and erratic assistance of additional cultch.

It is reasonable to predict that in the future man is going to encroach more and more on the environments suitable for oyster culture. Depletion will increase and the present bountiful spatfall will be decimated if remedial measures are not undertaken. Then, the only way the industry will be able to survive will be by the use of transplanted seed oysters as in New England now.

This is not intended as a gloomy prediction, it seems to me to be an obvious conclusion to be derived from the history of the oyster

industry in other regions both in the United States and abroad. We should profit by this knowledge of history and prepare now for the establishment of a permanent and controlled seed oyster program.

Since the need for seed oysters in the Gulf area is negligible now, we might inquire whether or not this superabundance of natural set can be of help to the northern industry in its dire need for seed. The answer is probably no, at least in the immediate future. There are many contributary reasons for this opinion among which I list the following: no state harvests at present an exportable surplus and only one state has an established seed oyster program; there is too much evidence that some oyster parasites are endemic in the Gulf area; the great density of fouling organisms in good setting areas will make production of a clean exportable product extremely difficult; and finally, we still have no evidence that Gulf oysters can flourish in any other geographical area where there is a seed shortage.

We should also inquire whether the oyster industry in the Gulf area can help itself by stimulating a widespread seed oyster program. The answer to this is a very definite yes---despite the fact that the demand for seed oysters is still negligible. Much of the uncertainty in the oyster industry here today stems from the unpredictable fluctuations in the annual harvest, which are due, in turn, to the failure of the natural set in particular areas. Too much of the industry is dependent on a single source for its oysters; individual shucking houses may obtain almost their entire harvest from a single reef. Creation of a seed oyster industry and an intensification of programs already started could do much to stabilize annual production and bolster the economy of the industry. Development of seed supplies in different regions would make it possible to compensate for local failures in the crop. This is practiced now to some extent in Louisiana.

Uncertainty of the labor supply is a second factor contributing to the weakness of the oyster industry in some areas. More and more workers, recognizing the importance of the guaranteed annual wage are reluctant to enter a business that is both seasonal and erratic.

There are many places where the establishment of a seed oyster program would not only increase the annual yield, but also would put many more acres of water bottoms into useful production and create steady work for the local labor force. Stabilizing production and establishing year-round cultural techniques would go a long way in solving this problem of an unpredictable labor force.

In Alabama, for example, there is an opportunity to initiate a seed oyster program which has manifest possibilities. Old oyster reefs in the upper part of Mobile Bay are only intermittently productive now because of the severity of spring freshets. It would be possible to plant cultch on these bottoms in early summer and harvest a crop of seed oysters in the late winter months. The heavy spatfall and early rapid growth in this area could produce a large crop of good-size seed in about

nine months time, and the oysters could be transplanted before there was any mortality from fresh water. Such a seed oyster program could utilize submarginal grounds in many areas along the coast to real advantage.

I should like to conclude this discussion by pointing out the ever-increasing importance of the state research laboratories to a flourishing oyster industry. It seems to me that now, before depletion becomes critical, additional research funds should be made available. Biologists could be locating the most advantageous setting areas, building them up, learning their good and bad points. They could be experimenting with transplanting seed into different areas along the Gulf coast to learn what seed survives best and where. They could be finding out how to improve the stock, and perhaps selecting strains which are more resistant to the various diseases that may be present here. The importance of a progressive seed oyster program on the part of both the laboratory and the industry can not be overemphasized.

SYMPOSIUM ON THE PRODUCTION AND UTILIZATION OF SEED OYSTERS

SUMMARY

Thurlow C. Nelson, Biologist

New Jersey Division of Shell Fisheries

For the first time in the history of our Association we have in this symposium a comprehensive view of the state of the oyster industry from Long Island Sound to the Gulf of Mexico. Much of what we learned is discouraging, but as in the solution of all problems the essential and first step is to establish the facts. Once these have been ascertained it should be possible to bring to bear whatever of our already substantial knowledge of the oyster may be helpful, and to determine wherein further investigation is essential. It is ironical that the 67 years since peak oyster production in 1890, during which we have witnessed its steady decline to the lowest point in history, also embrace the period in which we have learned more about the oyster scientifically than in all previous years. Although the oyster is now scientifically the best known marine animal in the world, we must agree with Dr. Coker (1956) of the University of North Carolina who recently concluded: "The oyster still calls for research."

From the great beds of shells of the fossil oysters, Exogyra and Gryphaea, the largest and most heavily shelled oysters the world has yet produced, we learn how nature alone with no exploitation by man some 60 million years ago wiped out these magnificent bivalves. Evidence points strongly to increasing deposits eroded from the land during rapid uplift of coastal areas during the Cretaceous period (Nelson 1938). So we can well appreciate the figures given by Joe Glancy for oyster production decline in the New England-New York area during the decade 1945-1955 from 5,045,000 to 1,354,000 pounds of meats. Had he chosen the latter half of this decade the figures would have been even more startling, for they would have begun with the very destructive hurricane of November 25, 1950, during which some seed storage beds in Long Island Sound lost three-quarters of their stock. Gusts up to 90 miles an hour created at a depth of 50 feet currents of sufficient magnitude to transport part of the seed two and one-half miles while burying the remainder. The final punch, delivered by hurricanes Connie and Dianne in 1955, caused such heavy losses among market oysters as to cause closing down of one of the oldest and finest oyster companies of the world, H. C. Rowe and Company of Connecticut. While deeply lamenting the demise of this outstanding company, it is heartening news from the climatologists that we have now passed the peak of hurricane probability in the northeastern area, a prediction fully substantiated in 1956.

Two other hazards, however, still face the industry of this area in addition to the ever-present sea stars. Increased industrial activity in the Bridgeport and New Haven areas is sending a greater load of

industrial wastes into some of the better seed-producing regions. It is hoped that increasing interest in recreational use of our coastal waters, which has grown by leaps and bounds in recent years, will assist in maintaining the necessary freedom from toxic wastes to permit local seed production. Recreation and the oyster industry have much in common. Mr. Glancy held out no hope of supplementing insufficient seed supplies of this area with oysters from further south even from an area as close as Delaware Bay. Dr. L. A. Stauber (1950) of Rutgers University showed that the oyster from Delaware Bay southward is a southern variety which must have water temperatures of 77° or above to spawn. Transplanted further north they fail to spawn or to grow after one or two years.

The second hazard, so important with failing spatfalls, is the oyster drill, Urosalpinx. The big question before the oyster grower is whether he can afford to add to the already peak price of oysters the extra expense of keeping drill populations in control through use of the suction dredge or by other methods. Of equal importance: can he afford not to fight the drill? We wish the staff of Milford Laboratory and any others working on the problem full speed ahead and all the luck in the world in finding a satisfactory method of chemical control. Meanwhile as full use as possible should be made of such methods of drill eradication as are available (Carriker 1955). Recent reports of success in burying drills through turning over the bottom are encouraging, although there are many valuable oyster grounds which have been developed only through shelling of soft mud incapable of holding oysters where such methods would destroy the surface. Of equal importance is the possible harmful effect of turning over the bottom on the supply of food on the surface. Fundamental studies by the Danes (Petersen & Jensen 1911) led them to the conclusion that the nutrition of bottom marine animals was intimately bound up with the brownish film a half inch thick covering the surface.

Delaware Bay though spared the overwhelming hurricane losses visited upon Long Island Sound is now faced with the most serious lack of seed of all time. Dr. Harold H. Haskin, Biologist in Charge of the New Jersey Oyster Research Laboratory, traced the history of the natural seed beds of this area. Fifteen years ago their yield was comparable to that from the beds of the James River, Virginia, probably the foremost natural oyster seed producing region of the entire world. At that time New Jersey beds yielded 1,000 to 2,000 spat per bushel, comparable to the spatfalls in James River. From 1936 to the late 1940's there were always at least 50 per cent oysters as contrasted with shells on the Delaware Bay beds. Spatfall in 1936 was 5,000 per bushel, while in 1949 occurred a maximum set at Beadon's Point bed with 10,000 per bushel. Since 1950 spatfall has been a failure.

With shift from sail to power in the mid 1940's small shallow-draft boats for the first time were able to dredge the inshore spawning beds which had for many years served as a great spawning sanctuary. In an effort to halt the declining production Shell Rock Bed was closed in 1953 for a three year period. In spite of dire predictions of fouling

and the necessity for "working the bed", sets there were as good as on the nearby dredged areas. Evidence was obtained that approximately 50 per cent of each season's set was being killed by spring-dredging operations. During the three-year closure Shell Rock ratio of oysters to shell rose from 63 to 89 per cent. Also, in spite of tradition against the heavy sets of the Cape May shore some 43,000 bushels of this was moved to Shell Rock where it showed excellent survival, reaching market size in three years. On the basis of observations, recommendation from the staff of the Laboratory to the State officials was to restrict dredging during the 1956 season to 150,000 bushels with Shell Rock opened. Actually, however, over half a million bushels were removed, and when the beds were closed after three weeks Shell Rock showed a fall in oysters from 85 to 18 per cent; Cohansey-Ship John, 33 to 12; Middle, 64 to 14; and Bennies, formerly the best of all the beds, from 30 to 6 per cent. The spawning stock present on these beds has been overestimated by at least four times. To get back to the full production of 15 years ago beds would have to be closed to dredging for 15 to 20 years with question whether the most seriously depleted beds could ever recover.

For the Chesapeake Bay area, Dr. J. D. Andrews presented a report by Dr. J. L. McHugh and himself on the survival and growth of South Carolina seed oysters in Virginia waters. Tracing the history of transplantation of southern oysters to northern waters since 1825 it appears that originally oysters of market size were purchased for relaying and prompt sale in nearby markets. As demand arose for seed the needs of Virginia planters for the seed crop of the James River resulted in banning its direct export out of the State. Substantial quantities of James River natural seed planted first on Virginia grounds, however, have been purchased by growers in northern waters, notably in Delaware Bay. Since the annual catch of seed in the James River is not sufficient even to supply all local needs, Virginia welcomed recent lifting of the ban on export of seed from South Carolina. Anticipating increased demand for such seed the Chesapeake Biological Laboratory at Solomons, Maryland, and the Virginia Fisheries Laboratory in cooperation with the Bears Bluff Laboratory, South Carolina, have been studying comparative growth and mortality of South Carolina seed and native stock in Chesapeake Bay.

These studies reveal the great value to the industry of the Atlantic Seaboard of the discovery by Dr. J. G. Mackin (1951) of Texas A. & M. College of the marine fungus Dermocystidium and the extensive mortality caused by it in waters of the Gulf. No better demonstration of the importance to the oyster industry of fundamental scientific research can be found than those brilliant investigations of Dr. Mackin, of his associates, and others (Ray 1954). The Virginia Fisheries Laboratory promptly put these findings to practical use and soon delineated the areas of infection by this fungus. Within the past two years the visiting Danish microbiologist, Mrs. Greta Christensen, working at the New Jersey Oyster Research Laboratory, found foci of infection in numerous restricted areas in Delaware Bay. In every instance it was determined that Chesapeake Bay oysters had been transplanted to these beds.

The Virginia Fisheries Laboratory kindly offered to cooperate with the New Jersey authorities and with the laboratory at Bivalve to the end that only uninfected oysters would be brought in. As the New Jersey Department of Agriculture now lays down embargoes against importation of farm crops and animals from areas of dangerous infection, so it was proposed that shellfish officials take similar moves to limit spread of this infection in Delaware Bay. Unfortunately, no advantage was taken of this opportunity hence New Jersey growers especially in unusually warm summers can expect a new and increasing cause of oyster mortality in Delaware Bay.

Studies of comparative mortality of local oysters with that of South Carolina seed carried on from 1951 to 1956 at the Virginia Fisheries Laboratory showed that the imported seed was relatively resistant to Dermocystidium with approximately only half as great mortality during hot summers as in local stock. Growth was almost as good but mortality during cold winters was serious. The Virginia scientists in reviewing these results reintroduce a new concept to our oyster growers: the advisability of marketing their crops at the time the total weight of meats is at its maximum. By waiting for growth to reach desired size heavy mortality may take such heavy toll as to cause serious losses to the grower at the time of harvest.

Dr. Philip A. Butler (1952), Pensacola, Florida, in a comprehensive report, "Shell growth versus meat yield in the oyster, C. virginica", first presented this concept to our Association. In summary: "The ratio of total volume to shell volume appears to possess certain advantages in estimating growth and meat yielding potential of oysters as compared to the customary dimensional measurements." In simple language: not how large are my oysters but how much meat have I on my beds? Dr. Caswell Grave (1912) in one of the most helpful practical reports ever made by a scientist to assist the oyster industry describes, pages 314-317, how any careful oyster grower can easily determine this for himself with no apparatus other than a dish large enough to hold 20 oysters, a one-liter and a 100 milliliter graduated cylinder, and a medicine dropper. In brief, the volume of the oysters is measured by water displacement in the dish. The oysters are then shucked, carefully saving all shell liguor. The meats and shells after draining are then measured separately by water displacement. The water displaced by the meats plus the shell liquor give total capacity of the shell cavities. The per cent of the shell cavities represented by the volume of the meats is a measure of the plumpness of the oysters. The per cent of the volume of water displaced by the unopened oysters represented by volume of the meats gives ratio of meats to unopened oysters. The ratio of water displaced by the empty shells to the volume of water displaced by the unopened oysters gives the proportion of the whole represented by the shells alone.

Dr. A. F. Chestnut reported on oyster seed production in North Carolina. The highest figure, some two million bushels, was attained many years ago. In recent years seed production has varied from 200,000 to 600,000 bushels. North Carolina has great potential capacity for producing

seed; in the Neuse River and south of Roanoke Island lie excellent lumps with oysters up to three inches long. Shipments have been made to northern waters, one dealer sending 50,000 bushels to Chesapeake Bay. South of Morehead City in the intertidal zone along the shores are extensive oyster bars with large reefs. All oysters below low water are killed by boring sponge and the oyster drill Urosalpinx. Setting intensity high, 1,000 or more spat per shell with peaks of intensity in June, July and September. Rehabilitation of state beds has been initiated with 80,000 to 200,000 bushels of shells annually with plans calling for half a million bushels eventually. Seed will be removed from crowded rocks to areas where there is adequate room. It is recommended that Pamlico Sound be opened to private leasing. At the present rate of planting it would require 10 years to cover just the mouths of the rivers and coves alone.

North Carolina is an area of great interest to the marine biologist since Cape Hatteras like Cape Cod in Massachusetts forms a boundary between northern and southern forms. It is sincerely hoped that progress will be made toward leasing at least substantial portions of bottoms, now barren for lack of cultch, which should produce excellent oysters. An inspection made in company with Dr. Chestnut revealed new growth on many of the oysters by the last week in March equal to the best to be expected in Delaware Bay during the course of an entire season. Also striking were the clean hard shells devoid of boring sponge attack in oysters growing in new territory. As yet we know little regarding the mode of infestation of the shells of living oysters by boring sponges. There is no doubt, however, that the presence on old beds of large amounts of heavily infested shells of dead oysters and clams constitutes a reservoir of infestation so extensive as to guarantee prompt invasion of the shells of any oysters planted thereon. Since Dr. Chestnut reports the death of oysters below low water through boring sponge and by the drill Urosalpinx it appears worth while to explore the results of catching spat upon clean shells which have been on land long enough to destroy all traces of sponge within them, and which have been planted on suitable bottom now devoid of any shells or oysters.

Why not take a lesson from the remarkable success of the Dutch oyster growers who under orders from Dr. Korringa dredged the cockle shells infested with shell disease and started anew with clean bottoms? By this piece of seaboard sanitation he saved the Dutch oyster industry from destruction. There are few oyster beds of the Atlantic seaboard, in higher salinities, which now are not literally paved with shells and fragments heavily infested with the sponge Cliona.

Dr. Philip Butler, F.A.W.S., completed the symposium with a brief description of the production and utilization of seed oysters in the Gulf area. With a coastline of over 3,500 miles only 350,000 acres within the entire Gulf area has been set aside for private leasing. While in states north of the Gulf the size of the annual oyster drop is roughly proportioned to the amount of cultch planted, the Gulf area has been productive for over 100 years with no real assistance from man. Of chief interest to the reviewer was Dr. Butler's statement to him several years ago that

at Pensacola water temperatures adequate for spawning exist for a month or longer before discharge of eggs and sperm begins. Only after the spring plankton bloom occurs do the oysters reproduce. As to the feasibility of supplying northern oyster beds with seed from the Gulf, Dr. Butler's answer is an unqualified "no", at least in the immediate future. His reasons are: (1) No Gulf state now has an exportable surplus of seed; (2) only one Gulf state has an established oyster seed program; (3) Gulf seed is infected and infested with dangerous parasites and predators; (4) the great density of fouling organisms renders very difficult production of a clean exportable product. In closing Dr. Butler stressed the ever-increasing importance of state laboratories to a flourishing oyster industry, with the need for biologists to locate the best setting areas prior to their critical depletion through removal of parent stock. In the future much must be accomplished in restitution of depleted areas while careful records should be kept of the results of transplanting.

Highly commended is Dr. Butler's emphasis on the importance of state laboratories to a flourishing oyster industry. To which may be added the further benefits to be secured through close cooperation of these laboratories with each other. In this, our Association must, and does, play a vital role in bringing the personnel of these laboratories together once a year, providing a program through which our problems and our findings can be presented and discussed.

Also important is Dr. Butler's conclusion that the time has arrived for the Gulf states to give serious consideration to the production of seed oysters for their own use. Of equal moment is his firm conviction that it is not feasible to use these oysters to supply any of the beds in northern waters.

In closing I wish to restate the following from the last paragraph of my annual review of the papers presented at this Convention as published in the Fishing Gazette for December 1956. We greatly missed our usual associations with Dr. Loosanoff and his associates from the Fish and Wildlife Service laboratory at Milford, Connecticut. They have much to give to our Association, but of even more moment is the stimulus received particularly by younger scientists from papers read and from associations with other scientists. These young and eager research men have the most to gain from our meetings. With relatively low salaries and often heavy financial responsibilities they are the least able to provide the necessary funds themselves. Let it be stressed with all possible emphasis that the most important ingredient in every research project is an idea. Far too often conventions are looked upon as joy rides at the expense of the taxpayer. Checking with the journalists who cover our meetings will reveal that in their opinion our Association stands at the very top in the percentage of our members who sit through long sessions in spite of lighter attractions just beyond the hotel doors. It is earnestly hoped therefore that administrators will make adequate allowance in their budgets for attendance at our meetings. No finer reward could a young scientist receive than the recognition from above that his work is worthy of reporting to fellow scientists. No surer investment could be made by his

superiors than to assure the enthusiasm, new ideas, and fresh outlook which the young researcher would bring back to his laboratory from these meetings.

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BIOLOGY

of

SHELLFISH

DETERMINATION OF HOW LONG OYSTERS HAVE BEEN DEAD BY STUDIES OF THEIR SHELLS

Gordon Gunter[1], C. E. Dawson[2]
and Wm. J. Demoran[3]

Oyster biologists are often called upon in cases of oyster mortality to determine what caused the damage, usually where there is conflict of opinion and contingent damage claims are involved. If the time of the mortality can be set, sometimes even within fairly broad limits, certain causes or claimed causes of the trouble can be eliminated. After a mortality has taken place, the remains afford the only clues to the period of time elapsing since death. Sometimes, if the mortality is recent, bits of meat are left, but usually only the shells remain. The age of these shells, as dead shells, is sometimes estimated by the amount of fouling organisms attached. However, these estimates vary widely and there are few data upon the matter. The authors attempted to set some limits upon the ages of dead shells as indicated by the fouling organisms.

The work was carried on at Port Aransas, Texas, and at Ocean Springs, Mississippi. Oysters (Crassostrea virginica) were killed with an oyster knife and placed overboard in Sea Rac baskets or in bags of chicken wire. One basket was lowered to the bottom and another was suspended at the surface at Ocean Springs where the depth was six feet. At Port Aransas where the water was twelve feet deep a third was suspended at mid depth. Sets of oysters thus treated were put down at every season of the year.

Several observations were made: the time of disappearance of all oyster meats, the time of first fouling, moderate fouling, and heavy fouling. Some of the results are given in Table 1. These are approximate for comparable objective measurements are difficult to make. The table relates to oysters on the bottom. Several interesting points are not amenable to tabular treatment and can only be recounted.

Oyster meats disappeared much more rapidly in bottom baskets than in suspended bags due to the nonswimming crabs and Thais, which were especially abundant while the meats lasted. Bits of muscle remained for several weeks in cool water. Preceding fouling of macroorganisms, and while putrefaction was still going on, a slime, doubtless caused by bacteria, formed over all the shells. Fouling by algae, barnacles, and Crepidula was noticeable before putrefaction was complete and while some of the meat still remained in the shells. The first visible fouling in shallow water or upper layers was often a patch of green unicellular algae. This was followed quickly by small barnacles. In the twelve foot depths at Port Aransas, few barnacles attached and even after long periods

1, 3) Gulf Coast Research Laboratory, Ocean Springs, Mississippi.
2) Bears Bluff Laboratory, Wadmalaw Island, South Carolina

fouling did not become as abundant. On this bottom the fouling complex was dominated by bryozoans, Crepidula, and worm tubes, and later small oysters, rather than by barnacles. In shallow water fouling was heavier and was dominated by barnacles.

At water temperatures of 10° C oyster meat remains fresh in the shell for three weeks and more, and fouling is slow. Thus, shells may remain white and shiny for a month after death or a little more. In contrast, during warmer months fouling on shells of recently dead oysters is noticeable in three to four days, and in a week to ten days fouling is extensive.

The rate of fouling is strongly dependent upon temperature. Therefore, widely separated areas with similar temperatures would be expected to have similar initial fouling rates. Similarly, the fouling complex varied more with depth than with locality of the two rather widely separated areas studied.

There was no erosion of shells or noticeable change or dimunition of the hinge ligament of shells which had been in the water 73 days.

Table 1. Approximate times of developments on the shells of oysters killed by stabbing with an oyster knife and set on bottom in wire bags or baskets.

	Disappearance of all meats	Initial fouling	Moderate fouling	Average temperature °C	Average salinity o/oo
Winter	4 weeks	3 weeks	2 months	10.0	
Spring	10 days	4 days	3 weeks	17.7	17.0
Summer	2 days	3 days	2 weeks	28.0	35.4
Fall	8 days	4 days	$2\frac{1}{2}$ weeks	18.5	26.1

ON THE SHELL OF BIVALVE MOLLUSKS [1]

Carl N. Shuster, Jr.

Department of Biological Sciences, University of Delaware

Introduction

The writer has been interested in mollusk shell growth for some time (Shuster 1951). However, attention was refocused on the subject this past winter when Robert Livingstone, Jr., Fish & Wildlife Service, Newark, Delaware, asked the writer to determine the age of surf clams he had collected. Since then, the problem and the scope of the inquiry has expanded from the original consideration of age determination to the comparative anatomy and ecology of bivalves and relation of shell growth to environmental conditions.

Methods

The methods of preparing shells for this study have not been elaborate. Generally, the left valve was embedded in gypsum and the hardened block cut into one or more sections with a hacksaw or a carborundum circular saw.[2] The cut edges of the valve were smoothed by scouring on a glass plate covered with water and a carborundum powder and then polished with a cleansing powder. These polished surfaces were studied for gross structure and sketched. The intact right valve was then compared with the sections of the left valve.

Some shells were treated with varying dilutions of hydrochloric or acetic acid to erode shell material. This erosion was controlled in some cases by coating parts of the valve with paraffin.

The observations are listed under each of the species studied. The taxonomy of Abbott (1954) has been followed. All of the specimens were from Delaware waters except where otherwise noted.

Observations

Eastern American Oyster, Crassostrea virginica Gmelin. (Plate I).[3]

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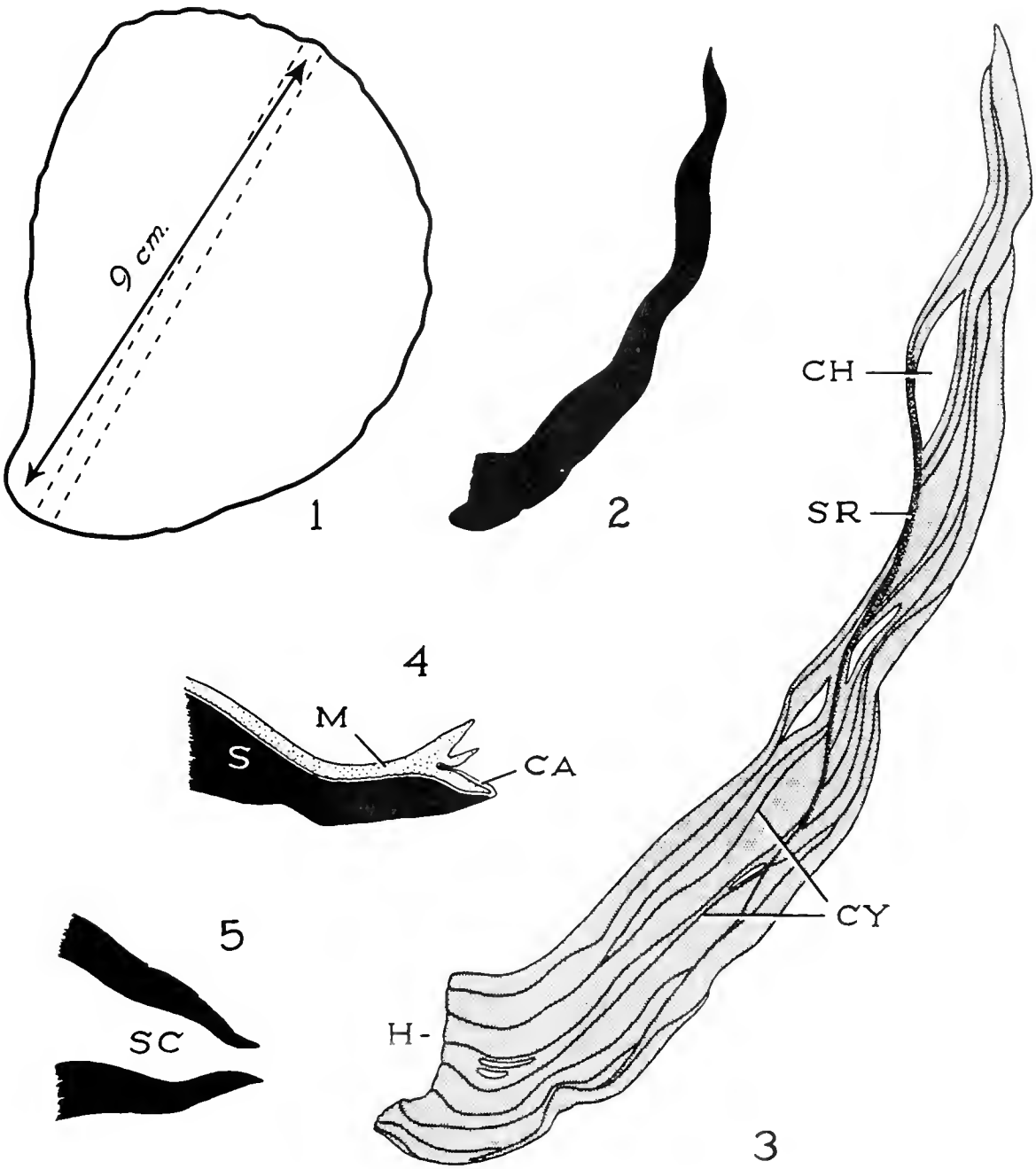
1) Contribution No. 5, University of Delaware Marine Laboratories.

2) Dr. Philip A. Butler, Shellfishery Laboratory, Fish & Wildlife Service, Pensacola, Florida, has communicated to the writer information on a copper wheel used to section snail shells for the tourist trade (6 August 1956). This copper saw, manufactured by the Felker Manufacturing Company of California, has a smooth outer edge with a series of low ridges on its lateral faces. A slow stream of water is directed over the wheel during cutting.

3) The paper by Galtsoff (1954) is excellent for detail of the oyster shell and chalk deposits.

Plate I. THE EASTERN AMERICAN OYSTER. 1. Outline of left valve, showing region of sectioning. 2. Silhouette of section. 3. Enlargement of section, showing gross structure: (CH) chalk lens, (SR) muscle attachment "line", (CY) prominent lines of conchiolin, and (H) hinge area. 4. Diagram of valve margin, showing "caisson" (CA), mantle (M), and shell (S). 5. Diagram of shell margin, showing the flaring of the valves and the "siphonal chamber" (SC).



Sheets of conchiolin are prominent, especially in the region of the hinge, where they can be traced from the hinge surface into the sectioned shell. Most of the shell material has a translucent nature and is present in varying thickness. The uneveness of shell deposition is further heightened by the inclusion of "chalk deposit" lenses. The migration path of the adductor muscle is seen as a diagonal, inward and posteriorly directed, purple-pigmented line through the shell. There is a tendency for the margins of the valves to flare forming a marginal chamber (Fig. 5).

Northern Quahog, Mercenaria mercenaria L. (Plate II).

The laminated structure of a Venus shell is clearly visible. The prominent laminae of translucent material in the inner portion of the shell pass diagonally to the outer shell surface through two regions of opaque shell of different coloration and texture. The outer opaque region is cream-colored and is not as hard as the inner one. Purple pigment, when present in the shell, is prevalent in the translucent material. Within the outer edge of the translucent shell, conchiolin and periostracum seemed to be joined. At least, the periostracum is deeply imbedded in the translucent material.

Disk Dosinia, Dosinia discus Reeve.

The laminated structure of the disk shell is not easy to follow macroscopically, yet it seems to be essentially the same as that of the quahog. Although "annual rings" are not as distinct as in other species, certain Dosinia shell characters related to growth are apparent, especially the change in shell shape. A tightly adhering periostracum covers the shell. Surface ornamentation of the shell has an interesting bifurcated pattern similar to that of circuli on a fish scale. An analysis of the variation in width and bifurcation of the "concentric" bands of the shell surface might give information on the growth of this species. This specimen came from South Carolina.

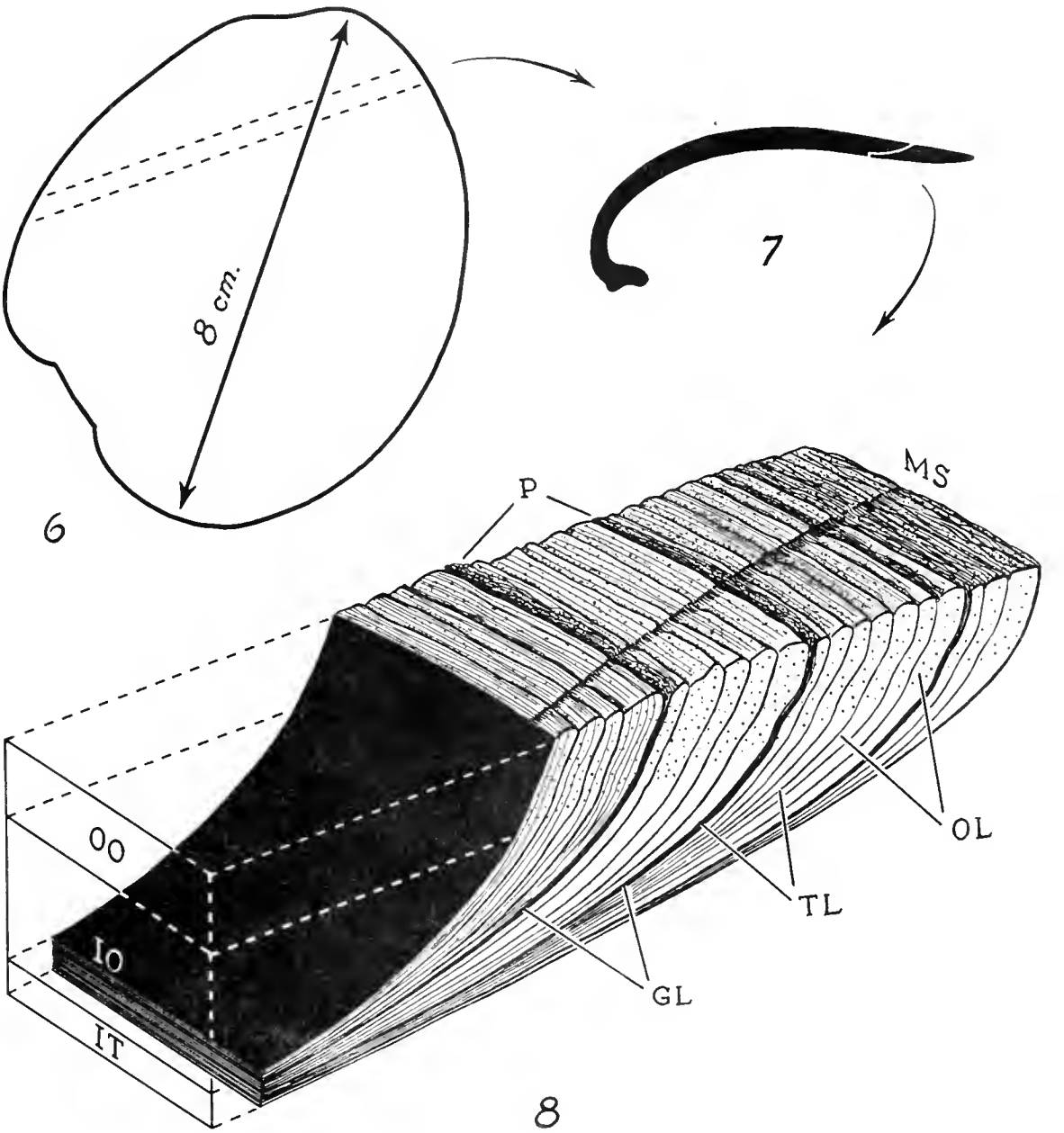
Blood Ark, Anadara ovalis Brugiuere.

The ark shell is comprised largely of translucent material which makes it difficult to see the laminations. Sections cut across the ribs reveal opaque ovals through which translucent lines traverse.

Surf Clam, Spisula solidissima Dillwyn.

In the bivalve shells studied, the laminated pattern of growth is most prominent in Spisula. The surface of the chondrophore and sections of the shell clearly show the continuity of the laminae. The opaque material, like that of the quahog, is of two colors. Many striations, fine lines crossing the translucent laminae, can be seen in the opaque shell.

Plate II. THE NORTHERN QUAHOG. 6. Outline of left valve, showing region of sectioning. 7. Silhouette of section. 8. Stereogram of valve margin, showing gross feature of shell: (OO) outer opaque region, (IO) inner opaque region, (IT) inner translucent region, (GL) prominent growth lines, (TL) translucent laminae, (OL) opaque laminae, (MS) margin of shell, (P) periostracum.



Blue Mussel, Mytilus edulis L.

Except where eroded, the periostracum tightly adheres to the shell. Large amounts of conchiolin, with a rubbery texture, remain after treating the shell with acid. The bluish pigment is more or less evenly distributed throughout, although, depending upon the section, the inner shell region may be white. Laminar lines can be seen but they are very faint.

Pismo Clam, Tivela stultorum Mawe.

A left valve of this clam, from the western shore of the Gulf of California, was given to the writer by Robert Livingstone, Jr. Although the valve broke unevenly during sectioning with a hacksaw, one piece, from umbo to shell margin, remained intact. This section revealed a structure similar to that of Spisula and Mercenaria. One large fragment -- the posterodorsal quadrat of the valve -- was coated exteriorly with paraffin and the inner surface of the shell eroded by acid. While an attempt to "cancle" the whole valve by Weymouth's method (1923) had been unsuccessful, the margins of the "annual rings" on the acid-eroded piece were revealed by "candling" as translucent shell material.

Discussion

The classical report of Weymouth (1923) on the growth of the pismo clam delved into the gross structure of a bivalve shell to an extent not matched by any other paper consulted. It is, therefore, the departure point for further discussion. On the basis of the present comparison of Tivela and Spisula shells, it is believed that if Weymouth had studied the latter he might well have been stimulated by the clearness of its macroscopic structure to study further this aspect of his work.

The account in this paragraph is condensed from Weymouth (1923). There are four layers in the Tivela shell: periostracum, vertical layer, oblique layer, and nacre. These layers are interrupted by more or less continuous laminae, which can be traced from the outer shell surface through the shell and even into the umbo and chondrophore. Microscopic sections show that the entire shell is composed of extremely thin lamellae, alternately translucent and relatively opaque. The closer together the translucent lines, the more apparent are "growth rings" on the shell surface. Conspicuous groups of translucent lines mark what once was the inner shell surface; and the "growth lines" on the surface, the corresponding margins of the shell. These prominent translucent lines were interpreted as annual rings and the fine lamellae as probably representing a short time rhythm of about the magnitude of individual days or tides, or some physicochemical periodicity. The thickness of the shell was related to the activity of the entire mantle. Variations in the level of the concentric rings on the outer shell surface were ascribed to slight variations in the extension of the mantle.

In their brief paper, Owen, Trueman, and Yonge (1953), describe three layers of the shell: a superficial periostracum and underlying outer and inner calcareous layers. The periostracum is secreted as a thin sheet within a groove between the outer and middle lobes of the mantle edge. Outer calcareous layers are produced by the general surface of the mantle. Thus, the periostracum and outer calcareous layers grow by increments from the periphery of the mantle, while the inner calcareous layers normally continue to increase in thickness throughout life. They reported that additional calcareous layers occur in some genera between the outer and inner layers of the valves, as in Tivela stultorum (Weymouth 1923) and Tellina tenuis (Trueman 1942). These additional layers are produced by the less active portion of the mantle edge between the extreme edge where growth is most active and the inner calcareous layer of the valves.

The present interpretation of gross bivalve shell structure differs somewhat from that of Weymouth (1923) on Tivela, and Trueman (1942) on Tellina. Agreement, and disagreement, is based chiefly upon the concept of shell growth as exemplified by the terms "laminae" and "lamellae," and "layers." These terms do not have the same connotation. The first two refer to increments of shell growth, the latter to parts of these increments.

Shell deposition from umbo to shell margin, if not simultaneous, appears to be at least regular enough to produce a marked degree of continuity. This continuity of deposition, from umbo to shell margin, forms laminae which appear to be the basic macroscopic unit of shell structure. These laminae are alternately opaque and translucent shell material. Prominent translucent laminae are sometimes referred to as "growth lines." Shell "layers," more pronounced in the opaque portion of the shell, are merely regions along the laminae which are deposited by the same representative portion of the mantle. Each "layer" grows, therefore, through the accumulation of related regions of the laminae (see Fig. 8).

Differences in the kind and amount of shell material deposited could be related to environmental conditions affecting the rate of shell deposition: the opaque shell being deposited under the more optimum growth conditions; translucent shell during periods of slow growth, when, it is possible, the carbon dioxide level in the mantle tissues might be higher than during rapid growth. Perhaps the laminated structure of bivalve shells is comparable to growth rings in trees; opaque shell material to spring wood, and translucent material to summer wood.

Korringa (1952) stated that "shell growth occurs periodically" in the oyster. He does "not believe in alternation of prolonged periods of tissue growth with periods of shell growth in the oyster, but" supposes "that both may occur simultaneously, and under favorable nutritional conditions, continually." Brief observations suggest that shell growth and tissue growth do indeed proceed at different rates. Just what the different growth rates may be is not known although it

has been shown that Ca^{45} in seawater is incorporated into inorganic shell material within a 24-hour experimental period (Bevelander 1952). In Delaware Bay bivalves the fastest shell growth does not occur during the periods of gonad ripening, gamete discharge, and fattening of the body. While development of gametes and glycogen stores may not be "growth" in the strictest sense, obvious changes in the meat weight of bivalves occur during the period of less rapid shell growth. Growth of bivalves may occur in a step-wise fashion, with shell growth followed by tissue growth. The great extensibility of the mollusk mantle makes it possible for shell deposition to occur without an accompanying and simultaneous addition to the mantle tissue.

The juxtaposition of the mantle and the inner surface of the shell suggest that the mantle, shell, and periostracum function as a "caisson" within which the deposition of shell material occurs unhindered by the direct action of the environmental seawater (see Fig. 4).

In comparing the oyster with those bivalves possessing siphons, it seems possible that the chamber formed by the flaring of the margin of the oyster shell may serve a distinct function. Within the confining "siphonal chamber," the mantle edge could function in a manner analogous to the sleeve-like siphons of clams (see Fig. 5).

A significant contribution from the field of paleobiochemistry helps to form a concept of the comparative anatomy of bivalve shells. Abelson (1955, 1956) has found a remarkable identify of amino acids between the quahog, Mercenaria mercenaria, and its fossil forebears of millions of years ago. This retention of the basic chemical nature of the organic matrix of the quahog shell is an indication of the "conservative" nature of the evolution of shell structure. Although it can be predicted that they also would have the laminar pattern of growth, it would be of interest to section fossilized bivalves.

The biochemistry of bivalve shell pigments and conchiolin is also of interest. According to Comfort (1950) the shell pigments of bivalves are intimately associated with the conchiolin of the shell and resist extraction. These appear to be chromoproteins, possibly with prosthetic groups related to the melanins, for which no successful technique of extraction has yet been devised. The dark pigment of Mytilus was determined to be a melanin. Observations in the present study on the distribution of shell pigments indicate that the colors are more prominent in the region of the translucent laminae. This could be due to the greater amounts of conchioloin in the translucent shell. Beedham's (1954) histochemical tests and chromatographic analyses on parts of the shell and ligament of several bivalves indicated a difference between the composition of the amino acid content of the conchiolin of the inner layers of the valve and ligament and that in the rest of the shell. Despite these differences in the amino acid content, the studies of Abelson (1955, 1956) indicate that the fundamental organic complex has remained essentially unchanged for millions of years.

Summary

In summation tentative statements on certain comparative aspects of bivalve mollusk shell growth can be listed.

1. The well-known laminar construction of bivalve shells is being examined to see if internal and external "landmarks' can be correlated with shell growth.

2. The laminar pattern of shell growth was common to all the species studies. Sections through the shells of Mercenaria mercenaria, Spisula solidisimma, Tivela stultorum, and Dosinia discus are remarkably similar. Laminations in the shell of Crassostrea virginica are most clearly evident in the hinge area.

3. The observed similarity in the "architecture" of bivalve shells indicates that comparative studies may enable biologists to reach a better understanding of the correlations between shell structure and the natural history of the mollusks. It is believed that information on the relationship of growth patterns to environmental factors will give additional insight into the lives of these mollusks, and thus, may be of practical value in the management of shellfish crops.

4. In this preliminary study, an attempt to relate the width of the laminae to environmental factors has not been made, although it is evident that the more prominent groups of translucent laminae have been interpreted as annual rings by other workers.

5. It appears that the translucent laminae are deposited during periods of slow shell growth; opaque layers, during rapid growth.

6. It may be that the periostracum in conjunction with the mantle and shell forms a "caisson" within which shell deposition can proceed unhindered by direct action of the environmental water.

7. The slight concave flaring of the inner surface of the marginal area of the oyster shell, may, by "shaping" the mantle edges, provide the mechanical advantage derived from the siphon in other bivalves.

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SURVIVAL AND GROWTH OF *VENUS MERCENARIA*, *VENUS CAMPECHIENSIS*, AND THEIR HYBRIDS IN SUSPENDED TRAYS AND ON NATURAL BOTTOMS[1]

Dexter Haven and Jay D. Andrews

Virginia Fisheries Laboratory, Gloucester Point, Virginia

Introduction

In the course of laboratory experiments on spawning of mollusks and propagation of larvae and young, Loosanoff and Davis (1950) of the Milford Laboratory of the U. S. Fish and Wildlife Service crossed the southern hard-shell clam, *Venus campechiensis* Gmelin, with the northern species *Venus mercenaria* Linné (Loosanoff, personal communication). To determine the ecological adaptations of the hybrids, groups of the parent species and their reciprocal hybrids were sent for testing to six laboratories from Maine to Florida. The northern quahog or hard-shell clam inhabits the shores of the Western Atlantic from the Gulf of St. Lawrence to Florida and the Gulf of Mexico; the southern quahog has been recorded from Chesapeake Bay to Florida (Abbott 1954) although it is doubtful that it occurs naturally in Chesapeake Bay for we have not encountered it. Since the two species cross easily in the laboratory, questions arise about the validity of the species and the amount of natural hybridization which occurs in areas south of Chesapeake Bay where the ranges overlap. The characters used by conchologists to distinguish *Venus campechiensis* are obesity, great width of lunule, thickness of shell, persistence of growth ridges, and absence of purple color internally.

The first series of clams, received in Virginia in May 1954, was planted in screen-covered boxes dug into the bottom at Gloucester Point near the Virginia Fisheries Laboratory. This experiment was a joint project with James B. Engle of the U. S. Fish and Wildlife Service. Although all four groups of clams were of the same age, the hybrids were distinctly larger when received from Milford. In the fall of 1954 when the boxes were first examined, mortality had been high, particularly in the groups containing the smaller clams; some predation was evident. Later in the fall hurricane Hazel dislodged some of the boxes and seriously curtailed the experiment.

After this experience, we conceived the idea of growing clams in boxes in trays suspended in the water; by this method oysters have been carried successfully through several hurricanes at Gloucester Point. Later it was discovered that Belding (1912) had used a similar method some 50 years earlier. The primary purpose of the tests was to compare

[1] Contributions from the Virginia Fisheries Laboratory, No. 74.

growth and mortality of the two species and their hybrids under identical environmental conditions. With all four groups in one tray, the habitat was essentially similar, and predation, type of substratum (Pratt 1953), and accessibility were easily controlled.

Methods and Procedure

In November 1954, Dr. Loosanoff shipped a second series of clams selected arbitrarily for uniformity of size from lots of the same age. The clams were grown in wooden boxes filled with sandy mud, suspended about one foot off the bottom in "Sea-Rac" trays. The wooden boxes, 37 x 16 x 4 inches and subdivided into four 9 x 16 inch compartments, were covered with a lid of one-fourth inch mesh hardware cloth. With lids on, the boxes were submerged in water and refilled; this removed mud snails, coarse shells, and rocks from the muddy-sand bottom. The substratum in the boxes was seldom eroded, but a layer of soft mud one-quarter to one inch thick accumulated between examinations. Examinations were made once a month during the growing season, but less frequently during the winter. The clams were washed from the boxes over a screen. Individual clams were measured but weights and volumes were obtained by groups. Length is defined as the greatest dimension of clams from the anterior to the posterior margin.

Mortality of Clams

Upon arrival in Virginia, each group of clams, containing from 125 to 145 individuals, was placed in one of the four compartments. In November 1954, therefore, the density was about 125 clams per square foot, and the mean length was approximately 11 mm in each group. In July 1955 the clams were rearranged in two boxes, which increased the space available and decreased the density by half. In late October 1955, the clams had reached such a size that crowding was again suspected and differential mortality had changed the density in the various compartments. At this time numbers were marked on all clams; 25 of each group were placed in boxes and the rest planted on natural bottom. The density of clams in the boxes was reduced to about 10 per square foot, and average lengths of the groups were from 25 to 33 mm.

Two years of observations revealed that the death rate of the native species, V. mercenaria, was low during all seasons (Table 1). At these early ages and small sizes, neither disease nor environmental factors caused much death among clams of the northern species, although they were bred artificially from brood stock obtained in Long Island Sound. During the warm seasons, all groups had low mortalities, and it may be surmised that in Virginia summer conditions are probably not limiting to the species or the hybrids. In winter, however, the southern species had heavy losses and the two hybrids had important losses (Table 1).

After 25 of each group had been placed in trays, the remainder of the numbered clams was placed on natural bottom. In June 1956 about two-thirds of these clams were recovered by diving. In all groups, boxes (empty shells) and dying clams comprised less than three per cent of the total recovered---except in V. campechiensis which had a death rate of 74 per cent. As the warm season progressed, all groups of clams were rapidly decimated. Shell fragments began to appear in June, increased in abundance in July, and a large quantity was recovered in August. Positive identification of predators was impossible, but the size and nature of shell fragments, higher losses in the groups of smaller clams, and the long period of predation cause us to suspect the blue crab (Callinectes sapidus).

Growth

Growth of clams began in April or early May and ceased in November each year. V. campechiensis and the two hybrids had very similar growth rates (Fig. 1). In trays these groups increased in weight from 0.5 to nearly 11 gm in the 1955 growing season and from 11 to 29 gm in the 1956 season. However, none of the clams of the southern species survived the second winter. The northern quahogs grew little more than half as fast as the others; they reached a length of 26 mm and a weight of six gm the first season and 38 mm and 17 gm at the end of the second season.

During the growing season of 1956, clams retained in the suspended trays outgrew their counterparts in natural bottom (Table 2) although relatively few of this last group survived. This supports our belief that boxes of muddy sand suspended off the bottom in trays provide a suitable habitat for growth and survival of clams.

Yield

The potentiality of these clams as seed for Virginia waters depends ultimately upon the yield to the clammer. The amount of crop obtained and rapidity of harvest after seeding or setting depend upon rates of growth and survival of clams before a marketable size is reached. All the southern clams died before reaching a marketable size. During the two years of the experiment, the hybrid clams usually have had a greater biomass or yield than the northern clams (Fig. 2). Relative yield or biomass has been discussed by Andrews and McHugh (1957). None of the clams has reached marketable size yet, however, and the slow growth of the northern clams is almost compensated by the high rate of survival.

Discussion

The causes of clam mortalities in Virginia waters are unknown, yet it is significant that when predation was prevented losses were very

Table 1. Mortality of clams in trays at Gloucester Point, Virginia

Group	Number (Nov. 1954)	Percentage dead		Number (Nov. 1955)	Percentage dead	
		Nov. 1954 to Mar. 1955	Apr. to Oct. 1955		Nov. 1955 to Mar. 1956	Apr. to Oct. 1956
Species						
V. mercenaria	145	4.8	8.4	25	0.0	0.0
V. campechiensis	130	66.9	6.0	25	96.0	- -
Hybrids						
V. mercenaria ♀ x *V. campechiensis* ♂	125	24.8	6.8	25	12.0	0.0
V. campechiensis ♀ x *V. mercenaria* ♂	130	5.4	5.7	25	24.0	0.0

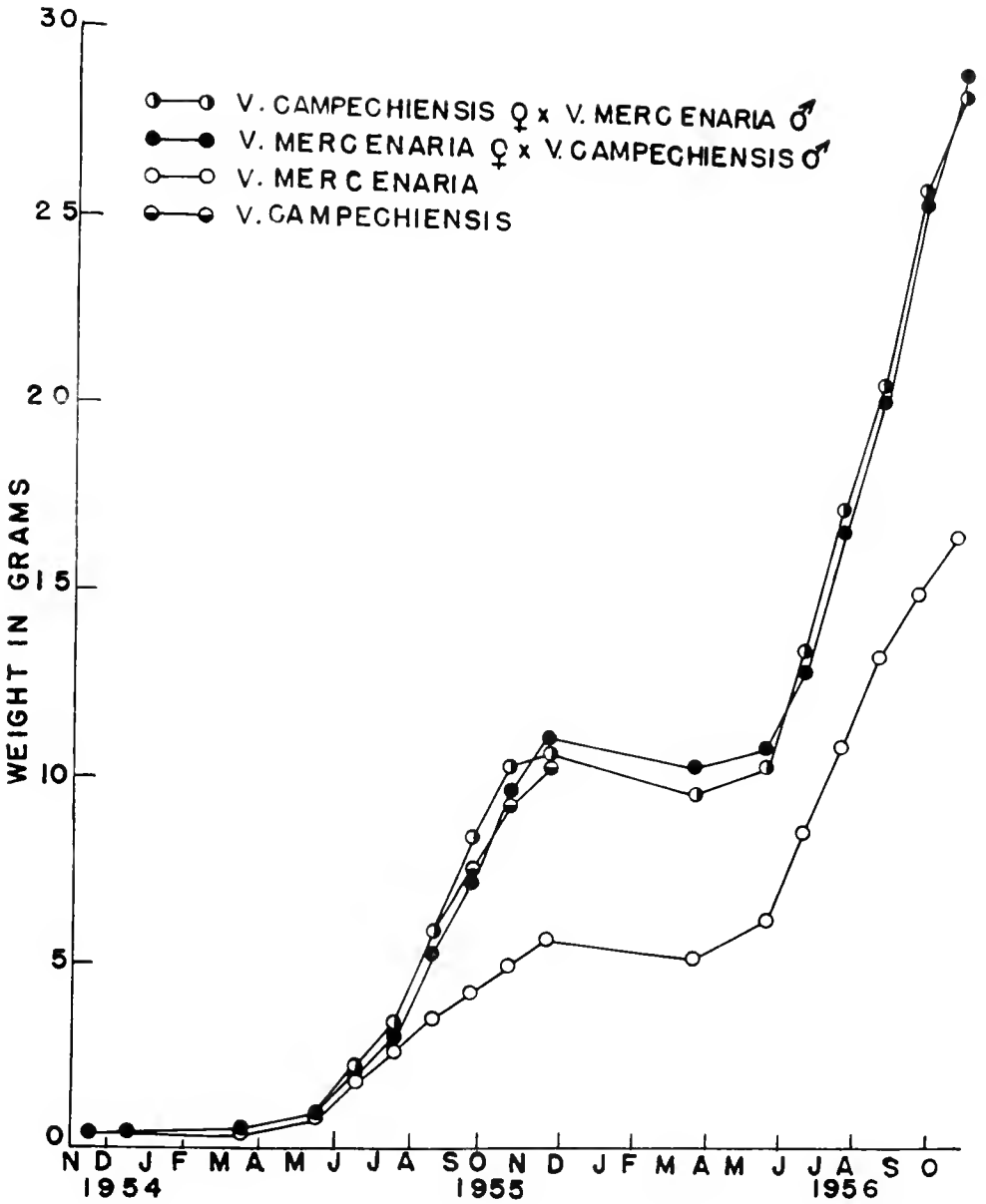

Fig. 1. Mean weight, including shell, of clams grown in boxes suspended in trays at Gloucester Point, Virginia.

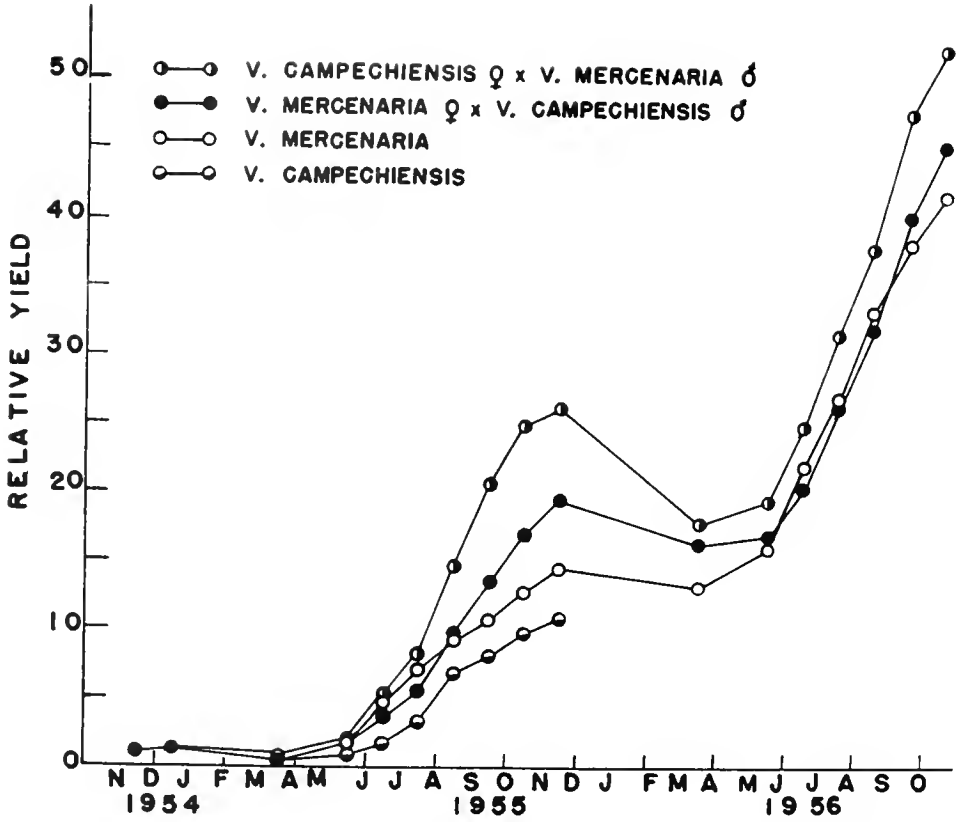

Fig. 2. Relative yield (biomass) of clams grown in boxes suspended in trays at Gloucester Point, Virginia.

Table 2. Mean Lengths and weights of clams in trays and in natural bottom, September 14, 1956[1]

Group	Length (mm)		Weight (gm)	
	Tray	Natural bottom	Tray	Natural bottom
V. mercenaria	37	33	15	13
V. mercenaria x *V. campechiensis*	44	40	26	19
V. campechiensis x *V. mercenaria*	43	39	26	22

[1] All clams were grown in trays until October 1955 when part of each group was planted on natural bottom. Subsequently, there were heavy losses in the bottom-living clams from predation.

low in the northern species at all seasons. Methods for reducing winter mortalities of northern quahogs in Maine have been discussed by Dow and Wallace (1951). The deaths of the southern clams and some hybrids in late winter suggest inability to withstand low temperatures. The experiments imply that V. campechiensis may be unable to persist in Chesapeake Bay long enough to breed and establish a population. The test in trays was fairly rigorous in respect to temperatures, for the water was shallow, and the winters of 1954-55 and 1955-56 were the coldest in a decade. The southern clams living in natural bottom also died at a high rate in the winter of 1955-56.

Growth of the hybrids was clearly superior to that of the northern clams. It appears that this desirable characteristic may be traced as much to inheritance from the southern quahog as to hybrid vigor, for V. campechiensis equalled the hybrids in growth in Virginia waters. It must be remembered that the progeny of V. mercenaria were obtained from brood stock native to the cold waters of Long Island Sound. Clams native to Chesapeake Bay may grow faster.

The relative yield of the hybrids and the northern clams at marketable size is undetermined. If growth becomes slower with age, and winter losses continue, then the hybrids may yet be exceeded in yield by the northern quahog.

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GROWTH OF YOUNG *VENUS MERCENARIA*, *VENUS CAMPECHIENSIS*, AND THEIR HYBRIDS.

A. F. Chestnut, W. E. Fahy, and
H. J. Porter

Institute of Fisheries Research University of North Carolina
Morehead City, North Carolina

Growth studies of the hard clam, *Venus mercenaria*, have been centered primarily on the large size groups. Studies in the northern part of the range of the species have shown that shell growth occurred during the months of April through November (Kellogg 1903, Belding 1912, Haskin 1949, Pratt 1953, Gustafson 1954, Pratt and Campbell 1956). Belding (1912) found in Massachusetts 51.7 per cent of the total growth for a year was attained during August and September. Gustafson (1954) showed the peak of growth in Maine to be between mid-July and mid-September; Pratt and Campbell (1956) reported that most of the growth in a year took place before mid-July in Rhode Island. In North Carolina Chestnut (1952) showed that greatest growth occurred in April and May and least in September.

The following studies were made with clams under 30 mm spawned from known parents and grown under laboratory conditions by Dr. Victor L. Loosanoff and his associates at Milford, Connecticut. Rate of growth was studied for 18 months in Bogue Sound, North Carolina, at the Institute of Fisheries Research. Grateful acknowledgement is made to Dr. Loosanoff for supplying the clams.

Methods

Two groups of hybrid clams, *V. mercenaria* ♀ x *V. campechiensis* ♂ and the reciprocal cross, were received from Milford in May 1954. A second shipment received in September 1954, contained four groups of clams, *V. mercenaria*, *V. campechiensis*, and their hybrids.

The clams were kept in wooden frames about four inches deep and 18 inches square with plastic screen on the top and bottom. Beach sand was sifted over the clams until each box was half filled. The boxes were partially buried and kept in the intertidal zone where they were exposed each low tide. Measurements of the greatest length and total weight were made a monthly intervals.

In previous studies where small clams under 15 mm in length were used mortalities frequently resulted among clams left undisturbed for longer than a month. Complete filling of the boxes with sand and heavy fouling of the screens prohibited the circulation of water. It was necessary to remove small predators, such as drills and crabs, which

passed through the mesh and were trapped inside the boxes. When sets of native clams occurred in the boxes, they could be distinguished from experimental clams by their smaller size; they were removed to prevent any masking effects.

Comparison of Growth of Hybrids

The first shipment of hybrid clams, V. mercenaria ♀ x V. campechiensis ♂ and the reciprocal cross, was planted on May 10, 1954. The average length of both groups at the beginning of the studies was 7.1 mm (range 4.0 - 11.0 mm). Data are found in Table 1. After seven months mercenaria ♂ x campechiensis ♀ averaged 23.9 mm in length and the other cross averaged 24.5 mm. This difference is not considered significant.

Approximately 80 per cent of the total increase in length occurred in May, June, and July with the greatest growth during June. The studies were discontinued in January 1955. A sudden freeze while the clams were left exposed following measurements resulted in a mortality of 81 per cent in one group and 42 per cent in the other.

Comparison of Growth of Five Groups of Clams

A second series of experiments begun on September 15, 1954, was concluded in January 1956. The hybrids ranged in length from 3.5 to 27.0 mm. Two size groups were established; all clams under 10 mm were separated from those 10 mm or larger. The five groups in this series were comprised of mercenaria, two groups of mercenaria ♀ x campechiensis ♂ and two groups of campechiensis ♀ x mercenaria ♂. Because of the large number of individuals in each group, random samples were measured and weighed from September through January 1955. All the clams in each group were measured in successive months beginning in February 1955. No data are included for campechiensis which suffered heavy mortalities by February 1955. A summary of the average length and weight at 6 month intervals of the five groups is shown in Table 2.

The greatest increase in length occurred in the smaller hybrids, which averaged 34.9 and 35.6 mm at the end of 15 months (see Fig. 1). During the first six months within the smaller size group fluctuations occurred in average length as shown in Figure 1. These merely reflect the normal variation in random samples. The two larger groups of hybrids averaged 40.4 and 41.2 mm, respectively. Venus mercenaria showed the slowest growth rate and averaged 26.2 mm. The total increase for both crosses in the smaller hybrid group was 29.2 mm, but mercenaria ♀ x campechiensis ♂ was 1.4 g heavier than the reciprocal cross. In the larger series of hybrids campechiensis ♀ x mercenaria ♂ showed a greater increase in total length and total weight. These differences in rate of growth as measured by total length or weight between the hybrid clams were not great enough to be considered important.

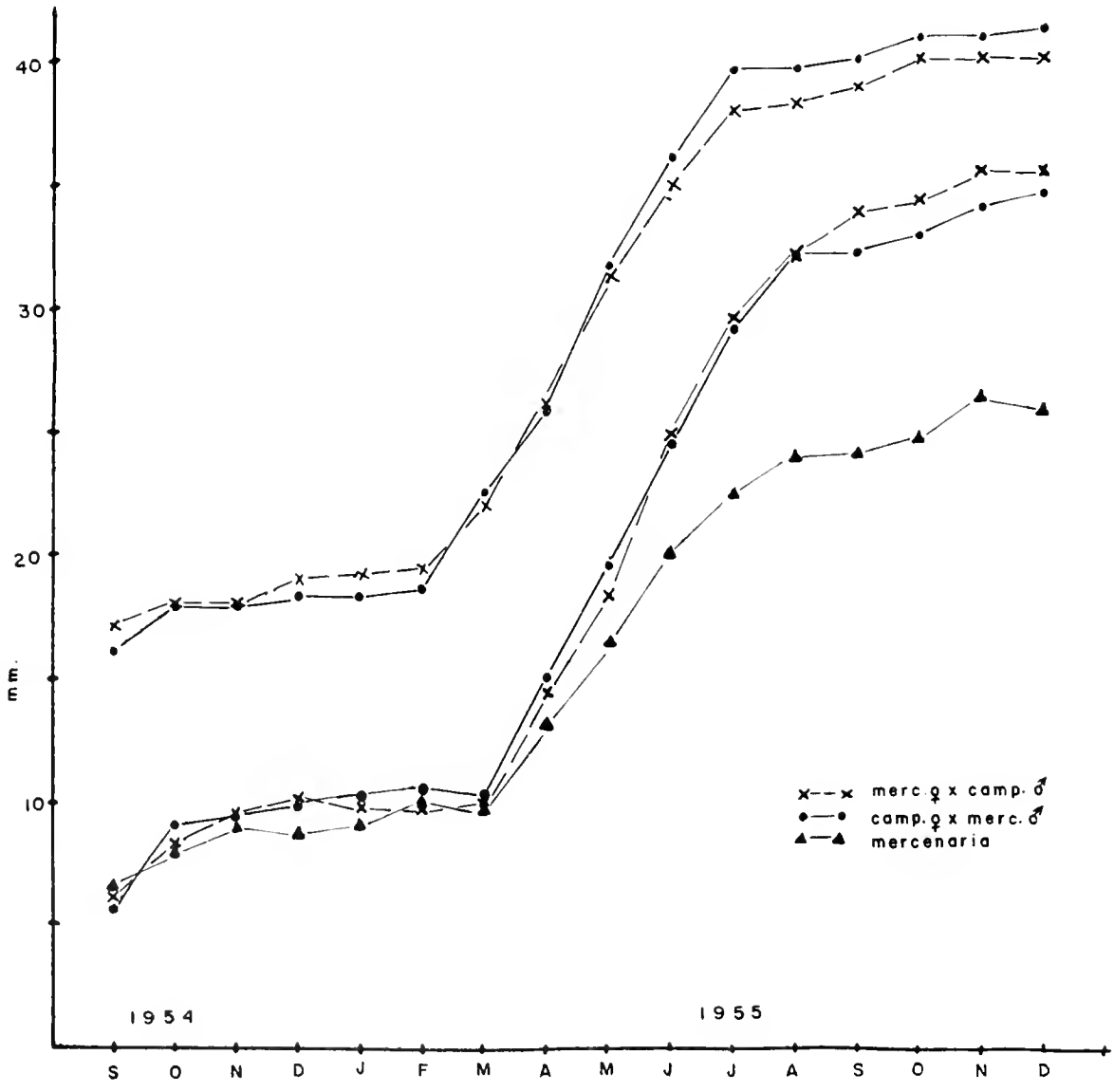
Table 1. Growth and survival of two groups of hybrid clams.

Date	Average Length mm.	Range mm.	No. of live clams
V. campechiensis ♀ x *V. mercenaria* ♂			
1954			
May 10	7.1	4.0-11.0	199
June 10	11.0	7.5-16.0	190
July 9	16.8	12.5-23.0	171
Aug. 10	20.4	15.0-27.5	170
Sept. 10	21.9	16.0-29.0	170
Oct. 11	22.9	16.0-30.0	169
Nov. 11	23.8	16.5-30.5	169
Dec. 9	23.9	16.5-31.0	169
1955			
Jan. 10	25.1	17.0-31.0	32
V. mercenaria ♀ x *V. campechiensis* ♂			
1954			
May 10	7.1	4.0-11.0	170
June 10	10.7	5.5-15.5	169
July 9	17.0	8.5-22.0	169
Aug. 10	20.8	13.0-26.0	168
Sept. 10	23.1	15.0-29.0	168
Oct. 11	23.7	15.0-29.0	168
Nov. 11	24.2	15.0-30.0	162
Dec. 9	24.5	15.5-30.5	154
1955			
Jan. 10	24.7	17.0-30.0	90
Feb. 10	24.9	17.5-29.5	81

Table 2. Average increase in length and weight of five groups of clams.

	Sept. 1954	March 1955	Sept. 1955	Dec. 1955	Total Increment
V. mercenaria					
av. length	6.7	9.9	24.5	26.2	19.5 mm
av. weight	0.09	0.3	5.0	6.9	6.8 g
V. camp. ♀ x *V. merc.* ♂					
av. length	5.7	10.4	32.4	34.9	29.2
av. weight	0.05	0.6	10.5	13.6	13.5
V. merc. ♀ x *V. camp.* ♂					
av. length	6.4	9.9	34.1	35.6	29.2
av. weight	0.07	0.2	12.8	15.0	14.9
V. merc. ♀ x *V. camp.* ♂					
av. length	17.2	19.5	38.4	40.4	23.2
av. weight	1.5	2.2	19.3	22.9	21.4
V. camp. ♀ x *V. merc.* ♂					
av. length	16.2	18.6	39.3	41.2	25.0
av. weight	1.2	1.9	21.7	24.1	22.9

Fig. 1. Increase in average length of Venus mercenaria and hybrids at monthly intervals.



Greatest increase in length occurred during the five month period April through August (75 to 84 per cent). Growth rate decreased in September, but showed a slight increase in October and November. The increment in total weight within each group followed the some pattern as that for total length.

Notes on Survival

Heavy mortalities were noted during the months of January and September. Some mortalities during the cold months were due to exposure of the clams following measurement before they were returned to the bottom. Heavy mortality during September 1955 may be attributed in part to a sharp reduction in salinity. Average rainfall per month from May 1954 through July 1955 varied from 0.6 to 6.2 inches. Salinity was rather stable during this period ranging from 31 to 37 o/oo. In August and September 1955 three hurricanes passed through the area accompanied by heavy rains. Salinities dropped to 15 o/oo when a total rainfall of 43.8 inches was recorded for the two months. Salinities increased in November 1955 to range between 27 and 32 o/oo and by December were above 30 o/oo.

A peculiarity was noted in the behavior of the siphons of the hybrid clams. During low tide the siphons frequently were observed lying extended on the bottom. This condition was never noticed in mercenaria, and such behavior might render the hybrids vulnerable to predators.

Discussion

Hybrid clams produced by crossing mercenaria and campechiensis grew more rapidly than the species mercenaria. Unfortunately, the species campechiensis did not survive for further comparison. Failure of campechiensis to survive the cold weather suggests a possible lack of resistance to cold. Native campechiensis are found in North Carolina growing in the intertidal zone, however, the clams used in this study were progeny of clams from Florida. Further studies to compare the survival of native campechiensis would be of interest.

The pattern of monthly growth rates as measured by increase in total length was similar for the small and large size groups of hybrids. These results are similar to the previous findings in the same area described by Chestnut (1952) for adult mercenaria.

A comparison of the growth of mercenaria with results of Gustafson (1954) for similar size clams in Maine show that growth occurred over a much longer period in North Carolina.

Further studies should be made in subtidal areas of this locality to determine the effect on growth rate and mortalities.

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BIOLOGY

of

SHELLFISH ENEMIES

OUR PRESENT KNOWLEDGE OF THE OYSTER PARASITE "BUCEPHALUS"

Sewell H. Hopkins

A. & M. College of Texas, College Station, Texas

The purpose of this report is to put on record, for oyster biologists, what parasitologists now know about the oyster-castrating flatworm "Bucephalus".

In 1827 the German zoologist von Baer discovered a larval trematode (cercaria) which developed from branching tubular parasites in the gonads of European fresh-water "clams". This cercaria had a tail with a bladder-like stem and two long flexible lateral branches. In its characteristic swimming position, hanging in the water, head down, with tail branches stretching to each side, it resembled the head of a steer, so von Baer named it Bucephalus, meaning "ox head".

This type of cercaria is now known to be the characteristic larval form of an entire family of trematodes or flukes, the Bucephalidae, whose numerous adult forms live in the intestines of marine and fresh-water fishes. The second cercaria of the Bucephalus type, Bucephalus haimeanus, was found by Lacaze-Duthiers (1854) in European oysters and cockles of the Mediterranean Sea. The third one, Bucephalus cuculus McCrady (1874) was found at Charleston, South Carolina, in American oysters, Crassostrea virginica. Both Lacaze-Duthiers and McCrady noted that the gonads of parasitized oysters were destroyed, so that these oysters were completely sterile. "Parasitic castration" of the hosts is caused by all the bucephalid larvae known to date, whether in oysters, cockles, clams, scallops, pearl oysters, mussels, or fresh-water clams.

Textbooks give the following account of the life history of the oyster Bucephalus: Cercariae emerge from sporocysts (the branching tubes in the gonad of the oyster) and swim around until they happen to run into a silverside minnow (Menidia). The cercaria then penetrates the skin of the silverside, encysts in its flesh, and grows but does not become sexually mature. When the infected silverside is eaten by a billfish or needle gar (Strongylura), the bucephalid comes out of its cyst and develops into an adult in the intestine of this third and final host.

There is good evidence now that this textbook account is not quite right. It is based partly on guesses by European parasitologists, who have never done any experimental work on Bucephalus to this day, and partly on a pioneer study by Tennent (1904, 1905, 1906, 1909) of Johns Hopkins University fifty years ago. Tennent was handicapped not only by being a pioneer, but also by a prejudice against "splitting species" which let him to believe that all bucephalids must be physiological varieties of one species. In consequence, he combined stages of at least two and probably three different species into one composite

life cycle, the one given in the textbooks. Tennent never proved that the immature bucephalids in silversides, or the adults in needle gars, had any connection with the larval forms in oysters. The only thing he did prove is that oysters could be infected (100 per cent, in his experiment) by injecting feces of bucephalid-harboring gars (Lepisosteus) into the mantle cavities of oysters. It is unfortunate that two different fishes called "gars" were involved in Tennent's account, for of course there is no kinship between the marine needle gar, Strongylura, and the true gar, Lepisosteus, of lakes, rivers, and estuaries. Unfortunately, Tennent paid no attention to the characteristics of the adult bucephalids of the gars (Lepisosteus) which he used in his oyster-infecting experiments, because he assumed that all bucephalids were the same species. Now, the only bucephalid known from true gars is very different from any of the several species in needle gars and silversides, and has its immature encysted stage not in silversides but always in mullets (Mugil cephalus and M. curaema). Our present knowledge of the life history of the oyster bucephalid rests here, with the probability but not yet the certainty that the cercariae from oysters penetrate the fins of mullets and encyst inside the fin rays. If this is true, then the adult forms develop in the intestines of gars (Lepisosteus) which eat the infected mullets. Oysters presumably become infected by contact with the feces of infected gars (Hopkins, 1954).

I have seen statements in the literature to the effect that Bucephalus cannot be an important oyster parasite because it is so uncommon. True enough, in most places hundreds of oysters may be examined without finding a single Bucephalus infection. But in some places a high percentage of the market-size oysters contain this parasite, and therefore contain no gonads and produce no eggs or sperm. In Tennent's time, fifty years ago, some oyster beds in Pamlico Sound and in Newport River above Beaufort, North Carolina, had Bucephalus infections in as high as 33 per cent of the oysters. Even higher percentages of infection in local areas have been reported to me by colleagues in recent years, and not all in the South; one such report concerns a small river in New Jersey. I have known bayous in Louisiana which could be counted on to show Bucephalus in 10 to 25 per cent of all oysters over one year old. The oyster beds with a high incidence of Bucephalus, whether North or South, seem to be found mostly in low salinity estuaries or in small marshland waterways. Bucephalus seems to be rare or absent in broad open bays or sounds where the salinity is higher and the oysters are surrounded by a much larger volume of water. This agrees with what would be expected if oysters are infected only by contact with fecal material from gars.

The effects of Bucephalus on oysters are no better known now than at the time of my last report (Menzel and Hopkins, 1955, 1955a). Except in the very early stages of the infection, bucephalid sporocysts always destroy the gonad completely; usually not a single egg or sperm cell can be seen in an oyster which contains mature sporocysts. There is some slight evidence that in the fairly early stages of infection, while sporocysts are still confined to the gonad region, Bucephalus may

stimulate growth of the oyster, as similar gonad parasites of snails have definitely been proved to do. There is much better evidence that older Bucephalus infections damage tissue other than the gonad and interfere with growth. Presumably the parasite would eventually kill the host, if the oyster did not die of old age or something else first, but this has not yet been proved. Gastronomically, Bucephalus may be considered a beneficial parasite in southern waters, not only because it gives the oysters some badly needed birth control, but because the sporocysts remain in the oyster the year around while the reproductive elements are mostly lost in the long spawning season (half the year, on the Gulf Coast). Bucephalus-infected oysters have an excellent taste and are fat-looking and full of glycogen when uninfected oysters are spawned out, thin, and tasteless. On several occasions, I have rather facetiously suggested that it might be a good idea to produce Bucephalus infections in a whole bed of oysters, and thus supply caponized oysters for a premium market. Even if that idea is not entirely practical, I still think that Bucephalus is a very interesting parasite and deserves further study.

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THE FLATWORM *PSEUDOSTYLOCHUS OSTREOPHAGUS* HYMAN, A PREDATOR OF OYSTERS

Charles E. Woelke

State of Washington, Department of Fisheries
Shellfish Laboratory, Quilcene, Washington

General Biology

In the spring of 1953 the Department of Fisheries of the State of Washington received a request from the Olympia Oyster Growers Association to investigate unusually heavy losses of spat of *Ostrea lurida* during the first year following setting. Investigation of these losses led to the discovery that the flatworm is a predator (Woelke, 1954).

Experimental lots of cultch were placed in water at staggered intervals through the setting season and removed for examination after periods ranging from two weeks to nine months. These experiments, together with observations on commercial cultch, were designed to ascertain the time and magniturde of losses. In late September 1953 heavy spat losses were noted on both experimental and commercial cultch. Over 70 per cent of the dead spat had very small holes of an unusual oval shape in the right valve ranging from 106 x 113 to 180 x 260 microns with an average of 147.4 x 189.9 microns (Plate 1).

Observations and laboratory experiments proved the causative organism was a flatworm of the Polycladida (Plate 2). The discovery that a flatworm possesses the ability to penetrate an oyster shell was new and was greeted with some skepticism by both oyster growers and scientists. Specimens were sent to Dr. Libbie Hyman for identification. She reported them to be an undescribed species of the genus *Pseudostylochus*. In 1955 Dr. Hyman published a description of this new species and gave it the specific name *ostreophagus*.

From September 1953 to September 1954 the number of spat per square inch on one commercial planting of cemented egg-divider cultch declined from 28.3 to 3.4 spat. From samples collected at least once a month it was found that over 90 per cent of the dead were victims of the flatworm. Population density of the worm in this particular area was in excess of 600,000 per acre. It has been found that flatworms will destroy spat from setting size to 4 mm at a rate of 85 per month and spat from 4 to 12 mm at a rate of 41 per month. In laboratory experiments the worm successfully attacked the spat of the Olympia oyster (*Ostrea lurida*), the Pacific and Kumamoto oysters (*Crassostrea gigas*), and the Virginia oyster (*Crassostrea virginica*) as well as Olympia oysters up to two years of age.

Destruction of the oyster by the flatworm is accomplished by penetrating the shell and in some manner separating the adductor muscle

Plate 1. Typical oval hole found in upper valve of dead oyster spat.

from the right valve. The worm then crawls between gaping valves and ingests the entire live oyster. Physical irritation has caused worms to regurgitate whole live oysters. The actual manner in which penetration of the shell is accomplished is not too well understood. However, repeated observation of worms attacking oysters, and the presence of gelatinous mucoid droplets on the incomplete shell perforations of attacking worms, suggest that penetration is accomplished chemically. There is some evidence that once the shell has been penetrated the worm extrudes the edge of its pharyngeal folds through the small hole into the shell cavity. The adductor muscle is then severed and the oyster gapes, becoming easy prey to not only the flatworm, but also other scavengers in the immediate vicinity. Experiments by Smith (1955) to determine whether flatworm homogenates would dissolve oyster shell gave negative results. His experiments showed also that Pseudostylochus ostreophagus was primarily a predator and not a scavenger.

Distribution of the worm on any given bed or in any general area is extremely variable. They are essentially subtidal and as such are very common in the Olympia oyster dikes. Diked areas located highest in the intertidal zone contained greater numbers than lower ones. The worm is present on nearly all oyster beds in Puget Sound.

Specimens of the most common flatworm found in the Pacific seed oyster producing areas in Japan were collected by the author and sent to Dr. Libbie Hyman. These were found to be the same species attacking the Olympia oyster. Samples of dead spat of Crassostrea gigas in Japan indicated that the flatworm caused losses as high as 49.1 per cent in some areas. The presence of live flatworms in the shipments of Pacific oyster seed arriving at Washington ports from Japan fairly definitely establishes that it is another exotic introduced to our waters with this oyster.

Life History

Life history studies conducted in 1954 and 1955 revealed that in Washington egg laying extends from March through October. Freshly laid eggs average 147 microns in diameter. Estimates of fecundity based on egg density and size and number of egg masses deposited by individual worms in one season gave a range of 3,373 to 84,332 eggs, an average of 31,472 per worm. Incubation time at 15-17° C ranges from 30 to 34 days before hatching. The typically polyclad larvae measure about 141 x 208 microns at hatching. Part or all of the larvae are free swimming for an unknown period of time. Worms as large as 1140 x 640 microns have been taken in plankton samples and worms up to 0.5 cm in length have shown swimming tendencies in the laboratory. There is some question as to whether a definite "settling size" actually can be found. Food of the larval worm is not known. A maximum of 16 larvae per 20-gallon plankton sample has been found.

In the laboratory worms grew from an "apparent settling size" of 1140 microns to 0.50 cm in 21 days and from 0.50 cm to 1.49 cm in 66 days.

Plate 2. Three specimens of Pseudostylochus ostreophagus on a Pacific oyster shell.

From November through July random samples of large numbers of worms show virtually all sizes up to the maximum found (3.20 cm) at any given time. The disappearance of adult worms in the field and loss of all laboratory stock in midsummer for three successive years probably indicates a life span of one year or less.

In laboratory experiments the worms definitely avoided strong light. Temperatures in excess of 35° C are fatal after a one hour exposure. Salinities below 10.2 °/oo are fatal after seven days, 7.9 °/oo at three days, 5.4 °/oo in 24 hours and fresh water after one hour. Relative abundance of a year class and salinity may be related since salinities of less than 28 °/oo prevailed with low populations and over 29.5 °/oo were accompanied by strong year classes.

Control

Fresh water treatment of oyster seed is the most obvious method of control and has been successful experimentally. Commercial scale dipping of seed oysters in a salt brine has been very successful. Japanese seed oyster growers have used chlorinated lime baths at a concentration of about 450 °/oo with moderate success. In one commercial operation the worms were forced off the cultch by placing seed oysters high in the intertidal zone for three weeks. Another grower claims to have had success driving the worms from his beds by burying perforated containers of creosylic acid in diked areas. Presumably the acid very slowly diffuses from the jar and stimulates the worms to move. Alternating electrical current at a density of about 0.95 milliamps per cubic cm caused an actual tissue breakdown and death of worms.

Summary and Conclusions

A new oyster predator Pseudostylochus ostreophagus Hyman introduced to Pacific Coast oyster beds from Japan has caused extremely heavy losses of Ostrea lurida spat. Life history studies indicate it follows a typical polyclad type of development. A life cycle of a year or less is indicated. Reproductive success or failure may be dependent on salinity. A number of promising control measures have been discovered which probably will result in the development of practical control measures.

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SOME EFFECTS OF HIGH-FREQUENCY X-RAYS ON THE OYSTER DRILL UROSALPINX CINEREA[1]

William J. Hargis, Jr.

Virginia Fisheries Laboratory, Gloucester Point

Mary F. Arrighi, Robert W. Ramsey, and R. Williams

Medical College of Virginia

Scientists of the Department of Agriculture (Bushland et al. 1955) recently announced the successful eradication of the screw-worm, Callitroga hominivorax, from the Dutch Island of Curacao. This was accomplished by releasing x-ray sterilized males, which competed successfully with normal indigenous males for the females. After such matings the monogamous females deposited only sterile egg masses. Although several releases were necessary, eventually no fertile eggs were detected at any of the numerous observation points. Subsequent checks failed to reveal any live flies.

Because existing information concerning ecology and reproduction of drills appeared favorable, our group was encouraged to investigate this technique as a possible control method for oyster drills. The present paper is a report of a series of experiments which were designed to determine the lethal dose.

Specimens collected from the York River, Virginia, were transported to Richmond wrapped in moist cheesecloth, and held in perforated plastic dishes in a covered, aerated, thirty-gallon aquarium of seawater which was constantly filtered. Locations of dishes in the all-wood rack were randomized in order to eliminate possible position effects. The animals were fed for several hours once a week by placing pieces of oyster meat in the dishes. During the irradiation period both control and experimental animals were transfered to small plastic boxes and handled in the same manner except for the actual x-ray exposure of the latter. Moist blotting paper was placed in each box to prevent desiccation. Following the last dose the blotting paper was removed and both controls and irradiated drills were returned to their regular containers in the tank.

The x-ray source was a beryllium window 1000 KVP machine located at the Medical College of Virginia. The snails were placed around the periphery of a circular wooden platform which was rotated at approximately two revolutions per minute. Dose rate measurements were made under the same conditions, with a thimble chamber substituted for one of the plastic

[1] This reserach was conducted under a contract with the U. S. Fish and Wildlife Service, No. 14-19-008-2372, Study of Oyster Drills in Chesapeake Bay. Contributions from the Virginia Fisheries Laboratory No. 75.

boxes. Two millimeters of aluminum filtration were added to remove the very soft components of the beam. The half-value layer in lead is 1.3 millimeters under these operating conditions. At a dose rate of 576 roentgens per minute the minimum dosage of 3,000 r required an exposure of 5 minutes and 12 seconds. Larger doses were secured by increasing, doubling, tripling, etc., the exposure time. For convenience higher levels were obtained by successive increments of 3,000 r each.

Series I

On February 3, 1956, six groups of drills not segregated by sex were irradiated at dose levels from 3,000 r to 18,000 r. Subsequent daily observations made over an 81-day period yielded the cumulative mortality data illustrated in figure 1. Mortalities exceeded 40 per cent only in the 6,000 r group. The others were near or below the level of the controls. Although there is this single exception to the general mortality curve pattern it seems evident that, under the conditions of the experiment, dose levels up to and including 18,000 r do not have a marked lethal effect on U. cinerea.

Series II

The cumulative mortalities of four groups of males and six groups of females irradiated in April were greater than those of the controls (Fig. 2). Of the dose range administered, from 21,000 r to 48,000 r, we are able to conclude that the lethal dose for this group of drills is from 24,000 r to 27,000 r. Although there are some slight discrepancies between these curves (e.g. the 48,000 r ♀♀ experienced lower mortalities than lower dose groups) we may assume that, given a longer observational period, all the drills receiving doses higher than 27,000 r would have died.

These data suggest that there may be a sexual difference in susceptibility to x-ray injury. All three of the high dose levels administered to males produced total mortality by the sixty-fourth day after irradiation. In comparison, only one group of females had been eliminated by the same time. None of the female groups which received doses in excess of 33,000 r was eliminated by the sixty-ninth day when the experiment was terminated. The experiments were not designed to test this point, however, and the data are not amenable to statistical analysis.

This phase of the experiment was terminated when the remaining irradiated drills and some of the controls were sacrificed for gonad smears. Although these smears appeared to indicate some adverse effects produced by radiation, the small number of subjects involved and the uncertainties of the interpretation render further conclusions unwise.

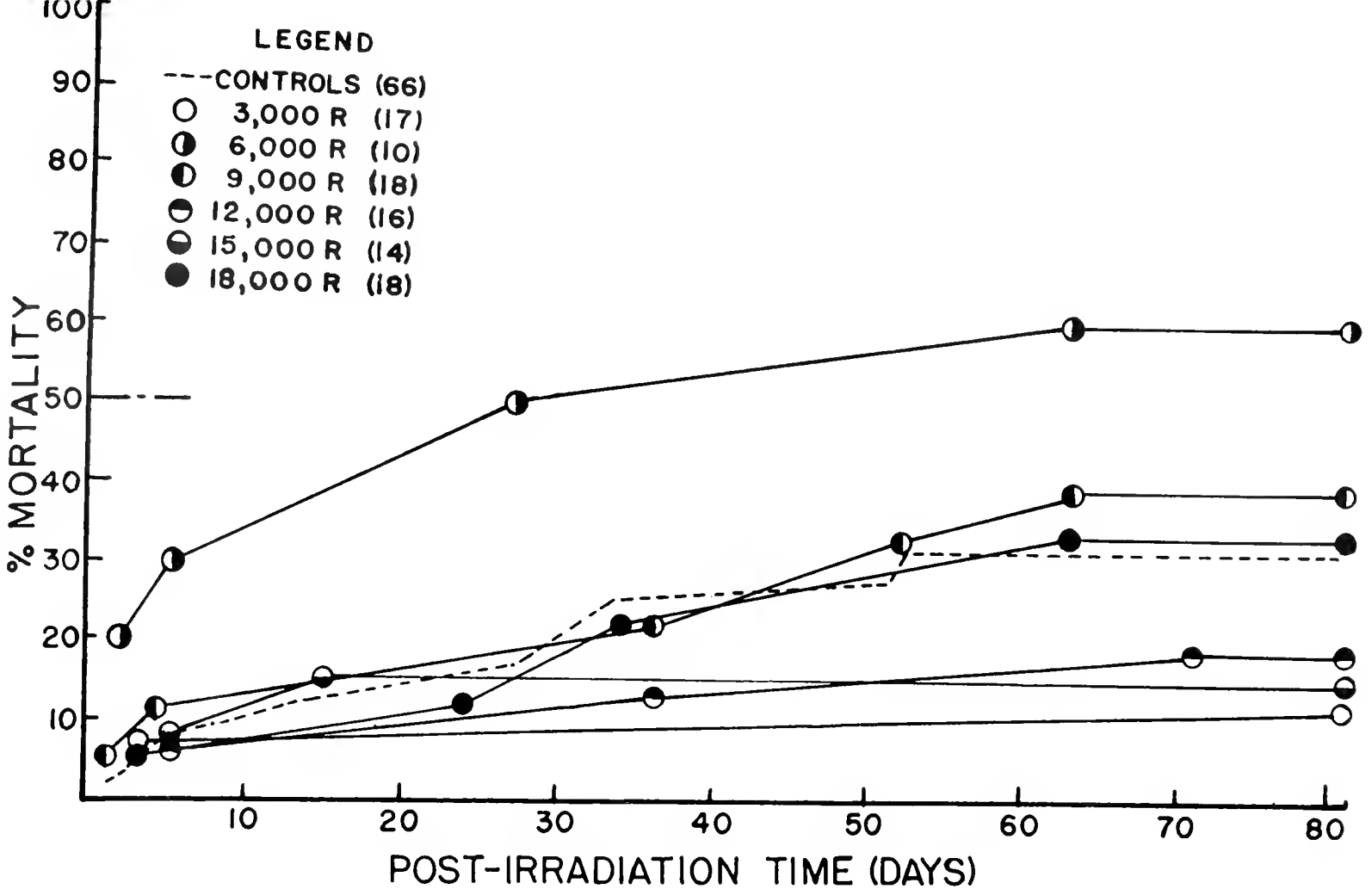

Fig. 1. Series I. Percentage mortality occurring in six groups of oyster drills, Urosalpinx cinerea, which were subjected to varying dosages of high-frequency x-rays. The numbers in parentheses are the individuals in each group.

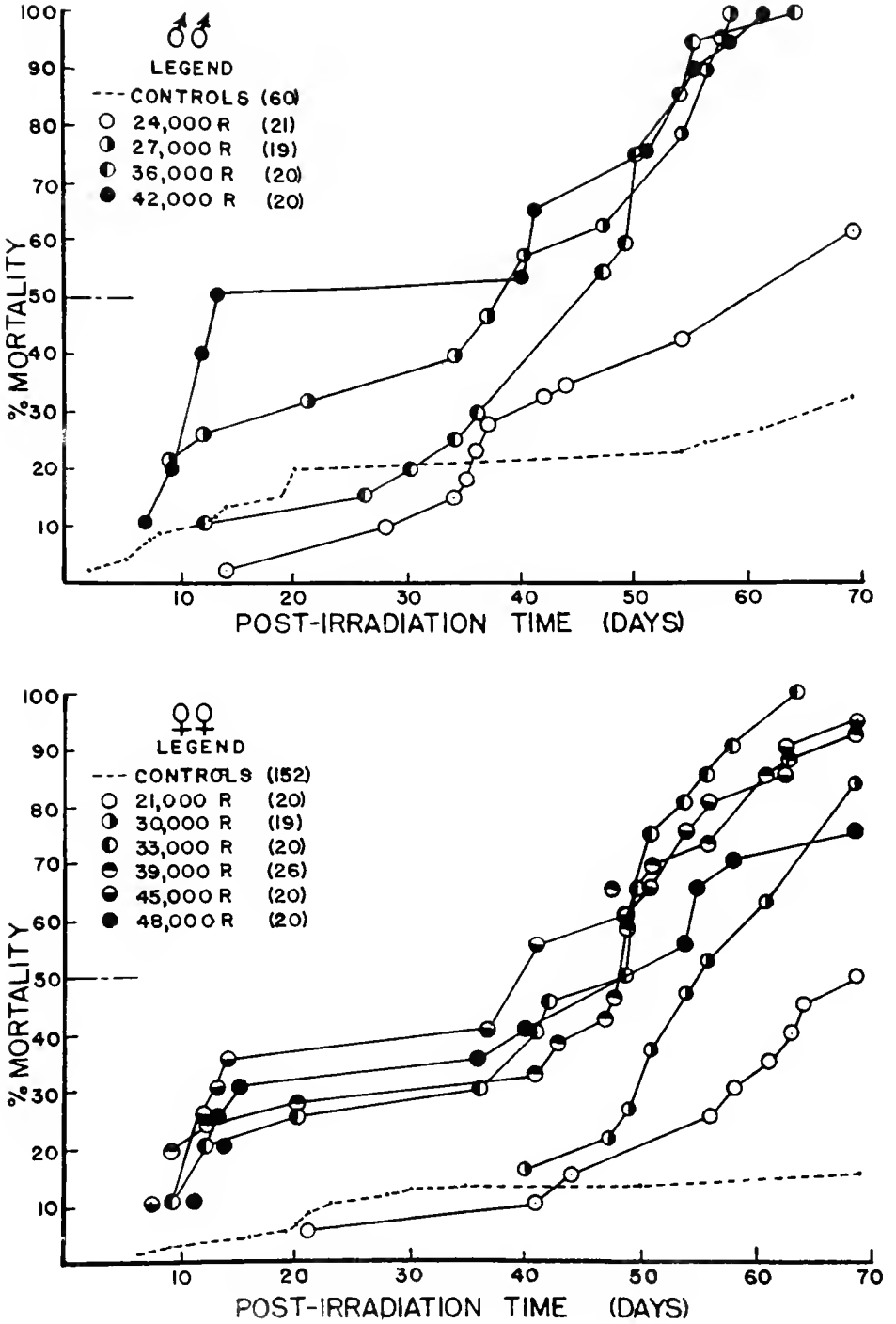

Fig. 2. Series II. Percentage mortality occurring in ten groups of oyster drills, Urosalpinx cinerea, which were subjected to varying dosages of high-frequency x-rays. The numbers in parentheses are the individuals in each group. Isolated points on graph indicate points of two or more curves which are identical.

We have shown that Urosalpinx cinerea from the York River, Virginia, can tolerate high dose levels of high-frequency x-rays. Like many invertebrates, drills survive irradiation for longer periods of time than mammals usually do. It is interesting that Bonham and Palumbo (1951) found that the gastropods Radix and Thais withstood large doses of high-frequency x-rays.

Even if irradiation is applicable as a control tool, the costs of handling and treating with an x-ray machine would be prohibitive. However, more economical sources, such as Cobalt-60, could probably be made available for commercial dosages.

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COPPER, A POSSIBLE BARRIER TO OYSTER DRILLS

John B. Glude

U. S. Fish and Wildlife Service
Annapolis, Maryland

The oyster drill (Urosalpinx cinerea), a small carnivorous gastropod with a healthy appetite for young oysters, has long been recognized as a limiting factor in oyster production in the saltier waters of the Eastern Coast of the United States (Carriker 1955).

Many investigators have searched for methods of controlling this predator, but few have considered seriously the possibility of fencing subtidal oyster beds to exclude drills. The recent development of the aqualung makes it possible to install low fences on oyster beds which occur at depths of 5 to 50 feet.

A project to design a fence which would stop oyster drills was begun at the U. S. Fishery Laboratory at Boothbay Harbor, Maine, in the autumn of 1955 with the assistance of Gareth W. Coffin and George W. Griffith. Drills from York River, Virginia; Chincoteague Bay, Maryland; and Milford, Connecticut, were shipped to Boothbay Harbor and kept in tanks of warm sea water so they would crawl actively.

Laboratory Experiments

The first approach was to determine if drills would cross all metals. Zinc and iron were tried first but the drills readily crawled across strips of these metals. Next, 10 drills were placed in a wooden tank of sea water and surrounded by a horizontal three-inch strip of 22-gauge (.024" thick) copper sheet. The drills did not cross the copper; in fact, each one retracted its foot and remained motionless as long as the copper plate was in position.

The experiment was repeated by placing 25 Urosalpinx inside a 24-inch square enclosure of eight-gauge (.128" diameter) copper wire on the bottom of a wooden tank and oyster spat just outside of the wire to serve as bait. During the first seven hours, while the water was gradually warmed from 5 to 25° C, nine drills crawled to the wire, touched it with a tentacle or foot, and turned back. Most moved only a short distance away from the wire, then retracted their foot and remained motionless for the duration of the experiment. After 24 hours the drills became inactive. Since there was no flow of water through the tank, it is likely that the copper concentration gradually increased until the drills were immobilized.

In a later experiment 25 drills were placed inside a 24-inch square enclosure made by laying a 10-gauge (.102" diameter) iron wire

on the bottom of a wooden tank containing clean warm sea water. Three of the drills immediately crossed the wire which proved that wire was not a mechanical barrier.

The iron wire was replaced with 14-gauge (.065" diameter) copper wire and 30 Urosalpinx were placed within a 15-inch square enclosure in a wooden tank without running water. Oyster spat were placed outside of the enclosure to attract the drills. No drill crossed the copper wire and at the end of 10 days all were dead. The increase in copper concentration resulting from ionization of five feet of 14-gauge copper wire in 45 gallons of sea water at 19.5 to 22.5° C proved lethal to oyster drills. A control lot of drills held in a similar tank without any copper had a mortality of less than 5 per cent during this period.

Two experiments showed that the oyster drill, Urosalpinx, and the mud snail, Nassa obsoleta, must touch the copper or brass or approach it very closely to be repelled. Both species failed to cross a 1/8-inch diameter copper wire placed on the bottom of a tank, but both succeeded when the wire was covered with a 1/8-inch of clean sand. In another experiment the 1/8-inch copper wire was bent upward to provide 5/8-inch to 3/4-inch clearance between it and the bottom of the tank and several Nassa crawled under the wire. Apparently the concentration of toxic ions is great enough to repel the snails only in the immediate vicinity of the copper.

Several experiments demonstrated that copper or brass effectively repels Nassa in running water. The water was too cold (2 to 5° C) to try similar experiments with Urosalpinx which is not very active below 10° C. Since Nassa was repelled by copper to about the same extent as Urosalpinx at higher temperatures, it was substituted in these experiments. In one experiment a clean eight-gauge copper wire was placed across the bottom and up the sides of a wooden tank which was 12 inches across the ends and 34 inches along the sides. Running sea water at a flow of 230 milliliters per minute entered at one end of the tank and left through a standpipe at the other which maintained a water depth of three inches. Twenty Nassa were placed at the downstream end of the tank and two cracked soft-shell clams, Mya, were placed upstream of the wire to serve as bait. After 18 hours the flow was increased to 1200 milliliters per minute, and three hours later to 3,540 milliliters per minute. Since no Nassa had crossed the wire, the flow was later increased to 4,800 milliliters per minute. Even at this flow a single copper wire effectively stopped Nassa. When the wire was removed the snails spread throughout the tank.

In a similar experiment a one-inch brass screen fence was placed across the width of a tank 12-inches wide and 34-inches long and a flow of 9,480 milliliters of sea water per minute was introduced at one end. None of the 25 Nassa placed downstream of the fence succeeded in crossing this barrier to reach the cracked clams which had been placed there as bait.

The effectiveness of copper and brass screen fences at greater current velocities was tested in a long trough eight inches wide by seven inches deep. Sea water at 3 to 4° C was introduced at one end of the trough through a two-inch diameter pipe, and the depth was maintained at 4½ inches which produced a surface velocity of 11 centimeters per second. Nassa obsoleta and Littorina littorea were placed inside two-inch high rectangular enclosures of brass and copper and their movements recorded. The snails were encouraged to escape by their tendency to move upstream and by the cracked clams which had been placed upstream as bait. Later the current velocity was increased to 23 centimeters per second (0.45 knots) at the surface and 16 centimeters per second (0.31 knots) at the bottom by reducing the depth of the water to three inches. None of the snails crossed the barriers at these current velocities which are similar to those found on subtidal oyster beds.

The possibility of reducing the cost of a drill barrier by using a narrow strip of brass screen at the top of a plastic screen fence was explored. An 18-inch square enclosure 1½ inches high was made of wooden lath and lined with Saran plastic screen. The plastic was fastened to the wood with iron wire staples and the fence was nailed to the bottom of a wooden tank filled with sea water warmed to 20° C.

Three of the 33 Urosalpinx placed inside of this enclosure crawled over the fence during the first 90 minutes which proved that the plastic itself is not an effective barrier for oyster drills. A 1/4-inch strip of brass screen was then fastened to the upper part of the fence with copper tacks; however, the brass was in contact with the iron staples which held the plastic in place. During the next 2½ hours three drills crawled up the plastic, over the brass, and down the outside of the fence.

All of the iron staples were then removed from the fence and the 33 drills were again placed inside the enclosure. During the next 24 hours we saw seven drills which crawled up the plastic to the brass and then crawled or fell to the bottom. None crossed the barrier.

For a further verification of these results iron staples were driven through the brass screen and into the wood lath at two-inch intervals, and 66 Urosalpinx placed inside the enclosure. In 24 hours seven crawled over the fence. This demonstrated that the iron, being more active, had ionized instead of the copper when the two metals were in contact. This had destroyed the effectiveness of the fence since the drills are not repelled by iron ions as they are by copper. It was found later that this effect had been reported in 1824 by Sir Humphry Davy in relation to preservation of copper sheathing on ships. Davy also showed that when metallic copper is coupled to iron or zinc it fails to prevent fouling.

Laboratory experiments similar to those with oyster drills described above were conducted using the snails Nassa obsoleta, Littorina littorea, Littorina obtusata, and Thais lapillus. The snails

Nassa and Thais were repelled by copper ions to about the same extent as Urosalpinx. The two species of Littorina proved to be more resistant to copper ions. The eight-gauge copper wire stopped Urosalpinx, Nassa, Thais; whereas a few Littorina of both species crossed it. A one-inch or two-inch brass screen fence of 10 to 20 meshes per inch contained all of these species in laboratory experiments even at a water velocity of 16 centimeters per second.

Field Tests of Copper Barriers During 1956

The first field trials of copper barriers were conducted at Mill Cove, West Bath, Maine during May and June, using the mud snail, Nassa obsoleta. This snail is extremely abundant in the intertidal zone and is easily attracted to any bait such as a cracked soft-shell clam or hard-shell clam. Since we had observed that Nassa was repelled by copper to about the same extent as Urosalpinx in laboratory experiments which had been conducted during the winter, this species seemed to present a suitable substitute for Urosalpinx for preliminary field trials.

The first experiment which was set up in Mill Cove consisted of a brass screen fence four inches high and five feet square. The brass mesh was pushed down into the soft mud flats so that it extended above the surface $2\frac{1}{2}$ inches. The mud snails were removed from the inside of this enclosure and placed around the outside along with several hundred other Nassa which were collected from the adjacent flats. A number of cracked soft-shell and hard-shell clams were placed inside of the enclosure to act as bait. The area was inspected daily or every other day during the period from May 4 to June 4, 1956. Only 20 Nassa were found inside the enclosure after the first three days, during which occasional Nassa were found that had been missed when the area was cleaned.

A control plot consisting of a four-inch high galvanized iron hardware cloth fence surrounding a baited enclosure was maintained during part of this time. The mean number of Nassa entering the control plot per day was 7.71 as compared to 0.74 for the test plot.

The second experiment consisted of two identical 15-inch square enclosures made of four-inch brass screen of four-mesh per inch. These fences projected above the flats approximately $2\frac{1}{2}$ inches. Cracked clams were placed inside of the "offshore" experimental area and large numbers of Nassa were collected and placed around the outside of the fence periodically. The "inshore" experiment was exactly the opposite in that the bait was placed around the outside of the enclosure and 100 Nassa were placed inside of the enclosure. Each plot was observed daily or every other day from May 6 to May 25, 1956. During this time, no snail was found inside of the "offshore" experiment. None of the 100 snails escaped from the "inshore" enclosure from May 6 to May 18, even though the experimental animals were replaced with newly collected Nassa twice during this period. On May 18 four pieces of iron wire were

laced into the brass screen, and by the following day only 13 Nassa remained within the enclosure. On May 22, 100 new Nassa were placed inside of the enclosure with the pieces of iron wire still in place. By the next day only 27 remained inside of the enclosure, and by June 4 all of these had escaped. The results of this experiment substantiated laboratory observations that a copper barrier is rendered ineffective if a piece of iron is in contact with the fence. In this case the copper is protected from ionization by the more active iron; and the iron ions are not toxic to these gastropods.

The third field experiment using Nassa consisted of a 36-inch square enclosure eight inches high constructed of 12 mesh per inch Saran plastic screen fastened to a wooden lath frame and inserted three inches into the sediment. A one inch wide strip of new 8 mesh per inch brass screen was fastened to the outside of the top lath on this fence. Bait was placed inside of the enclosure upon each observation, and Nassa which had been collected from the adjacent flats were placed around the outside of the test area. No Nassa crossed this barrier during this experiment which ran from May 8 to May 25.

The fourth copper barrier experiment was set up in the intertidal zone immediately in front of the Boothbay Harbor Laboratory, using the snail Littorina littorea. The test plot consisted of a 15-inch square enclosure formed by inserting a four inch high, six mesh per inch brass screen into the flats $1\frac{1}{2}$ inches. The control fence was the same size but was made of four mesh per inch galvanized iron hardware cloth. The two experimental areas were approximately four feet apart and were at about the same tidal level and in the smae sandy bottom. One hundred medium-to-large-size Littorina were placed inside each enclosure on May 14. After three days only 47 Littorina remained within the control area while the original 100 remained within the test area. The experiment was repeated by placing 50 Littorina in each enclosure on May 21. Eight days later only two Littorina remained within the control area, whereas the original 50 remained within the test area.

The fifth experiment which was conducted at Mill Cove, West Bath, Maine, using the mud snail Nassa, consisted of a control plot surrounded by a four inch barrier of galvanized iron hardware cloth and four experimental areas. One experimental area used the same plastic screen fence which had been described under Experiment 3, except that a brass mesh strip at the top was replaced with a strip of copper, 3/4 inch wide. The second experimental plot utilized the same 15-inch square brass enclosure which was described above under Experiment 2, "offshore", except that the mesh was inverted so that the shiny part of the brass which had previously been below the surface of the flats was now exposed. The third experimental area utilized an identical brass mesh enclosure which had previously been used in the Experiment 2, "inshore", and this fence was again used in its original position. The fourth experimental area was enclosed by a four inch high plastic screen fence with a one inch wide strip of used brass screen around the outside of the top lath. Each area was baited with cracked clams upon each observation, and the number of Nassa which had entered the enclosure was recorded.

During the 28 days of this experiment 691 Nassa entered the control area, or an average of 24.7 per day. Only eight Nassa entered the plastic-fenced enclosure with the copper strip around the top, or an average of 0.35 Nassa per day. Only one Nassa surmounted the second experimental plot which consisted of the four inch brass fence which had been inverted before use.

During the first four days, 108 Nassa (27 per day) crossed the second experimental brass fence which had not been inverted, and it seemed likely that the brass had become corroded enough that too few copper ions were being released to repel Nassa. This fence was then inverted to expose uncorroded brass, and the mean number crossing it each day dropped to 0.94. After about two weeks, however, this fence became less effective once again.

The fourth experimental plot which was enclosed by a plastic fence, topped with a one inch wide strip of used brass, was nearly ineffective since the mean number of Nassa crossing it each day was 16.6 as compared to 24.7 in the control. It has been reported by Edmonson and Ingram (1939) that copper alloys release less copper ions after they have been dried in air following an immersion in sea water. Since the brass screen used in this experiment was treated in this way, it seems likely that this may explain the poor results.

The first field experiments using Urosalpinx are now being conducted at Chincoteague Bay on the oyster grounds of Mr. Richard Kelly. Since these beds are exposed at low tide and have a tremendous population of large drills, they provide an ideal location for copper barrier experiments. The present experiment consists of three plots: a control three feet by six feet in area surrounded by a four inch high strip of galvanized iron hardware cloth; a similar plot fenced with a four inch high brass screen; and a third plot fenced with an eight inch high 12 mesh per inch plastic screen with a 3/4 inch wide copper strip around the outside of the fence near the top. Each area was baited with about a half bushel of small oyster spat, and large numbers of Urosalpinx were gathered and placed around the outside of each enclosure to supplement the natural population. During the period from June 14 to July 25, 593 Urosalpinx entered the control area, or an average of 14.5 per day. Only 14 Urosalpinx were found inside of the enclosure fenced by a brass screen, and two of these were believed to have crawled under the fence through a hole dug by crabs. Excluding these two, the average number entering this plot was 0.3 per day.

The other experimental area surrounded by the plastic and copper fence has been extremely successful. Only two Urosalpinx have been found inside of this plot, and both of these are believed to have crawled under the fence when one corner was washed clear of the bottom by wave action. The effectiveness of this fence after 40 days is very encouraging.

Discussion

The toxicity of copper to aquatic plants and animals has been reported by many authors. Harvey (1955) states that the concentration of cupric ions which is poisonous to marine plants and animals varies around 1,000 milligram per cubic meter. Monier-Williams (1950) quotes Atkin's (1932) statement that most marine mollusks will not tolerate more than 0.1 to 0.2 ppm of copper, but that estuarine species tolerate copper more readily. Marks (1938) lists copper tolerance of some gastropods and shows that 0.05 to 0.15 ppm of copper added to sea water was lethal to some species. Hale 1948) states that fresh water snails were destroyed in most cases within 48 hours by use of copper sulfate in doses of 0.5 to 2.0 ppm. The value of copper in the prevention of marine fouling is well documented by Woods Hole Oceanographic Institution (1952) in its publication describing research conducted for the U. S. Navy. Chow & Thompson (1952) describe an improved method for determining the concentration of copper in sea water. None of these authors mention Urosalpinx or its close relatives.

Several American investigators, including Engle, Newcombe, Lindsay and McMillin, have found that copper sulfate kills the prehatching stages of oyster drills in the egg cases (Carriker 1955). None of these authors considered it an effective control for adult drills.

Only three references to the use of physical or chemical barriers for control of drills have been found. Ota (1946) states that is is possible to protect oysters grown by the "umbrella" type of oyster culture against the attacks of Rapana, a Japanese oyster drill. Ota recommends a special guard of metal consisting of a tin plate six inches in diameter with the edges turned downward and inward fastened to the supporting pole. In this application the metal serves as a mechanical barrier to these large drills. Suehiro (1948) quoted by Korringa (1952) described how Rapana with its soft foot is unable to climb over a spiny object like a chestnut bur clasped over ropes and poles. Carriker (1955) describes Glancy's method of preventing Urosalpinx from crawling up a vertical pipe by interposing an inverted cylinder in which foul gases are supposed to collect.

No published report has been found which describes the repelling effect to Urosalpinx or other oyster drills of ions released from metallic copper in sea water which were observed in the experiments described above.

Application

Further research is necessary before copper can be recommended for protection of commercial oyster beds from drills. Laboratory results must be checked by additional field tests to determine the length of time a copper barrier will remain effective. Fouling by organisms and

silting must be controlled and the effect of copper ions on oysters and other marine species adjacent to the fence must be determined. An economical and practicable fence must be designed, and better methods for releasing copper ions must be explored.

Nevertheless, the discovery that ions from metallic copper repel the oyster drill Urosalpinx may point the way toward effective control of this predator in many areas. A vertical mesh fence a few inches high seems to be the most promising design to decrease silting, and the use of plastic screen may help to reduce the cost. The fence might be installed around a seed oyster bed by divers using aqualungs after all drills have been removed. The fence might be left in place until the oysters were large enough to resist the drills, or possibly until they were ready for transplanting, or for harvesting.

The eastern oyster drill, Urosalpinx, and the Japanese drill, Tritonalia (Ocenebra) japonica, are both serious predators of the native oyster, Ostrea lurida, in the Pacific Northwest. Since this small oyster is grown in diked pools in the intertidal zone, a simple fence which released copper ions might be installed on top of the concrete dikes to exclude Urosalpinx. If Tritonalia is also found to be repelled by copper ions, and if the oysters are not harmed by the copper, this method may solve the drill problem for this valuable industry.

Additional experiments are planned to test the effect of copper ions on the other oyster predators, Eupleura, Thais, and Busycon.

Summary

1. Laboratory tests showed that the oyster drill Urosalpinx would not crawl across clean copper or brass although it would cross iron, zinc, and Saran plastic.

2. A clean copper wire as small as 14 gauge repelled Urosalpinx in still-water laboratory experiments.

3. In slow currents an eight-gauge copper wire repelled Nassa obsoleta which was substituted for Urosalpinx because it is more active at low temperatures and repelled by copper to about the same extent.

4. Brass and copper screen fences two inches high were not crossed in the laboratory by Nassa obsoleta, Thais lapillus, Littorina littorea or Littorina obtusata at current velocities of 0.31 to 0.45 knots. Urosalpinx could not be tested under these conditions because of low water temperatures which rendered them inactive.

5. The copper or brass fence loses its effectiveness if it is in contact with a more active metal such as iron. Under these conditions iron ions which do not repel drills are released instead of copper ions.

6. The results of the field tests of copper barriers in Maine, using Nassa, have corroborated the laboratory observations. The great difference in the number of Nassa entering the control areas and those entering the experimental areas demonstrated that these gastropods are definitely repelled by copper ions.

7. The decrease in effectiveness of brass fences after three to four weeks in the intertidal zone suggests that better methods of releasing copper ions over a longer period of time should be sought. The possibility that brass or copper fences would remain effective for a longer period in subtidal areas is being explored through additional experiments which are now being conducted in Chincoteague Bay.

8. Practical applications might include installation of low fences which release copper ions around subtidal seed oyster beds by divers using aqualungs, but cannot be recommended until problems of biofouling, silting, economy, and effect of copper on oysters and other marine species are solved.

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TRAPPING OYSTER DRILLS IN VIRGINIA

III. The Catch per Trap in Relation to Condition of Bait[1]

J. L. McHugh

Virginia Fisheries Laboratory, Gloucester Point, Virginia

INTRODUCTION

In the course of trapping experiments previously described (Andrews 1955, McHugh 1955), a question arose concerning deterioration of bait with time. It is fairly obvious to those who fish the traps that the condition of the bait changes. The smallest oysters die first, through predation by drills, crabs, and other enemies, and through smothering in the muddy bottom. Barnacles and other organisms on the shells also die from various causes. The valves of the dead oysters soon separate, and some are lost through meshes of the trap, so that the volume of bait also decreases. Stauber (1943) found that efficiency of traps decreased as the interval between lifts increased. He found also that the catch increased significantly after rebaiting.

A series of 20 traps was fished from the Virginia Fisheries Laboratory pier from July 1953 to December 1955. Although the traps were not rebaited until early October 1954, the catch per trap was greater during the second summer. If bait does deteriorate, as Stauber (1943) and others have concluded, this increased catch must reflect an increase in abundance or availability of Urosalpinx in 1954. But by October 1954, the bait consisted mainly of isolated valves, and the few surviving oysters were thick-shelled and blunt. It was decided to conduct a controlled experiment with these traps to test the effect of rebaiting. This experiment began in October 1954 and continued through the summer of 1955.

The rebaiting experiment seemed to show that both Urosalpinx cinerea and Eupleura caudata preferred fresh bait to old oysters and shell as Stauber (1943) already has contended. It was realized, however, that the amount of bait in the traps might also influence catch, and that the quantity had not been well controlled in previous experiments. If the catch of drills should be a function of amount of bait rather than kind of bait in traps, then the results of the previous experiment would be open to question. Consequently, in 1955 a more extensive experiment was conducted, in an area offshore from the Laboratory pier, in which both kind and amount of bait were controlled.

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[1] Contributions from the Virginia Fisheries Laboratory No. 76.

REBAITING EXPERIMENTS

Methods

The traps fished from the Laboratory pier were arranged in two series of ten each, one on each side of the pier, as illustrated by McHugh (1955). Five traps from each series were selected (using a table of random numbers), and these were rebaited with fresh seed oysters from the James River. Bait in the remaining ten control traps was augmented where necessary with old bait discarded from the randomly-selected experimental series, so that volumes of bait in each trap were approximately the same.

Catch in Experimental and Control Traps Prior to Rebaiting

These traps were fished continuously, at intervals of one day to one month, beginning July 9, 1953. On July 29, 1954, the arrangement was altered by moving traps 1 and 2, at the offshore end of each series, to the inshore end of the pier, and renumbering them as 11 and 12. Only catches made after this date were used in estimating performance of the experimental and control traps before rebaiting.

Control traps caught 780 Urosalpinx and 21 Eupleura; those selected later for rebaiting caught 640 Urosalpinx and 28 Eupleura. The ratio of the two Urosalpinx catches differed significantly from 1:1 (χ^2 = 13.80, P much less than 0.01), therefore this difference was considered in analysing the results of the rebaiting experiment. The ratio of the two Eupleura catches did not differ significantly from 1:1 (χ^2 = 1.00, P about 0.6).

Catch in Experimental and Control Traps After Rebaiting

By the end of the second week, rebaited traps had caught 470 Urosalpinx and 32 Eupleura, whereas the controls had taken only 315 and 2 respectively. Within three weeks, however, the initial advantage had been lost. In experimental and control traps, from November 1954 to April 1955 inclusive, catches of both species maintained approximately the ratios observed before the experiment began. In May 1955, however, both species were caught in larger numbers in rebaited traps, and this superiority was maintained, with occasional deviations, until the experiment was terminated early in December 1955. From May to December, 572 Urosalpinx and 54 Eupleura were caught in rebaited traps, but only 440 and 17 respectively in controls. By this time bait in all traps was in poor condition.

From October 12, 1954, to December 2, 1955, experimental traps caught about 1.3 Urosalpinx for each Urosalpinx caught in controls. This catch differed significantly from the expected catch (χ^2 = 105.4, P very much less than 0.001). During the same period experimental traps

caught about 4.4 Eupleura for each Eupleura caught in the controls. This differs significantly from the expected ratio of 1:1 (χ^2 = 47.2, P very much less than 0.01).

Sizes of Drills Caught on Old and New Bait

As mentioned previously, new bait caught more drills than old. It would be of value to know whether the sizes of drills caught on the two kinds of bait differed, and the data suggest that new bait caught relatively more small drills (Table 1). Indeed, in the period from October 18 to December 1, 1954, the total catch of Urosalpinx 14 millimeters in length and over apparently did not differ in the two kinds of bait (χ^2 = 3.25, P greater than 0.05), and the excess catch in the rebaited traps was made up of drills 13 mm and smaller (χ^2 = 22.08, P much less than 0.001). The arbitrary division between 13 and 14 mm was chosen because it gave the best separation between yearling and older drills.

From April to November 1955 the total catch on new bait exceeded the catch on old (χ^2 = 13.05, P less than 0.001). This excess catch in rebaited traps was distributed evenly over all sizes, and frequency distributions of shell height of drills from the two kinds of bait were almost identical.

To determine whether placement of rebaited traps was random with respect to shell height of drills available to them, the frequency distributions of shell height of Urosalpinx on the two sets of traps were compared for the period August 12 to October 11, 1954, prior to rebaiting. As shown in Table 1, traps that were later rebaited had been catching fewer large drills than those that were not changed, and this difference was statistically significant (χ^2 = 15.72, P less than 0.001, for Urosalpinx 14 mm in shell height and larger). There was no great difference in frequency distributions of shell height of drills 13 mm and under (χ^2 = 0.40, P greater than 0.5).

The excess catch of small drills in rebaited traps therefore probably has no biological significance. The same traps caught a higher ratio of small to large Urosalpinx before rebaiting, and new bait simply increased the frequency of capture of all sizes.

CONCLUSIONS FROM REBAITING EXPERIMENT

It has been demonstrated that the catch of oyster drills by traps in the York River, Virginia, can be increased substantially by rebaiting traps. New bait apparently maintains its superiority over old for at least a year after rebaiting, and therefore it probably follows that seed oysters are superior to older oysters, and older oysters are superior to shell, for attracting drills. This is not unexpected, in view of the findings of Stauber (1943), Haskin (1950), and others.

Table 1. Frequency distributions of shell height in Urosalpinx cinerea caught in experimental and control traps before and after rebaiting

	Experimental (rebaited)		Control (not rebaited)	
	13 mm and less	14 mm and over	13 mm and less	14 mm and over
Before rebaiting 12 Aug - 11 Oct 54	202	427	215	551
After rebaiting 18 Oct - 1 Dec 54	161	379	87	331
April - Nov 55	179	523	130	443

Eupleura seems to respond to new bait more vigorously than Urosalpinx. This could be interpreted in at least two ways, either Eupleura is more destructive of young oysters than its fellow-predator, or it deserts oysters more readily for other food when young oysters are not available. It has been observed repeatedly at Gloucester Point that although Eupleura is not uncommon in eel-grass beds near shore, it does not climb pilings of piers as Urosalpinx does. This may help to explain the relative scarcity of Eupleura in traps, and the large increase in catch when desirable bait is introduced.

For both species the similarity in catches in experimental and control traps in winter and early spring may be primarily a temperature-controlled phenomenon. In other words, although both drills may move about when water temperatures are relatively low, their sensitivity to differences in bait may be repressed. The observations of Janowitz (1957), that rapidity of shell growth rather than age of oysters is the significant factor in attracting drills, are suggestive, for the growth of oysters in Virginia practically ceases in the period December to March.

EXPERIMENTS WITH VARIOUS KINDS AND AMOUNTS OF BAIT

Methods

On July 14, 1955, an experiment was set up to test the relative merits of seed oysters, adult oysters, and oyster shell, each in three different quantities by volume, as bait in chicken-wire traps. Seed oysters were obtained from the James River, adult oysters were taken with tongs in shallow water near the Virginia Fisheries Laboratory pier, where they had been placed at various times during the past two years, shell likewise was tonged from the bottom near the pier.

Volumes of bait were selected to correspond with 6, 12, and 18 adult oysters, which measured about one, two and three quarts respectively. Seed oysters and loose valves of dead adults were measured in these volumes.

Thirty-six traps of galvanized chicken wire, of the usual dimensions, were baited in equal numbers with different combinations of kinds and amounts of bait. Three kinds and three amounts gave nine combinations, thus each combination was given four replications.

Four long stakes were driven in the river bottom to form a right-angled cross around a central stake. Each arm of the cross extended 100 feet on each side of the central stake, and the arms were roughly parallel with and at right angles to the river bank. The center of the cross was about 400 feet from shore and water depth ranged from about five to seven feet at mean low water.

Tarred hemp line, one-quarter inch in diameter, was cut in 100-foot lengths and attached to large wrought-iron rings which were free to move up and down each stake. Traps were attached to these main lines at 10-foot intervals with snoods of three-eights inch tarred hemp line 10 feet long. On each main line the trap nearest the center was attached five feet from the center stake. Placement of various combinations of bait was chosen using a table of random numbers.

Analysis of the Catch

Urosalpinx cinerea. The 36 traps were fished at weekly intervals until September 15, 1955 inclusive. On the next fishing date, September 22, because lines were beginning to rot, one trap was lost. The experiment continued until October 28, inclusive but for the original purpose of the experiment the results were progressively less satisfactory, because bait, particularly seed oysters, deteriorated with time, various traps were lost and replaced, or lost and recovered at a later date, and the catch was declining, probably because water temperatures were dropping.

For these reasons, the experimental observations were separated into three periods for analysis. The results are summarized in Table 2, in which catches have been grouped so that each number represents total catch in four replicate traps over a period of several weeks. The last period includes all observations in which one or more traps were missing. The durations of the first two were chosen to include approximately the same total catch in each.

In the first period, bait was fresh, and it would be expected that differences in attractive power of baits, with respect to kinds and amounts, would be at a maximum. In the second and third periods, differences might decrease or disappear.

The frequency distribution of individual catches was skewed strongly to the right, and more than half the catches contained no drills. A transformation therefore was necessary before the analysis of variance could be applied. The square-root transformation was chosen, but first each individual catch was increased by adding 3/8.

The transformed data for the first period were treated by analysis of variance (Table 3). None of the interactions between factors was significant, and the variance ratios computed for different quantities of bait and successive weeks of fishing were no greater than would be expected by chance. The catches in different kinds of bait, however, differed by amounts greater than usually would be expected by chance ($F = 5.52$, $F_{0.01} = 4.74$). Under the conditions of this experiment, it appears that seed oysters are superior to adult oysters, and adult oysters superior to shell, as bait for Urosalpinx cinerea.

Table 2. Catch of Urosalpinx per trap in the period July 21 to October 28, 1955 inclusive, on three kinds and three quantities of bait. The four replicate treatments have been grouped, and catches have been grouped by periods according to the condition of the bait. Traps were fished weekly.

Inclusive dates	Number of weeks	Amounts of bait	Kinds of bait			Totals
			Seed	Adults	Shell	
21 July to 18 Aug.	5	1	10	12	13	35
		2	29	8	5	42
		3	38	22	8	68
Totals			77	42	26	145
27 Aug. to 15 Sept.	4	1	18	19	19	56
		2	18	12	7	37
		3	22	21	16	59
Totals			58	52	42	152
22 Sept. to 28 Oct.	6	1	8	20	28	56
		2	20	16	5	41
		3	32	18	24	74
Totals			60	54	57	171

Table 3. Summary of analysis of variance of the transformed catch of Urosalpinx per trap in the period July 21 to August 18, 1955, inclusive.

Nature of effect	Source of variation	Sum of squares	Degrees of freedom	Variance estimate
Main factors	Weeks (W)	0.98	4	0.24
	Amounts (A)	1.11	2	0.56
	Kinds (K)	2.77	2	1.38
First order interactions	K x W	0.60	8	0.08
	A x W	2.05	8	0.26
	K x A	1.93	4	0.48
Second order interaction	K x A x W	2.73	16	0.17
Residual	Replication	35.45	135	0.26
	Total	47.62	179	---

The data for the second period showed evidence of heterogeneity only with respect to the catches of successive weeks (Table 4). The relatively large catches of August 27 following Hurricane Hazel were primarily responsible for this result. Catches in traps commonly increase substantially after storms. There was no evidence that catches on different kinds of bait, or on different quantities of bait, differed significantly in the second period.

Catches on missing traps in the last period were each assumed to be zero for purposes of analysis. Most of the lost traps were recovered at a later date by careful searching with a hooked pole, and catches on recovery were never inconsistent with the assumption that catches in missing weeks were zero. Records of the catch show that during the period in question about half the catches contained no drills, 32 per cent contained one, and about 18 per cent contained two or more. There was no significant difference in distribution of catches on seed oysters, adults, or shell, nor on the three quantities of bait. Therefore, the assumption that all missing catches were zero has an even chance of being correct, and there is no evidence that any other distribution of estimated catches would fit the facts better. As illustrated in Table 5, there was no good evidence of heterogeneity in catches recorded for the third period.

Eupleura caudata. Only 15 Eupleura were caught during the entire experiment. Catches were too small to justify an analysis of variance, but it is interesting that the largest total catch (9) was made in traps baited with seed oysters, and the smallest (2) on shell. Catches on different quantities of bait were similarly inconclusive.

Deterioration of Bait

If it be assumed that the characteristics of shell as bait did not change during the experiment, catches on shell can be used to test rates of deterioration of seed and adult oysters. The total catches of Urosalpinx per week on shell in the three periods were 5.2, 10.5 and 9.5 respectively. The increase from the first to the second period was caused by an increase in abundance of drills by recruitment of young born in the summer of 1955. The increased availability persisted through September and early October, but catches declined again, probably influenced by falling temperatures, toward the end of the third period.

In the first period, both seed (χ^2 = 100.0, P very much less than 0.01) and adult oysters (χ^2 = 9.85, P much less than 0.01) were superior to shell. In the second period, seed oysters probably were still superior (χ^2 = 6.10, P less than 0.02) but catches on adult oysters could not with any great confidence be said to exceed catches on shell (χ^2 = 2.38, P about 0.2). In the third period catches on seed, adults, and shell did not differ significantly (χ^2 = 0.16, P about 0.7).

Table 4. Summary of analysis of variance of the transformed catch of Urosalpinx per trap in the period August 27 to September 15, 1955, inclusive.

Nature of effect	Source of variation	Sum of squares	Degrees of freedom	Variance estimate
Main factors	Weeks (W)	7.38	3	2.46
	Amounts (A)	0.66	2	0.33
	Kinds (K)	0.44	2	0.22
First order interactions	K x W	0.52	6	0.09
	A x W	2.84	6	0.47
	K x A	0.39	4	0.10
Second order interaction	K x A x W	2.61	12	0.22
Residual	Replication	25.22	108	0.23
	Total	40.06	143	---

Table 5. Summary of analysis of variance of the transformed catch of Urosalpinx per trap in the period September 22 to October 28, 1955, inclusive.

Nature of effect	Source of variation	Sum of squares	Degrees of freedom	Variance estimate
Main factors	Weeks (W)	3.63	5	0.73
	Amounts (A)	1.09	2	0.54
	Kinds (K)	0.00	2	0.00
First order interactions	K x W	1.58	10	0.16
	A x W	0.80	10	0.08
	K x A	2.91	4	0.73
Second order interaction	K x A x W	5.71	20	0.28
Residual	Replication	28.23	162	0.17
	Total	43.95	215	---

Deterioration of bait with time is illustrated in Figure 1. Formulae for the two lines, computed by the method of least squares, were as follows: for seed oysters $\log Y = 0.662 - 0.00812X$, for adult oysters $\log Y = 0.236 - 0.00287X$. Both lines intersect the axis $Y = 1$ in the vicinity of 82 days after the experiment began. This signifies that on October 4, under the conditions of this experiment, seed oysters and adult oysters were no longer superior to shell as bait for Urosalpinx. For practical purposes, of course, bait becomes inefficient long before it loses its potency completely. Consequently, it might be worth while to compute the period in which bait loses half its attractive power. For seed oysters the half-life was about 27 days, and for adults about 36 days.

It is interesting also to compare these results with results of the rebaiting experiment at the Laboratory pier. Control in the pier experiment was established by retaining old bait in half the traps. For purposes of comparison, this old bait can be considered as adult oysters. The lower regression line in Figure 2 was fitted by the method of least squares to points representing the ratio of total weekly catch on new bait to total weekly catch on old. The upper regression line represents the ratio of catches on seed and adult oysters, computed from data illustrated in Figure 1. The lower level, and greater slope of the line representing the pier experiment probably reflects the relatively greater numbers of drills near the pier, and decreasing water temperature. New bait no longer exhibited a significant advantage over old bait after about 40 days, and the half-life under these conditions was about 19 days.

Variation in Catches of Individual Traps

Some traps consistently caught more drills than others with similar bait. For example, trap number 17 took 47 drills during the experiment, and the weekly catches of this trap included the three largest catches of all traps. Trap number 7, on the other hand, contained the same amount and kind of bait, but caught only seven drills altogether.

Because kind of bait influences the catch, comparisons of individual catches are legitimate only within replications. Testing against expected catches based on average catch in each of the replications of four, the pooled chi-square values summarized in Table 6 were computed. Although tests at the lowest level did not always produce evidence that the variation was greater than would be expected by chance, the summed chi-squares for the three kinds of bait all showed evidence of heterogeneity at the one per cent probability level or better, two of the three amounts of bait produced equally conclusive results, and one gave less than one chance in twenty that a larger value of chi-square could result by chance. The sum of all chi-square values also strongly favored the view that chance was not the only factor influencing the catch in replicate traps.

Such undue variation could come about through uncontrolled variations in the attractability of the traps themselves, but it would

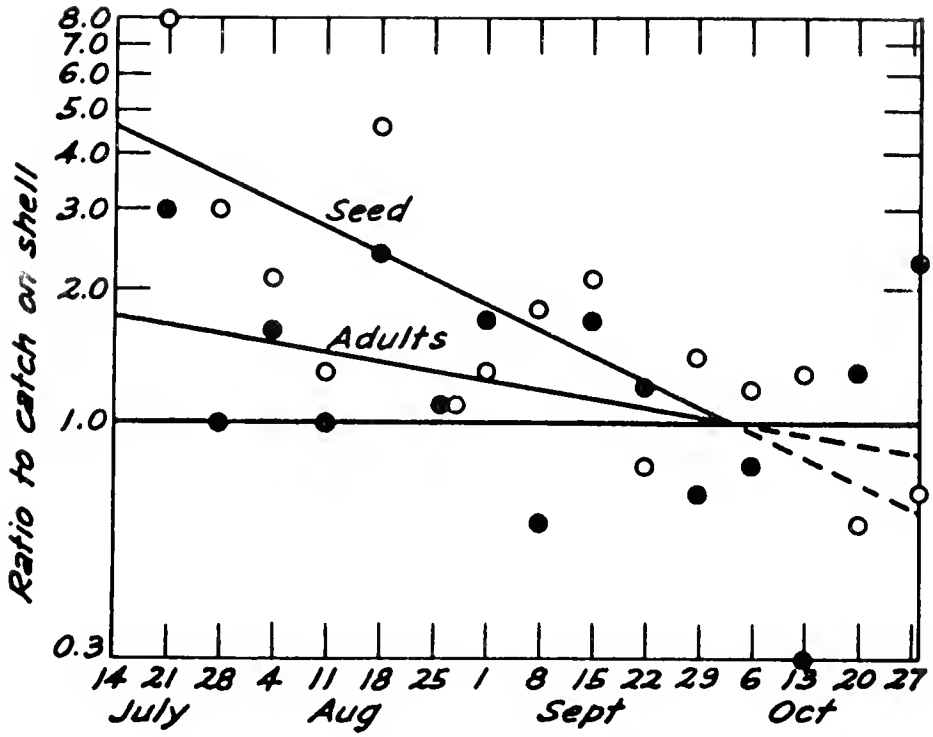

Fig. 1. The ratio of the catch on seed and adult oysters to the catch on shell in the offshore experiment of 1955. Open circles: seed-shell ratio; black circles: adults-shell ratio.

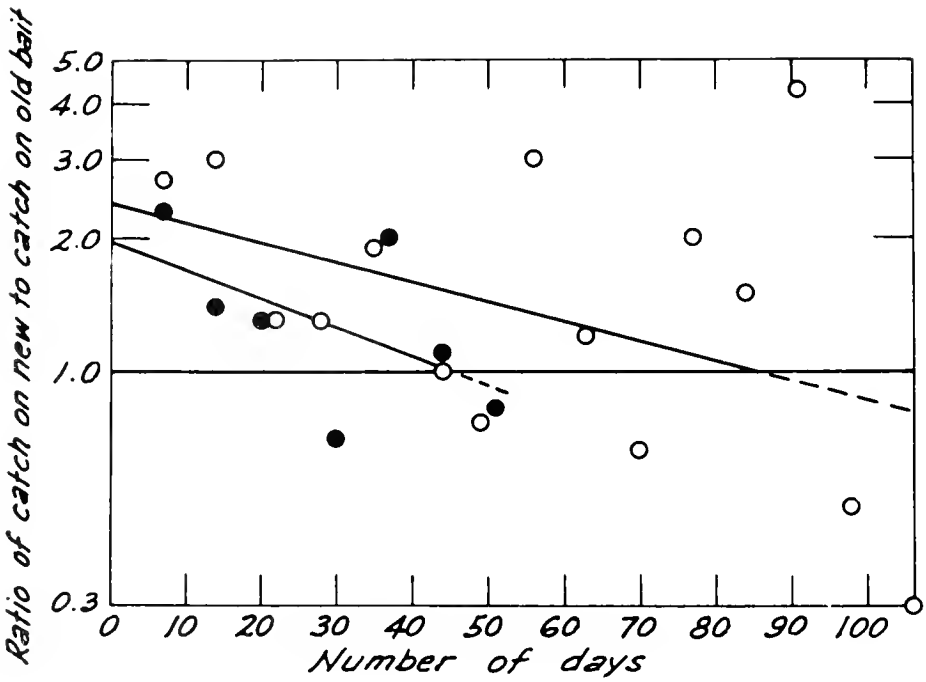

Fig. 2. The ratio of the catch on seed oysters to the catch on adult oysters at the Virginia Fisheries Laboratory pier in 1954 and in the offshore experiment of 1955. Open circles: offshore experiment; black circles: Laboratory pier.

seem logical to search first for evidence of non-random distribution of drills over the trapped area. The two lines of traps were oriented parallel to shore and at right-angles to it, and depth of water and character of bottom fluctuated. As shown in Figure 3 catches tend strongly to decrease in an offshore direction. In constructing Figure 3, allowance was made for differences in catch by the three kinds of bait by adjusting catches by appropriate factors.

Parallel to shore, smallest catches seemed to occur at the two ends of the line, highest near the center. The two ends respectively were not far from the Laboratory pier and a pier on adjacent residential property downriver. The proximity of these piers, the pilings of which harbored a rich community of fouling organisms, may have constituted a disturbing element. The trend was quite irregular, and perhaps not biologically significant.

Sizes of Urosalpinx Caught on Different Kinds of Bait

In view of the previous conclusion that no differences of biological significance appear to exist in the frequency distribution of shell height of drills caught on new and old bait, it is worthwhile to examine the shell height distribution of Urosalpinx caught on the three kinds of bait used in these experiments (Table 7). It is interesting that the difference in total catch on the three kinds of bait is confined entirely to adult drills (χ^2 = 26.70, P much less than 0.001). Total catches of Urosalpinx 13 mm in height or smaller (58, 58, and 59 drills respectively) were essentially identical.

This experiment suggests that although adult Urosalpinx are sensitive to differences between seed oysters, adult oysters, and shell, young drills are not. This may indicate a difference in food preference between young and adult drills. Or, as Dr. Thurlow Nelson has suggested, young drills are inveterate climbers, and this favors their wide distribution on materials that are moved across the bottom by currents. This could account for their relatively greater abundance on shells and adult oysters.

SUMMARY AND CONCLUSIONS

Ten traps, of a series of 20 that had been fished for about a year without replacing or augmenting bait, were selected at random and rebaited with seed oysters in October 1954. The catch of Urosalpinx and Eupleura increased significantly immediately, but the superiority of new bait over old declined steadily on successive fishing dates. Nevertheless, rebaited traps remained more attractive to drills for more than a year, except for a six-month period in winter and early spring, when the catch of Urosalpinx was about equal in new and old bait. There is no evidence that drills caught on the two kinds of bait differ in size. Eupleura responded more vigorously to new bait than did Urosalpinx.

Table 6. Tests of variations in the catch of individual traps, represented by summation of chi-square values at the various levels. Figures in parentheses represent the numbers of degrees of freedom.

Amount of bait	Kind of bait			Pooled χ^2
	Seed	Adults	Shell	
1	1.10 (3)	7.26 (3)	11.48** (3)	19.84* (9)
2	25.85** (3)	14.00** (3)	2.53 (3)	42.38** (9)
3	37.91** (3)	2.55 (3)	9.16* (3)	49.62** (9)
Pooled χ^2	64.86** (9)	23.81** (9)	23.17** (9)	111.84** (27)

* Probability of a larger value of chi-square 0.05 or less.
** Probability of a larger value of chi-square 0.01 or less.

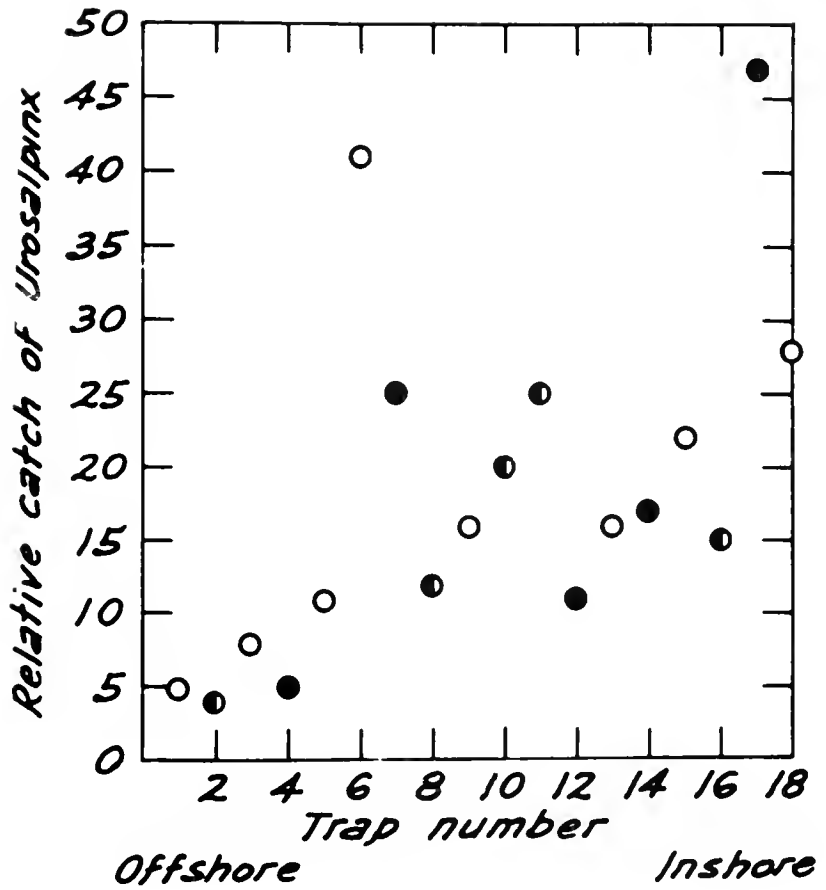

Fig. 3. The total catch of Urosalpinx in a series of traps arranged in a line at right angles to the shoreline in the York River at Gloucester Point. Black circles: seed oysters; divided circles: adult oysters; open circles: shell. The catches on adults and shell were weighted by appropriate factors so that the average catch per unit of effort was equal to that on seed oysters.

Table 7. Numbers of small and large Urosalpinx cinerea caught on seed, adult oysters, and shell in 1955.

Shell height	Kinds of bait		
	Seed	Adults	Shell
13 mm and less	58	58	59
14 mm and over	137	90	66
Grand totals	195	148	125

Thirty-six traps were set out in July 1955 to test the relative catching power of seed and adult oysters and oyster shell, and to measure the relative merits of different amounts of bait. In the first five weeks the greatest catch of Urosalpinx was made on seed oysters, and the smallest on shell, and odds were less than one in 100 that these differences could occur by chance. For the next ten weeks also, the greatest catch was made on seed and the least on shell, but these differences were not significant statistically. There was no evidence, at any time during the experiment, that quantity of bait affected the catch. Only a few Eupleura were taken, and catches on the different kinds of bait did not differ significantly, but total Eupleura catch followed the sequence demonstrated for Urosalpinx greatest on seed and least on shell.

The rate of deterioration of bait can be expressed as the time in days during which it loses half its power of attraction. In the experiments described here this was determined in relation to catch on shell, and gave values ranging from 19 days at the Laboratory pier to 36 days for adult oysters in the offshore experiment. Undoubtedly rate of deterioration is a function of the abundance of drills, kind of bait, water temperature and salinity, and many other things. Ignoring environmental effects for the moment, the results here obtained apparently fit a logical pattern, for the relatively short half-life of new bait at the pier is linked with a greater abundance of drills, and the greater half-life of adult oysters as compared with seed oysters in the offshore experiment matches the greater attraction of seed for drills. On the other hand, it must be noted that both experiments, but especially that conducted at the pier, covered periods in which water temperatures declined appreciably from the late summer maximum, and declining catches probably were hastened by falling temperatures. This is confirmed by increased catches on new bait at the Laboratory pier in the summer of 1955.

Available evidence suggests very strongly that catches of individual traps in the offshore experiment varied to a degree much greater than chance alone would allow. Apparently distribution of drills over the trapped area was non-random, and the pattern of catches suggests that abundance decreased rather regularly from the inshore to the offshore part of the experimental area. This is consistent with previous observations that beds of eelgrass near shore harbor a large natural population of drills.

With respect to shell height of Urosalpinx caught on seed, adults, and shell, the results of the offshore experiment are at variance with those of the experiment at the pier. Catches of drills 13 mm or less in height were identical on the three kinds of bait, but larger drills were most strongly attracted to seed, and least strongly to shell. This suggests seasonal or local differences in habits of young and adult Urosalpinx, possibly related to food or depth preferences, and reactions to gravity.

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ENVIRONMENTAL CONDITIONS

SHELLFISH FOOD

SHELLFISH POISON

SOME FEATURES OF THE HURRICANE PROBLEM

Gordon E. Dunn

Weather Bureau Office
Miami, Fla.

How is a Hurricane Formed?

In the past there have been two principal theories of hurricane formation: (1) the convection theory, and (2) the frontal theory. Advocates of the convection theory believed that calm moist air of the doldrums was favorable for convection either because of surface heating or high moisture content of the air or both. Heated air began to rise and resulted in numerous cumulo-nimbus clouds and widespread showers and thunderstorms. Then pressure mysteriously began to fall, cumulo-nimbus clouds gradually coalesced, and if the area was sufficiently far from the equator for rotation of the earth to be effective, a cyclonic circulation was initiated. Development of the circulation continued until full intensity was reached through release of heat by condensation. While it is true that small hurricanes appear to be one huge convective cloud and there is no doubt that the hurricane is essentially a convective mechanism, the convective theory of formation left too many questions unanswered, among them: What is the starting mechanism, what causes theinitial fall in pressure, and why are there so few hurricanes?

One of the notable advances in meteorology was the introduction of air mass analysis and the frontal theory of storm formation by the Norwegian meteorologists after World War I. In the early 1930's, some meteorologists attempted to apply these theories to hurricane formation, that is, they formed along a front or boundary between the southeast trades of the southern hemisphere and the northeast trades of the northern hemisphere, in the same manner as in the temperate zone. Unfortunately there is no density discontinuity here and the large majority of tropical storms in the Atlantic, at least, do not form along the so-called tropical front or intertropical convergence zone but rather in the trade wind zone. The frontal theory of hurricane formation is no longer in vogue.

We do not yet have a complete explanation of the formation of hurricanes but we do know the following:

(1) Hurricanes will develop only over comparatively warm water with temperature 80-81° F and higher. The energy for increasing kinetic energy must come from the warm ocean.

(2) Hurricanes form only in pre-existing disturbances, the most common of which in the Atlantic is the easterly wave. A deep easterly current is usually present over the developing tropical storm.

(3) There must be high-level divergence to remove accumulated air from the system and to permit pressure to fall at the surface.

Now these three conditions are present almost every day somewhere over the tropical Atlantic Ocean although not necessarily in the proper juxtaposition. Since there have been several years when only two tropical storms were noted over the whole Atlantic Ocean, it is obvious that some rather unique combination of weather conditions is required for hurricane formation. The exact starting mechanism has eluded us so far.

What Moves a Hurricane?

Once a tropical storm has formed and has been detected, the principal forecast problem is one of movement -- both rate and direction. Accuracy of our warnings from the standpoint of timeliness and geography will depend upon our ability to handle this problem. Many of the techniques in use in the temperate zone are ineffective here. Hurricanes are, to a large extent, steered by the basic current in which they are imbedded; that is, the stream of air surrounding the storm from the surface up to 60,000 feet or so. Since these storms are located out over the oceans, evaluation of this steering current poses a difficult problem to the forecaster. Our only source of information is that provided by observations from land stations which are often too far from the hurricane to be useful and from the Air Force and Navy hurricane reconnaissance planes. Our present procedures entail 'boxing' of the hurricane at approximately 20,000 feet; that is, sending planes around the storm outside the hurricane circulation itself to measure, if possible, this steering current. We are making an assumption here that the 20,000 foot level is representative of the steering current from sea level up to 50 or 60,000 feet and we know that this assumption is often incorrect. There have been some good results with this technique but at the same time, it is ineffective when the regional circulation of the atmosphere is changing rapidly with time. Also there is probably a significant internal contribution to the storm's movement from the storm itself which cannot at the present time be calculated.

Some meteorologists have advanced the theory that the course of the hurricane might be changed slightly by seeding a certain quadrant of it with dry ice or silver iodide. They reason that the hurricane, at least to some extent, will move in the direction of the greatest concentration of energy which could be induced in some desired quadrant by seeding there. In this way the hurricane could be detoured for a few hours, long enough perhaps for it to miss some concentration of population. It is believed that the majority of meteorologists are very pessimistic of the practicality of the theory.

What was the Steering Pattern in 1954 and 1955?

In both 1954 and 1955 all major circulation features were displaced north of normal. This included the jet stream, temperate zone westerlies and sub-tropical and tropical easterlies. The principal warm

season weather control for the Atlantic and eastern U. S. is the Azores-Bermuda anticyclone, or HIGH. Warm tropical air and tropical storms form on the underside of this HIGH and move first westward and then northward around it. During the past two years, and indeed frequently since the middle 1930's, this HIGH has been displaced north of its normal position and has been stronger than usual. This forces hurricanes inland across the Atlantic coast which would normally recurve harmlessly northeast and out over the open Atlantic. In addition, a trough of low pressure forms underneath the HIGH which seems to be favorable for above-normal hurricane frequency.

What are the Prospects for 1956?

Several hundred more years of hurricane statistics will be needed before averages will mean anything. In the Cape Fear area of North Carolina, there were no more than eight destructive hurricanes between 1740 and 1953, or an average of one every 27 years. Hazel came along in October 1954 and three more in 1955 making four in 11 months. I would be foolish to make any definite prediction how many hurricanes there will be this year or where they will strike. However, people living along the North and Middle Atlantic coasts have some basis for optimism. The circulation this year is just about opposite to that of last year. This summer, so far, mid-latitude westerlies and sub-tropical easterlies have remained south of their normal position, which means westerly winds aloft have been prevailing as far south as Jacksonveille and, for as long as they continue, will shunt any hurricanes, which form, away from the coast from the Carolinas northward. Of course I would emphasize neither I nor any other forecaster can tell now what the circulation may be by September. Certainly we would expect some retreat northward to a more normal position.

What are the Causes of the Hurricane Tide?

Mr. David H. Wallace tells me that the 1955 hurricanes alone caused $10,000,000 damage to the oyster industry in the area of North Carolina, Chesapeake Bay, and Delaware Bay. Probably wave action and sub-surface currents are more important in producing oyster damage but I will take a few minutes to discuss the hurricane-produced tide which may only indirectly affect the oyster beds.

Of all the destructive agents of the hurricane, sea action is responsible for the most of the damage and most fatalities. Hurricane induced floods are second and direct effect of the wind third.

The greatest concentration of destructive power unleashed by most hurricanes occurs as they move inland from the sea. It is here that so much of the energy stored in the swells and waves from way out at sea is expended. The added surface frictional drag begins to extract energy from the air at a greater rate as the storm crosses the

coastline. This concentration of destructive energy is reflected in the record of destruction and death wrought by hurricanes along coastal areas back through history. Bay far the majority of deaths attributed to hurricanes occur at the coast where tides rise to abnormal heights. One storm wave on the shore of the Bay of Bengal in 1881 is reported to have caused the death of 300,000 persons.

Storm tides, rising on occasions to 12 or 15 feet or more above normal, and pounding waves have proved to be most difficult for man on shore to cope with. A rapid rise of the water suddenly places man in an unnatural habitat. Pounding waves and lashing winds are too much for the average structure to withstand.

Rapid change of conditions brought about by a storm tide affects not only man but all living creatures within reach of the flood waters and the tidal range. At least temporarily, the ocean overruns its usual bounds and new boundaries are established. Adjustment to new boundaries involves considerable erosion, cutting new channels, and relocation of sandbars. All these changes are rapid with the rising tides of a hurricane; adjustments to new surroundings must be rapid. Those who can't keep the pace in adjusting suffer. Many small creatures whose natural homes are buried or washed away must seek locations and build new homes after these changes in coastal topography. Many others become casualties.

The problem of forecasting the storm tide which a given hurricane will create is complicated by many factors which are as variable as the coastlines which the storms affect. The first requirement in forecasting the tide is to predict very accurately the point where the center of the storm will move across the coast. Ten or 20 miles displacement of a storm track to the reight or left of a point on the coast can mean a difference between severe flooding and tides below normal. On a nearly straight coast the highest tides occur 20 to 50 miles to the right of the track of the storm center depending upon the size of the storm. To the left of the storm center the wind circulation is such that water is blown away from the shore thus creating tides below normal. Such extremely low tides should be watched with suspicion because occasionally the wind is holding the water away from the coast and then, stopping rather abruptly, allows the water to return with enough momentum to create abnormally high tides. This is a definite danger in bays and estuaries particularly. If there is a deficiency of water on one side of an estuary, there is usually an excess on the other side. Then when the force that holds water out of gravitational equilibrium is suddenly decreased or removed, the effect may be likened to tipping a pan of water and starting a natural oscillation. There have been occasions when this return of water coincided with the astronomical high tide and resulted in serious flooding.

In hurricane Hazel of 1954 tides up to 18 feet above (MLW) mean low water, the highest tides of record along the North Carolina coast normally were reported just to the right of the "eye". A section of the coast about 60 miles long experienced tides ranging from 6 to 12 feet above the normal high. The highest water occurred where the "eye" crossed the

coast and 20 to 30 miles to the right of its track. The configuration of the coast where Hazel crossed is concave; however, it is not certain how much if any influence this had in the development of excessive tides. Some other factors currently believed to be favorable to the generation of high storm tides and which were present in Hazel were: (1) this was a severe hurricane, that is, high winds and low barometer; (2) the trajectory of the storm was only slightly curved and this curvature was to the right; (3) the angle of incidence to the coast was large, about 60 degrees; (4) the continental shelf here has a very gradual slope to about 50 fathoms 50 miles off shore; (5) the storm moved forward at about 25 to 30 miles per hour and accelerated as it approached and crossed the coast; and (6) the storm literally "rode in" on top of the normal high tide. The portion that each of the enumerated conditions contributed to the total tide is not known but seldom would chance bring such concerted conditions for generation of a high storm tide.

The highest hurricane tide on record at Atlantic City, which is considered representative of the New Jersey coast, is approximately 5.5 feet above normal and recorded in the hurricane of September 14, 1944. This tide would have been some 11 feet above mean low water if it had occurred at the time of high spring tide. Flooding begins along the New Jersey beaches at about 6 feet above mean low water. If an intense hurricane moved inland over New Jersey on a course perpendicular to the coastline at time of a high spring tide, water levels 12 to 18 feet above mean low water would be likely. However, the chances of such an occurrence would appear to be considerably less than once in a hundred years.

A CONTINUOUS WATER SAMPLER FOR ESTIMATION OF DAILY CHANGES IN PLANKTON

Philip A. Butler and Alfred J. Wilson, Jr.

U. S. Fish and Wildlife Service Laboratory
Pensacola, Florida

The growth rates of oysters held in experimental trays suspended above the bottom at two stations only 1000 feet apart on either side of our laboratory island have differed markedly for the past several years (Butler 1953). At the east station, the oysters grow faster, mortality is somewhat lower, and more young oysters attach to test cultch. Although we have not checked other marine animals as closely, this station appears to be equally favorable for clams and scallops as well as such sedentary forms as barnacles, mussels and sea-squirts.

During the past year when we have maintained continuous records, average monthly salinity levels have differed by less than 0.5 °/oo and temperatures have differed by less than 1.0° C at the two stations. In the absence of other data, quantitative or qualitative differences in food appear to be the most plausible explanation for this situation. Knowledge of the fundamental causes for such differing growth patterns under apparently similar conditions would be of great practical importance to the oyster industry, and for this reason, we have undertaken an investigation of the problems involved.

Since shellfish are filter feeders, the logical starting point in our investigation was to obtain estimates of plankton concentrations and fluctuations at the two stations over a period of time. An accurate method for the collection and estimation of plankton was essential. Enumeration of the plankton was not considered feasible because of the time involved, but there are several methods for estimating chlorophyll, which is an indirect measure of phytoplankton abundance. In the past, chlorophyll concentrations have been reported in terms of the arbitrary Harvey Unit, but with the advent of the quartz spectrophotometer, it is possible to measure accurately and quickly the different chlorophylls as well as other plant and animal pigments. The remaining requirement for this investigation was, then, a suitable method for collecting valid plankton samples.

The discontinuous pattern of the plankton biomass in the sea from day to day and even from hour to hour creates real difficulties in sampling methods. Spot sampling may produce an entirely erroneous picture. Samples collected hourly from the waters near our laboratory may vary tenfold in volume of plankton on the same day. Consequently, we required some method for collecting continuously a relatively small amount of water which could be analyzed periodically for its plankton content.

Capillary bleeders are described in the literature to fulfill this need, but we found them impractical. They clog easily, are tedious to calibrate, and their rate of flow varies with changes in water depth. When this type of collector is submerged, an unknown amount of water surges into the sample bottle until a pressure equilibrium is reached. It is almost impossible to evaluate the error introduced in this way.

After much trial and error we have developed the arrangement of equipment shown in Figure 2, which overcomes the disadvantages of a capillary bleeder collector and offers some distinctive advantages. We are using this apparatus routinely in our investigation of plant pigments at the two stations.

As shown in the diagram, the essential feature of the apparatus is the connection of both vacuum and pressurelines to the six-liter collecting flask. The degree of vacuum is controlled by the stopcock. Pressure can be applied to the system by means of the three-way valve. Water is admitted to the sample bottle through the inlet tube, which has a seven to eight millimeter bore.

In use, pressure is applied to the system, and the sliding rack holding the collecting flask is lowered to the desired level under water. The rack, weighted to counterbalance the buoyancy of the flask, slides down a galvanized pipe set firmly into the bottom.

Pressure within the collecting flask prevents any initial surge of water through the inlet tube. When the rack is in position, the three-way valve is turned to close the pressure line. Excess pressure is relieved by the escape of air out of the inlet tube, and equilibrium with the outside wateris established almost immediately. The three-way valve is turned again to apply suction to the system, and water flows into the flask at the pre-regulated rate. The relatively large bore and slight current make clogging of the inlet tube unlikely. At the same time, they may hinder the collection of long chains of phytoplankton and elongate or highly motile macroscopic zooplankton. At the end of the collecting period, the rack is vigorously shaken to mix the sample thoroughly. As the rack is raised and pressure decreases on the system, there is a surge of water out of the collecting flask. Since this is an aliquot of the sample, its loss is of no importance.

The rate of collection is dependent on the adjustment of the stopcock: it may be varied so as to fill the collecting flask within any desired interval from a few minutes to 96 hours. We adjust the stopcock by trial and error so that approximately 20 drops of water per minute flow through the inlet tube. At the end of 24 hours the flask is approximately 80 per cent full and the sample is representative of the entire mass of water that has flowed past the station during that period of time. Since the rate of flow in any one collecting flask stay uniform, differences in the flow rate and total volume collected at two different stations are of no significance providing, further, that the collections are simultaneous and each sample is less than the capacity of the flask.

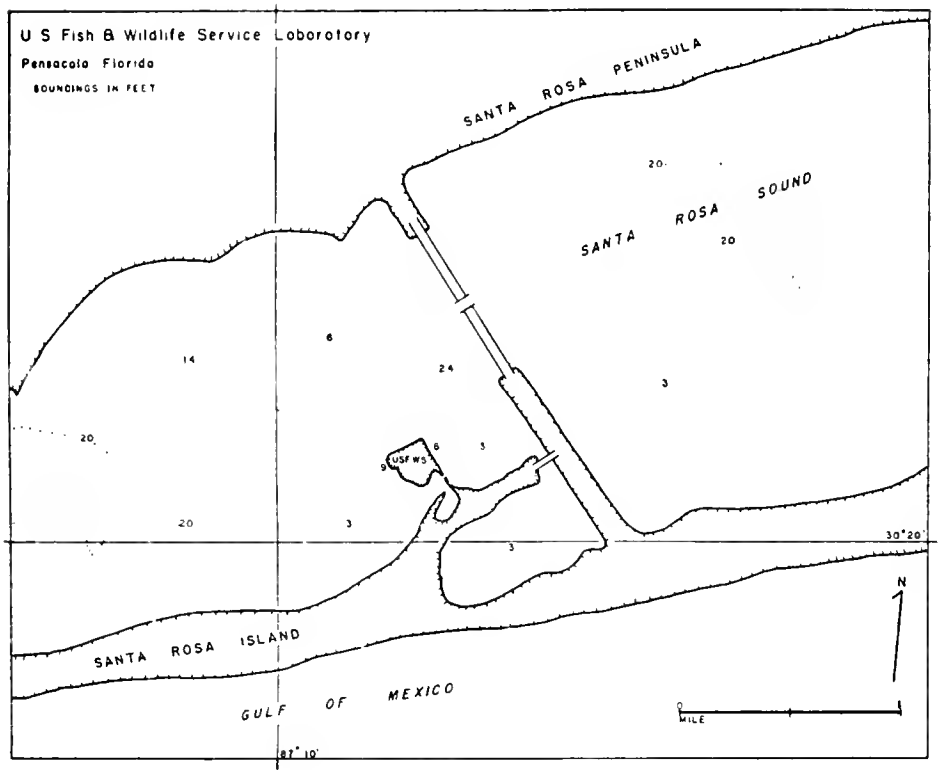

Fig. 1. Chart of the laboratory island, U. S. Fish and Wildlife Service, showing the location of the two sampling stations at the nine and six foot soundings on either side of the island.

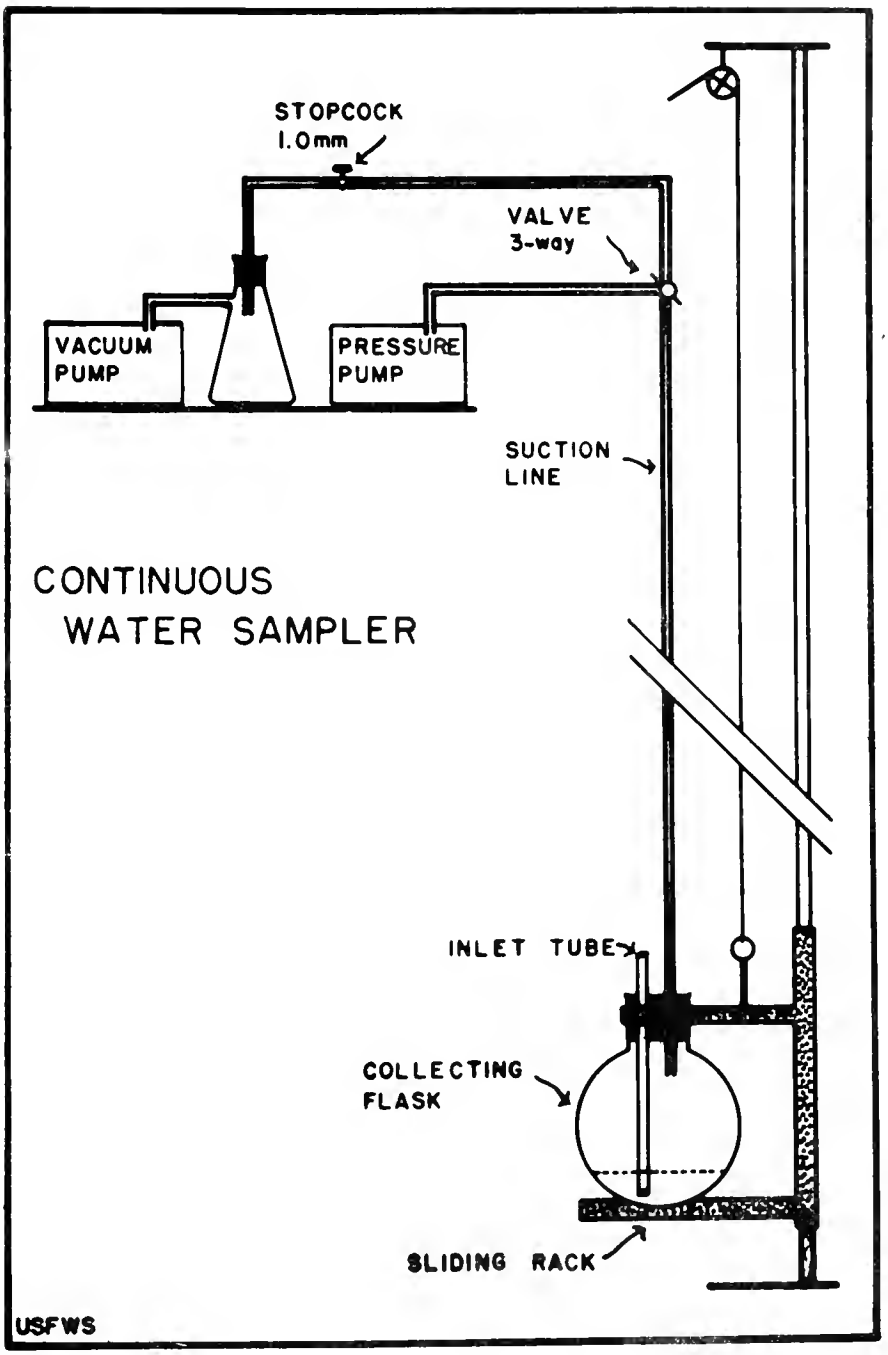

Fig. 2. Diagram of equipment used in collecting water samples.

With this arrangement of the collecting equipment, there is no necessity for the tedious calibration ordinarily required when using duplicate collectors.

The critical point in the entire system is the original adjustment of the stopcock on the vacuum line. This is most easily accomplished by transferring the suction line temporarily to a trial collecting flask fitted with an inlet tube. With this flask submerged in a large battery jar, the stopcock can be adjusted by observing the rate of flow into the trial collecting flask.

Our analytical procedure for estimating concentrations of plant pigments depends on a combination of techniques fully described in the literature. Briefly, plankton is removed from the water samples by suction filtration using Millipore AA filters, as described by Goetz and Tsuneishi (1951). Such filters retain particles having a diameter of 0.8 micron or greater as well as many smaller ones. Depending on the concentration of plankton, samples can be filtered at the approximate rate of one liter per hour. The cellulose ester filters are dried *in vacuo*, dissolved in acetone and absorbencies of the samples are read with a Beckman DU spectrophotometer. Concentrations of chlorophylls *a*, *b* and *c* as well as astacin and non-astacin carotenoids can then be calculated using formulae published by Richards and Thompson (1952).

At present, we collect one sample per station per day for four consecutive days each week and analyze them individually for plankton pigment concentrations. We consider these four-liter samples to be valid aliquots of the millions of gallons of water flowing past each station daily. The effectiveness of this apparatus for collecting representative samples may be judged by comparing the average monthly salinity of its samples with data obtained from another water sampler used routinely at this laboratory (Collier et al. 1953). This second device collects a small sample of water at hourly intervals from the continuously flowing salt water system of the laboratory. Although salinity levels may vary from day to day during the month by as much as 15 parts per thousand, average monthly data obtained using these two collecting methods are in frequent agreement and, during the past year, havenot differed by as much as one part per thousand.

We have been conducting this program of plankton pigment analyses for several months now and find that initial results are consistent and show characteristic trends at the two stations. Chlorophyll *a* appears to be the most significant component of the pigment complex and ranges in concentration from approximately 1.0 to 12.0 mg/M^3. Both chlorophyll *a* and *c* fluctuate greatly from day to day, but changes in their average concentrations seem to be directly related to changes in average water temperatures.

Summary

The water collecting device described here has special features which may be of value to other investigators. Its application is limited to fixed installations such as docks. Its chief advantage lies in its capacity to collect a relatively small aliquot from a large mass of water during a period of one or more days. Such samples have obvious value for estimating average concentrations of both particulate matter in the plankton and dissolved ions including nutrient salts and trace elements.

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PUBLIC HEALTH SIGNIFICANCE OF PARALYTIC SHELLFISH POISON:

A REVIEW OF LITERATURE AND UNPUBLISHED RESEARCH

E. F. McFarren, M. L. Schafer, J. E. Campbell, and K. H. Lewis

Robert A Taft Sanitary Engineering Center,
Cincinnati, Ohio

E. T. Jensen

Milk and Food Program
Public Health Service
Washington, D. C., and

E. J. Schantz

Chemical Corps, Ft. Detrick, Frederick, Md.
Consultant to the Robert A. Taft Sanitary Engineering Center

The prevention of poisoning due to the ingestion of toxic shellfish has been a problem of mutual concern to public health and fishery authorities in Canada and United States for many years and has been recognized for over a century as a clinical entity. From the standpoint of public health, paralytic shellfish poisoning or so-called "mussel poisoning" cannot be classified as a major problem; however, it has caused considerable concern because of its extreme toxicity and the fact that there is no known antidote. Less than one millionth of a gram is sufficient to kill a mouse, and the fatal dose for man is only a few milligrams.

During the 1945-46 clam canning season, which was cut short by the regulatory action of the Food and Drug Administration, Southeastern Alaska operators produced packs of frozen and canned butter clams, Saxidomus, valued at over $170,000. As this industry was a winter operation, offering employment and income during an otherwise slack season, it was of special importance to resident Alaskan economy. Likewise, the Maritime Provinces of eastern Canada are an important producing area for soft shell clams, Mya, normally exporting about four million pounds per year to the United States.

Along the Pacific Coast of North America and the Canadian Atlantic Coast, as well as a few other parts of the world, mussels, Mytilus, may also become poisonous. The chief danger in these areas is that individuals may gather the shellfish and roast them on the beach. Because of this popular summer sport of beach parties or "clam bakes" and the commercial operations mentioned above, it is obvious that unless adequate control measures are maintained mass intoxication would result.

Of the many naturally occurring poisons, there are only two general types which resemble shellfish poison in some respects. One of these is the so called "waterbloom poisoning" (Fitch et al. 1934) which may be elaborated by profuse growth of fresh water algae or by their subsequent decay. Farm animals have died within an hour after drinking from lakes where the plankton grows, and it has been possible to demonstrate the poison in solution in the water. Deer and ducks and other wild birds have also reportedly been killed by this poison in various parts of the world. There are no reported cases of humans being poisoned by drinking water containing large quantities of algae. However, during the great droughts of the 1930's there was a widespread unexplained outbreak of gastroenteritis in several eastern cities. Some investigators thought that this outbreak might have been related to tremendous growth of algae which occurred in rivers used as a source of raw water, even though the water was purified in efficient water treatment plants and was heavily chlorinated.

The other related group of poisons is found in the flesh of fish. Three different types of fish poisoning have been intensively investigated by Halstead (1951), but little is known concerning the origin or nature of these poisons. One of the types, Ciguatera, is common in the Caribbean and in several instances has been reported as causing intoxications in Florida. The great barracuda is one of several fish species involved, and is most frequently associated with poisoning in Florida. A second type of fish poisoning, the Pacific, is found throughout the South Pacific areas and is generally associated with the coral belt. The third type of poison, Tetrodon, is found primarily in the Japanese areas. Of the three types, the Tetrodon is the most deadly and causes symptoms (Sommer and Meyer 1937) most similar to those of shellfish poisoning in man and animals.

Source of the Poison

The original source of the poison in shellfish is certain species of unicellular microscopic marine organisms of which the dinoflagellate, Gonyaulax catenella is perhaps the best known (Sommer and Meyer 1948). It is a free-swimming organism, multiplying by formation of chains of two, four, or even eight individuals of dark orange or greenish brown color, and living like a true plant cell by photosynthesis. It is most abundant in the summer. At times it may increase to 40 million per liter. At such times the water for miles is a deep rust color---the so-called "red water" in the day time and a beautiful luminescent spectacle at night. Other dinoflagellates or diatoms reach similar population densities in the ocean without being poisonous. Gonyaulax catenella may vary considerably in number and perhaps, in its poison content, because small numbers which are not visible as red water may cause shellfish to become poisonous.

Plankton serves as food for many animals of the seashore, and various plankton feeders may at times become poisonous. There is only

one known case in which oysters (Hunter and Harrisoh. 1928) may have been toxic. Scallops may become highly toxic, but the danger is not great because ordinarily only the nontoxic adductor muscle (Medcof et al. 1947) is eaten. The principal species of edible shellfish which reach dangerous levels of toxicity are mussels and clams. After the mollusks ingest plankton, the poison tends to accumulate in the digestive gland of mussels (Sommer et al. 1937) and clams, although gills of the soft shell clam (Medcof et al. 1947) are a secondary site of accumulation and the siphons of butter clams (Chambers et al. 1952) have been found to contain over 50 per cent of the poison. The poison evidently does not harm the shellfish. Toxicity is proportional to the number of Gonyaulax ingested and to their poison content. If a large number of Gonyaulax is present in the water, the toxicity of the bivalves may rise to dangerous levels within a few days. In the absence of the organisms the stored poison is slowly eliminated.

The reasons advanced by Sommer et al (1937) for believing that Gonyaulax catenella is the primary source of poison on the west coast of the United States are: (1) In the stomachs of toxic mussels this species of plankton was in considerable numbers, whereas it was either absent or present in very small numbers in the stomach of nontoxic mussels. (2) Years of investigation indicate that the yearly maxima of certain species of the genus Gonyaulax occurs preceding and during each poison period. (3) When poisonous mussels were kept in the laboratory in clean aerated sea water without food, the toxicity of the mussels dropped about one-half in 10 days. On the other hand, when mussels were kept in the laboratory in fresh sea water at a time when Gonyaulax catenella was abundant, the poison content rose as much as 20 times. (4) When surface water to the amount of 150 liters was filtered through a No. 25 net and the residue collected in a volume of 72 ml, a count of the organisms revealed a total of 2,100,000 Gonyaulax catenella. Extraction of these organisms with acid revealed that 2,050 of these organisms yielded one lethal dose (mouse). Riegel et al. (1949) describe an experiment in which about 500,000 mouse units of poison were centrifuged from 5,000 liters of red sea water containing Gonyaulax.

In studying paralytic poisoning associated with mollusks from the Bay of Fundy, Needler (1949) concluded that toxicity was caused by Gonyaulax tamarensis. Koch (1939) found that another dinoflagellate, Pyrodinum phoneus was responsible for extreme toxicity in Belgian mussels. In contrast to mussels from California and New Brunswick, the toxic Belgian mussels originated from estuaries and inner harbors, and it must be assumed that an organism is responsible which has its habitat in brackish waters. Further taxonomic studies are needed to establish the relationship of these species to Gonyaulax catenella.

In an attempt to correlate shellfish toxicity with other periodic manifestations, one naturally turns to the meteorological and oceanographic data. From a study of the weather records in California it is apparent that the maxima of shellfish toxicities occur in the summer at times when the water and air are relatively cool (Sommer and Meyer 1937).

The temperatures of the water along the coasts of California and Oregon indicate that there are upwellings of colder water to the surface. Consequently the average temperatures of the water along the central coast of California are 3 to 4° C lower than the average to be expected from the latitude of the locality. In fact, average temperatures of the water along the entire region in which mussel poisoning occurs, from central California to Alaska, are remarkable similar in summer time, ranging from 10 to 14° C.

In regard to the tides (Sommer and Meyer 1937) no clear relationship can be observed. As a general rule toxicity may be expected to reach a maximum on or immediately after the second big tide in early summer, but outbreaks have occurred at time of the autumnal equinox when tide differences were at a minimum. Evidently tides are not a primary cause of variations in the toxicity of shellfish.

In studying the effect of water conditions on Gonyaulax found in the Bay of Fundy, records were kept showing stage of the tide, time of day, condition of the weather, and surface and bottom temperature of the water. On these the only factor correlated with occurrence of Gonyaulax tamarensis was the temperature of the surface water. Comparison of numbers of Gonyaulax tamarensis and water temperatures, especially at the surface, indicate that Gonyaulax tamarensis may appear any time after the surface water reaches 10° C. Small peaks in the counts of Gonyaulax tamarensis often occur at the same time as temperature peaks during late July or early August, but the highest count of Gonyaulax tamarensis for any given year corresponds to the peak temperature (13.9° C) in the latter half of August. Although these findings have provided a useful basis for local control measures, specific relationships between water temperature and toxic plankton are not characteristic of the St. Lawrence River estuary and perhaps other areas.

In the Bay of Fundy the principal enemy of Gonyaulax is a ciliate Favella ehrenbergii (Needler 1949). The ciliate occurs at about the same time of year as Gonyaulax, sometimes in enormous numbers. It was found feeding on small dinoflagellates, including Gonyaulax tamarensis, and investigation showed a relationship between the counts of the two species, large numbers of Gonyaulax tamarensis occurred only when Favella ehrenbergii was rare or absent. A survey of the diatom production in this area also indicated that the number of diatoms (Needler 1949) may have some effect on the number of Gonyaulax tamarensis. It is suggested that when many diatoms are present in late July or August they may compete with Gonyaulax for food or reduce the light and so check production.

During the summer of 1949 the Hooper Research Foundation of the University of California [illegible]perating with the Fishery Products Laboratory in Ketchikan, Alaska, successfully identified Gonyaulax catenella in waters from several areas of Southeastern Alaska (Magnusson and Carlson 1957). The organisms were never found in numbers even approaching the concentration needed to cause a visible red tide. Red tides do occur during the summer, but they have been found to contain concentrates of

other plankton which do not exhibit the toxic qualities of Gonyaulax. In Alaska, however, shellfish are toxic the year around (Chambers et al. 1952) with a slight indication of a period of high toxicity in the fall followed by a decrease during the winter and a rise again in the spring. It is, therefore, probable that the water temperature does not fluctuate so greatly in Alaska, and that there Gonyaulax are present most of the time although the temperature never becomes high enough for blooms of these organisms to occur.

Occurrence and Distribution of Toxic Shellfish

The first recorded death ascribed to paralytic shellfish poison on the North American Continent occurred on June 15, 1793, on Vancouver Island, British Columbia, when one of Captain Vancouver's seamen died after eating roasted mussels. There is reason to believe that this was not the first death due to paralytic shellfish poison, for Vancouver wrote that his crew members had some idea of how to treat the stricken seamen, thus indicating prior knowledge.

In 1799 a troupe of Aleut hunters from Unalaska and Kodiak stopping at a place now known as Peril Way (near Sitka, Alaska), consumed mussels and, according to Petroff (1884), 100 men died in less than two hours.

In 1953 Meyer reported that the Hooper Foundation had collected histories of more than 400 cases of mussel or clam poisoning recorded in the literature (Sommer and Meyer 1937, Meyer et al. 1928, California State Department of Public Health 1951, and Public Health Service 1951). There were 35 deaths among the group. Most of these poisonings occurred at irregular intervals along the central California coast with a sprinkling of extensive outbreaks from Juneau, Alaska, to southern California. All cases occurred between May 15 and October 26.

Medcof and his associates (1947) and Needler (1949) reported a total of 28 cases of paralytic poisoning due to the soft shell clam in the areas of the Bay of Fundy, New Brunswick, and Nova Scotia in 1945. In 1936 Murphy reported five cases with two deaths caused by eating mussels in Nova Scotia. Twenty-four cases (Fish and Wildlife Service 1946) were reported in Maine in 1943.

Several cases attributed to the ingestion of the white mussel (Donax serra) or the black mussel (Mytilis edulis) have been observed near Cape Town, South Africa (Saprika 1948, von Bonde 1948). There were 12 cases with four deaths due to these shellfish in Belgium (Koch 1940).

Mussel poisoning has also occurred in New Zealand (Meyer 1953) and before 1915 Germany, England, Ireland, and France had reported over a period of 20 years approximately 110 cases with 24 deaths (Meyer et al. 1928).

In 1948 two children died from eating mussels collected at Les Boules, Quebec, on the south shore of the St. Lawrence River, and in July 1954 two out of seven persons died from eating soft shell clams collected at Metis Beach. (Tennant et al. 1955).

One fatal case of shellfish poisoning was also reported in July 1954 at False Pass, Alaska (Meyers and Hilliard 1955). Six other eating mussels from the same source four days earlier became sick. A summary of known outbreaks by geographical location, year of occurrence, and number of cases is given in Table 1. The disastrous outbreak in Alaska in 1799 has been omitted from the table because description of the clinical manifestations is not sufficiently detailed to establish conclusively that mussel poison was the cause of illness.

In the above cases of shellfish poisoning it has been assumed that fresh mussels and mussels kept for a few days were equally toxic. In California (Sommer and Meyer 1937) all of the mussels Mytilus californianus were derived from the open shore of the ocean. Not a single poisoning occurred from eating mussels of the species Mytilus edulis, gathered in San Francisco Bay or other bays. On the west coast nearly all clam poisonings were caused by Saxidomus nuttalli, Washington clam, (Sommer and Meyer 1937) from Bodega Bay. However, Siliqua patula (razor clams) on several occasions have contained only a little less poison than mussels from a nearby source. The toxicity curve of sand crabs has also been found to parallel that of mussels.

The data accumulated along the Pacific coast (Sommer and Meyer 1937) of the United States indicates that the time of danger from shellfish poisoning is from May until October, with peak toxicity about the middle of July. Variation in toxicity of mussels in a given bed is not large, i.e., not more than $\pm$ 50 per cent, and is negligible compared with the variability in samples from different beds, which amounts to as much as 1:3500. Severaly assays were also made of male and female mussels and toxicity was always found the same within the limits of experimental error. These constancies held, however, only if mussels from approximately the same tide level were compared. Specimens gathered from the lowest possible locations, which are swept by the waves most of the time, are on the whole more poisonous than those from the upper limits of their habitat where the water supply may be scarce.

In studying the presence of poison in the butter clam, Saxidomus giganteus, in Alaska, it was concluded that the clams were toxic throughout the year in certain areas, although there was slight indication of a high toxicity period in the fall, followed by a decrease during the winter and a rise again in the spring. In most cases there was no significant difference in samples taken at the same time from high and low plots (Chambers et al. 1952). It was further shown that bodies of the clams do not vary greatly in toxicity from month to month but toxicity of the siphons showed marked fluctuations.

Table 1. Known cases of paralytic shellfish poisoning 1798 to 1954

Location	Years of occurrence	No. of cases	No. deaths
California	1903, '15, '17, '18, '27 '29, '32, '36, '39, '43 '44, '46, '48, '54	373	30
Oregon	?, 1933	22	1
Vancouver, B.C.	1793	3	1
Alaska	1934, '54	19	3
Nova Scotia & New Brunswick	1936, '45	33	2
Maine	1943	24	-
Quebec	1948, '54	9	4
England	1857, '72, '88, 1904	7	4
Wales	1909	19	1
Scotland	1827	30	2
Ireland	1872, '90	11	8
Norway	1901	5	2
Prussia	1885, '87	22	5
France	1907	13	2
Belgium	1940	12	4
South Africa	1948	several	-
New Zealand	1951	several	-
	TOTAL	602	69

In Alaska none of the beaches (Chambers and Magnusson 1950) from so-called outside waters contained toxic clams, but beaches on or near the open water of the wide straits characteristic of southeastern Alaska yielded the most toxic clams. As sampling was continued toward the head of the bays toxicity decreased.

Poisonous shellfish gradually lose their toxicity if they are put into sea water (Sommer and Meyer 1937) in nontoxic surroundings. With strongly poisonous shellfish, excretion of the toxic substance into the water can be demonstrated directly. Experimental work in California showed a drop to one-half of the original toxicity in 10 days when mussels were kept in filtered aerated sea water at room temperature. However, the Alaskan butter clam (Magnusson and Carlson 1951) and the east coast soft shell clam (Medcof et al. 1947) lost their toxicity much more slowly in transplantation studies. On the other hand, by transplanting nontoxic shellfish to toxic areas, the shellfish become poisonous in three to four days (Sommer and Meyer 1937); in fact, some poison was detected after 24 hours. In California, in one area, mussels showed a hundredfold increase in toxicity in about 12 days. At Metis Beach, Quebec on the south shore of the St. Lawrence River it was shown in 1954 that within six days toxicity of Mya arenaria increased from 542 to 26,180 mouse units per 100 grams (Tennant et al. 1955). These facts indicate that slightly poisonous shellfish may become dangerous in the course of a few days and demonstrate the importance of the problem from the standpoint of public health.

Physiology and Toxicology

Symptoms of poisoning (Meyer et al. 1928) in human beings are primarily peripheral paralysis which may vary from slight tingling and numbness about the lips to complete loss of strength in muscles of the extremities and neck, and to death by respiratory failure. In a moderately severe case the tingling, stinging sensation around the lips, gums and tongue develops about five to thirty minutes after consumption of the mussels. This is regularly followed by numbness or a prickly feeling in finger tips and toes, and within four to six hours, the same sensation may progress to the arms, legs and neck, so that voluntary movements can be made only with great difficulty. In all cases of moderate severity this toxic weakness and stiffness of locomotion is accompanied by a peculiar feeling of lightness. Some patients have declared that they experienced a floating or flying sensation and even heavy objects appeared to them very light. The reflexes are normal and active. It is stated that in one of the fatal cases the deep reflexes were affected. The mentality remains clear, although dizziness, staggering, and drowsiness may be noted. A few patients complain of a gripping sensation around the throat and slight respiratory distress. Incoherence of speech was noted in one of the fatal cases. Vomiting is an inconstant symptom, but diarrhea and abdominal pain have not been recorded in untreated cases. In fact, there is a tendency to constipation which persists for several days. Records of carefully observed cases

show average temperature to be slightly subnormal (mean 98° F). The pulse is firm and slightly accelerated (80-100 per min.). During recovery some patients have chilly sensations in their limbs and may feel slightly stupefied or become easily fatigued for a number of days. The longest recorded interval between eating poisonous shellfish and death is 10 hours and the shortest interval is three hours. Intoxications may be mild or fail to develop in those who have consumed shellfish in conjunction with a heavy meal, have boiled the mollusks with rice and garlic, or have fried them in oil. When shellfish are taken into an empty stomach intoxication rate seems to be higher. As soon as intoxication is recognized emptying of the stomach by an emetic and purging by brisk laxatives has been the usual practice, but in severe cases these measures canot be relied upon to prevent adsorption of a fatal dose of poison. As soon as difficult breathing develops artificial respiration should be applied. Clinical observations indicate that even in severe cases this procedure may prevent a fatal issue if extended over several hours.

The lethal dose of mussel poison for man was not known until 1946, when an epidemiologic investigation (Meyers 1953) furnished leading information. In most instances the amount of poison eaten by victims cannot be estimated reliably, but in this episode, involving two men and a woman, the exact number of mussels eaten by each was known from the number of empty shells left after the meal. Furthermore, adequate samples of cooked and raw mussels for assay purposes were left over from the beach picnic. It was estimated that one member of the party consumed about 42,000 mouse units; this man died in less than four hours. The woman ingested about 22,000 mouse units, became seriously ill and was put in a respirator for four and a half hours. This supportive measure was probably responsible for her recovery. The second man, who had eaten mussels containing about 17,000 units, had mild symptoms and recovered. It this is an accurate estimate, the lethal dose for man is probably between 20,000 and 40,000 mouse units.

A more recent epidemiological investigation of an outbreak involving seven persons at Metis Beach, Province of Quebec, gives further information on the amount of poison which may be necessary to cause intoxication (Tennant et al. 1955). One death resulted from the ingestion of not less than 3,300 or more than 31,300 mouse units. A second fatality resulted from ingestion of not less than 1,200 or more than 11,700 mouse units, but another person who ate a similar quantity survived. Four other persons survived but had severe symptoms from ingestion of 428 to 3,200 mouse units each.

Medcof et al. (1947) also investigated clam poisoning in Canada and concluded that people involved showed great variation in severity of reactions to approximately equal doses. In general, however, those who ingested the most poison suffered most. Mild poisoning, with symptoms sometimes including numbness of legs and arms, arose from doses of poison estimated at 2,000 to 10,000 mouse units. Those severely poisoned, who suffered prostration and paralysis in addition to mild reactions, generally ingested 10,000 to 20,000 units. Respiratory difficulty resulted

from ingestion of heavier dosages estimated at about 30,000 units. Inquiry further showed that picnickers (city people) eat clams only on rare occasions. In contrast, people living in many of the shore communities eat them frequently, especially in winter when other types of protein food are often not readily available. The 88 different persons for whom records of consumption of toxic shellfish were obtained included 35 residents and 53 nonresidents of shore communities. One case of poisoning arose in the former and 23 in the latter group. The maximum dosage ingested without producing any symptoms was estimated at 17,000 units, which is sufficient to produce severe illness in the most susceptible persons. From these records (Medcof et al. 1947) it may be deduced that: (1) some people have a natural tolerance to the poison; (2) some people in the shore communities who consume shellfish more or less regularly may acquire a tolerance to doses of poison which would produce severe symptoms in susceptible persons. The investigators were unable to demonstrate development of tolerance in experiments with guinea pigs and mice. Their research indicated that the poison is not antigenic, and that repeated exposures do not increase resistance.

In connection with establishment of the human lethal dose, it is of interest to compare estimates for man with results obtained using various laboratory animals. In 1945, 1946, and 1947 several shipments of frozen clams from Alaska to Seattle, Washington, were detained by the Food and Drug Administration (Woodward 1955) because they contained shellfish poison. Toxicity tests were made to determine the LD_{50} for several different animals. The comparative data in Table 2 were obtained by using material from a commercially frozen pack containing from 800 to 2,000 mouse units per 100 grams. From 40 to 80 animals of each species were used, except for the monkeys and dogs, where smaller numbers were tested.

As indicated in Table 2, these workers, using a crude acid extract of clams, found an oral LD_{50} for rats of 1,060 mouse units per kilo. After a supply of purified paralytic shellfish poison became available to the Public Health Service, the LD_{50} of this solution was determined at the Sanitary Engineering Center, Cincinnati, Ohio. In one experiment with 40 white albino rats from Hamilton Laboratory Animals, Inc., Cincinnati, Ohio, an oral LD_{50} for male and female animals weighing 100-150 grams was 1,715 mouse units per kilogram. In another experiment, with 80 adult male and female albino rats of the Sprague-Dawley strain weighing 200-250 grams, the oral LD_{50} dose was established as 3,270 mouse units (Davis and Campbell 1956) per kilogram of body weight. In still another experiment, in which 90 rats of the Sprague-Dawley strain were pretreated with a nonlethal conditioning dose of 1,000 mouse units per kilogram two weeks prior to determination of the LD_{50} dose for these rats, the LD_{50} dose was 4,718 mouse units per kilogram. From these preliminary results summarized in Table 3, it can be concluded that the oral LD_{50} dose for rats may vary, depending upon strain and/or weight of rats used, and that a nonkilling oral dose of paralytic shellfish poison renders rats less sensitive to subsequent doses. If a comparison can be made between the LD_{50} for rats determined by the Food and Drug

Table 2. LD_{50}* of shellfish poison for various animals

Animal	Oral LD_{50} per kilogram**	Estimated lethal dose for adult animal**
Pigeons	500	25C
Mice	2,100	63
Rats	1,060	320
Rabbits	1,000	3,500
Guinea Pigs	640	260
Cats	1,400	3,500
Dogs	1,000 approximately	11,000
Monkeys	2,000 to 4,000	15,000
Man	500	36,500

* Woodward. 1955

** In mouse units.

Table 3. Variation in response of rats to oral doses of paralytic shellfish poison

Strain	Weight g	No. tested	Conditioning dose*	Oral LD_{50}*
Hamilton	100-150	40	0	1,715
Sprague-Dawley	200-250	80	0	3,270
Sprague-Dawley	200-250	90	1,000	4,718

* In mouse units

Adminstration and the LD_{50} determined by the Sanitary Engineering Center, it would appear that an LD_{50} dose determined by using purified shellfish poison is higher than when a crude extract of poison is used. This latter result would seem to confirm the finding of an effect of salt (Schantz et al.) on the bioassay of clam meat extracts. It would appear that salt in the extract lowers the bioassay by causing a decrease in the rate of adsorption of the poison when injected intraperitoneally into mice or fed orally to rats.

A number of physiological and pharmacological studies have been made on shellfish poison in order to investigate its mode of action. In autopsies on humans (Meyer et al. 1928) no significant findings were recorded in the abdominal or chest cavities except a slight pulmonary congestion. In every case folds of the stomach were studded with small hemorrhages.

Injection of small or intermediate amounts of mussel poison into dog or rabbit (Prinzmetal et al. 1932) causes a definite slowing of respiration with gradual recovery. In the dog there is a fall in blood pressure followed by a secondary rise. With respiratory depression, a secondary slowing of the heart occurs. Large doses cause an immediate speeding up of the heart followed by marked secondary slowing. When fatal doses are given, the heart continues to beat after respiration has stopped. In one rabbit the heart beat 45 minutes after respiration had stopped, and when the thorax had been opened, it was found that the auricles were beating three to five times faster than the ventricles. It is thought that this heart block is probably due to the direct action of the poison on the heart muscle.

In a study of the excretion (Prinzmetal et al. 1932) of the poison, urine was taken from a dog which had been fed 100 mouse units over a period of two hours. Of the 100 mouse units, 40 were recovered in the urine. It was concluded that excretion takes place probably almost as fast as absorption, thereby preventing a high concentration of the poison in the body fluids.

Frogs (Prinzmetal et al. 1932) are quite resistant to shellfish poison. Only a slight transitory stimulation of the respiratory movements is caused by 7.5 mouse units. Fifteen mouse doses are required to cause severe symptoms. The general symptoms resemble closely those caused by curare and consist of progressive paralysis, which is quite general, with the exception of the heart action. Respiration is slowed and may stop, and the heart is slowed, but the animal may recover after several days depending upon its individual resistance and environmental conditions.

On macroscopic examination of the central nervous system of poisoned animals, softening and edema of the brain was observed with moderate congestion of surface vessels (Covell and Whedon 1937). There was no evidence of hemorrhages into the spinal cord or brain. The lungs revealed large and small areas of hemorrhage, and the abdominal viscera were congested with blood. Microscopic examination of the organs revealed

the usual changes accompanying agonal death (Covell and Whedon 1937). Acutely poisoned animals revealed no alterations in the nerve cells of the central nervous system. The large nerve cells of the ventral horn of the spinal cord and the ganglion cells of the medulla of the chronically poisoned animal showed alterations in certain cytologic constituents. The only striking evidence within the organs is the altered condition of mitochondria of the convoluted tubules of the kidney. The gradual elimination of the poison by the kidneys may be responsible for this effect.

From a group of experiments on frog muscle and nerves, Kelloway (1935) concludes that shellfish poison is a neurotoxin with central effect upon the cardiovascular and respiratory centers and peripheral effect upon the nerve endings, both motor and sensory.

Fingerman et al. (1953), however, conclude that shellfish poison has a strong effect on both peripheral nerves and skeletal muscles of the frog. Action of this poison on muscle is similar to that of curare. Furthermore, shellfish poison has an even more general action than curare, for it not only decreases excitability of muscle to direct stimulation but also blocks nerve conduction. In view of these actions, this poison must be considered a neuromuscular toxin rather than a neurotoxin.

Characteristics of the Poison

On the occasion of the Wilhelmshaven mass intoxication in 1885, Salkowski (1885) classified mussel poison as belonging to the group of the chemical poisons of the highest potency. Stability to heat, instability to alkali and solubility in alcohol were established. At the same time, Brieger (1889) isolated a gold salt of the base $C_6H_{15}NO_2$ called "mytilotoxin" which he considered a quaternary ammonium base and the pure poisonous principle. According to Muller (1935), Thesen in 1902, although obviously dealing with the same type of toxin, could not identify his poison as "mytilotoxin".

Muller in 1935 also attempted to repeat Brieger's method for isolation of "mytilotoxin" but concluded that even in highly concentrated and purified solutions no precipitate of the poison could be formed by either gold or platinum salts. As a result, Muller was inclined to believe that the toxic properties of Brieger's mytilotoxin were simulated by traces of poison adherent to an inactive substance. Muller's experiment supports the opinion that "mytilotoxin" does not, as stated in many textbooks, represent chemically pure poison responsible for paralytic type of shellfish poison.

Mussel poison has been shown to be soluble in water, methanol, ethanol, glacial acetic acid, and aqueous acetone (Meyer et al. 1928). Effectiveness of these solvents for extracting the poison appears to be increased if small amounts of hydrochloric acid are added. The poison is insoluble in ether, chloroform, ethyl acetate, butanol, and toluene.

It cannot be extracted from an aqueous solution by any of these solvents. The isolation of betaine from a partially purified extract of mussel poison indicated that the properties of betaine and the poison were enough alike that the substances tended to accompany each other. This fact and the solubility behavior suggested that it might be a nitrogenous base.

Muller (1935) further characterized shellfish poison as being of relatively small molecular weight. The purified poison did not give any color reactions for alkaloids and was not removed from solution by the customary precipitants. The poison was purified by the following steps. Drying of the toxic organs, extraction of lipoids with ether, extraction of the poison with acid methyl alcohol, adsorption of impurities and pigments on Norit A, adsorption of poison by permutit, elution by KCl, separation from inorganic salts by the alcohols, acetone, or HCl-ether, and precipitation of poison by rufianic and reinecke acids. The most purified preparation had a potency of 590 mouse units per mg of solids but still contained 35 per cent ash. Analysis of this purified preparation showed it to contain 10.73 per cent carbon, 5.31 per cent hydrogen, and 8.59 per cent nitrogen. Two atoms of nitrogen were present for each three atoms of carbon. The preparation also gave negative carbohydrate and protein tests, was dialyzable, and was not affected by ultraviolet irradiation. Catalytic hydrogenation, as well as attempts at methylation did not change the physiological activity, but acetylation completely inactivated the poison. Hydrolysis in moderate concentrations of mineral acid did not affect the poison. Boiling in strong acid solution destroyed its potency. Oxidants attacked the poison in acid solution only at high temperatures causing instantaneous destruction at 100° C when treated with H_2O_2. No adsorption of the poison on aluminum hydroxide occurred at various pH values. Kaolin adsorbed about 50 per cent of the poison from an aqueous solution. Brief shaking with Lloyd's reagent removed the poison quantitatively from aqueous or acid alcohol solutions, but no satisfactory method for elution was found. Common sea sand, washed free of electrolytes, adsorbed approximately 90 per cent of the poison from aqueous solution and practically none from alcoholic solution. Diatomaceous earth did not adsorb the poison or pigments from a crude extract.

Bendien and Sommer (1941) investigated the use of chromatography for further purification and found active carbon the most satisfactory adsorbent. In preliminary tests to determine optimum conditions for chromatography, four different carbons were tested. Of these Norit A (Sommer et al. 1948) seemed to be most suitable. Mussel poison was not adsorbed completely from solutions containing less than 1.0 N acid. Traces of ethanol in the extract also prevented complete adsorption of the toxic material. The mussel poison hydrochloride was retained by the carbon, and the major portion of the inorganic, as well as organic, contaminants passed through at once. The use of distilled water to develop the chromatogram was sufficient to carry the poison through into the filtrate. By repeated treatment, as above, the ash content of highly toxic material obtained from carbon columns was reduced to three

or four per cent. By chromatography on carbon of a concentrate obtained by ion exchange on Decalso, a mussel poison hydrochloride was obtained with a toxicity greater than 1,000 mouse units per mg of solids (Sommer et al. 1948).

Mussel poison extracts, even after preliminary purification, retain varying amounts of other bases. Choline was found in Decalso eluates. After chromatography of the eluates on Norit A, it was found in the primary acid filtrates. Betaine was also found in acid filtrate from the chromatography of crude extracts on Norit A. A study of the nitrogenous bases (Riegel et al. 1949) was made because their presence in partially purified poison indicated they possessed chemical properties similar to the poison, and because the removal of these bases would be made easier if they were identified. In addition to betaine and choline, homarine, taurine, and tyrosine have been isolated and identified. Choline and homarine, both quaternary bases, remain closely associated with the poison through the preliminary steps in its purification. An additional base has been detected by its characteristic ultraviolet absorption spectrum but has not been identified.

Recently, Schantz et al. and Mold et al. have obtained the poisons from California mussels and Alaska butter clams in pure form. These poisons were purified by adsorption on Amberlite IRC-50 followed by chromatography on XE-64 and on acid washed alumina. The potency of the preparations obtained in this way ranged between 5,500 and 6,000 mouse units per mg of solids. The purified poisons were subjected to frontal analysis and counter-current-distribution and were pure. Repeated chromotography on alumina and recrystallization of the poisons as a helianthate did not produce a product of higher toxicity. The chemical and physical properties of the two poisons indicate that they are identical.

Studies (Sommer et al. 1948) were also made of the effect of pH and temperature on the stability of the poison in aqueous solution as measured by its toxicity. The decrease in toxicity with increases in pH and temperature, as illustrated in Figure 1, shows that the poison must be handled in acid or, under certain conditions, in neutral solution, and that the temperature should be kept low. For example, there is a loss in toxicity of only six per cent in six days at 25° C, but at a pH of 6.6 this loss increases to 35 per cent, and to 74 per cent at pH 11.5. Likewise an increase in temperature from 25 to 100° C at a pH of 5.0 results in increased loss in toxicity from six per cent in six days to 85 per cent in one day. No change in toxicity of shellfish poison is observed in aqueous solution at room temperature if the solution is more acid than pH 4.5, nor in 3 N hydrochloric acid solutions up to a temperature of 55° C. These studies, on the other hand, indicate that commercial processing of clams and mussels by heating above 100° C may be expected to reduce the toxicity considerably unless the product is acidic.

Medcof et al. (1947) reported the effects of domestic cooking

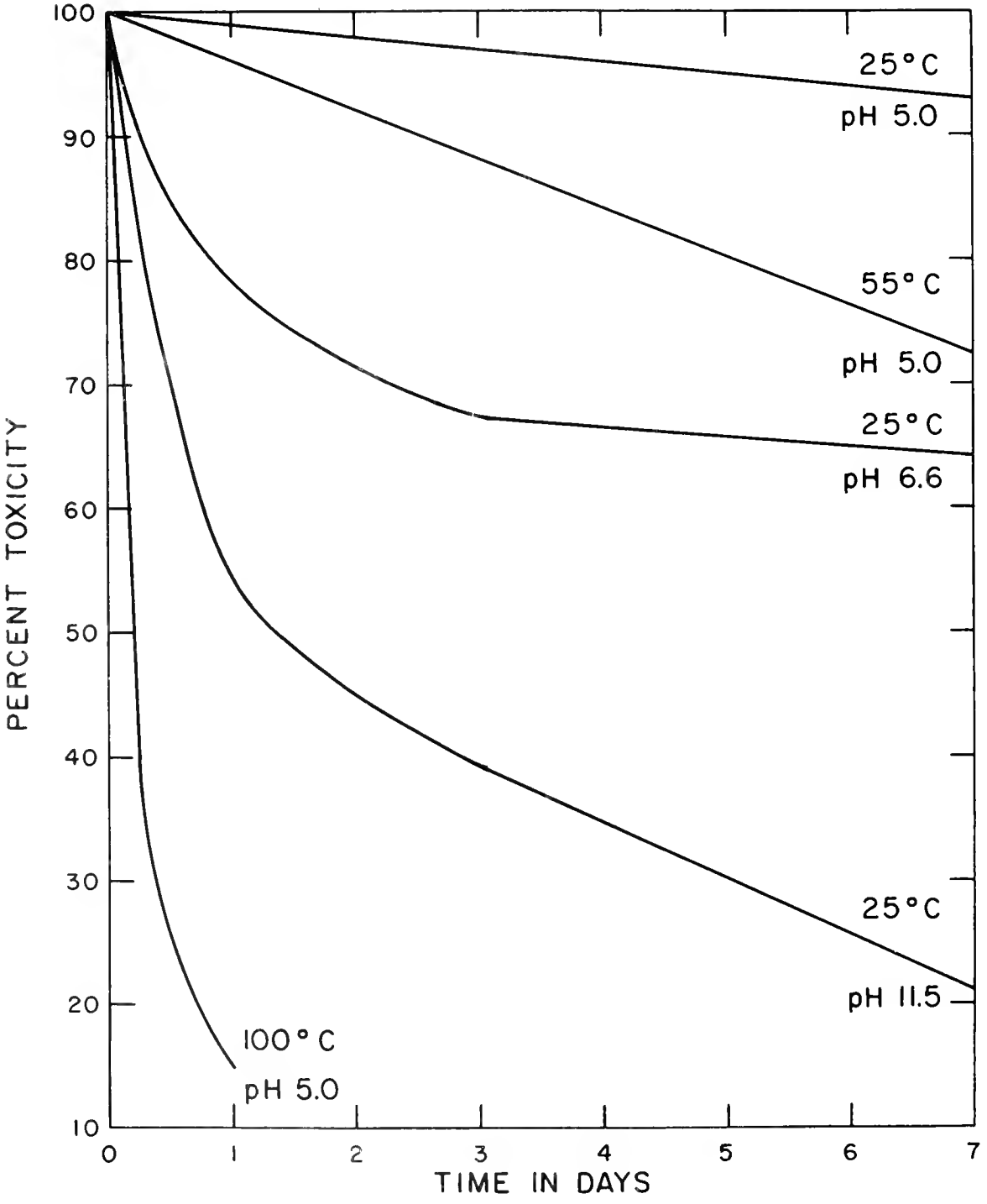

Fig. 1. pH and temperature effects on shellfish poison in aqueous solution (Sommer et al. 1948).

on toxicity. Their results show that ordinary cooking of shellfish in a little water, even for brief periods, provides the consumer with some degree of protection. On the average, 100 grams of steamed, boiled, or pan-fried meat contained only about 30 per cent of the poison in 100 grams of raw meat. Cooking may actually reduce toxicity of the meat by considerably more than 70 per cent because part of the poison is expressed in the juices. However, when clams are steamed or boiled, varying amounts of the juices may be ingested with the meats, either in the form of bouillon or broth. Temperatures involved in pan frying are considerably higher than in steaming or boiling and seem to be somewhat more effective in destroying the poison even though none of the extractives is discarded.

In 1944 and 1945 a cannery (Medcoff et al. 1947) processed 15 different lots of poisonous clams following its established procedure. This involved precooking for 15 to 20 minutes in barrels with loose-fitting heads into which live steam was fed. The clams were then shucked, siphons trimmed off, meats washed in warm fresh water, and packed in cans. Next a portion of the hot bouillon resulting from steaming was used to completely fill the cans. The bouillon was seasoned with vinegar lowering the pH from about 8.2 to 6.5. The cans were sealed without being exhausted and were retorted at 250° F for 45 minutes. The results showed that under the alkaline condition of steaming and the slightly acid conditions in cans during retorting, 84 to 94 per cent of the poison is destroyed. Presumably canning without adjustment of the pH by addition of vinegar would result in even greater destruction of the poison, since it is less stable in alkaline than in acid media. Further tests showed that the bouillon contains considerable poison. In commercial canning preliminary cooking with steam produces much more bouillon than is ordinarily used to fill the cans, and surplus has to be discarded. Thus, much of the poison present in raw clams never enters the commercially canned product. To illustrate this point, raw shellfish meat was packed in cans without adjustment of pH, sealed and processed by two methods---retorting at 230° F for 55 minutes and in a boiling water bath for three hours. The lesser reduction in toxicity (64 to 84 per cent) in these tests indicated that drainage of bouillon from meats is highly important in producing the low toxicities consistently observed in commercial packs. It was concluded that precooking clams with steam for 10 minutes reduced the poison content by about 90 per cent, but extending the period produced no significant additional reduction. Retorting at 250° F for 45 minutes lowered toxicity only by an additional three per cent.

Although perhaps not true of the soft shell clam, it should be pointed out that with the Alaska butter clam the commercial practice of removing the siphon is highly significant in reducing the toxicity of the canned product, since about one-half to two-thirds of the poison contained in the butter clam is found in the siphon.

Numerous studies on transplanting and on storage in air, on ice, and in the frozen state have been conducted in an effort to find a means of detoxification of raw shellfish. None of these methods has resulted in development of feasible industrical practices. Studies have also been

made of variations in commercial canning and processing in an effort to find ways to destroy all of the poison. Until more effective methods are found for detoxifying clams and mussels, commercial exploitation of this valuable food supply is not possible in many areas. Recently, preliminary experiments on destruction of shellfish poison by ionizing radiations have been conducted cooperatively by the Robert A. Taft Sanitary Engineering Center and the Massachusetts Institute of Technology. Through the courtesy of Dr. B. E. Proctor, Head of the Department of Food Technology at M.I.T., both toxic clam meat and the purified poison have been exposed to 3,000,000 roentgen-equivalents-physical. Although the data now available are too limited to be conclusive, the poison appears to be so little affected that irradiation offers no ready means of detoxifying shellfish on a commercial scale.

Prevention and Control

Prevention of shellfish poisoning involves education and legislation in addition to technical control measures. The plain fact that any type of clam or mussel may be a potential source of poison should be readily understood. Along the Pacific shore of North America and the Canadian Atlantic Coast a safe rule is: Do not eat the viscera (dark meat) of mussels or the siphons of clams, or drink the juice of mussels, clams, and scallops from the open coast unless the shellfish from that specific area have been tested recently. The white meat must be thoroughly washed before cooking. The addition of baking soda to cooking shellfish has been advocated to help reduce the toxicity, but it is no safeguard against poisoning if highly toxic whole shellfish are prepared, and it may reduce their palatability.

During the poison season of 1939 in California (Sommer and Meyer 1948) it was shown that warning signs posted along the beaches and across the highways at county lines are effective in reducing the number of persons who dig clams. The same applies to publicity in the local press and through radio stations along the coast. The health officer or local physician has an excellent opportunity to dispel in the minds of the people the many erroneous notions (Sommer and Meyer 1948) concerning the cause of the shellfish poisoning. Among the points to be emphasized are the following: (1) Paralytic shellfish poison is not a post-mortem product of decomposition. (2) Temporary exposure to the sun does not harm living shellfish, nor does it make them poisonous. (3) Mussels below the tide lines are, if anything, more poisonous than those above water. (4) Copper in the rocks, oil on the beaches, and stagnation or pollution of the water are in no way connected with paralytic shellfish poisoning, although they may render the shellfish otherwise unfit as food. (5) Toxic mussels or clams cannot be distinguished from normal shellfish without laboratory tests. (6) Discoloration of a piece of garlic or of a silver spoon in the pot is no indication of poisonous shellfish. The plain story of the chain of intoxication from the microscopic plant through the bivalve to man should be clearly understood by people along the coast, in order that they may avoid exposure to this type of food poisoning.

As for legislative control, various food and drug, fishery, and public health agencies in both Canada and the United States have taken measures for many years to prevent the occurrence of shellfish poisoning. Wherever poisonous clams are known to exist, digging is prohibited whenever tests demonstrate the meats to be toxic. In Alaska this may include nearly all seasons, because clams in certain localities remain toxic the year around. In California, mussels, being the most common source of the poison, are put under quarantine by the State Department of Public Health (Sommer and Meyer 1948) from May 1 to November 1 and at such other times as laboratory tests show them to be dangerous.

During the last war, commercial canning of clams in Alaska grew rapidly from a negligible operation to one of significance to the territory (Mangnusson and Carlson 1951). During the 1945-1946 season the operation was cut short when poison was found in packs of frozen clams. In January 1949 a tolerance level (Magnusson and Carlson 1951) was established for the amount of toxin in whole and minced clams. As modified in February 1951, marketing of fresh, frozen, or canned clams is permitted only when they have an average toxicity of less than 400 mouse units per 100 grams of contents (Magnusson and Carlson 1951). No individual unit package is permitted to exceed 2,000 mouse units per 100 grams. In the case of minced or chopped clams, which are subject to less variation, the average toxicity must be less than 2,000 mouse units per 100 grams of contents and no unit package should materially exceed that figure.

Canning of clams in Canada around the Bay of Fundy and the mouth of the St. Lawrence River has also been practiced for many years. Almost 5,000 toxicity tests (Public Health Service 1955) have been made in this region since 1943, and the indicate that most of the growing areas in the Bay of Fundy can be readily classified for control purposes. Class I areas are not affected by paralytic shellfish poison at all. Class II areas are affected only in late summer for a short period each year, if at all. Class III areas are usually affected the year around. Furthermore, there is a consistent order in the time of appearance of poison in different areas. Mussels in areas exposed to the open Bay of Fundy regularly show poison about 10 days before first traces appear in the tributary inlets. Accordingly, regular sampling at a "key station" shows when the time has come to impose quarrantine.

Up to and including 1945 canning of softshell clams was permitted in Canada at all seasons, but when toxicities of raw stock were above 400 mouse units per 100 grams the pack was released for sale only after systematic sampling showed that it contained not more than 200 units per 100 grams of canned meat (Medcof et al. 1947). Since processing normally destroys about 70 per cent of the poison, such a product was regularly obtained (Medcof et al. 1947) from raw shellfish containing 1,000 mouse units or less per 100 grams. After U. S. authorities relaxed their restrictions in 1951 on packs of whole Alaskan butter clams, Canadians again adopted the 400 mouse unit quarantine level and now permit the marketing of clams (fresh, frozen, or canned) when average score of

representative samples from each shipment are less than 400 and scores of all samples are less than 2,000 mouse units per 100 grams (Public Health Service 1955).

Commercial harvesting of mussels of any species from the Bay of Fundy (Medcof et al. 1947), either for canning or for sale as raw food, has been prohibited at all seasons since the autumn of 1943, when their toxicities were found to be several times higher than those for clams. Likewise the marking of beaches or quarantine of an area to prevent local residents and tourists from digging clams or mussels has been generally instigated when toxicity of the shellfish exceeds 400 mouse units per 100 grams of meat.

These restrictions have interferred seriously with the shellfish industry in Nova Scotia, New Brunswick, and Alaska (Medcof et al. 1947). Nevertheless, they have proved necessary and do provide significant protection, as shown by the fact that sufficient poison (1,000 to 5,000 units) to produce the mildest symptoms in the most susceptible persons would require an individual to eat a whole can, about 300 ml of meat and bouillon combined, with a toxicity of 300 to 1700 units per 100 ml.

For control purposes, the present accepted technique for determining toxicity of shellfish is a bioassay using mice; briefly, this method (Medcof et al. 1947) involves the following steps. Clams are shucked, washed in fresh water, drained on a sieve for five minutes and minced in a meat grinder. A 100 gram portion of the ground meat is suspended in 100 ml of 0.1 N hydrochloric acid and boiled gently for five minutes with continuous stirring. The mixture is cooled, adjusted to a pH of 4.0 or 4.5 by addition of a few drops of 5 N acid or 0.1 N sodium hydroxide, and then made up to 200 ml with distilled water. The supernatant liquid is clarified by settling or centrifuging and used for injection into a few test mice to obtain a preliminary estimate of potency. With high toxicity clams, dilutions of the extract may be necessary to obtain solutions which give death times within the desired range. The death times corresponding to various numbers of mouse units may be determined by referring to Sommer's Table*. When injection of 1 ml into each of three mice produces a mean death time of 10 to 20 minutes, each milliliter is said to contain one mouse unit. The toxicity per 100 grams of meat is the number of mouse units contained in 1 ml of extract multiplied by 200, which is the total volume of 100 grams of meat plus 100 ml of dilute acid.

In 1949 the Fishery Products Research Laboratory sponsored a collaborative study (Public Health Service 1955) on the assay of toxic clam extracts. This study indicated considerable variation when several

*Furnished by Sommer to other workers in this field. These tables are based on graphs (Sommer and Meyer 1937) recorded in the literature. The essential portions have been reproduced for distribution by the Public Health Service (1956).

strains of mice are used, and that results from one laboratory may differ from the assays of another by as much as 60 to 70 per cent under these circumstances.

In 1950 some changes in the assay procedure (Public Health Service 1950) were suggested, and further progress was made in 1955 when the Public Health Service sponsored a one-day conference to discuss the most recent developments in assay of shellfish poison. At the conference, the use of a purified shellfish poison as a reference standard was proposed by Dr. E. J. Schantz. Dr. Schantz and his associates at Fort Detrick have been engaged in this field of research for several years and have succeeded in isolating (Schantz et al.) the poison in pure form (Mold et al.). On the basis of the information presented at the conference, the conferees agreed: (1) that purified poison should be used as a reference standard for obtaining uniform bioassays, and (2) that results of future bio-assays should be reported in terms of weight of the poison. As a result of the conference, the Robert A. Taft Sanitary Engineering Center cooperated with Dr. Schantz in determining practical requirements for use of the purified poison as a reference standard. Briefly, it was found (Schantz, McFarren et al.) that: (1) a median death time between five and seven minutes gives the most reliable estimate of potency; (2) in using the reference standard to determine the factor for conversion (CF value) of mouse units to micrograms of poison, the results should not vary by more than $\pm$ 20 per cent; (3) the CF value obtained in one laboratory may differ significantly from that obtained in another laboratory through the use of different strains of mice or variations in technique of assay, but once the CF value has been determined in each laboratory it is expected that comparable results will be obtained in separate laboratories assaying clams if CF values are used to calculate micrograms of poison; (4) in assaying low-toxicity clams containing around 400 mouse units per 100 grams, which is the present level of acceptability, the bioassay procedure may underestimate total toxicity by as much as 60 per cent.

The Public Health Service has recently issued an interim plan (Public Health Service 1956) for standardization of the bioassay and has secured a quantity of purified poison from the Chemical Corps for use as a reference standard. These items are now ready for distribution, and individuals or organizations desiring to obtain them should address their requests to the Department of Health, Education, and Welfare, Public Health Service, Washington 25, D. C., attention, Shellfish Sanitation Section.

As the result of determination of the micrograms of poison equivalent to one mouse unit, and the information, also presented at the conference in May 1955, that the poison gives a color reaction (Schantz, Mold, and Lynch) in the Jaffe Test, a chemical method for the quantitative determination (McFarren et al.) of the poison has been devised. Briefly, this method (Fig. 2) consists of making an acid extract of clam meats, adsorption of the extract on an ion-exchange resin, elution of the poison and measurement of it colorimetrically by the Jaffe Test.

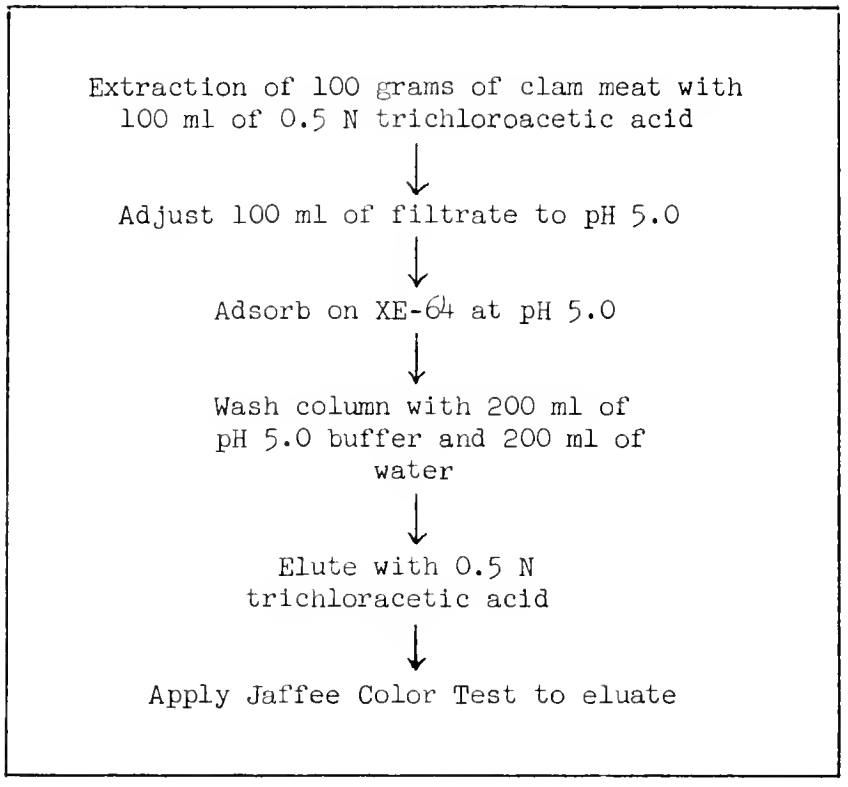

Fig. 2. Flow sheet for chemical assay of paralytic shellfish poison

Table 4. Comparison of the bioassay with the chemical assay for determination of the toxicity of clams

Clam sample	Bioassay ug/100 g	Chemical assay ug/100 g
A	115	159
B	147	198
C	160	207
D	328	337
E	1093	1054

In Table 4 are presented some data comparing the toxicities of various clam samples as determined by chemical and bioassay procedures. The data indicate that in testing low-toxicity clams the bioassay may underestimate the poison content by a considerable amount. This is as to be expected, since, as mentioned previously, in recovery studies in which poison was added to non-poisonous clams, the bioassay underestimated the toxicity by as much as 60 per cent. In assaying clams of greater toxicity the bioassay becomes progressively better, until at high toxicities the recovery appraoches 100 per cent. In this case, as can be noted in Table 4, the bioassay agrees with the chemical assay. The chemical test may not be suitable for use in the field, but in the laboratory has accuracy equal to or greater than that of the bioassay, and should be useful as a control test particularly in areas where it is difficult to obtain and keep mice.

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SHELLFISH TECHNOLOGY

and

PUBLIC HEALTH ASPECTS

THE EFFECT OF AUREOMYCIN[R] CHLORTETRACYCLINE IN THE PROCESSING AND STORAGE OF FRESHLY SHUCKED OYSTERS

Anthony Abbey
A. Richard Kohler
Sidney D. Upham

American Cyanamid Company
Fine Chemicals Division
Princeton, New Jersey

Abstract

The use of Aureomycin[R] chlortetracycline, in concentrations from one to 30 ppm, was evaluated in the processing and storage of freshly shucked oysters. Several trials were conducted in cooperation with commercial processors using production facilities and schedules whenever possible. The proportions of oysters and treating solutions and the exposure times were varied. Although unfavorable storage conditions were usually employed, microbial counts and organoleptic tests favored the oysters processed with Aureomycin[R] chlortetracycline.

FREEZING AND PROCESSING SOUTHERN OYSTERS

This topic was organized for the Convention in the forms of a panel discussion moderated by Charles F. Lee. Abstracts of the discussions are recorded in the following section.

INTRODUCTION TO PANEL DISCUSSIONS

Charles F. Lee

U. S. Fish and Wildlife Service, College Park, Maryland

The first technological project approved under the Saltonstall-Kennedy Act was for research on Southern oysters. "Southern" oysters as here defined refers to the product of the Gulf Coastal States, North and South Carolina, Georgia, and Florida. Problems of handling these oysters differ considerably from those of the oyster industry north of North Carolina.

The necessity for working with such a highly perishable product as raw oysters required work to be done by contract groups located near the producing area. Research teams located at Florida State University in Tallahassee, Florida; Tulane University in New Orleans, and Louisiana State University in Baton Rouge, Louisiana, received these contracts in February 1955. All of these contracts were renewed and research is well advanced in the second year. The results of this research will be presented by representatives of each group.

The Fish and Wildlife Service has coordinated the work and provided liaison between the groups. The staff of the Fishery Technological Laboratory at College Park, Maryland, has also determined the dry solids, protein, fat, ash, chlorides, and carbohydrates (by difference) of a large number of samples of raw shucked oysters collected throughout the season and in most of the states in the area.

Samples of shucked oysters, as prepared for market by the plants as well as samples of unwashed oysters shucked from the shell into the sample can were obtained in most instances. This material has been published in Southern Fisherman, Vol. 16, April 1956 and in Commercial Fisheries Review, Vol. 18, July 1956.

A large degree of variability for all constituents was found as a result of the seasonal, plant process, and geographical differences in the samples, making interpretation of the results a somewhat difficult matter.

An impression of the degree of variability may be obtained from listing the ranges in average values obtained for the various constituents with the shell samples only. Dry solids ranged from 7 to 17 per cent, so for better comparison, all other data have been converted to a dry basis. Protein on a dry basis ranged from 35 to 65 per cent; fat 5.5 to 15.5 per cent; mineral matter 5 to 35 per cent; chlorides (salt) 2.5 to 29 per cent; and carbohydrates (by difference) 8.5 to 45 per cent.

The data for shell samples indicated that dry solids and fat increased from low values in September and October to maximum values in March and April, then decreased rapidly to low summer values during the spawning season. On the other hand, mineral matter and salt content of the oyster decreased at a uniform rate from October to a seasonal low in March and April and increased during the summer months.

The carbohydrate content increased irregularly as the oyster season advanced, and exhibited quite a marked inverse relationship to the protein content: when one was low the other was high.

The problems of the southern oyster industry are numerous and there is no promise of simple or rapid solution. The research groups have completed the preliminary stages of acquiring familiarity with the industry, and accumulating basic information concerning its product and problems. During the coming oyster season they expect to concentrate to a greater extent on practical phases of the problems and anticipate results of both interest and value to the industry.

INVESTIGATIONS OF THE BODY FLUID AND "BROWN-SPOTTING" OF THE OYSTER

Milton Fingerman and Laurence D. Fairbanks

Tulane University, Louisiana

Since January 1955 the study of oyster biology at Tulane University has been concerned chiefly with the physiology of the body fluid and the phenomenon of "brown-spotting" in the American oyster (Crassostrea virginica).

Several experimental approaches have been utilized in this study. Physical stimuli such as shucking, wedging open of the shells, and heat cause considerable fluid losses. As much as 40 per cent of the oyster weight may be lost within 30 minutes after shucking. A comparison of oysters in March 1956 from New Jersey and Maryland with oysters from Louisiana has shown that there is no difference in weight losses following shucking at this time. In the fall, however, Louisiana oysters lost 26.6 per cent more body weight than Delaware Bay oysters.

Oysters exposed to heat above 41° C lost body weight progressively with increase in temperature. Mortality closely paralleled body weight losses. However, death appeared to be due to the direct effects of heat and not to any certain percentage weight loss of fluids. No significant weight loss or death occurred at temperatures below 35° C. Oysters could be killed by short exposure to high temperatures (45-55° C) or long exposure to lower temperatures (40-42° C). Apparently the lethal temperature varies with the conditions of the experiment.

Elimination of water and conservation of chloride ions are effected in great extent by excretion of a hypotonic urine through the organ of Bojanus by way of the pericardium. Determinations of chloride concentration of fluid from the mantle cavity (shell fluid), mantle tissues (mantle fluid), pericardial fluid, blood, and fluid from the organ of Bojanus indicate that the latter fluid generally has a chloride concentration about 18 millimoles per liter below that of any of the other body fluid components of oysters in estuarine water (chloride concentrations of 241 to 270 millimoles per liter). The average chloride concentrations of the other fractions of the body fluid generally remain about the same as that of the environment in the range of 241 to 270 millimoles per liter.

Determinations of concentrations of proteinaceous material of oyster body fluids in June and July 1956 showed that the blood has an average concentration of 1.93 per cent; mantle fluid, 1.56 per cent; pericardial fluid, 0.96 per cent; and shell fluid, 0.30 per cent. A correlation between concentrations of chloride ion and proteinaceous material was evident. Oysters with greater concentrations of proteinaceous material tended to have greater concentrations of chloride ion.

Average blood cell counts of fractions of the body fluid of individual oysters are as follows: blood, 1,715 cells per cubic millimeter; mantle fluid, 1,048; shell fluid, 535; and pericardial fluid, 348. Centrifugation of the blood cells showed that they constitute no more than 0.10 per cent of the blood and generally much less than this. Apparently the blood cells contribute very little directly to protein concentration of the body fluids.

Microscopic observation of histological sections of "brown-spotted" oyster tissues showed that the "brown-spot" material is composed of numerous golden brown granules restricted to the epithelial cells of the mantle. The brown material may be easily scraped from the surface of the mantle. The pattern of spotting found on the mantle often matches the pattern of coloration on the inner side of the shell against which that particular part of the mantle lies, suggesting that the colored material is laid down in the shell by the overlying mantle. The intensity of coloration on both the mantle and shell ranges from light tan to intense purple and black. Often the intensity of coloration on the mantle matches the intensity of coloration on the shell. Sectioning of a shell whose inner surface is colored may reveal that the coloration extends two to three millimeters into the prismatic layer from the inner surface of the shell. There may or may not be deeper layers of pigment, indicating that the formation of "brown-spot" material need not be a continuous process.

Oysters receiving implants of "brown-spotted" oyster tissue and oysters maintained for a week in sea water containing homogenized "brown-spotted" oyster tissues showed no significant increase of intensity of "brown-spotting". There seems to be no particular time of year when "brown-spot" is more frequently found and no particular size of oysters that is more frequently spotted.

RESEARCH ON HANDLING AND PROCESSING

SOUTHERN OYSTERS

Arthur Novak and E. A. Fieger

Louisiana State University
Baton Rouge, Louisiana

In the first series of experiments extra-select oysters were frozen. Treatments prior to freezing included the following:

1. Water washed, packed in Marathon cartons with overwrap of Tyton.

2. Washed with either water or salt solution, packed in Marathon cartons, and after freezing glazed with water or salt solution.

3. Washed with water or with salt solution and vacuum packed in sealed cans.

All samples were stored at 0° F.

Brief summary of results:

1. Rancidity developed in the overwrapped samples after six months storage, in the glazed samples after eight months storage. Vacuum packing gave the best product and rancidity was not evident until after nine months frozen storage. We believe, therefore, six to nine months is the maximum storage life under the conditions of our experiments.

No loss of protein or glycogen occurred during frozen storage, while the pH of the drip and of the meats decreased slowly.

Bacterial counts increased during the first eight months and then remained constant.

Although the frozen oysters retained a satisfactory flavor during six to nine months frozen storage, other changes occurred which will have to be prevented if freezing is to become a satisfactory means of preserving oysters on a commercial scale.

Detrimental changes:

1. During the first two months of frozen storage the loss in weight upon cooking increases and after two months storage fluctuated between 50 and 60 per cent. Accompanying this change is a pronounced decrease in the size of the cooked

oyster in comparison with cooked oysters which had not been frozen. We are of the opinion neither the institutional trade nor the housewife would continue to buy frozen oysters unless this change in size upon cooking can be greatly reduced.

2. The second change we noted was the development of a black to green spot on the body of the oyster. These spots varied in intensity of color and size and generally after several months of frozen storage were about the size of a dime. Upon cooking, these spots remain as dark spots. Again we believe this condition will have to be corrected before frozen oysters can be successfully marketed commercially.

3. When frozen oysters are exposed to air, as is the case with an overwrapped package, or through improper glazing, the surface of such oysters become lemon-yellow in color, and when cooked become an intense orange color. Also such oysters have a definite rancid flavor.

4. A definite darkening of the edges of the mantle occurs during frozen storage. While not too serious it does decrease the attractiveness of the product.

5. A small percentage of the oysters developed a pink discoloration which spread out from the edge of the adductor muscle. We have not been able to associate this discoloration with yeasts or molds.

6. With increasing length of storage the thawed product becomes very fragile and must be carefully handled to prevent tearing.

The above results were obtained with extra-select Louisiana oysters. Similar results are being observed on select Mississippi and Alabama produced oysters.

In late June we undertook experiments to determine whether it would be possible to decrease the loss in weight and reduce the shrinkage upon cooking. It is too early to predict the outcome of this work.

In a brief summary on this phase of the work we can only say we have shown what detrimental effects must be overcome or solved before a satisfactory frozen oyster can be had.

Analysis of Louisiana Oysters Supplied by the Louisiana Wild Life and Fisheries Commission

Samples were obtained monthly from several beds located both east and west of the Mississippi River. Samples from both high and low salinity areas were obtained.

A summary of the results follows:

The amount of solids, fat, and glycogen decreased during the summer and reached a minimum during September-October. With the advent of cool weather in the fall the amounts of all these constituents increased. The content of the B complex vitamins was quite variable and decreased slightly in late summer and early fall. The salt content of the oyster meats was quite variable and tended to parallel the salt content of the water from which they were dredged. The pH of freshly shucked oyster meat samples varied between pH 6.17 and 5.80 and, therefore, precludes its use as a test of quality of commercial stored samples.

The Use of Chlorotetracycline for the Preservation of Freshly Shucked Oysters

Freshly shucked oysters were washed in water or in a solution containing 10 ppm of the antibiotic chlorotetracycline (aureomycin). After draining they were placed in pint cans and stored in ice. The oysters washed in antibiotic had lower bacterial counts from the 8th to the 15th day of storage than the water washed samples, with extension of storage life by about five days, or from 10 to 15 days. No loss of B complex vitamins occurred during 19 days storage for either series of samples. the pH of the meats decreased progressively with increasing length of storage.

OYSTER RESEARCH FROM FLORIDA STATE UNIVERSITY[1]

Betty Watts, Harvey Lewis, Mark Schwartz

Florida State University, Tallahassee

I. CORRELATION OF pH AND QUALITY SHUCKED SOUTHERN OYSTERS

The spoilage pattern of raw whole Southern oysters stored at 5° C has been found similar to that reported for oysters in other locations. This spoilage is characterized by a gradual and continuous decrease in pH and the development of a sour odor. A seasonal variation in pH, initially and after storage, has been observed, with the values being lowest during the summer (June 6.02) and the highest during the winter (February 6.38).

The initial pH values of liquors, both after shucking and after washing, were higher than those of oyster meats. However, the pH of the liquors fell much faster than that of the meats, presumably because of the lower buffer capacity of the liquor.

It was possible to produce the characteristic sour odor during storage with very little or no fall in pH by heating the oysters just enough to partially inactivate the enzyme catalase.

II. DETERIORATION OF COOKED OYSTERS

The type of spoilage occurring in cooked oysters (immersed in water at 90-95° C for 2½ minutes) in which the enzyme catalase is completely inactivated is different from that which occurs in uncooked oysters. A characteristic "sour" odor develops in uncooked oysters stored at 4° C. The type of spoilage occurring in cooked oysters stored at 4° C appears to be of an oxidative type characterized by a "rancid-fish" odor. The addition of various antioxidants, including butylated hydroxyanisole (BHA), nordihydroguaiaretic acid (NDGA), and commercial liquid smoke, to the cooking water has retarded this type of spoilage.

Weight losses during cooking were influenced more by length of cooking time than by any of a number of types of cooking methods used. Further losses of liquid took place upon subsequent storage (4° C) of the cooked oysters. The total weight losses of cooked oysters ranged from 17 to 59 per cent, whereas uncooked oysters held under the same conditions lost 10 per cent.

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[1] This report is a brief summary of work reported in greater detail elsewhere (Gardner & Watts 1956, Gardner & Watts 1957, Schwartz & Watts 1957, Gardner & Watts, in press).

III. QUANTITATIVE MEASURE OF RANCIDITY IN OYSTERS - THIOBARBITURIC ACID TEST

The thiobarbituric acid test has been applied to oysters in an attempt to establish a rapid objective test for the determination of oxidative rancidity. This test was selected for two reasons. First, it can be applied directly to the oyster tissue without the necessity of extracting the fat. Second, the fat decomposition product responsible for the test is obtained in much greater amounts from highly unsaturated fatty acids such as are present in marine fats.

The test measures oxidation products of unsaturated fatty acids. It depends upon the spectrophotometric estimation of a pink-red compound produced when the acidified sample is heated in the presence of 2-thiobarbituric acid.

As measured by this test, refrigerated cooked oysters have a definite induction period during which the TBA values do not increase over those for freshly cooked samples. At the end of the induction period there is a very rapid increase in the TBA values which corresponds closely with the development of "rancid-fish" odors.

The "rancid-fish" odor, typical of oysters, cooked enough to inactivate catalase, has been retarded by the addition of an antioxidant added in sufficient quantity to give a final concentration of 0.1 per cent butylated hydroxyanisole (BHA) in the cooking water. Other experiments, using a variety of antioxidants on frozen cooked oysters, are now being carried out.

IV. DEVELOPMENT OF FROZEN PREPARED OYSTER PRODUCTS

A. Fried Oysters

Breaded oysters may be frozen either raw or after frying. In either case it was found necessary to dip the oysters in a batter before breading. Direct coating with breading mixtures, without the use of batters, resulted in poor adherence.

Problems encountered with breaded raw frozen oysters are mainly concerned with exudation of liquor during preliminary stages of freezing and during thawing. This results in clumping. The problem of clumping was less pronounced when the product was subjected to quick freezing (-30 to -40° C).

The chief disadvantage of the prefried frozen oysters was the relatively rapid development of an oxidative type of deterioration in the freezer, resulting in stale or rancid fish odors and flavors within a few weeks. Experiments on preventive measures for this type of deterioration are now in progress.

B. Oyster Stew

The problems encountered in oyster stews are those of excessive shrinking of the oyster and curdling of the milk.

The use of raw oysters to overcome the excessive shrinkage problem in frozen oyster stews is presently being explored. A number of different types of milks, (homogenized, evaporated, and dried) have been used in an effort to overcome curdling. In the results so far obtained dried milk yields the most suitable product. Presently, alkaline phosphate is being used in an attempt to inhibit undesirable changes.

Past experiments have shown that cooked oysters utilized for stew yield a tough product, and after a few weeks of storage in the freezer development of an oxidative type of spoilage results.

V. NUTRITIVE VALUE

Oysters from Apalachicola Bay were assayed for niacin, riboflavin, and total solids from February 1955 through August 1956. Several relationships between vitamin contents and other factors were found.

Total solids varied from period to period during the study. An inverse relationship was found between niacin content and total solids during the summer months. The seasonal changes in niacin values were almost the inverse of the changes in riboflavin content. Riboflavin content tended to decrease as spawning activities increased. An increased need for riboflavin in spawning may account for the decreased storage of the vitamin during the spring and summer months.

Some factor in oyster homogenate was found to destroy thiamine during freezer storage. A loss of 64 per cent was observed in two samples studied for four weeks. This loss is believed to be caused by the enzyme thiaminase.

Ranges of vitamins present in mg per 100 gm dry matter were niacin, 6.9 to 15.9; riboflavin, 0.5 to 1.87; and thiamine 0.36 to 1.02.

VI. PRESERVATION WITH IONIZING RADIATIONS

Although much investigation of food preservation by cold sterilization through ionizing radiations is now in progress, no one has yet reported any such investigation with oysters. The present study was undertaken to determine whether or not preservation of oysters can be effected by radiation treatment. This involved the determination of whether or not oysters can be irradiated at dosages high enough for partial or complete sterilization without producing undesirable side reactions and also the determination of the extent to which souring and pH changes may be inhibited by irradiation of the oysters.

Irradiation of raw oysters with sterilizing doses of gamma rays produced an odor described as "grassy". Neither free sulfhydryl groups nor catalase activity were noticeably reduced. Subsequent souring and fall in pH occurred both in irradiated and unirradiated controls.

The irradiation of cooked oysters produced a somewhat different type of odor described as "oxidized". The off odors were not eliminated by the addition of various antioxidants and free radical acceptors.

The most acceptable irradiated products from an odor standpoint were those irradiated raw but subsequently heated sufficiently to destroy enzymes. The heating eliminated the grassy odor and prevented subsequent enzymatic souring. However, the small size of samples which could be irradiated with the limited source available precluded adequate storage studies.

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ANNUAL CONVENTION

The 1956 Annual Convention of the National Shellfisheries Association was held jointly with the Oyster Growers and Dealers Association of North America and the Oyster Institute of North America, July 30-August 2, at the Algiers Hotel, Miami Beach, Florida. A special feature of the program consisted of a "Symposium on the Production and Utilization of Seed Oysters", and a panel discussion on the "Freezing and Processing of Southern Oysters". In addition there were two half-day sessions of contributed papers, the majority of which have been published in this volume of the Proceedings.

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EDITORS' NOTES

The cost of typing duplimat masters for Volumes 45 and 46 of the Proceedings was borne by the Oyster Institute of North America, and preparation of plates of illustrations and final publishing of the volumes was performed by the Fish and Wildlife Service, U. S. Department of the Interior. The present publication, Volume 47, was handled in the same manner, except that an improved method reproduction, the "Xerox Process" was employed.

Some 500 copies of the Proceedings are published each year for distribution to Association members and to marine laboratories and other institutions concerned with research in marine biology in the United States. A number of scientists and institutions abroad have also requested copies and have been placed on the Association mailing list. This past year the Secretary-Treasurer of the Association distributed copies of Volume 46 of the Proceedings to 55 libraries in this country and abroad without charge. This makes the Proceedings available in at least this many permanent key locations. Papers appearing in the Proceedings are now regularly abstracted in Biological Abstracts.

The Editorial Committee has functioned smoothly in its task of reviewing all papers submitted to it for publication in the Proceedings during the past year. In some cases the aid of additional reviewers was sought. In most instances papers were returned to authors for rewriting before final acceptance for publication. We believe this procedure has materially improved contributions. Proofs were distributed to authors for final proof reading before publication. Reprints are available to authors at cost.

INFORMATION FOR CONTRIBUTORS

Scientific papers delivered at the Annual Association Convention and additional papers submitted by members of the Association will be considered for publication, in entirety or in abstract form. Papers appearing in print elsewhere are not acceptable.

Manuscripts will be judged on the basis of the original data, ideas, and interpretations which they contribute. They will be examined by the Editorial Committee and by other competent reviewers. Each paper should be ready for publication before submission to the Editorial Committee.

Manuscripts should be typewritten and double-spaced; carbon copies are not acceptable. Tables should appear on separate sheets; most footnote material should be incorporated in the text. Scientific names should be underlined. Use the following style in lists of literature citations: "Galtsoff, P.S. 1955. Recent advances in the studies of the structure and formation of the shell of Crassostrea virginica.

Proc. Natl. Shellfish. Assoc. 45: 116-135". Reference to literature citations in the text should be made as follows: "Loosanoff (1955)". Abbreviations for the names of serial publications will be patterned after those employed by Biological Abstracts (for special list see Biol. Absts. 29(5): v-xxxv, 1955). Abbreviations for units of weight and measure, and fundamental rules for the use of these, will be patterned after those given in the Handbook of Chemistry and Physics, 36th. Edition, pages 3108-3134. The present publication should be used as a guide for general format.

Illustrations should be reduced to a size to fit on paper 8 x $10\frac{1}{2}$ inches with ample margins; photographic copies of high quality are preferred to originals. Illustrations smaller than page size should be loosely attached to plain white paper with rubber cement, and the legend typed in the proper position under the illustration. More than one illustration may appear on a sheet. If the illustration is page size, the legend, properly spaced, should be typed on a separate sheet of paper. No illustrations should appear on text pages.

Every paper should be accompanied by an author's summary, complete in itself and understandable without reference to the original article, for submission to Biological Abstracts by the Editors. Address all manuscripts and correspondence concerning editorial matters to the Editor, M. R. Carriker, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina. All manuscripts should reach the Editor prior to October 1 for inclusion in the Proceedings of that year.

Masters used in the reproduction of the Proceedings will be retained for one year. Reprints can be made at cost, expense to be borne by the author. Authors desiring reprints should communicate directly with Mr. J. Nelson Callahan, Head, Duplicating Department, Bingham Y, University of North Carolina, Chapel Hill, N. C.

TITLES OF OTHER TECHNICAL PAPERS PRESENTED AT THE CONVENTION

Augello, William. The fishery exemption --- one of your industry's most valuable possessions.

Engle, James B. Design and function of a suction drill dredge for small boats.

Glancy, Joseph B. The supply of seed oysters in the New England-New York area.

Haskin, Harold H. The seed supply in Delaware Bay.

Ingle, Robert M. Florida's potential beckons.

Janowitz, Edward. Further studies in the attraction of Urosalpinx by oysters.

Mackin, J. G. Effects of graded dosage on infection and rate of development of fungus disease of oysters caused by Dermocystidium marinum.

Mackin, J. G. Miscellaneous new diseases and parasites of oysters.

Menzel, Winston, N. C. Hulings, R. R. Hathaway. Oyster research in Apalachicola Bay, Florida.

St. Amant, Lyle S. Some trends in the biological investigations of various oyster problems in Louisiana.

Udell, Harold F. Handling of southern oysters for sale in New York markets.

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Aldrich, F. A., Assistant Curator of Limnology, Academy of Natural Sciences, 19th & The Parkway, Philadelphia 3, Pa.

Allen, J. Francis, Department of Zoology, University of Maryland, College Park, Md.

Andrews, Jay D., Oyster Biologist, Virginia Fisheries Laboratory, Gloucester Point, Va.

Atlantic Biological Station, Fisheries Research Board of Canada, St. Andrews, N. C., Canada.

Ayers, Robert J., Oregon Fish Commission, 1236 W. Marine Drive, Astoria, Ore.

Ball, Eric T., 212 Summit Street, New Haven 13, Conn.

Baptist, John P., U. S. Fish & Wildlife Service, Shellfish Laboratory, Beaufort, N. C.

Beaven, G. Francis, Oyster Biologist, Maryland Department of Research & Education, Solomons, Md.

Berry, W. R., Bureau of Shellfish Sanitation, 5709 Sellger Drive, Norfolk 2, Va.

Blake, John W., Department of Zoology, University of North Carolina, Chapel Hill, N. C.

Brittain, G. J., Jr., Shellfish Consultant, 5643 Flamingo, Houston 21, Tex.

Butler, Philip A., Chief, Gulf Oyster Investigations, Shellfish Laboratory, U. S. Fish & Wildlife Service, Gulf Breeze, Fla.

Carriker, Melbourne R., Department of Zoology, University of North Carolina, Chapel Hill, N. C.

Carver, Thomas C., Jr., Fishery Research Biologist, U. S. Fish & Wildlife Service, Gloucester Point, Va.

Chanley, Paul E., Fishery Research Biologist, U. S. Fish & Wildlife Service, Biological Laboratory, Milford, Conn.

Chapman, Charles R., Fishery Research Biologist, U. S. Fish & Wildlife Service, Shellfish Laboratory, Gulf Breeze, Fla.

Chestnut, A. F., Director, University of North Carolina, Institute of Fisheries Research, Morehead City, N. C.

Chipman, Walter, Director, Fishery Radiobiological Laboratory, U. S. Fish & Wildlife Service, Beaufort, N. C.

Collier, Albert, Fishery Research Biologist, U. S. Fish & Wildlife Service, Fort Crockett, Galveston, Tex.

Cronin, L. E., Director, Maryland Department of Research & Education, Solomons, Md.

Currier, Wendell, Ass't. to Vice President, Research & Development, Campbell Soup Co., Camden, N. J.

Darling, J. S. & Son, P. O. Box 412, Hampton, Va.

Dassow, John A., Technological Laboratory, U. S. Fish & Wildlife Service, 2725 Montlake Blvd., Seattle 2, Wash.

Davis, Harry C., Fishery Research Biologist, Biological Laboratory, U. S. Fish & Wildlife Service, Milford, Conn.

Dawson, C. E., Biologist, Bears Bluff Laboratory, Wadmalaw Island, S. C.

Deiler, Frederick G., Biologist, Freeport Sulphur Co., Port Sulphur, La.

Dow, Robert L., Director, Marine Research, Department of Sea and Shore Fisheries, Vickery-Hill Building, Augusta, Me.

Dumont, William H., U. S. Fish & Wildlife Service, Department of Interior, Washington 25, D. C.

Dunnington, Elgin W., Oyster Biologist, Department of Reserach & Education, Solomons, Md.

Edwards, Malcolm B., Pacific Coast Oyster Growers Association, South Bend, Wash.

Engle, James B., U. S. Fish and Wildlife Service, Clam and Chesapeake Oyster Investigations, 800 Dreams Landing, Annapolis, Md.

Fahy, William, University of North Carolina, Institute of Fisheries Research, Morehead City, N. C.

Fingerman, Milton, Newcomb College, Tulane University, New Orleans, La.

Florida State Board of Conservation, W. V. Knott Building, Tallahassee, Fla.

Flower, Frank M. & Sons, Growers of Pine Island Oysters, Bayville, Long Island, N. Y.

Fox, Leo, Associate Sanitary Biologist, Department of Public Health, 511 A State House, Boston 33, Mass.

Galtsoff, Paul S., Director, Shellfish Laboratory, U. S. Fish & Wildlife Service, Woods Hole, Mass.

Ganaros, Anthony E., Fishery Research Biologist, Biological Laboratory, U. S. Fish & Wildlife Service, Milford, Conn.

Gibbs, Harold N., A-71 Sowams Road, Barrington, R. I.

Girard, John G., State Department of Health, Smith Town, Seattle, Wash.

Glancy, Joseph B., Shellfish, Inc., Box 212, West Sayville, Long Island, N. Y.

Glude, John B., Chief, Clam and Chesapeake Oyster Investigations, U. S. Fish & Wildlife Service, P. O. Box 151, Annapolis, Md.

Grice, George D., Fish and Wildlife Service, Juneau, Alaska.

Gunter, Gordon, Director, Gulf Coast Research Laboratory, Ocean Springs, Miss.

Gustafson, Al, Professor Biology, Bowdoin College, Brunswick, Me.

Hanks, James E., Department of Zoology, University of New Hampshire, Durham, N. H.

Hargis, William J., Jr., Associate Biologist, Virginia Fisheries Laboratory, Gloucester Point, Va.

Harrison, George T., President The Tilghman Packing Company, Tilghman, Md.

Haskin, Harold H., Director, New Jersey Oyster Research Laboratory, Department of Zoology, Rutgers University, New Brunswick, N. J.

Haven, Dexter, Virginia Fisheries Laboratory, Gloucester Point, Va.

Hedrick Brothers Oyster Company, 730 Auster City Street, New Orleans, La.

Hewatt, Willis G., Biology-Geology Department, Texas Christian University, Fort Worth, Tex.

Heydecker, Wayne D., Atlantic States Marine Fisheries Commission, 22 West First Street, Mount Vernon, N. Y.

Hofstetter, Robert P., Rt. #1, Box 132, La Porte, Tex.

Hopkins, Sewell H., Biology Research Laboratory, Texas A & M Research Foundation, College Station, Tex.

Huber, L. Albertson, Hydrographic Engineer, 297 E. Commerce Street, Bridgeton, N. J.

Hulings, Neil C., Oceanographic Institute, Florida State University, Tallahassee, Fla.

Jensen, Eugene T., U. S. Public Health Service, Shellfish Branch, Washington 25, D. C.

Kahn, Archie M., Executive Director, Texas A & M Research Foundation, College Station, Tex.

Kelly, C. P., Shellfish Sanitation Laboratory, U. S. Public Health Service, Gulf Breeze, Fla.

Lamson, P. G., Publisher, "Atlantic Fisherman", Goffstown, N. H.

Lednum, J. M., Town Engineer, Town of Islip, N. Y.

Lester & Toner, Inc., c/o Royal Toner, 208 Front Street, New York 38, N. Y.

Lindsay, Cedric, Director, Shellfish Laboratory, Fisheries Department, Washington State, Quilcene, Wash.

Littleford, Robert A., Seafood Processing Laboratory, Crisfield, Md.

Loesch, Harold, Marine Biologist, Alabama Department of Conservation, Bayou La Batre, Ala.

Logie, R. R., Department of Zoology, Rutgers University, New Brunswick, N. J.

Loosanoff, Victor L., Director U. S. Fish and Wildlife Biological Laboratory, Milford, Conn.

Lunz, G. Robert, Director, Bears Bluff Laboratories, Wadmalaw Island, S. C.

Mackin, J. G., Director, Marine Laboratory, Texas A & M Research Foundation, Galveston, Tex.

Macomber, Ronald, 11 Prescott Avenue, Montclair, N. J.

McConnell, James L., P. O. Box 1034, Bay Towing & Dredging Company, Mobile, Ala.

McConnell, James N., Director, Division of Oysters and Water Bottoms, Department of Wildlife and Fisheries, 126 Civil Courts Building, New Orleans 16, La.

McHugh, J. L., Director, Virginia Fisheries Laboratory, Gloucester Point, Va.

Manning, Joseph H., Clam Biologist, Chesapeake Biological Laboratory, Solomons, Md.

Mansueti, Romeo, Fishery Biologist, Maryland Dept. of Research & Education, Solomons, Md.

Marshall, Nelson, Dean of Liberal Arts, Alfred University, Alfred, N. Y.

Menzel, R. Winston, Oceanographic Institute, Florida State University, Tallahassee, Fla.

Nelson, J. Richards, The F. Mansfield & Sons Co., 610 Quinnipiac Ave., New Haven, Conn.

Nelson, Thurlow C., 8 North Main St., Cape May Court House, N. J.

New Jersey Department of Conservation, Trenton, N. J.

Pellissier, Carroll E., Editor of Fishing Gazette, 461 Eighth Ave., New York 1, N. Y.

Perlmutter, Alfred, Senior Aquatic Biologist (Marine), D.-J. Fish Research Unit, New York Conservation Department, 65 West Sunrise Highway, Freeport, N. Y.

Pomeroy, Lawrence, Marine Biology Laboratory, Department of Biology, University of Georgia, Sapelo Island, Ga.

Porter, Hugh J., University of North Carolina, Institute of Fisheries Research, Morehead City, N. C.

Price, T. J., Fishery Radiobiological Laboratory, U. S. Fish & Wildlife Service, Beaufort, N. C.

Pritchard, Donald W., The Johns Hopkins University, 121 Maryland Hall, Baltimore 18, Md.

Quayle, Daniel B., Nahcotta, Wash.

Ray, Sammy M., 3127 Avenue R, Galveston, Tex.

Rice, Theodore R., Fishery Radiobiological Laboratory, U. S. Fish & Wildlife Service, Beaufort, N. C.

Rego, John L., Director, Department of Agriculture & Conservation, Veterans Memorial Building, 83 Park Street, Providence 2, R. I.

Ropes, John W., U. S. Fish & Wildlife Service, 29 Linden Street, Salem, Mass.

Russell, Henry D., Springdale Avenue, Dover, Mass.

Sangree, John B., Glassboro State Teachers College, Glassboro, N. J.

Sayce, Clyde S., Fishery Biologist, Box 205, Ocean Park, Wash.

Sellmer, George, Department of Biology, Upsala College, East Orange, N. J.

Shuster, Carl N., Director, Marine Laboratory, Department of Biological Sciences, University of Delaware, Newark, Del.

Sieling, Fred W., Department of Research & Education, Box 186, Snow Hill, Md.

Silliman, Ralph P., Chief, Section of Anadronous Fish, Interior Department, U. S. Fish & Wildlife Service, Washington 25, D. C.

Smith, F. G. Walton, Director, The University of Miami Marine Laboratory, Coral Gables 46, Fla.

Sollers, Allen A., 1305 Park Avenue, Baltimore 17, Md.

Sparks, Albert K., Chief Biologist, Assistant Director, Box 203, Thibodaux, La.

Sprague, Victor, Hiawassee, Ga.

St. Amant, Lyle S., Wildlife and Fisheries Commission, 126 Civil Courts Building, New Orleans 16, La.

Stern, Joseph A., School of Fisheries, University of Washington, Seattle, Wash.

Trezise, William R., Fishery Aide, 318 - 12th Street, Raymond, Wash.

Truitt, Reginald V., Great Neck Farm, Stevensville, Md.

Udell, Harold, N. Y. Conservation Department, Bureau of Marine Fisheries, Freeport, Long Island, N. Y.

Virginia Commission of Fisheries, Newport, News, Va.

Wallace, Dana E., Department of Sea & Shore Fisheries, Vickery-Hill Building, Augusta, Me.

Wallace, David H., Director, Oyster Institute or North America, 6 Mayo Avenue, Bay Ridge, Annapolis, Md.

Webster, John R., U. S. Fish & Wildlife Service, P. O. Box 151, Annapolis, Md.

Weiss, Charles M., Department of Sanitary Engineering, School of Public Health, University of North Carolina, Chapel Hill, N. C.

Welch, Walter R., U. S. Fish & Wildlife Clam Investigations, R.F.D., Boothbay Harbor, Me.

Westley, Ronald E., Fishery Biologist, Washington State Department of Fisheries, Shellfish Laboratory, Quilcene, Wash.

Whaley, Horace H., The Johns Hopkins University, 121 Maryland Hall, Baltimore 18, Md.

Wilde, Frank W., Box 5, Shady Side, Md.

Woelke, Charles E., Fishery Biologist, Box 323, Quilcene, Wash.

Wolman, Abel, The Johns Hopkins University, Whitehead Hall, Baltimore 18, Md.

Wurtz, Charles B., 610 Commercial Trust Building, Philadelphia 2, Pa.

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