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INTRODUCTION OF HONORARY MEMBERS

DAVID H. WALLACE

Director, Bureau of Marine Fisheries,
New York Conservation Department
Former Director, Oyster Institute of North America

David Wallace was born 17 February 1916 at Barclay, Maryland, where his father was postmaster. At the age of 15 he entered Washington College, Chestertown, Maryland. There Professor Kathleen Carpenter, the famous Welsh fresh-water biologist, inspired him to choose biology as a career. After taking a B.S. degree at Washington in 1935 David entered the University of Maryland, where he studied ichthyology under Professors V. Vladykov and R. V. Truitt and won the M.S. degree in zoology in 1937. During the summers of 1936 and 1937 he did field and laboratory work on the rock (striped bass) and shad at Chesapeake Biological Laboratory, Solomons, Maryland. In 1938 he was promoted from assistant biologist to biologist in charge of fisheries research, and extended his investigations to croakers. Eight publications were based on his researches during this period.

In 1940 Dave moved to Annapolis to become administrative assistant in the Maryland Department of Tidewater Fisheries, rising to executive secretary in 1941 and director in 1946. In 1949 he became chairman of the Maryland Board of Natural Resources.

Upon the death of Dr. Lewis Radcliffe, the Oyster Institute of North America chose David Wallace to replace him. From 1951 to 1962 he served with distinction as director of the Oyster Institute and as executive secretary of the Sponge and Chamois Institute.

Since 1962 he has been Director of the Bureau of Marine Fisheries of New York's Conservation Department at Oakdale, N. Y., with responsibility for research, management, shellfish sanitation, and law enforcement programs. As a member of the staff of the University of New York at Stony Brook, he is planning the permanent offices of the Conservation Department and developing a graduate marine science program for the University.

While mainly an administrator, Dave Wallace has found time to do research, and to serve on several state and federal committees concerned with research. He has also served a term as secretary-

treasurer of the National Shellfisheries Association. Furthermore, he has found time for leadership in his church and PTA, the Civitan Club, and community activities so important that he was named Man of the Year for Anne Arundel County (1951). He is admired by all who know him for his ability to get along with people, for his tact and ready sympathy, and for his integrity as well as for his ability.

DAVID H. WALLACE

VICTOR L. LOOSANOFF

Bureau of Commercial Fisheries
U.S. Fish and Wildlife Service

Dr. Loosanoff has held offices including the Presidency in this Association and has always been active in its affairs. Since 1931, he has vigorously pursued the study of the oyster and other shellfish, advancing our knowledge in the economic as well as in the academic and scientific fields. Because of his eagerness to present his useful findings to science and industry, his research is well documented in our literature. Those of us who had the privilege of working with him caught the spirit of his intensive research and made his influence felt in this country and abroad as we separated to work elsewhere.

Victor Loosanoff was born in Kiev, Russia, 3 October 1899, into a military family. He received his early schooling in Russian military academies. During World War I, he served as an artillery officer in the Royal Russian Army. He managed to escape the purge of the 1917 revolution and the next few years found him on the fluid battlefront against the Red armies. The fortunes of this war brought him to Siberia, China, Japan, and finally the United States. These experiences form an exciting period in his life which only he can tell about.

What prompted him to switch from the military to the life sciences is not known, but in 1927 he graduated with honors from the University of Washington with a B.S. degree. His professional career started with the State of Washington and in 1931 he came east as Chief Marine Biologist of Virginia. The U. S. Bureau of Fisheries claimed him in 1932 as an aquatic biologist when he began his distinguished career at Milford, Connecticut. From 1935 to 1963 he served as Director of the Milford laboratory. He earned a Ph.D. degree from Yale University in 1936.

The development of control measures for one of Long Island Sound's principal shellfish predators, the starfish, was among his first objectives on the east coast. This led to a vigorous warfare on other enemies of shellfish. His work with larval shellfish and their artificial propagation is monumental and shows a way to controlled mollusks. The energetic, persistent, and often ingenious way he approached the problems of our industry has made him one of our most distinguished and honored shellfish scientists.

VICTOR L. LOOSANOFF

THE EUROPEAN OYSTER, OSTREA EDULIS, IN MAINE

Walter R. Welch

Bureau of Commercial Fisheries Biological Laboratory
Boothbay Harbor, Maine

ABSTRACT

Favorable temperature and salinity characteristics of the Maine coast encouraged several introductions of Ostrea edulis from Holland. The most successful transplant was in Boothbay Harbor and surveys indicated that oysters have set annually on suitable bottom below low tide level, in and near this area, persistently increasing in abundance and range. Spat set also in the intertidal zone, but most did not survive the winters. Using shell bags on bottom, and raft-suspended shell strings, initial set was found to be greatest in shell bags, but survival through winter was greater on suspended shell strings. Not all transplants were successful, and recruitment rate is low, but the persistent expansion of the oyster population in Boothbay Harbor is encouraging.

INTRODUCTION

The decline of soft-shell clam stocks in northern New England from 1946 to 1959 emphasized the economic need for an additional fishery in intertidal or shallow subtidal environments. The successful introduction of the European oyster, Ostrea edulis L., into northern New England appeared theoretically possible. Although the coastal waters are often too cold to allow spawning, the temperature and salinity characteristics of many inshore areas are superficially similar to those in parts of the natural European habitat. According to Korringa (1940) this species thrives in the Oosterschelde of Holland, with salinity varying from 25.1 to 32.9 o/oo; also, spawning can occur at temperatures of 15 to 16 C with more successful spatfall resulting from more prolonged higher temperatures. Detailed information on temperature and salinity was not available prior to introduction of the species, but data for 1959 will serve as an example of conditions occurring in Boothbay Harbor. Daily salinity readings at the Bureau of Commercial Fisheries Biological Laboratory varied from 16.7 to 34.2 o/oo, but monthly averages of daily readings varied only from 29.0 to 32.8 o/oo. Salinities below 22.5 o/oo, a minimum for normal growth and setting of Ostrea edulis larvae (Davis and Ansell, 1962), were recorded on only

three separate days. All variations were well above the 15.0 o/oo minimum found by Chanley (1958) to be suitable for Ostrea edulis juveniles. Boothbay Harbor water temperatures during the spawning season of 1959 rose well above the 15 C minimum for spawning of this oyster.

The first European oysters were introduced in 1949 and Loosanoff (1951, 1955, 1962) reported on their initial survival, gametogenesis, and spawning. There has never been a coordinated study made of the first and subsequent introductions, but this report brings together all known additional information on the existence of the European oyster in Maine. Since there has been little effort made to improve oyster setting or growing conditions in Maine, the natural increase and spread of this introduced species is of considerable ecological interest. The data have been obtained principally through observations of personnel of the Bureau of Commercial Fisheries Biological Laboratory at Boothbay Harbor, Maine, and the Maine Department of Sea and Shore Fisheries. Age or year class was determined by a combination of relative size and counts of annual rings. Unless otherwise indicated, all references to oysters in this paper are to the European species, Ostrea edulis.

TRANSPLANTATION OF OYSTERS

Over a period of 12 years, several attempts were made to introduce oysters from Holland into Maine waters. These attempts and other related transplants were as follows, with locations shown in Figs. 1 and 2.

Group 1, Basin Cove, Harpswell

This group and the two following made up the original introduction in October 1949 (Loosanoff, 1951, 1955, 1962). The oysters had been shipped from the Oosterschelde in Holland to the U. S. Fish and Wildlife Service Biological Laboratory in Milford, Connecticut, and had been carefully screened for diseases, parasites, and other potentially dangerous organisms. The lot included representatives of the 1947, 1948, and 1949 year classes and was distributed in Maine to determine survival and ability to mature sexually and spawn. In Basin Cove, 1,060 were held in cages.

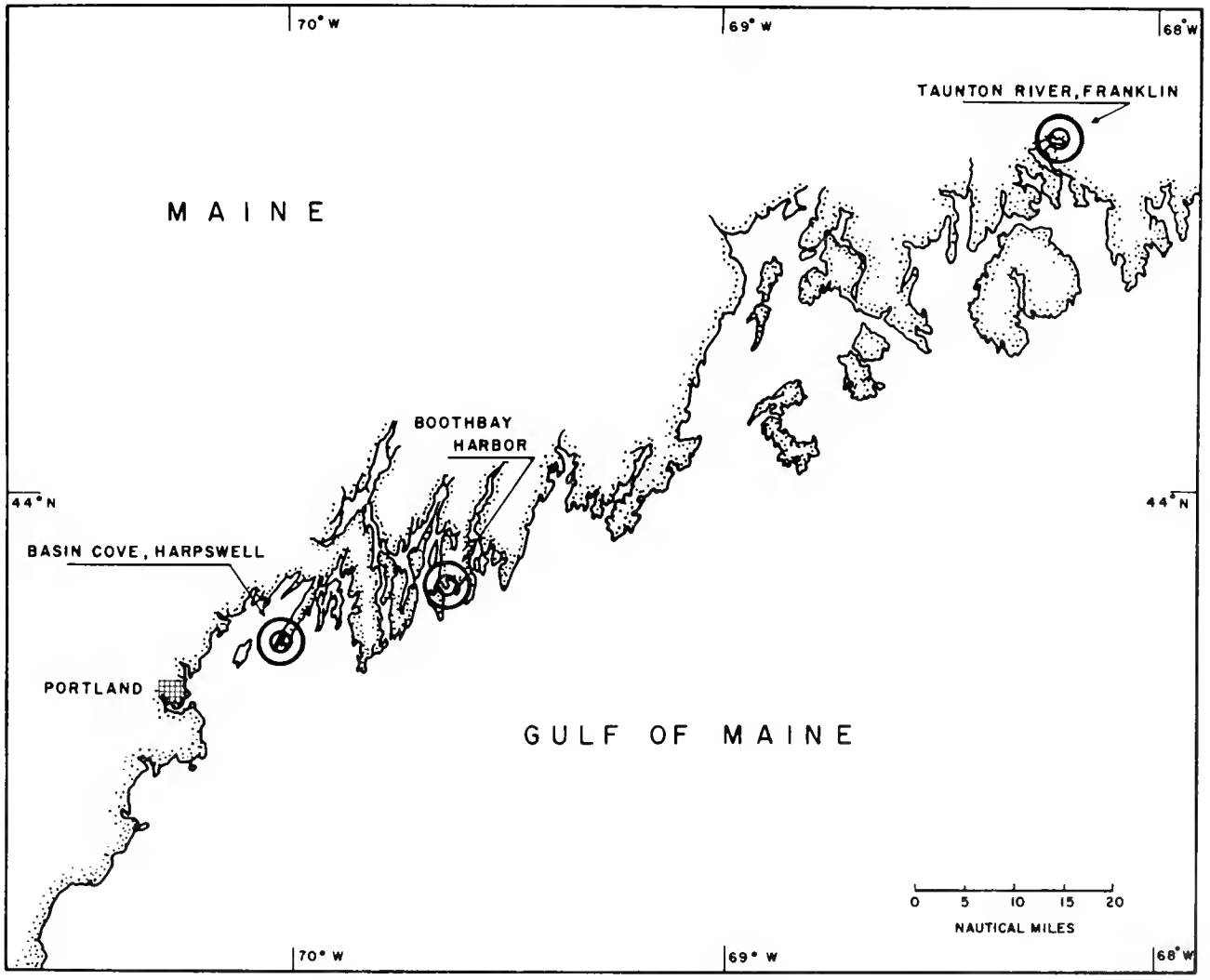

Fig. 1. Locations of the original introductions of European oysters in Maine, 1949.

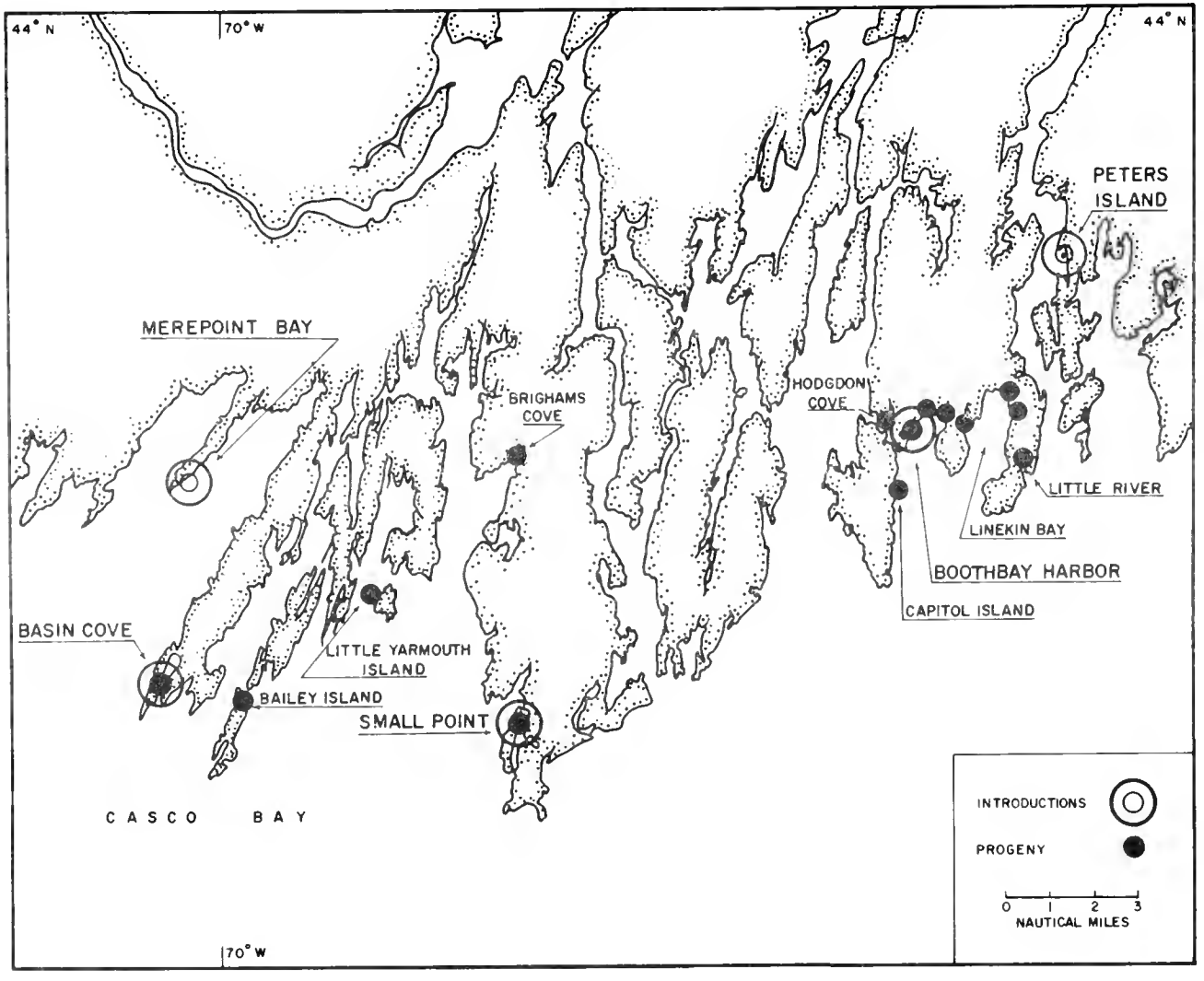

Fig. 2. Locations of introductions of European oysters and known locations of progeny, 1949-1962.

Group 2, Boothbay Harbor

Part of the same lot as Group 1; 3,600 were held in cages.

Group 3, Taunton River, Franklin

Part of the same lot as Groups 1 and 2; 1,060 were held in cages.

Group 4, Boothbay Harbor

In June 1954, the Maine Department of Sea and Shore Fisheries imported 390 lbs of adult oysters from Holland. They were held at the laboratory wharf in an effort to augment the local spawning population of oysters (progeny of the original introductions).

Group 5, Small Point, Phippsburg

In December 1955, an estimated 4,000 Dutch oysters were privately imported in cooperation with the Maine Department of Sea and Shore Fisheries. These oysters were placed in a lobster pound as the nucleus ot a spawning population.

Group 6, Peters Island, Bristol

In April 1959, personnel of the Maine Department of Sea and Shore Fisheries transferred 50 adult Boothbay Harbor oysters (progeny of the original introductions) to Peters Island to test the possibility of extending the local range more rapidly by dispersing groups of mature oysters.

Group 7, Merepoint Bay, Harpswell

In May 1961, approximately 2 bushels of oysters of various sizes from the Milford laboratory were placed in cages in shallow water. These oysters had been propagated at the Milford laboratory from Dutch stock and were placed in Merepoint Bay to serve as spawning stock.

Group 1, Basin Cove, Harpswell

A large proportion of the oysters survived and spawned during the first 2 years when they were under close observation. By July 1954, there were 120 of the original stock alive in the cages; and in July 1962 SCUBA divers reported 55 in the immediate area of the original cages. Seven of these, examined at the laboratory, appeared to be very old and are believed to be of the original stock.

Group 2, Boothbay Harbor

A large proportion of this group survived, spawned, and produced set during its first 2 years. In May 1952, all survivors of the group, except 15 retained in laboratory tanks, were returned to the Milford laboratory. The remaining 15 were also returned in May 1953.

Group 3, Taunton River, Franklin

This group suffered heavy mortality and by November 1953 no live oysters could be found.

Group 4, Boothbay Harbor

These oysters spawned as expected during their first summer, but during the following winter and spring all were lost because of damage to the holding cages.

Group 5, Small Point, Phippsburg

Few observations were made on this group and the oysters were reported as all dead or missing the following spring.

Group 6, Peters Island, Bristol

No observations were made on this group until August 1961, when only dead shells were found.

Group 7, Merepoint Bay, Harpswell

This group was examined occasionally and dead oysters were removed. Although there was no initial count, there did not appear to

be a very heavy mortality. In October 1962, 1,181 remained alive. These were removed from the cages and scattered on hard bottom about 3 feet below mean low water.

EVIDENCE OF SETTING

Table 1 summarizes the results of all known reports of oysters other than those accounted for by known introductions. In the few cases where oysters were reported by persons other than federal or state biologists, specimens were obtained and identification verified.

During most of the 11-year period, reports of oysters resulted from chance observation or from casual searches. Data for 1957, 1958, 1961, and 1962, however, were obtained by means of systematic search.

The first such systematic search was conducted in Boothbay Harbor in June 1957. At low tide, two men in a skiff examined the clearly visible portion of the bottom from mean low water to a depth of minus 4 feet (datum is mean low water) in a 2.4-mile band, averaging 30 feet in width, extending around the three major coves in the western and northern portion of the harbor (Fig. 3). Where conditions of visibility permitted, the survey occasionally included depths to approximately minus 8 feet.

In addition to the numbers of oysters given in Table 1, the results of the survey showed that: 1) only occasional small dead oysters were found between mean low water and the minus 2-foot level of extreme low tides; 2) most oysters were found in the minus 2- to minus 4-foot unexposed zone, where gravel, shell, and other firm substrate are commonly available, and 3) oysters at greater depths were found only in the rare instances where firm substrate existed.

In October 1958, during a period of extremely low tides, a more thorough survey was conducted using essentially the same methods as in 1957. In addition to examining the submerged bottom by boat, the exposed intertidal zone was explored on foot. The areas surveyed included a 1.2-mile section of the west shore of Boothbay Harbor, the area between Southport and Capitol Islands, and the northeastern portion of Linekin Bay.

This survey was carried out to gain more information on the relative abundance of oysters in the occasionally exposed zone between mean low water and minus 2 feet; and in the unexposed zone between

Table 1. Observations of progeny of European oysters in Maine, 1952-1962

Location	Number of oysters										
	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962
Casco Bay											
Basin Cove	0	0	0	-	-	-	-	-	0	-	2
Bailey Island	-	-	-	-	-	-	-	1	-	-	-
Little Yarmouth Island	-	-	-	-	-	-	-	-	2	-	-
Brighams Cove	-	-	-	-	-	-	-	1	-	-	-
Small Point	-	-	-	-	-	-	-	-	-	-	3
Boothbay Harbor Area											
Hodgdon Cove	-	Few	-	-	-	-	-	-	-	2	-
Boothbay Harbor—Total	Several	91	-	Many	Many	407	920	-	-	1,250	-
West Side	Several	91	-	-	-	322	920	-	-	1,032	-
North End (2 coves)	-	-	-	-	-	85	-	-	-	218	-
Capitol Island	-	-	-	-	-	-	20	-	-	32	-
Linekin Bay—Total	-	-	-	2	12	58	586	-	-	753	-
Cove, West Side	-	-	-	2	12	-	-	-	-	537	-
North End	-	-	-	-	-	-	558	-	-	48	-
Cove, Northeast Side	-	-	-	-	-	58	28	-	-	118	-
Little River	-	-	-	-	-	-	-	-	-	-	14

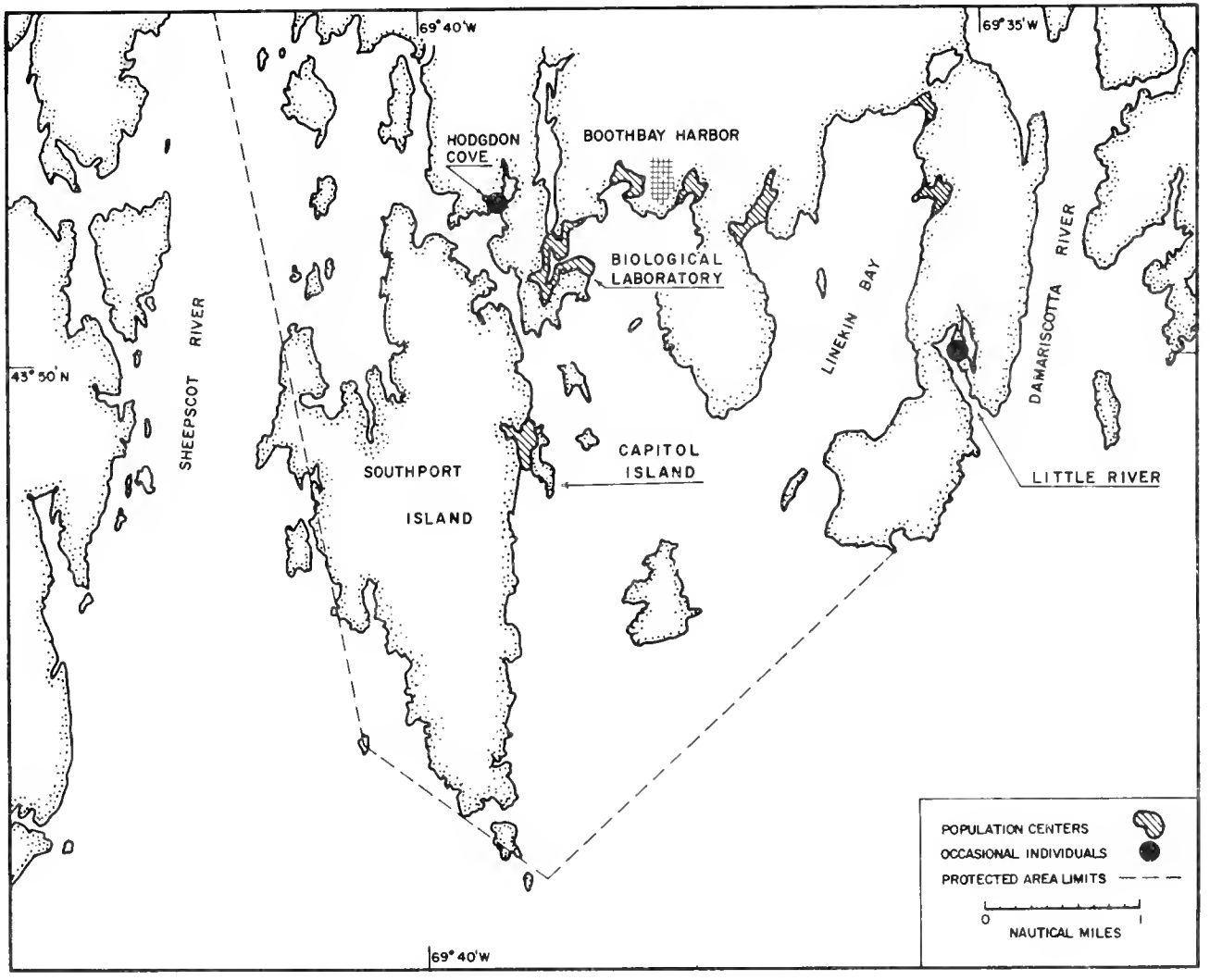

Fig. 3. Known locations of European oysters in the Boothbay Harbor region and extent of the area within which they are protected by law.

minus 2 and minus 4 feet. Observations were also made on the relative abundance of spat, yearlings, and older oysters in the same two zones.

Total count of oysters in each area are shown in Table 1. In addition, Table 2 gives the number of oysters in each of the two bottom zones and in each of the three-year class groups. The data confirm the observation made in 1957 that abundance is generally very low in the 0- to minus 2-foot zone subject to periodic exposure. The Boothbay Harbor data, however, indicate that setting does sometimes occur in this zone.

Table 2. Results of survey of European oysters in the vicinity of Boothbay Harbor, Maine, October 1958

Location	Year Class	Number of oysters	
		From 0 to -2 feet	From -2 to -4 feet
Boothbay Harbor	1958	3	0
	1957	19	16
	Older	58	824
Capitol Island	1958	0	0
	1957	0	0
	Older	0	20
Linekin Bay	1958	0	63
	1957	0	128
	Older	0	395
Total		80	1,446

Table 1 shows that the 1958 total for Boothbay Harbor is nearly three times that for the comparable area in 1957. The increase was not a result of the 1958 setting, since Table 2 shows that the greatest proportion of the oysters had set prior to 1957. Many oysters were undoubtedly overlooked in 1957 but counted during the more intensive search in 1958.

All data for 1961 and Little River data for 1962 were obtained through survey methods similar to those used in 1958, except that only total counts for each area were obtained. The two young oysters found in Basin Cove in 1962 were located by a cooperating group of SCUBA divers who searched for 13 1/2 man-hours in the subtidal zone of the cove.

EXPERIMENTAL SHELL PLANTING

During 1959 and 1960 an experiment was conducted in shell planting in Boothbay Harbor and Linekin Bay to gain information on the use of cultch and the success of setting. On 19 June and 10 July, while daily mean water temperatures were still below the minimum for spawning (Fig. 4), samples of gonads from Boothbay Harbor oysters were found to be insufficiently developed for spawning. On 22 July, after daily mean water temperatures had been above 15 C for 5 days, all six oysters of a Boothbay Harbor sample spawned soon after being placed in standing water in trays in the laboratory. On 5 August, a sample of six Boothbay Harbor oysters included three which had spawned but no longer contained larvae and three which contained black or well-advanced larvae in the mantle cavity, nearly ready to be released into the water. With the prospect of additional spawning and swarming, and, according to Korringa (1940), possible setting in 2 to 3 weeks at the prevailing temperatures, cultch was placed as follows:

On 11 to 13 August in each of two coves, one on the west side of Boothbay Harbor, the other at the northeast tip of Linekin Bay, 40 to 50 shell bags filled with soft-shell clam cultch were placed on bottom between mean low water and minus 3 feet. Approximately 4 bushels of loose clam shells were scattered just below the zone where the shell bags were placed. Seven strings, 6 to 8 feet long, of hard clam shells were suspended from a raft in Boothbay Harbor.

The cultch was examined occasionally to determine when the first set occurred, but none was found until 9 September. The first spat, two in number, were from Linekin Bay shell bags and measured 2.0 and 3.5 mm.

The final examination of cultch for the season was made during October to determine the success of setting. Five shells were removed from each shell bag for examination, and the shell bags were then moved into deeper water, well below the extreme low tide level, for protection from winter air temperatures. All of the shells on the remaining five raft-suspended strings (two had been lost) were examined. Those shells having spat on them were returned to the raft and left

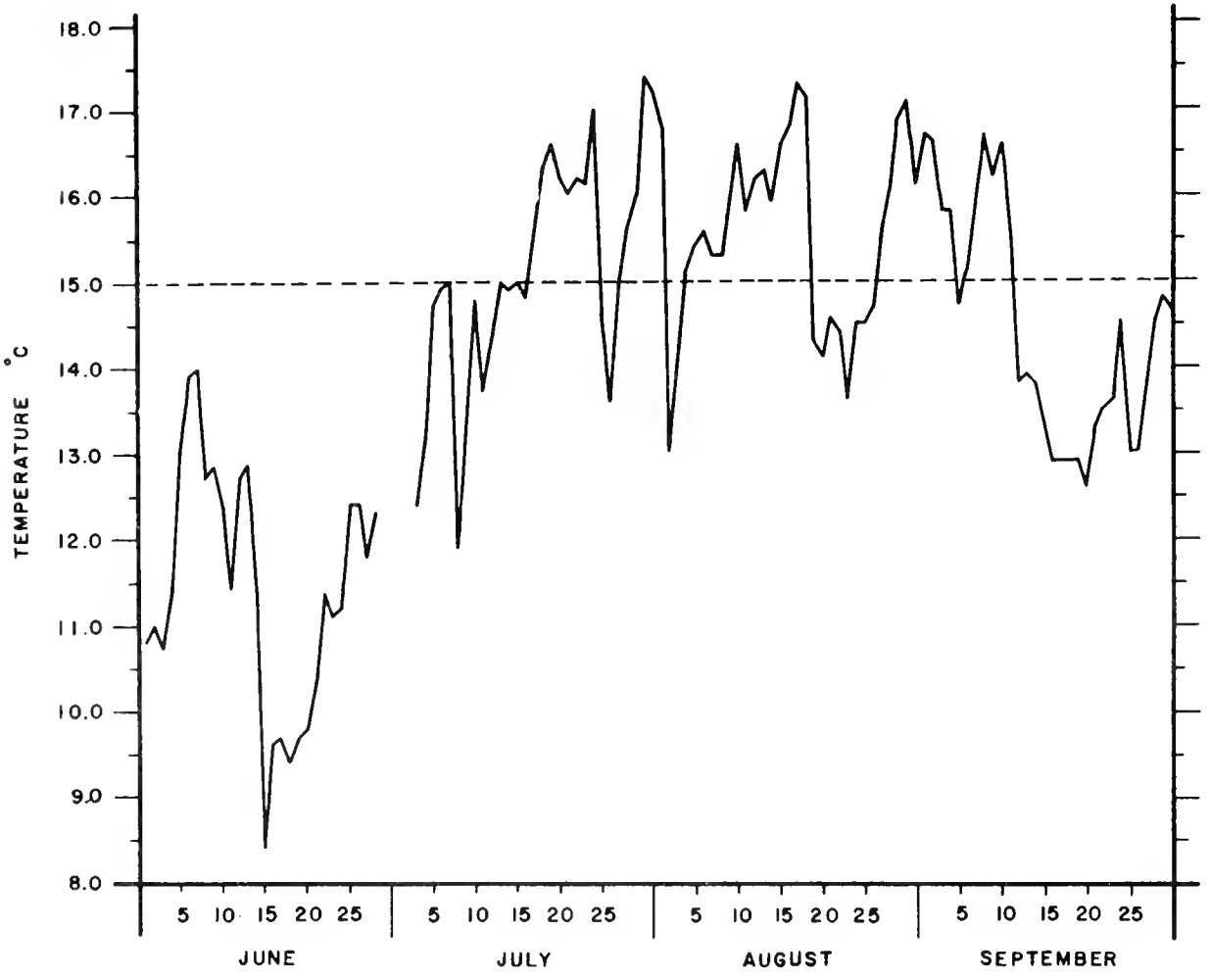

Fig. 4. Daily mean sea water temperatures at Boothbay Harbor, Maine, from June through September 1959. Dashed line at 15.0C is minimum temperature for Ostrea edulis spawning.

suspended through the winter. The loose shell on bottom was sampled, but since no set was found and the shells were heavily silted, no further examinations were made.

The results of the shell counts are shown in Table 3. The data indicate: 1) the success of setting of cultch in shell bags in Linekin Bay was of the same order of magnitude as that in Boothbay Harbor, which had been regarded as both the center of the spawning population and the area of heaviest setting; 2) the success of setting on raft-suspended shell strings was less than half as great as on cultch in shell bags in the immediate vicinity (shell strings had accumulated far more silt and fouling organisms than had the shell in bags), and 3) ranges in size were typical of set occurring over an extended period of time.

Table 3. Setting of European oysters in the vicinity of Boothbay Harbor, Maine, October 1959

Type of cultch and location	No. of shells examined	No. of spat	Ratio, spat per 100 shells	Size (mm)		
				Min.	Max.	Mean
Shell bags in Boothbay Harbor	210	97	46.2	1	13	—
Shell bags in Linekin Bay	220	115	52.3	1	10	—
Shell strings in Boothbay Harbor	105	23	21.9	3	15	6

During the following June and July (1960), the shell strings and all the shell bags that could be found were retrieved to obtain data on survival and size of the spat. As Table 4 indicates, the spat in shell bags in both Boothbay Harbor and Linekin Bay suffered extremely heavy mortalities. The spat on the raft-suspended shell strings fared better, with nearly half surviving. Percentage survival was based on the number of spat per 100 shells in October 1959 (Table 3).

The shell bags had been placed at a level where, although totally submerged, they were subjected to wave action at low tide. All shells were practically devoid of animal or plant growth. The abrasive action of the shells scouring each other and the turbidity in this zone of wave disturbance may have contributed to the mortality of spat.

Table 4. Survival and size of European oyster spat after one winter in the vicinity of Boothbay Harbor, Maine, June-July, 1960

Type of cultch and location	No. of shells examined	No. of spat	Ratio, spat per 100 shells	Survival %	Size (mm)		
					Min.	Max.	Mean
Shell bags in Boothbay Harbor	3,972	108	2.7	5.8	3	26	8
Shell bags in Linekin Bay	3,045	11	0.4	0.8	4	14	8
Shell strings in Boothbay Harbor	105	10	9.5	43.4	4	11	7

DISCUSSION

The successful culture of the European oyster could be economically important to northern New England. As far as is known, the American oyster, Crassostrea virginica, exists in New England north of Cape Cod only in the Piscataqua River between New Hampshire and Maine; in a single, small bed in the upper Sheepscot River estuary in Maine; and as rarely-found individuals in the Damariscotta River in Maine. Ecological conditions apparently do not permit spawning nor survival of larvae or spat outside these areas. Except for the bay scallop (Aequipecten irradians) just north of Cape Cod, the soft-shell clam (Mya arenaria) and the hard clam (Mercenaria mercenaria) are the most important commercially-used mollusks living in the intertidal or shallow subtidal environments. Both of the latter species are generally most abundant in areas not suitable for oysters; therefore, the ecological competition of the oyster with these species appears to be negligible.

The fact that Ostrea edulis has lived and reproduced successfully in the Boothbay Harbor, Maine area since 1949 indicates that the environment is basically suitable. Water temperature has been sufficiently high to bring about annual spawning, since setting has occurred each year, and the range of salinities observed in the area does not seem to constitute a problem. However, since the oyster population has yet to reach commercial abundance and the rate of recruitment appears to be low, there is need for a better understanding of the ecological

requirements of the species and its adaptibility in the area. It should be noted that introductions of Ostrea edulis from Conway, North Wales, into Canada at St. Andrews, New Brunswick, and Ellerslie, Prince Edward Island, were made in 1957, 1958, and 1959. This move was encouraged in part by the success of the Maine introductions, but extremely heavy mortalities occurred, apparently associated with low winter water temperatures (Medcof, 1961).

In Maine the results of the 1957 and 1958 surveys indicate the existence of two conditions which seriously limit the increase in oyster abundance. First, the relative scarcity of oysters in the zone occasionally exposed at extreme low tides indicates that the oysters cannot withstand winter air temperatures. According to Gaarder and Bjerkan (1934), Ostrea edulis in sea water can tolerate temperatures slightly below 0C, but those exposed to freezing temperatures in air will be seriously weakened and damaged, if not killed. In northern New England, subfreezing air temperatures coincide with extreme low tides often enough to virtually eliminate the entire intertidal zone from year-round production of oysters. Second, oysters were found only on firm substrate. Unfortunately, a large proportion of the bottom examined during the surveys and most of the bottom at greater depths, was covered with silt and completely devoid of the shell, rock, or gravel necessary for setting.

A possible third limiting set of conditions is of hydrographic nature but no specific information has been obtained. Korringa (1940) indicated that Ostrea edulis set may not reach commercial quantities in areas where water temperatures are not higher than 18 C, or where tidal exchange may reduce the numbers of planktonic larvae. He pointed out that in such circumstances proper use of cultch is necessary to make maximum use of the available set. Both of these limiting conditions of temperature and tidal exchange prevail in many New England areas.

The encouraging results of the trial use of cultch in 1959 indicate that, through employment of improved methods and materials, stocks of oysters might be increased far beyond those that have accumulated under natural conditions. Suitable subtidal growing ground is limited so that raft or rack culture, or relaying, might be necessary for a commercial operation.

Over a period of 13 years, sufficient encouragement has been gained from observations of the natural growth of the oyster population in the Boothbay Harbor area to recommend that further research be conducted on the European oyster. A research program should include:

1) determination of the physiological requirements of the oyster; 2) ecological studies of areas where the oyster has become established; 3) determination of the setting potential and satisfactory use of cultch, and 4) determination of the degree of physiological adaptation of the Dutch strain of Ostrea edulis to Maine waters since it was first introduced.

Future growth of the oyster population and any possible future research will be aided by the protection now afforded the species in the Boothbay Harbor area. In June 1960 the Maine Department of Sea and Shore Fisheries recognized the potential importance of this oyster and established an extensive closed area (Fig. 3), within which the taking of European oysters is forbidden by law. The boundaries of the closed area include all major known populations and the localities where continued spread and increase in abundance may be expected.

CONCLUSIONS

1. Temperature and salinity characteristics of parts of the Maine coast are basically suitable for survival, growth, and reproduction of Ostrea edulis from Holland.

2. In the Boothbay Harbor, Maine area, oysters have spawned and set annually since 1949 and numbers and range have shown progressive increases.

3. The greatest numbers of progeny exist only in the zone below the range of extreme low tides.

4. Mortality caused by low winter air temperatures is believed to limit abundance of oysters in the intertidal zone, and setting at lower levels is limited by a scarcity of suitable substrate.

5. More set occurred in shell bags on bottom than on raft-suspended shell strings, but winter and spring survival was better on the shell strings.

6. The persistent increases and spread of the Boothbay Harbor oyster population warrants further research.

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A BACTERIAL BASIS FOR THE GROWTH OF ANTIBIOTIC-TREATED BIVALVE LARVAE

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ABSTRACT

Routine addition of the proprietary antibiotic formulation "Combistrep" (dihydrostreptomycin-streptomycin sulfates) to larval cultures of clams, Mercenaria mercenaria, or oysters, Crassostrea virginica, usually results in a significant increase in growth rate of larvae. It had been assumed that this increase was effected by the elimination or suppression of bacterial flora, but plate counts show that the total number of marine bacteria increases in almost direct proportion to the added Combistrep up to 2,000 parts per million. Bacteria-free clam larvae showed no growth when cultured in autoclaved sea water to which Combistrep had been added. In Combistrep-treated cultures inoculated with a mixed flora of marine bacteria, the larvae showed significant growth, while cultures that received the bacterial inoculum but no Combistrep showed little or no growth. These results suggest that the antibiotic-induced bacterial flora in the Combistrep-treated cultures may be utilized by larvae as a food source.

INTRODUCTION

Since the development of methods of rearing larvae of clams, Mercenaria mercenaria, and oysters, Crassostrea virginica, the role of bacteria and dissolved substances in their nutrition has been a matter of conjecture. It has been shown that supplemental live algal foods are primarily responsible for growth and development of these larvae (Davis and Guillard, 1958). On occasion, however, other factors have been observed or suspected to play an important part in nutrition of larvae. Davis and Chanley (1956) reported an increase in the growth rate of clam and oyster larvae on the addition of several vitamins and antibiotics but offered no suggestion on how the increased growth was achieved. Carriker (1956) reared clam larvae to metamorphosis on an extract of cereal, Pablum, and concluded that the good growth of larvae was the result of increased microbial populations stimulated by the addition of the Pablum filtrate. Loosanoff, Davis, and Chanley (1955) stated that clam larvae seem able to utilize sulfur bacteria. Coe (1947) and Rodinca (1948) believed that bacteria played a part in the diet of adult mollusks. On the other hand, Davis (1953) fed pure cultures of nine species of marine bacteria to larval oysters with no success. Loosanoff, Davis, and Chanley (1955) stated that

lack of success in growing oyster larvae on several species of marine bacteria contradicts the generally accepted view that marine bacteria constitute an important part of the oyster diet.

The present paper reports experiments which show the growth-producing effect on clam and oyster larvae of a commercial antibiotic preparation Combistrep[1] (dihydrostreptomycin and streptomycin sulfates). It is furthermore demonstrated that the increase in growth of larvae is associated with a stimulation of marine bacterial populations by the Combistrep. This antibiotic preparation was originally used at the Milford laboratory in an attempt to prevent mortalities of larvae resulting from bacterial diseases, and its effect in increasing growth rates of bivalve larvae was first noted by Chanley (personal communication).

MATERIALS AND METHODS

Methods of conditioning and spawning adult bivalves and rearing larvae in the laboratory have been described in detail (Loosanoff and Davis, 1950). To determine effects of Combistrep on clam and oyster larvae, fertilized eggs were cultured 48 hours at concentrations of approximately 30 per ml in filtered ultraviolet-light-treated sea water. Forty-eight-hour veliger larvae were then collected on stainless steel screens and diluted to a known volume. After the number of larvae per unit volume was determined, appropriate volumes were used to set up experimental cultures with about 10 larvae per ml.

The culture medium, including test materials, was renewed every second day by collecting the larvae on a stainless steel screen and transferring them to new media. Temperatures were held to 24 $\pm$ 1 C throughout. After 10 days' exposure to the experimental conditions clam veligers were sampled quantitatively. Oyster veligers were similarly sampled after 12 days. Effect of experimental treatment on growth of larvae was determined by measuring the long axis of 50 clam or 100 oyster larvae. The generally uniform size of clam larvae permits good accuracy with the lesser sample size.

Methods used in determining the effects of Combistrep on marine bacteria and the effects of the stimulated bacterial populations on bivalve larvae are included briefly within the respective result sections.

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[1] Reg. U. S. Pat. Off. Chas. Pfizer & Co., Inc.

Combistrep is a proprietary compound of Chas. Pfizer & Co., Inc., and has the following composition:

dihydrostreptomycin base (as sulfate)	125 mg/cc
streptomycin base (as sulfate)	125 mg/cc
phenol	0.25%
sodium citrate	1.3%
sodium bisulfate	0.2%
water	77.4%

RESULTS

A compilation of data from experiments over the past several years demonstrates that both clam and oyster larvae receiving Combistrep have consistently shown more rapid growth than larvae in comparable cultures that did not receive Combistrep (Figs. 1 and 2). At concentration ranges of 100 to 300 parts per million (ppm), Combistrep generally increased the rate of growth of clam larvae by more than 100% in cultures not receiving a supplemental feeding of algae (Fig. 1). Growth of these untreated, unfed larvae varied considerably, i.e., the mean length at 10 days varied from about 115 to about 148μ. Such differences in growth are certainly due to variations in the amount of food present in the filtered ultraviolet-light-treated sea water. In all cases, however, above average growth in the untreated, unfed cultures was accompanied by a correspondingly more rapid growth of larvae in the Combistrep-treated unfed cultures.

Clam larvae receiving 200 to 400 ppm of Combistrep and no supplemental algal feeding when reared beyond the usual 10-day experimental period in all cases grew to metamorphosis with negligible mortality within 20 days at 24 C. In all such instances, of course, it required a longer time for these larvae to reach metamorphosis than for larvae receiving algal food supplements, but untreated, unfed larvae in parallel cultures never progressed beyond 140μ in length.

Combistrep also increased growth of clam larvae receiving live flagellates as food (Fig. 1). The increase in the growth increment averaged about 25μ at 12 days of age at optimal Combistrep concentrations. This was about a 25% increase in growth over larvae fed live flagellates without Combistrep. The optimum concentrations of Combistrep appeared to be higher in cultures receiving algal foods than in those not receiving the algae.

Growth of oyster larvae was increased nearly 100% by the addition of Combistrep at concentrations between 200 and 300 ppm

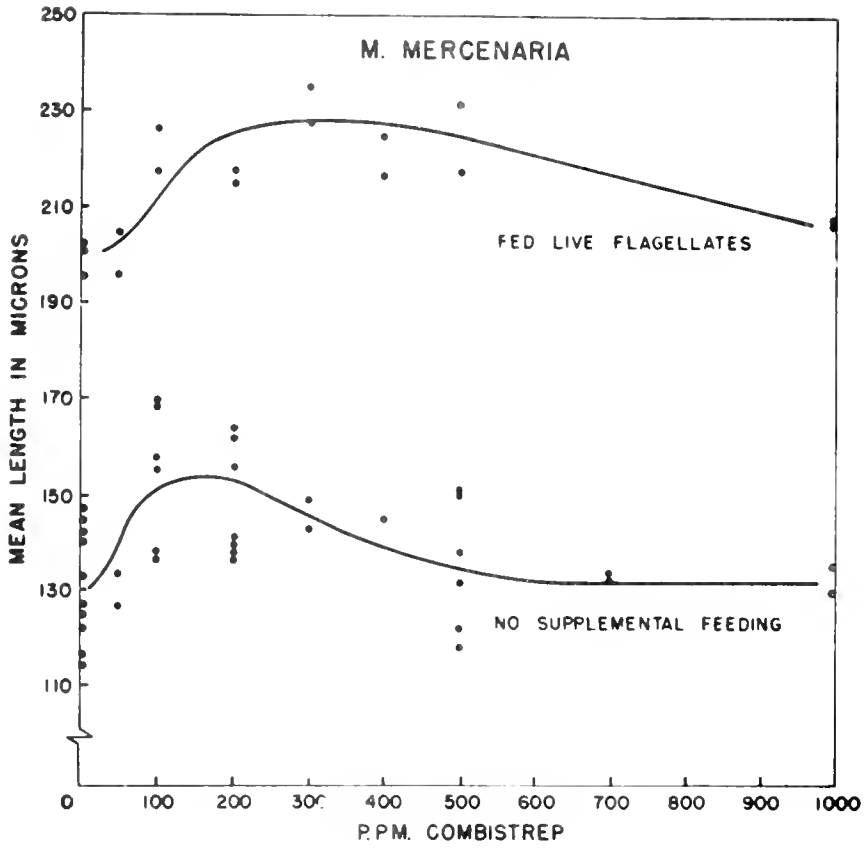

Fig. 1. Mean lengths of clam, M. mercenaria, larvae at 12 days of age receiving several dosage rates of Combistrep, with and without supplemental algal feeding. Data are a composite of several experiments which included Combistrep-treated cultures, together with suitable untreated controls. Each point represents a mean length of 50 larvae from a single population of approximately 10,000 larvae.

(Fig. 2). Figure 2 represents a composite of Combistrep-treated cultures, some of which received algal food supplements while others did not. Controls not receiving Combistrep are included in all cases, however. Because of the sensitivity of oyster larvae to variations in algal food quality, it is often difficult to distinguish larvae receiving algal food from those not receiving the food. The composite of mean lengths of fed and unfed larvae, however, clearly shows the value of Combistrep in increasing growth rates.

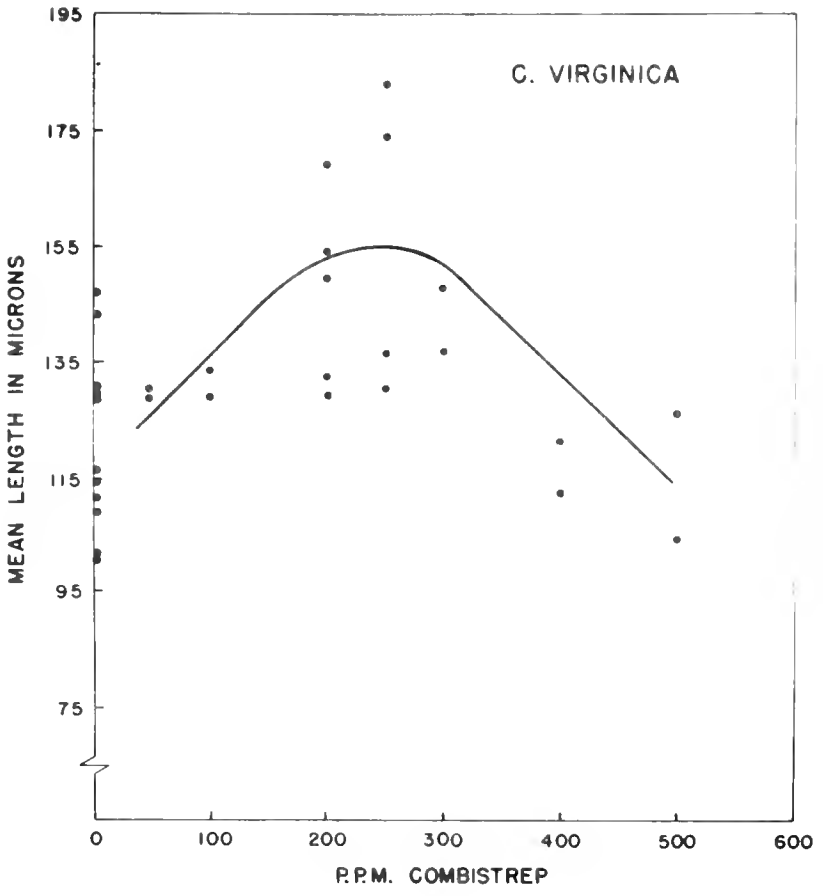

Fig. 2. Mean lengths of oyster, C. virginica, larvae at 14 days of age receiving several dosage rates of Combistrep. Data are a composite of several experiments which included Combistrep-treated cultures, together with suitable untreated controls. Each point represents a mean length of 100 larvae from a single population of approximately 10,000 larvae.

Effects of Combistrep on Bacterial Populations

It was noticed in the above experiments that, although Combistrep did help to prevent mortalities in cultures of clam and oyster larvae, the bacterial population of larval cultures treated with Combistrep and some other antibiotics became actually higher than bacterial populations in untreated cultures. Experiments were then designed to determine the effect of different concentrations of Combistrep on bacterial populations in sea water.

One-liter cultures of filtered ultraviolet-light-treated sea water were set up and given several different concentrations of Combistrep but without larvae and algal food. After 48 hours at 24 ± 1 C samples for plate counts of bacteria were taken. The 48-hour cultures were plated at several dilutions using standard plating techniques on Trypticase Glucose Yeast Extract Agar made up with sea water. Bacterial colonies were counted; no attempt was made to determine the species of bacteria represented.

Increasing dosages of the sterile antibiotic resulted in almost directly proportional increases in bacterial numbers (Fig. 3). This

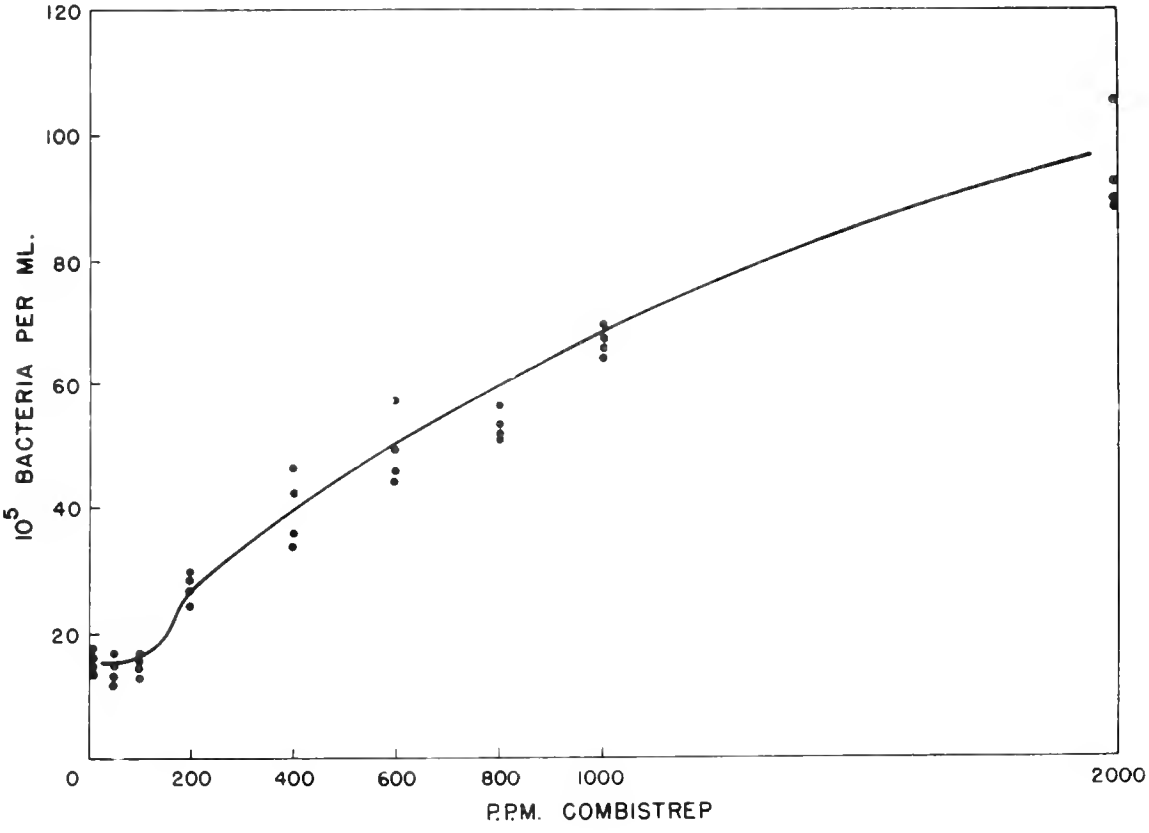

Fig. 3. Numbers of bacteria per ml of filtered sea water 48 hours after application of several concentrations of Combistrep. Duplicate sea water cultures were used at each test concentration. Points represent each of duplicate plate counts made of each sea water culture.

effect was not noted at the lowest concentrations (50 to 100 ppm). At concentrations of Combistrep that have been especially beneficial to larvae (200 to 400 ppm), there was approximately a two-fold increase in bacterial numbers over Combistrep-free cultures. Although the actual number of bacteria differed somewhat from experiment to experiment, the percentage increase with increasing dosages of Combistrep remained relatively constant.

Effect of Bacteria on Growth of Larvae

At this point it was important to determine whether the bivalve larvae were deriving benefit from the Combistrep directly or possibly from the increased bacterial populations that resulted from its addition to sea water. To do this we were eventually able to compare growth of larvae in aseptic and in bacterized Combistrep-treated cultures. Before we had developed methods for obtaining sterile larvae for aseptic cultures, however, several experiments were run in an attempt to correlate the rate of growth of larvae with the number of bacteria present.

It was postulated that if bacteria here were important in growth, then larvae receiving Combistrep and sea water with a fully developed bacterial population should grow more rapidly than larvae receiving Combistrep and sea water which were sterile. Consequently, non-sterile clam larvae receiving Combistrep were reared in sea water (1750 larvae in 500 mls) pretreated as follows:

1. Autoclaved and aged one week with sterile Combistrep added at the time of addition of water to cultures of larvae (sterile water supply).

2. Autoclaved, then aged one week after Combistrep plus a non-sterile sea water inoculum had been added (fully developed initial bacterial populations).

Larvae in control cultures, not receiving Combistrep, were reared in sea water with the following pretreatment:

1. Autoclaved, then aged one week.

2. Autoclaved, then aged one week after a non-sterile sea water inoculum had been added.

3. Autoclaved with live flagellate food added at the time of feeding to cultures of larvae.

The water in each culture was completely renewed every 24 hours and temperatures held to 20 C to minimize buildup of bacteria in treatments receiving the initially sterile water supplies. Duplicate cultures were used to test each treatment in each of the three replicate experiments. Results are shown as mean lengths of larvae after eight days of culture (Table 1). Mortality of larvae in all cases was negligible and thus is not included.

In the first experiment larvae grown in the presence of Combistrep with a fully developed initial bacterial population reached a mean length of 149.65μ, while those grown with Combistrep and sea water which were initially sterile reached only 117.90μ. Control cultures, i.e., those reared in sterile sea water and those kept in aged water plus the bacterial inoculum, also showed poor growth. Although the 149.65μ mean length was appreciably less than the 175.15μ mean length attained by larvae receiving live flagellates as food, it represents a significantly faster rate of growth than was achieved in control cultures.

The second experiment (Table 1), although conducted in exactly the same manner as the first, produced quite different results. In this trial 500 ppm Combistrep actually retarded clam growth, both when given with a sterile water supply and with a fully developed bacterial population. Since 500 ppm Combistrep is above an optimum dosage for clam larvae (Fig. 1), variable results might be expected. Growth was not reduced in a control culture within this experiment given only 250 ppm Combistrep. In a third trial Combistrep concentrations were adjusted to 250 ppm, which is a more nearly optimum concentration for clam larvae.

At 250 ppm (Experiment 3, Table 1) Combistrep-treated sea water with a fully developed initial bacterial population again produced good growth of clam larvae. Larvae kept in this water attained a mean length of 166.53μ, a length not much smaller than the 189.05μ achieved by larvae receiving live flagellate food. Larvae receiving sterile Combistrep and sterile sea water showed some growth (135μ mean length), while larvae kept in the sterile sea water and those kept in non-sterile sea water without Combistrep showed very little growth (121.20 and 116μ mean lengths, respectively).

Plate counts were made immediately after a change of sea water and again 24 hours later in this experiment to determine the typical numbers of bacteria at 0 and at 24 hours with each treatment (Table 2).

Table 1. The effect of several sea water treatments on the growth of non-sterile clam larvae. Larvae were cultured for 8 days at 20 C with water supplies renewed every 24 hours.

Sea water culture supply	Mean length (μ)		
	Exp. #1	Exp. #2	Exp. #3
Autoclaved (sterile)	113.00 ± 0.84[1]	121.25 ± 1.30	120.20 ± 0.83
Autoclaved + Combistrep (500 ppm)	117.90 ± 0.76	118.25 ± 0.94	No trial
Autoclaved + Combistrep (250 ppm)	No trial	126.65 ± 1.40	135.00 ± 1.18
Autoclaved + non-sterile inoculum (aged)	122.90 ± 1.09	125.55 ± 1.42	116.00 ± 0.77
Autoclaved + non-sterile inoculum + Combistrep (500 ppm) (aged)	149.65 ± 1.09	119.15 ± 1.66	No trial
Autoclaved + non-sterile inoculum + Combistrep (250 ppm) (aged)	No trial	No trial	166.53 ± 2.73
Autoclaved + flagellate food supplement	175.15 ± 1.68	190.35 ± 2.21	189.05 ± 2.64

[1]Indicates 95 per cent confidence limits, ± 1.98 SEm

Table 2. Numbers of bacteria present in non-sterile larval clam cultures receiving different treatments (Experiment 3, Table 1). Counts were made at times of water renewal (0 hours) and at the end of each change cycle (24 hours).

Sea water culture supply	Bacteria per ml	
	0 Hours	24 Hours
Autoclaved	19,000—21,000	550,000—580,000
Autoclaved + Combistrep	33,000	4,000,000
Autoclaved + non-sterile inoculum (aged)	50,000—100,000	65,000—150,000
Autoclaved + non-sterile inoculum + Combistrep (aged)	100,000—180,000	145,000—500,000

Although bacterial numbers were low at 0 hours in the cultures receiving the sterile water (19,000 to 33,000 bacteria per ml), there was a rapid buildup in the cultures by 24 hours (550,000 to 4,000,000 bacteria per ml). On the other hand, numbers of bacteria were initially high in cultures receiving the non-sterile water treatments (50,000 to 180,000 per ml) and showed a slower rate of increase in these cultures (65,000 to 500,000 per ml) at 24 hours. The aged sea water that had received the non-sterile inoculum plus Combistrep had considerably more bacteria per ml, both at 0 and at 24 hours, than the aged sea water that had the bacterial inoculum only. These results are in general agreement with experiments in which we determined the effect of Combistrep on the number of bacteria in sea water as previously described.

In subsequent experiments bacteria-free larvae were used to test the effect of Combistrep in aseptic vs. non-sterile cultures. Fertilized clam eggs collected on sterile stainless steel screens were washed several times with autoclaved sea water. These eggs were then permitted to develop into 48-hour veliger larvae in the trivalent antibiotic solutions described by Guillard (1959). Five

to ten of these sterile larvae were then transferred aseptically to 10 ml of autoclaved sea water in each of 16 test tubes for each of the following treatments:

1. No treatment (sterile control)

2. 250 ppm sterile Combistrep (Combistrep only)

3. 0.1 ml non-sterile sea water (bacteria only)

4. 250 ppm sterile Combistrep + 0.1 ml non-sterile sea water (Combistrep + bacteria).

After sterile larvae had been added cultures were held 11 days at room temperature. Sterility tests in sea water-thioglycollate were run on treatments 1 and 2 (above) at the end of the experimental culture period. Larvae in all vials were then killed and length measurements were taken of all larvae that had survived the test period.

Of several such experiments to measure growth of clam larvae receiving Combistrep under septic and aseptic conditions, only one was successful. In this experiment many of the culture tubes of the sterile groups remained sterile to the end of the test period, permitting valid measurement of the effect of treatment. Larval survival in this experiment was variable, but due to the low initial larval density survival did not appear to affect growth rates. The results expressed as mean lengths of larvae are listed in Table 3. In addition to the four original experimental treatments, two additional categories developed. These were the mean lengths of larvae from several cultures of the two originally sterile treatments which proved non-sterile by the end of the culture period. Although all larvae grew poorly, differences in mean lengths between treatments were highly significant statistically. An analysis of variance gave an F value of 21.82, indicating an overall difference of means significant far greater than the 99% confidence level (Snedecor, 1962).

Tests of significance of differences between individual treatments within this experiment showed that (a) mean length of sterile larvae (102.82μ) was not significantly different (at the 99% confidence level) from sterile larvae receiving Combistrep (103.68μ); (b) sterile larvae (102.82μ) did not differ significantly from those receiving the non-sterile inoculum but no Combistrep (105.54μ); (c) larvae receiving Combistrep plus the non-sterile inoculum (110.84μ) were significantly larger (at the 99% confidence level) than either those receiving Combistrep under sterile conditions (102.82μ) or those that had received only the non-sterile inoculum (105.54μ).

Table 3. Mean lengths of clam larvae after 11 days of culture receiving several different treatments

Treatment	Mean length (μ)
1. Sterile	102.82
2. Sterile + 250 ppm Combistrep	103.68
3. Bacterized non-sterile	105.54
4. Non-sterile + 250 ppm Combistrep	110.84
1a. Sterile (contaminated)	106.14
2a. Sterile + 250 ppm Combistrep (contaminated)	108.53

Also notable was the fact that the larvae receiving the sterile-Combistrep treatment, which accidentally became contaminated (108.53μ), were significantly larger than the other replicates within the treatment which remained sterile to the end of the culture period (103.68μ).

DISCUSSION AND CONCLUSIONS

The mechanism by which Combistrep induces greater bacterial populations in sea water can only be speculated upon at this time. Experiments thus far have only measured increase in gross numbers of bacteria without regard to possible species selection. It is possible that Combistrep is acting to inhibit certain toxin-producing species, thus allowing greater total numbers. The possibility of the minimal quantity of citrate present in dilute Combistrep acting as an energy source seems remote.

The series of experiments in which non-sterile clam larvae were exposed to Combistrep-treated sea water both sterile and with fully developed bacterial populations initially present indicated that the rate of growth of larvae was associated with the number and

probably the species of bacteria present and suggested that the larvae were using these bacteria as foods. In the two experiments in which Combistrep was beneficial to larvae (Exps. 1 and 3, Table 1), those larvae cultured in Combistrep-treated water with high initial populations of bacteria present in each case showed markedly greater growth than those receiving the initially-sterile water plus Combistrep. Although clam cultures receiving Combistrep with low initial bacterial numbers contained appreciable populations of bacteria by the end of the 24-hour water change cycle, there undoubtedly were significantly fewer bacteria present in these cultures throughout most of the 24-hour change cycle than in those receiving Combistrep with initially high bacterial populations. The fact that larvae in cultures receiving the bacterized aged water without Combistrep showed poor growth even though there were considerable bacterial populations present, throughout, would indicate that Combistrep was selecting and accelerating the growth of only certain beneficial species of bacteria.

The experiment using bacteria-free larvae showed that clam larvae in bacterized Combistrep-treated cultures grew, whereas those kept in sterile Combistrep-treated cultures and those kept in sterile, non-treated cultures showed little or no growth. This again demonstrated that it was the bacteria associated with the Combistrep treatment, not the Combistrep, itself, that caused the more rapid growth of bivalve larvae. Again, larvae in bacterized cultures that did not receive Combistrep showed less growth than in similar cultures containing Combistrep. This supports the above contention that the Combistrep-induced bacteria are perhaps of preferential utility to the larvae.

It is conceivable that the increased numbers of bacteria may at times be too great (over 500 ppm Combistrep). Such a biological mass might nullify beneficial effects by the creation of adverse environmental conditions for the larvae, such as reduction of dissolved oxygen, creation of toxic metabolites, etc. The variable results measured at 500 ppm Combistrep between experiments (Table 1) may be the result of this.

These studies, of course, do not show beyond all doubt that bacterial populations are utilized directly as food by bivalve larvae but do very definitely associate increased and probably selected bacterial populations with larval growth. As we learn more about these relationships, it may be possible to control bacterial populations to such an extent that they may become generally useful in future shellfish hatcheries, possible circumventing the presently difficult culture of relatively fastidious live algal food cells.

SUMMARY

1. Routine addition of the proprietary antibiotic formulation, "Combistrep" (dihydrostreptomycin-streptomycin sulfates) to cultures of clam or oyster larvae has consistently resulted in 25 to 100 per cent increases in the rate of growth of the larvae.

2. The addition of sterile Combistrep to filtered ultraviolet-light-treated sea water has produced an increase in numbers of bacteria roughly proportional to the concentration of Combistrep.

3. Tests with non-sterile Combistrep-treated cultures showed that clam larvae grew faster in Combistrep-treated cultures with a high initial bacterial count than in cultures with a low initial count.

4. In bacteria-free cultures the addition of Combistrep did not increase the rate of growth of clam larvae.

5. All data indicate that the increased rate of growth of larvae receiving Combistrep treatment is associated with the increase in numbers of a possibly selected group of bacteria.

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A METHOD FOR INCREASING SURVIVAL OF LOCALLY-CAUGHT PACIFIC OYSTER SEED IN WILLAPA BAY, WASHINGTON

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ABSTRACT

To enable the oyster industry to increase survival of local Pacific oyster spat through the first winter, it is recommended that shell strings be piled on hard bottom above low tide level until spring. This technique is basically that used in Japan. In the first commercial trial in Willapa Bay, 7,000 local shell strings held 6 ft above mean lower low water level from September 1957 to April 1958, then crushed and planted, yielded 3.68 gallons (13.9 liters) of oyster meats per shell string in the 1961–1962 season, substantially above usual production (less than 1 gallon per string).

Seed growers of Miyagi Prefecture, Japan, catch Pacific oyster spat on hanging cultch in July and August (Imai et al., 1951). The cultch strings are placed on flat hardening racks high in the intertidal zone during September after the major summer heat is past but before spat growth makes them too large for export. These racks are 12 to 18 inches above the bottom, and the strings are laid horizontally on them in layers two to four strings deep where they remain until selected and boxed for shipment to Washington State growers during February, March, and early April of the following year. The hardening process retards growth of spat and causes them to develop tightly closing valves which aids their survival during shipment.

In Willapa Bay it is customary to catch oyster seed in July and August, then remove the cultch strings from racks and put the shells on growing beds during fall months before winter storms begin. Because this bay is turbid, much silting occurs during winter, to the detriment of these small oysters. Since the Pacific oyster is very hardy and can withstand the harsh Japanese weather during the seed hardening period, it can survive the milder climate of Willapa Bay without undue mortalities, providing the seed is kept off the bottom and out of silt. Experiments in which seed oysters are held in trays from two to six inches above the bottom indicated that, in the absence of silt, spat mortalities during the first winter were due to space competition for growth.

Quayle (1957) showed that tray culture gave 64.4 per cent survival against 37.1 per cent survival on the ground for Pacific oyster seed from 28 April 1956 to 17 November 1956, a period of seven months. In a study at the Willapa laboratory, one tray containing 1,278 Pacific oyster seed had 513 (40.2 per cent) survival from 25 April 1956 to 18 March 1957. In another tray containing 1,017 oysters (seed), 563 (55.4 per cent) survived from 29 May 1956 to 19 March 1957. These survival percentages are not as high as Quayle's, but support his findings that in the absence of silting, space competition is the most important cause of spat mortalities, although predation or disease may be significant in certain areas or in specific cases. Quayle's experiments and those at Willapa Bay indicate that few mortalities occur after the first year. Annual growth and mortality surveys of Pacific oysters in Washington waters have shown that, in Willapa Bay at least, significant mortalities in older year classes of oysters do not occur. In his productivity experiments, Quayle (1954) found no difference between broken and unbroken seed because greater numbers of oysters in broken seed cases were offset by higher mortalities suffered in the first year from burying and fouling. Using numbers of oysters as an indicator of yield, his experiments showed productivity ranging from 2,168 to 6,295, with an average of about 4,000 oysters per case. This is in agreement with commercial production figures and indicates that maximum survival to harvest for Japanese oyster seed with a minimum of 12,000 spat per unbroken case and 16,000 spat per broken case is not very high.

In the Willapa Bay cultch survey (Sayce, 1958), counts of spat per equivalent case for local seed ranged from 81,000 to 108,000 when six shell strings per case were used as a basis of comparison. This may be considered as high-count seed, yet no oyster company operating in Willapa Bay has reported a production in excess of one gallon of oysters per shell string. From this, it is apparent that the potential oyster production from locally-caught seed is not being realized. To increase production from local seed, more young oysters must survive their first winter, and of the two important causes of early mortality, space competition and silting, the latter seems to be the easier to control.

When good or excellent local Pacific oyster sets occur, competition for space on the mother shell is lessened by breaking, crushing, or otherwise fragmenting the shell before placing it on oyster ground. However, if this is done in fall or winter months, silting mortalities negate the advantage unless the seed is placed directly on hard, silt-free ground. By holding seed oyster strings, after spatfall, on suitable silt-free ground until the following spring, then placing

them broken or unbroken on growing ground, locally-caught Pacific oyster seed will provide production comparable to that of imported seed.

To insure high survival of seed, the holding area must be carefully chosen. In Willapa Bay, this area must lie less than 7.0 feet above mean lower low water and should be no higher than about 5.5 feet above MLLW level so that shell strings may be piled two to four strings deep and still be adequately covered with water during high tides. Any suitable area below this level may be used if uncovered at low tide. Higher levels will retard spat growth, and if shell strings are held below mean lower water level, some spat growth will occur. This will cause undue spat mortalities during movement to oyster beds in the spring. Small spat withstand shell breaking or crushing operations with fewer mortalities than do large spat. Therefore, the tide level at which shell strings are held should be picked with this in mind.

Holding shell strings up out of silt may be accomplished by placing them on a natural oyster reef, on a graveled area, or on an area which has been prepared with several inches of oyster shell. Polyethylene sheeting may be used in place of, or under, oyster shell to prevent shell strings from gradually settling and becoming silted. These types of silt-free areas have been successfully used to hold locally-caught Pacific oyster seed through the first winter on a commercial scale.

The first commercial trial of this method was carried through to harvest with success. In this operation, 7,000 local shell strings were held at the 6.0 foot level above mean lower low water from late September 1957 until April 1958. At this time, the shells were crushed and planted in the Stackpole Harbor area of Willapa Bay. At completion of harvest in the 1961-62 production season, the company realized 3.68 gallons of oysters per shell string. This was a substantial increase of production over local shell strings handled in the usual manner.

Too often local seed is treated as bonus seed, planted when least able to survive, never broken and scattered into singles, seldom moved from growing areas to a good fattening bed, and often placed on marginal fattening ground resulting in low production. In order to fully utilize the production potential of locally-caught Pacific oyster seed, it must be protected from silting mortalities during the first winter and be placed on good growing ground the following spring. The increased survival of oysters will be measured by increased production in gallons of oysters at harvest time.

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NOTES ON THE OCCURRENCE OF *DERMOCYSTIDIUM MARINUM* ON THE GULF OF MEXICO COAST DURING 1961 AND 1962

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ABSTRACT

In October 1961 this parasitic fungus was found in 8 of 9 Florida samples of oysters from Tampa, Apalachicola, and Pensacola Bays. During the period October 1961–May 1962 it was found in all of 12 oyster samples from a Louisiana area extending from Bay Denesse (east of Mississippi River) to Bayou Du Nord Ouest (north of Lake Chien). During the period January–May 1962 it was found in 15 of 18 samples of Texas oysters from 10 stations in the Galveston area. The only uninfected samples were one from St. Vincent's Reef in Apalachicola Bay, and three from a low salinity station near Smith's Point in Galveston Bay.

INTRODUCTION

During 1961 I received reports of very low incidences of the oyster fungus parasite, *Dermocystidium marinum*, in the Gulf of Mexico. Some reports suggested that the parasite had practically disappeared from areas that in previous years were heavily infested. In view of the reduced water salinities during 1959, 1960, and 1961 in many Gulf Coast oyster-growing regions, especially in Louisiana, a drop in the incidence of *D. marinum* in some oyster populations would be expected but not to a point approaching "disappearance" in such areas as lower Barataria Bay.

These reports led me to conduct a survey in Florida, Louisiana, and Texas to obtain data on the occurrence of *D. marinum*, and to test modifications in the original thioglycollate culture technique, which preliminary studies indicated would improve this method for detecting *D. marinum*. This paper presents the results of the survey conducted during late 1961 and early 1962. The parasite was found in 35 of 39 samples of live oysters taken in three Gulf states.

MATERIALS AND METHODS

The thioglycollate culture method (Ray, 1952a and 1952b) for diagnosing D. marinum was used. In some cultures the original procedure, which utilizes penicillin G and dihydrostreptomycin to retard bacterial growth, was modified with regard to antibiotics. In one modification mycostatin (nystatin) was combined with these two antibiotics; in the other modification mycostatin and chloromycetin (chloramphenicol) were substituted for penicillin and dihydrostreptomycin. The details of antibiotic concentrations employed will be presented in the paper following this one.

Both rectal and mantle tissues were cultured to obtain comparative data on the original and modified methods. The rectum (that portion of the intestine extending over the adductor muscle) was split longitudinally into approximately two halves; one piece was cultured in original medium and the other in modified medium. This difficult and time-consuming procedure was discontinued after October 1961. During 1962, pieces (approximately 5 x 10 mm) of the anterior portion of the right mantle (referred to as mantle "A" in the text and in Tables 3, 4, and 5) just lateral to the palps were cultured for comparative purposes. Unpublished data gathered by the author had indicated that often more D. marinum cells are found per unit of mantle tissue in the region adjacent to palps than in the region near the adductor muscle. Care was taken to alternate the tubes representing each method in order that the anteriormost piece of mantle tissue from about half of the oysters in the sample would be tested by each method.

Data on the relative infection intensity in mantle A and the rectum were obtained for several samples. In such cases a piece of the mantle (approximately 5 x 10 mm) and the rectum were cultured in the same tube.

Some samples of oysters were cultured shortly after being removed from the water and others remained out of water for two or three days before being processed. All Louisiana oysters cultured 21 October 1961 had been stored in the boat slip of the Louisiana Department of Wild Life and Fisheries Laboratory on Grand Terre Island for several days before cultures were started. Since this storage occurred during October, I do not believe that the levels of D. marinum infections were increased.

The largest oysters were selected from most samples since older oysters are more likely to be infected with D. marinum than

younger ones. Some samples were rather small and most of the oysters in the sample were cultured in spite of the selectiveness.

RESULTS

The incidence and weighted incidence (Mackin, 1962) of D. marinum in oysters from widely-scattered areas in Florida, Louisiana, and Texas are presented in Tables 1-5. Eight of the nine Florida samples were positive for D. marinum. The negative sample came from St. Vincent's Reef, Apalachicola Bay. The incidence of infection in positive samples varied from 20 to 100 per cent, with weighted incidence ranging from 0.60 to 2.85.

Twelve samples taken in Louisiana were positive for this parasite; the incidence obtained by the original method varied from 19 to 90 per cent and the weighted incidence ranged from 0.23 to 1.95.

All of the Texas samples were infected with D. marinum except three samples taken at well 48 (near Smith's Point) in Galveston Bay. The salinity is generally lower there than at other Galveston Bay sampling stations because water from Trinity River tends to flow along the eastern shore of the bay. Incidence in positive samples varied from 25 to 100 per cent, and weighted incidence varied from 0.39 to 2.00. Weighted incidence of all positive samples from West Bay and Galveston Bay exceeded 1.00, even during January. The average infection level for the oysters in these samples varied between "light" and "light to moderate," according to Mackin's weighting system.

EXPLANATION OF TABLES 1-5

The antibiotics used in the culture medium are identified in the tables by the following abbreviations: "P+S" for penicillin and dihydrostreptomycin; "M+P+S" for mycostatin, penicillin, and dihydrostreptomycin; and "M+C" for mycostatin and chloromycetin.

Information on the sources of the oysters and the extremes of length (right valve) as well as the mean length are also presented in the tables. Samples taken from leased oyster beds (either planted or natural populations) are identified by using the name of the lease owner, except the sample from Bay Denesse, Louisiana, which is a composite sample from six leases. All other oyster samples were collected directly from natural growing areas, except the East Bay (Pensacola, Florida) stock, which was transferred to Santa Rosa Sound

Table 1. Incidence and weighted incidence of *D. marinum* in live oysters from Florida

Source of oysters	Date (1961)	No. of oysters	Min. & max. length (mm) (avg length)	P + S (Rectum)		M+P+S (Rectum)	
				Incidence (%)	Weighted incidence	Incidence (%)	Weighted incidence
Tampa Bay, Weedon Island (Leverock)	10/26	15	71-125 (90)	87	1.03	93	1.50
Tampa Bay, Weedon Island (Kirtsinger)	10/26	15	86-125 (107)	20	0.30	27	0.40
Tampa Bay, Rocky Point	10/26	15	78-125 (100)	67	0.97	80	1.60
Tampa Bay, Power Plant Bar	10/27	10	95-130 (107)	70	1.35	70	1.65
Apalachicola Bay, St. Vincent's Bar	10/26	10	65-87 (80)	0	0	0	0
Apalachicola Bay, Cat Point Bar	10/26	10	72-105 (87)	100	2.85	100	2.75
Apalachicola Bay, Green Point Flat	10/26	13	87-125 (102)	77	1.85	77	1.88
Santa Rosa Sound, Boat Slip, Sabin Is.	10/23	15	72-119 (91)	80	2.27	80	2.40
Santa Rosa Sound, Sabin Is. (East Bay Stock)	10/23	10	54-76 (60)	20	0.60	20	0.80

Table 2. Incidence and weighted incidence of D. marinum in live oysters from Louisiana

Source of oysters	Date (1961)	No. of oysters	Min. & max. length (mm) (avg length)	P+S (Rectum)		M+P+S (Rectum)	
				Incidence (%)	Weighted incidence	Incidence (%)	Weighted incidence
Bay Denesse (6 leases)	10/30 10/31	50	73-135 (96)	78	1.94	86	2.34
Bay Pizati (R. & L. Cognevich)	10/21	10	102-141 (121)	20	0.60	20	0.70
Bay Jaque(s) (R. & L. Cognevich)	10/21	10	89-126 (109)	90	1.95	100	2.20
Billet Bay (K. Tesvich)	10/21	6	91-150 (112)	67	1.50	67	1.67
Grand Terre Island, Turning Basin	10/21	10	102-136 (121)	80	1.50	90	1.85
Barataria Bay, Sugar House Bend (A. Pitre)	10/21	20	67-120 (80)	80	1.83	85	2.20
Caminada Bay, west side (Collins)	10/21	20	68-107 (84)	30	0.63	30	0.73
Timbalier Bay Lake Raccourci, so. Philo Brice Light, (N. Callais)	10/21	20	73-120 (92)	35	0.83	35	1.00

Table 3. Incidence and weighted incidence of *D. marinum* in live oysters from Louisiana

Source of oysters	Date (1962)	No. of oysters	Min. & max. length (mm) (avg length)	P+S (Mantle A)		M+C (Mantle A)		M+C (Rectum)	
				Incidence (%)	Weighted incidence	Incidence (%)	Weighted incidence	Incidence (%)	Weighted incidence
Grand Bayou (J. Jurisich & Popich, 2 leases ea.)	4/4 4/5	60	70/101 (88)	52	0.82	52	1.30	53	1.26
Billet Bay (J. Tesvich)	5/31	26	63-117 (87)	19	0.23	27	0.37	23	0.31
W. Champagne Bay (D. Collins, 3 leases)	1/9	60	72-118 (89)	-	-	-	-	40	0.72
Bayou DuNord Ouest (A. Naquin)	3/6	25	87-131 (104)	-	-	-	-	36	0.78

Table 4. Incidence and weighted incidence of D. marinum in live oysters and gapers from Galveston Bay, Texas

Source of oysters	Date (1962)	No. of oysters	Min. & max. length (mm) (avg length)	P+S (Mantle A) Incidence (%)	P+S (Mantle A) Weighted incidence	M+C (Mantle A) Incidence (%)	M+C (Mantle A) Weighted incidence	M+C (Rectum) Incidence (%)	M+C (Rectum) Weighted incidence
Well 48 (near Smith's Point)	1/30	8	74-153 (104)	-	-	-	-	0	0
	1/30	7[1]	47-163 (82)	-	-	-	-	0	0
Well 48	5/28	10	84-155 (117)	0	0	0	0	0	0
Well 87	1/30	8[1]	48-83 (63)	-	-	-	-	38	1.25
Well 87	1/30	10	90-130 (105)	-	-	-	-	100	1.65
Well 87	5/28	10	82-160 (109)	80	1.45	90	1.90	100	2.15
Small natural bar	1/30	10	70-140 (89)	-	-	-	-	60	0.95
Well 5[2]	5/28	12	72-130 (95)	100	1.29	100	2.38	100	2.38
Well 37	1/30	10	78-158 (113)	-	-	-	-	90	1.35
Well 37	5/28	10	83-126 (105)	90	1.30	90	1.90	90	1.90

[1] Gapers
[2] Sample taken from well 5 because small natural bar had been destroyed by shell dredgers between 1/30 and 5/28/62.

Table 5. Incidence and weighted incidence of D. marinum in live oysters from Texas

Source of oysters	Date (1962)	No. of oysters	Min. & max. length (mm) (avg length)	P+S (Mantle A)		M+C (Mantle A)		M+C (Rectum)	
				Incidence (%)	Weighted incidence	Incidence (%)	Weighted incidence	Incidence (%)	Weighted incidence
Well 66 (Galveston Bay)	1/30	10	83-117 (97)	-	-	-	-	70	1.15
Well 66	5/28	10	87-165 (105)	60	1.05	60	1.60	70	2.00
Redfish Bar (Galveston Bay)	1/30	10	71-115 (90)	-	-	-	-	70	1.15
Redfish Bar	5/28	10	70-92 (82)	80	1.50	90	2.65	90	3.00
Todd's Dump (Galveston Bay)	5/28	10	88-112 (98)	80	2.00	90	2.70	90	2.90
West Bay, Galv. (Martin, 306A)	3/5	18	85-165 (120)	89	1.11	100	2.25	100	2.14
West Bay, Galv. (Martin, 304)	3/5	16	72-108 (88)	88	1.25	94	2.60	94	2.34
West Bay, Galv. (Martin, 304)	4/12	38	70-140 (100)	76	1.71	79	2.74	79	2.61
Cow Trap Lake (west of Freeport)	5/23	28	83-130 (98)	25	0.39	29	0.61	29	0.77

about August 20, 1961. The oysters taken at Galveston Bay sampling stations identified by "well numbers" (Humble Oil and Refining Company) were from natural populations growing on shell pads placed on the bottom to support drilling equipment. The Galveston Bay stations are approximately in mid-region of the bay and are arranged in Tables 4 and 5 to indicate their location generally along an east-to-west line. The eastern-most station is well 48 near Smith's Point and the western-most station is Todd's Dump just west of the Houston Ship Channel.

DISCUSSION

If the data obtained during this survey are representative, it appears that D. marinum is still prevalent in many Gulf Coast areas, especially in moderate to high salinities. Lower Barataria Bay, Louisiana, was one of the areas from which low incidences of D. marinum were reported during 1961. The data obtained during this study indicate that the oyster populations sampled from lower Barataria Bay were highly infected during October 1961. High mortality and high levels of D. marinum infections are characteristic of market-sized oysters that spend the summer and fall in this area. I have no data on D. marinum from this area between March 1960 and October 1961. This period covers much of the time during which the low levels of D. marinum were reported.

During March 1960 live oysters from three experimental trays that were maintained at Sugar House Bend, lower Barataria Bay, for approximately one year were checked for D. marinum. The incidence of infection varied from 46 to 100 per cent and weighted incidence varied from 0.56 to 1.33. Live oysters from three experimental trays maintained in Bayou Rigaud, Louisiana, during the same period showed incidences of infection ranging from 70 to 90 per cent and the weighted incidence varied from 0.83 to 1.10. The oysters in all six trays had extremely low levels of D. marinum infection when they were transferred from Bay Chene Fleur, Louisiana, to Sugar House Bend and Bayou Rigaud during February and March of 1959. Based on the limited data presented above, it seems likely that oysters in lower Barataria Bay had ample opportunity to become infected during the summer of 1959. Furthermore, those oysters surviving through the winter (1959) and spring (1960) probably carried infections that became elevated during the summer and fall of 1960. A sharp drop in D. marinum infection levels may have occurred during the winter (1960) and spring (1961), but the high level of infection found in many Louisiana samples in October 1961 suggests that the parasite should not have been difficult to detect in market-sized oysters during the summer of 1961.

Although I do not have any direct evidence to account for the reported low incidences of D. marinum during 1961, I believe that faulty technique may account for some of them. This conclusion is based on the data presented in this paper and subsequent discussions with some of the individuals who had originally reported the scarcity of D. marinum in areas where oysters are often highly infected.

The suspected errors in technique as well as modifications, based on the comparative data obtained during this survey with regard to antibiotics, for improving the thioglycollate culture method will be presented in the following paper.

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A REVIEW OF THE CULTURE METHOD FOR DETECTING DERMOCYSTIDIUM MARINUM, WITH SUGGESTED MODIFICATIONS AND PRECAUTIONS

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ABSTRACT

Tissues from Gulf of Mexico oysters cultured with mycostatin (nystatin) and chloromycetin (chloramphenicol) often showed more and larger cells of the parasitic fungus Dermocystidium marinum than did tissue cultured with penicillin and dihydrostreptomycin. The difference was greater in spring than in October. The thioglycollate culture method for diagnosing D. marinum is reviewed and improvements are suggested. Mycostatin and chloromycetin, 200 units and 200 micrograms, respectively, per ml of medium, are recommended instead of penicillin and dihydrostreptomycin.

INTRODUCTION

The thioglycollate culture method for detecting Dermocystidium marinum in oysters was developed about 10 years ago (Ray, 1952a and 1952b). Although the method is not complicated, I believe that occasionally the parasite is either overlooked or found in less than actual abundance because of faulty technique. This belief is due to reports received during the summer of 1961 (Ray, 1966) that D. marinum was almost impossible to detect in some Gulf of Mexico areas, where previously it had been prevalent.

My first reaction to these reports was that D. marinum had "disappeared" because it seemed unlikely that errors in technique could be grave enough to allow moderate and heavy infections to escape detection with such consistency. During the summer of 1961, however, I encountered an apparent error in technique associated with a reported failure to find D. marinum in the Gulf. When the suspected error was corrected, high levels of infection were found consistently in oysters from the area in question.

My increasing concern regarding the validity of some D. marinum incidence data, new data concerning antibiotics, and several years of additional experience, have prompted me to present modifications

and precautions that should increase the reliability of the culture method for detecting D. marinum in oysters.

A step-wise synopsis of the procedure is presented below for rapid reference. The steps that contain modifications of the original procedure (Ray, 1952a and 1952b) are indicated by an asterisk.

1. Rehydrate 29.3 grams of Fluid Thioglycollate Medium (Difco, No. 1.0256-02 or Baltimore Biological Laboratory, No. 01-140) in 1 liter of distilled water containing 20 grams of NaCl.

2. Dispense rehydrated medium in 10-ml amounts in culture tubes and autoclave. Store sterile tubes of medium in the dark at room temperature until needed.

*3. Fortify each tube of medium with 200 units of mycostatin (nystatin) and either 200 micrograms of chloromycetin (chloramphenicol) or 500 units of penicillin G and 500 micrograms of dihydrostreptomycin per ml of medium just prior to use.

*4. Plant test tissues (gill, mantle, and/or rectum) in the fortified tubes of medium and incubate in the dark at room temperature for at least one week.

*5. Blot the incubated tissue on absorbent paper toweling, flood it with 2 or 3 drops of diluted Lugol's solution, and then tease tissues into fine bits.

6. Examine stained tissues microscopically at 25X to 100X magnification for brown, green, blue, and blue-black spheres.

CULTURE MEDIUM

The use of thioglycollate media that do not contain dextrose appears to have been the cause of at least one consistent failure to detect D. marinum. More than one laboratory had such a medium on its shelf and had possibly used it to check for D. marinum. Dextrose is one of the constituents of Fluid Thioglycollate Medium that is required for consistent enlargement of D. marinum cells in some oyster tissues (Ray, 1954a).

A Fluid Thioglycollate Medium without dextrose, such as Baltimore Biological Laboratory, No. 01-394, may be used if 5 grams of dextrose and 24.3 grams of dehydrated medium are used to prepare a liter of medium. Except as noted above and in the synopsis, prepare and store the medium according to the instructions on its container.

ANTIBIOTICS

Antibiotics are added to the sterilized tubes of medium to prevent excessive bacterial growth and tissue putrefaction. The enlargement of D. marinum may be inhibited if the cultures become excessively putrid during the early stages of incubation. Furthermore, after prolonged incubation in putrid cultures some tissues may become too badly decomposed for proper examination. Since some antibiotics are readily inactivated at room temperatures and/or by light, these agents should not be added to the medium until shortly before inoculation and the cultures should be incubated in the dark.

Recent studies, which will be elaborated upon in this report, indicated, however, that the culture method may be improved by employing antibiotics other than penicillin G and dihydrostreptomycin. The data suggest that mycostatin (nystatin), a broad-spectrum, antifungal agent and chloromycetin (chloramphenicol) are more suitable than the originally recommended antibiotics as antimicrobial agents. In this paper the abbreviation "P+S" indicates the use of both penicillin (500 units/ml of medium) and dihydrostreptomycin (500 micrograms/ml of medium) in the cultures. The abbreviation "My" indicates either the combination of mycostatin, penicillin, and dihydrostreptomycin or the combination of mycostatin and chloromycetin. In the former combination, used during October, 1961, about 100 units of mycostatin were added per ml of medium; and in the later combination, used during 1962, about 200 units of mycostatin and 500 micrograms of chloromycetin were added per ml of medium.

In general, chloromycetin and the combination of penicillin and dihydrostreptomycin have about the same effect on the enlargement of D. marinum. Occasionally in tissues from the same oyster, chloromycetin will inhibit enlargement of the parasite to a greater extent than the P+S combination; and in some cases the opposite effect occurs. The inhibition of enlargement by either chloromycetin or the P+S combination is "spared" in the presence of mycostatin. The influence of mycostatin on enlargement of D. marinum is shown in Figure 2. The author prefers the use of chloromycetin since it appears to have a slightly broader spectrum for the bacterial flora associated with oyster tissues than the P+S combination.

The data presented in the previous paper in this volume (Ray, 1966) show a slightly greater incidence and greater weighted incidence of D. marinum in My cultures than in P+S cultures for several samples. The differences, especially in weighted incidence, were greater in the spring samples than in the October samples. In some spring samples the weighted incidence value for My cultures was about double that of P+S cultures. The same data (Ray, 1966) are used to prepare Fig. 1 and Table 1, but the data are presented on the basis of individual oysters to give an idea of how the infection intensity ratings and cell counts differed with the two treatments. These methods of presenting the data also show generally that more D. marinum cells were observed in My cultures than in P+S cultures.

In many cultures, especially during the spring, numerous cells were observed at 25X to 30X magnification in My-treated tissues, whereas either no cells or very few cells were observed in P+S-treated tissues. Subsequent examination of the P+S-treated tissues at increased magnifications (100X to 430X), however, frequently revealed very small D. marinum cells that were visible only after careful search. These observations led me to make comparative cell counts of My- and P+S-treated tissues.

The D. marinum cells were actually counted in all tissue preparations that were estimated to have less than 800 cells visible at 100X magnification. This concentration of cells in a piece of oyster tissue (about 5 x 10 mm) is about the upper limit of my estimation of a "light to moderate" infection.

The data obtained from the comparative cell counts were used to compute the cell-count ratios: $\frac{\text{No. cells in My culture}}{\text{No. cells in P+S culture}}$, which are presented in Fig. 1

An examination of Fig. 1 reveals generally that more D. marinum cells were observed per unit of tissue in My cultures than in P+S cultures. The ratios exceeded 1 for about 80 per cent of the October cell counts and for about 95 per cent of the spring counts. Furthermore, it is evident that the disparity in the cell counts for the two methods is considerably greater in the spring samples than in the October samples. For example, about 75 per cent of the spring cell-count ratios exceed 5, whereas only approximately 25 per cent of the October cell-count ratios exceed this value. The maximum ratios were 20 to 30 for the October oysters in contrast to maximum ratios of 100 to 300 for the srping oysters. In view of this marked seasonal difference, mycostatin very likely enhances the enlargement of the proliferating stage(s)

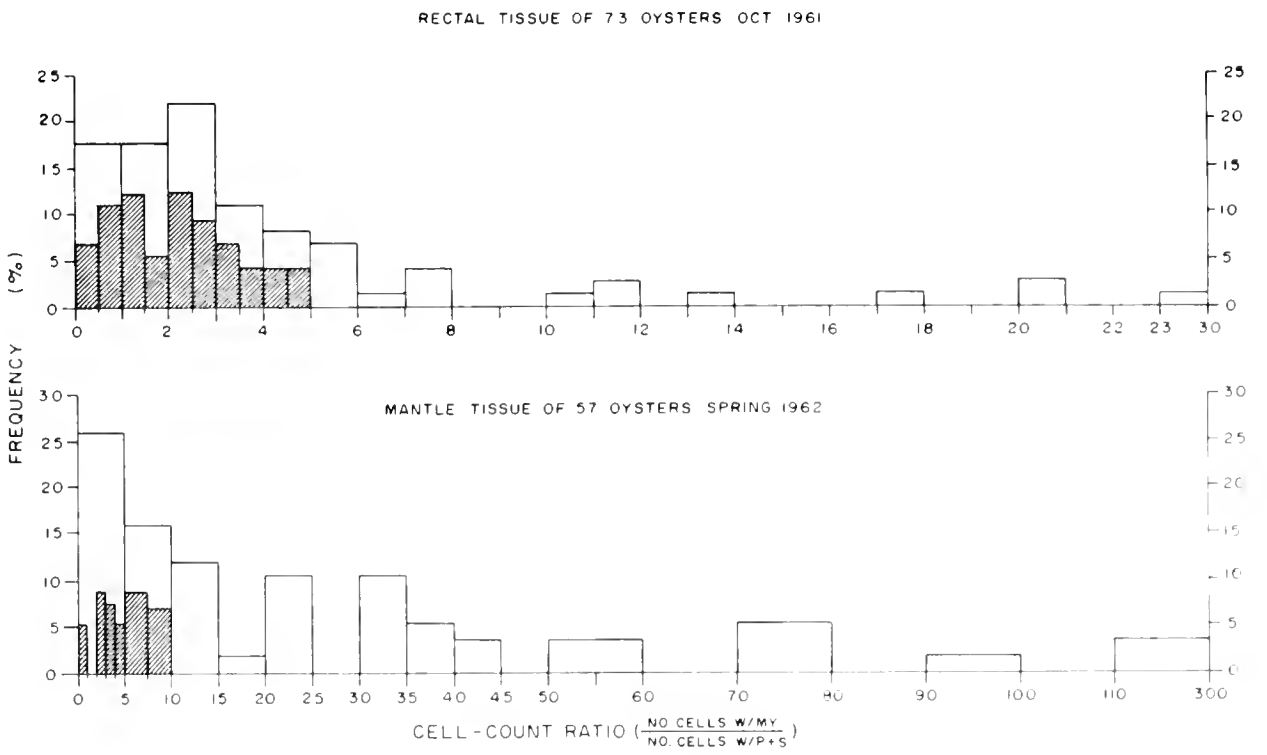


Fig. 1. Comparison of D. marinum cell counts in tissues cultured with mycostatin (My) and without mycostatin (P+S). The crosshatched bars represent a finer breakdown of the values indicated by the open bars, to permit more accurate evaluation of cell-count ratios close to a value of 1. See footnotes 1 and 3, Table 1, for details regarding treatment of rectal and mantle tissues, respectively.

Table 1. Summary of data on the effect of mycostatin on the intensity rating (IR) of D. marinum infections

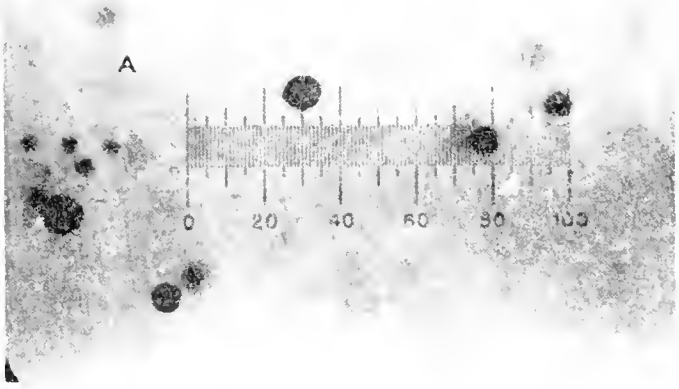
Source of oysters	Total no. pos. w/or w/out My	No. times IR w/ P+S > My	No. times IR w/ My > P+S	No. pos. w/ P+S - neg. w/My	No. pos. w/ My- neg. w/ P+S
Florida & Louisiana Oct. 1961[1] (259 Oysters)	170 (66%)[2]	8 (4.7%)	77 (45.3%)	2 (1.2%)	14 (8.2%)
Louisiana & Texas March, April & May, 1962[3] (248 Oysters)	163 (63%)[2]	4 (2.4%)	137 (84.0%)	0	9 (5.5%)

[1] Approximately one-half of the rectal tissue was cultured in medium containing penicillin and dihydrostreptomycin (P+S) and the other half was cultured in medium containing mycostatin, penicillin, and dihydrostreptomycin (My).

[2] Percentage based on total number of oysters sampled. All other percentages based on total number of positive cases.

[3] A piece of mantle (approx. 5 x 10 mm) was cultured in medium containing P+S and another piece of mantle was cultured in medium containing mycostatin and chloromycetin (My).

Fig. 2. Comparison of effects of mycostatin on the enlargement of D. marinum in thioglycollate culture. A. and E. Penicillin (500 units/ml) and dihydrostreptomycin (500 micrograms/ml). B. Mycostatin (200 units/ml), penicillin and dihydrostreptomycin. C. Chloromycetin (200 micrograms/ml). D and F. Mycostatin and chloromycetin. A-D. Mantle tissue from same oyster; photographed at 100X magnification, value for each scale unit is 6.6 microns. E-F. Gill tissue from same oyster; photographed at 35X magnification, value for each scale unit is 21.5 microns. All preparations stained with diluted Lugol's solution.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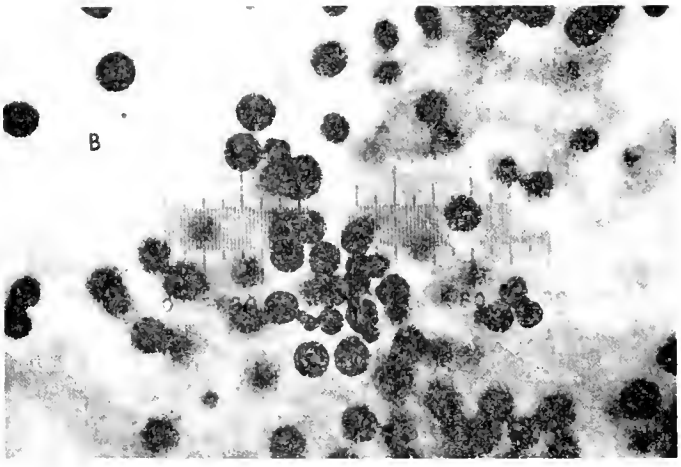
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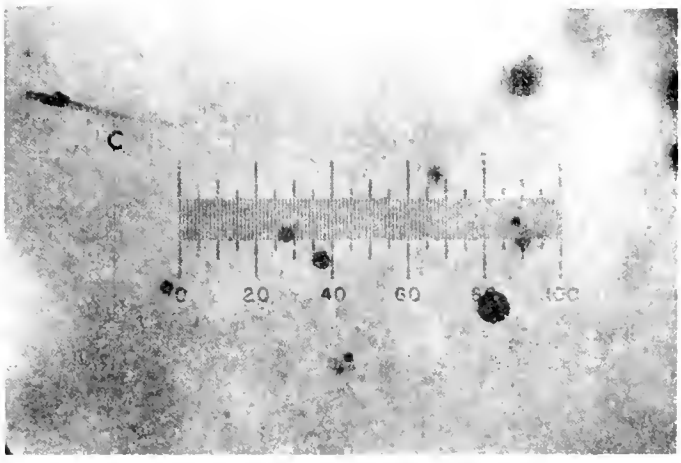
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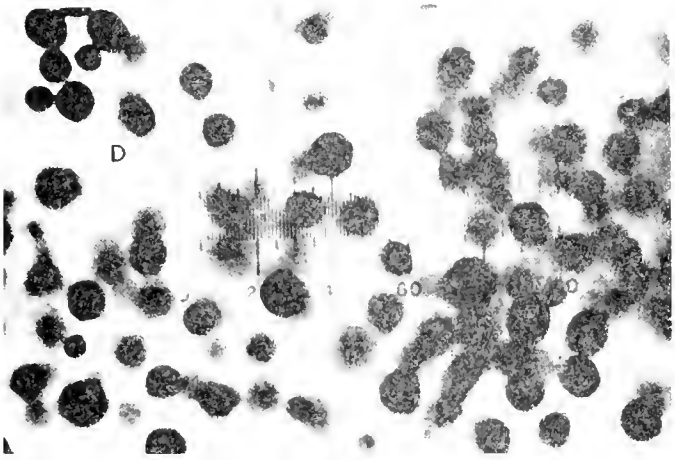
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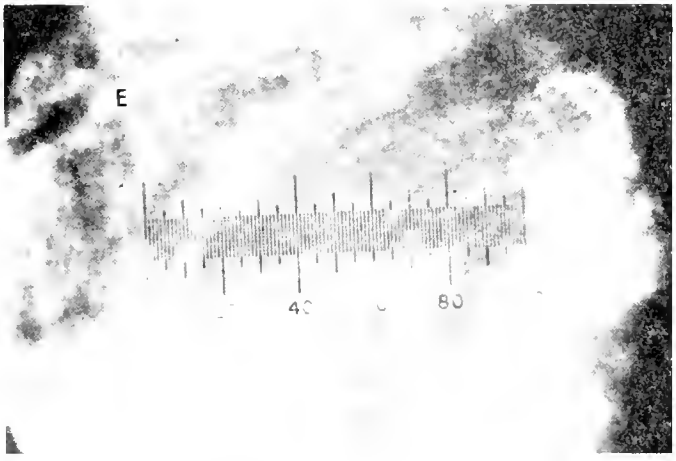
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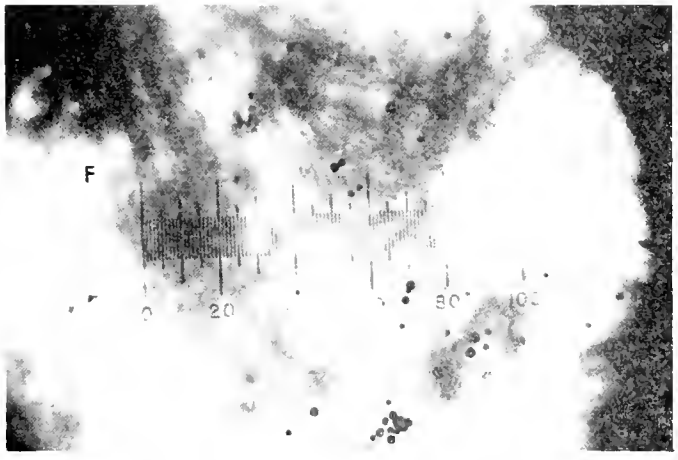
F

of D. marinum to a greater extent than the non-proliferating stage(s). Possibly the stage(s) affected by mycostatin is either the "amoeboid" stage of Mackin (1952) or some stage(s) that has not been associated with this parasite.

Before discussing the data presented in Table 1, some comments should be made regarding D. marinum infection intensity rating (IR). The IR system (Ray, 1954a, and 1954b; Mackin, 1962) employs six categories, ranging from "very light" through "heavy," to indicate the intensity of D. marinum infections. Infection intensities estimated to fall in the upper and lower portions of a category were rated as plus (+) and minus (-), respectively. These subratings, however, were treated as one category for data analyses. Each category is arbitrarily assigned a numerical value ranging from 0.5 for "very light" infections to 5.0 for "heavy" infections. These values are used in determining the weighted incidence (Mackin, 1962). Since intensity rating is a subjective procedure and since the D. marinum cells often average a larger size in My cultures than in P+S cultures, there may be a tendency to rate an infection higher for tissues with large cells than for tissues with a similar concentration of small cells. Care was taken to consider the number of cells rather than total mass of D. marinum cells in estimating intensity. As an additional precaution in rating infection intensities, the entire tissue preparation was systematically searched at 100X magnification, which is the highest magnification that is practical for routine detection of D. marinum, to reduce the chances of overlooking small cells.

Table 1 shows the frequency with which the IR of D. marinum infections in either My or P+S cultures exceeded the other. In the October samples the IR differed with the two treatments in 85 (50 per cent) of the oysters showing D. marinum infection; and in 78 of these cases (about 90 per cent) the IR for the My cultures exceeded the IR for P+S cultures. Furthermore, as in the case of cell-count ratios (Fig. 1), the difference between the two types of treatment was more pronounced in the spring samples than in the October samples. In the spring samples the IR of 141 (about 85 per cent) of the positive oysters differed with the two treatments; and in 137 of these cases (about 97 per cent) the IR was greater for My cultures than for P+S cultures. The cell-count data (Fig. 1) indicate that increased intensity ratings associated with the use of mycostatin are due, at least in part, to an increase in the number of cells becoming visible, and is not due simply to a further enlargement of the visible cells.

The cause for the disparity between My and P+S cultures is not known. Initially, I assumed that inhibition of fungal growths by mycostatin accounted for this difference, since many more P+S cultures

than My cultures were contaminated with molds. However, subsequent examination of many My and P+S cultures that showed no macroscopic evidence of mold growth after prolonged incubation did not substantiate this assumption. In many cases the tissues in My cultures showed either a greater IR or a higher cell-count than tissues in P+S cultures.

An edematous condition of tissues in cultures containing mycostatin is quite noticeable when compared with tissues cultured in its absence. The swelling phenomenon may have either a direct effect by influencing the permeability of the D. marinum cell wall or an indirect effect by distributing the nutrients more uniformly through the tissues (especially mantle and rectum). Frequently, I have noted that tissues from My cultures tease and stain more readily than tissues from P+S cultues; this difference is atrributed to the swelling phenomenon.

Although I prefer to use 200 units of mycostatin[1] and 200 micrograms of chloromycetin[2] per ml of medium for routine diagnostic work, the use of penicillin and dihydrostreptomycin instead of chloromycetin is satisfactory. Since both mycostatin and chloromycetin are practically insoluble in water, one must be sure that these antibiotics are properly suspended while they are being dispensed. To avoid great variations in the concentration of antibiotics, it is advisable to fortify only two or three tubes at a time because these materials, especially chloromycetin, settle rapidly.

SELECTION OF TISSUES

The rectum was originally recommended for routine survey work because this tissue often appeared to contain more parasites than three other tissues (heart, gill, and mantle) that were tested extensively. Biologists in the Cheasapeake Bay area usually culture three

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[1] Mycostatin, 500,000-unit vial (List 5915); E. R. Squibb & Sons.

[2] Chloromycetin, 1-gram vial (S.V.65); Parke Davis & Co. This recommended concentration of chloromycetin, only 40 per cent of that used in the studies reported herein, is based on data obtained from extensive studies conducted since the submission of this manuscript. Enlargement of D. marinum cells is occasionally inhibited by chloromycetin levels of 250 to 500 micrograms per ml of medium. Inhibition occurs much more frequently with chloromycetin alone than in the presence of mycostatin.

tissues (rectum, gill, and mantle) from each oyster. These three tissues are examined as a composite preparation and probably give a better picture of the overall infection of each oyster than using a single tissue. D. marinum is more easily observed in gill tissue than in any other tissue except heart. Furthermore, in recent studies with Virginia oysters, extremely light infections, presumed to be newly established, have been found occasionally in gill tissue but not in either mantle or rectal tissue. This observation indicates that D. marinum may on occasions, at least, initially invade oysters by way of the gills rather than through the digestive epithelium as suggested by histological studies (Mackin, 1951). In well established infections mantle and rectal tissues often appear to contain more parasites than gill tissues.

Rectal tissue has a decided disadvantage when the oysters have well developed gonads. The presence of large amounts of gonadal material makes the tissue difficult to stain with iodine; consequently, the parasites may be overlooked in light infections. Furthermore, other organisms and artifacts found in rectal preparations may confuse inexperienced users of this technique. The inclusion of appreciable amounts of adductor muscle in the preparation of rectal tissue makes intensity estimating more difficult since this tissue usually contains fewer cells than the rectum. Also, excess adductor muscle increases the difficulty of compressing the tissue uniformly.

Recent comparative studies (Ray, 1966) with regard to relative infection intensities in mantle and rectal tissues showed a slight but probably insignificant higher incidence and weighted incidence in rectum than in mantle. Mantle tissue was taken from the most anterior portion of this organ (mantle "A") just lateral to the palps, because earlier studies often showed noticeably more parasites in mantle tissue near the palps than in that near the adductor muscle.

I now favor using "mantle A" instead of rectum for the reasons stated. Also, mantle tissue is easier to dissert out. The rectum is often damaged in opening the oyster. The entire rectum, that portion of intestine passing over the adductor muscle, should be used except in very large oysters; and in small oysters the expanded portion of the intestine that passes over the pericardium should be included with rectum. In culturing gill and mantle tissues, pieces about 5 x 10 mm should be used.

INCUBATION AND EXAMINATION OF TISSUES

Previously, I recommended incubating thioglycollate cultures for at least 48 to 72 hours at room temperature. However, various workers, including myself, have noted occasionally that D. marinum cells do not reach maximum enlargement within this minimum period. Therefore, I suggest incubating the cultures for at least one week. Inasmuch as my cultures and probably those of most workers usually are at least a week old when they are examined, I do not believe that many infections are overlooked because of insufficient incubation.

Tissues usually remain suitable for examination after several weeks and even months of incubation unless badly decomposed when first cultured. Occasionally, tissues from live oysters disintegrate after several weeks' incubation in cultures that contain heavy growths of certain molds, yeasts and bacteria. Tissues tend to remain suitable for examination for much longer periods when mycostatin and chloromycetin are used instead of penicillin and dihydrostreptomycin. Occasionally tissues from badly decomposed gapers disintegrate more readily in My cultures than in P+S cultures, despite the absence in My cultures of the marked microbial growth and putrefaction exhibited by the companion (paired) P+S cultures. Such tissue disintegration in the absence of culture putrefaction is tentatively attributed to the swelling caused by mycostatin. When large numbers of cultures accumulate and I anticipate long delays in examining them, tubes that have been incubated two or three weeks are placed in the refrigerator. This prolongs the time the tissues will remain suitable for examination. Nevertheless, best procedure is to examine cultures as soon as conveniently possible after one week of incubation.

The possibility of overlooking light D. marinum infections may be reduced by exercising certain precautions in preparing the cultured tissues for microscopic examination. Unless tissues show signs of disintegration, place a piece of absorbent paper toweling on a slide to receive the tissue to be examined; do not remove the toweling until the excess medium is absorbed. This procedure makes it easier to stain tissues and D. marinum properly, and reduces the need for restaining preparations. Tissues from "watery" or poor oysters usually stain readily and require little teasing for penetration of the iodine solution. On the other hand, tissues from "fat" oysters (containing either much glycogen or much gonadal material) should be shredded into very small pieces to insure proper staining. Such tissues may be very difficult to stain and often the brown color fades rapidly.

For easiest examination, all or most of the preparation should be stained light brown. Wait about five minutes before placing a cover slip over the preparation. Poorly stained tissues may be restained conveniently if the preparation has not been covered. Before tissues are restained, remove excess fluid with absorbent paper toweling. Confirm negative findings associated with poorly stained tissues by restaining the preparations, but avoid over-staining. Over-stained tissues (extremely dark brown) are difficult to examine.

For microscopic examination of tissue preparations many workers use low magnifications (25X to 35X). Although such magnifications are adequate for detecting most D. marinum infections, negative preparations should be examined, at least cursorily, at 100X magnification for unusually small cells.

GENERAL COMMENTS

In my opinion some workers do not adequately record details of procedural changes that they may have made in the culture method. Information that indicates when and in what manner the routine procedures had been altered should be recorded for future reference. Furthermore, I believe it will be helpful in comparing the data obtained by various workers if certain details of procedure are presented in the materials and methods section of publications that contain D. marinum data obtained by the culture method. Such details should include the kinds of antibiotics employed, the tissues cultured, and other details of technique that might be significantly different from those generally used by workers in the field.

ACKNOWLEDGMENTS

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RADIATION PASTEURIZATION OF OYSTERS

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ABSTRACT

Gamma (^{60}Co) irradiation of pint cans of fresh oyster meats (0.2 Mrad) extended by several days the time that acceptable quality was retained under conditions simulating commercial handling and storage, as shown by organoleptic tests, trimethylamine content, bacterial counts, pH, and color.

Many problems have plagued oyster packers during the past five years. One results from the rejection by state health departments of oysters in transit from one growing and packing area to another, which are held for extra days to be repacked before final shipment to the ultimate distributor and consumer.

Although the excessive bacterial counts in such oysters might not have posed an actual health hazard, they were above the counts allowed in the standards. Most rejected samples could have been retained as acceptable if some simple method had been available to reduce the original bacterial counts in the oysters, thus giving them a few days' extension of quality.

Exploratory investigations on gamma radiation of fresh Gulf oysters suggest that such treatment of oysters can provide advantages for the fisherman, processor, distributor, and consumer. If such a method of processing were developed to successful commercial application, fresh oysters could be made available to consumers who presently are able to obtain only the canned or frozen product. Market prices of oysters, presently subject to fluctuations due to overabundances or scarcity, would tend to be stabilized.

Low dose gamma irradiation of oysters with Cobalt 60 resulted in an extension of their storage life of five or more days beyond that of non-irradiated oysters from the same batch. When the oysters were shucked under supervision and irradiated within six hours the storage period was extended for several days longer.

The oysters used in these experiments were treated as follows: freshly shucked oysters, which had been brought in from the oyster beds the previous night, were washed in running tap water (50F) for two minutes, and allowed to drain for five minutes. All draining was done on FDA-approved stainless steel skimmers, which had an area of not less than 300 square inches per gallon (201 cm^2 per 6) of oysters, drained, and which had perforations of at least 0.25 inch (6.35 mm) in diameter located not more than 1.25 inches (31.75 mm) apart. The oysters were distributed evenly over the draining surface of the skimmer but were not otherwise agitated during the draining period. After the oysters were washed and drained, they were packed into pint cans and stored in crushed ice. Within six hours, 24 pint cans were subjected to gamma radiation (0.2 Mrad) in the Nuclear Science Center on the Louisiana State University campus. After irradiation, they were stored in crushed ice, along with an equal number of non-irradiated pints of oysters which were employed as controls. Samples were removed at intervals for bacterial counts and trimethylamine, pH, and organoleptic tests.

Results are presented in the following tables.

Similar results were obtained with other batches of oysters treated and tested in the same manner.

Table 1. Organoleptic scores of irradiated (0.2 Mrad) and non-irradiated ice-stored oysters

Sample treatment	Score after listed storage period			
	Initial	7 days	14 days	21 days
Non-irradiated	9.5	6.0	3.8	Spoiled
Irradiated	9.5	8.0	6.5	5.3

Values are averages for 25 participants on taste panel for the attributes of odor, appearance, flavor, and texture.

Code of Scores:

(10) No change from fresh product of highest quality

(8) First noticeable slight change in attributes

(6) Moderate degree of changed attribute: increased in intensity and occurrence from score of 8

(4) Definite or strong degree of changed attribute

(2) Extreme degree of changed attribute

Table 2. Chemical tests on shucked oysters stored in pint cans packed in ice (comparison of non-irradiated, with irradiated at 0.2 Mrad)

Storage time	Initial		1 week		2 weeks		3 weeks	
Conditions / Quality test	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated
*Trimethylamine mg N/100g	0.68	0.74	1.18	1.15	1.36	1.30	Sample was putrid and inedible	1.37
Bacterial count x10^6	0.038	0.004	0.426	0.029	1.92	0.38		1.10
* pH (oyster meat)	6.14	6.12	5.85	5.96	5.63	5.80		5.73
Discoloration	None	None	Slight	None	Black areas	Slight		Slight

* Based on original drained weight

DIFFERENTIATION OF EFFECTS OF TWO PESTICIDES UPON UROSALPINX CINEREA SAY FROM THE EASTERN SHORE OF VIRGINIA

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ABSTRACT

Adult Urosalpinx were exposed to various combinations of two pesticides ("Sevin", a methyl carbamate, and "Polystream", a mixture of chlorinated benzenes) recommended for oyster predator control by the Milford Biological Laboratory of the U. S. Fish and Wildlife Service. Concentrations used were within the recommended range, and the field procedure suggested was modified by us for application in laboratory trays. Under controlled laboratory conditions, Polystream used alone killed half the animals within a period of 5.5 to 6.8 days. The use of Sevin, which is highly toxic in crustaceans, is therefore questionable.

INTRODUCTION

Several recent publications (Loosanoff, MacKenzie and Davis, 1960; Loosanoff, MacKenzie and Shearer, 1960; Loosanoff, 1960, and MacKenzie and Gnewuch, 1962) have reported the effectiveness of toxic chemicals on oyster predators, particularly Urosalpinx. The chemical agents recommended by these writers are "Polystream" and "Sevin." The former is an aggregate of chlorinated benzenes produced by the Hooker Chemical Company; "Sevin" is the trademark of an established insecticide manufactured by the Union Carbide Corporation.

It has been suggested that these two agents be mixed with sand and broadcast over oyster grounds, and considerable success has been reported (Davis et al., 1961) with this procedure in field tests in Long Island Sound. In light of this it seemed necessary to obtain additional basic information on these toxic chemicals and their possible effects upon the marine habitat.

Two immediate questions were proposed. First, we wanted to know something about the specific effects of the recommended dosage of the two agents upon the large, Eastern Shore Urosalpinx. For instance, does the mixture kill them directly, or does it simply cause

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[1]Contribution 163 of The Virginia Institute of Marine Science

them to swell so that they are easy prey for some roving larger animal which is itself unaffected by the poison? The second question grew out of the report (Loosanoff et al., 1959) that "Sevin" killed crabs. Since we were considering using "Sevin" in an area that supports a blue crab fishery, it seemed vitally necessary to establish the relative effectiveness of the Polystream-sand treatment with and without "Sevin."

MATERIALS AND METHODS

All experimental animals were adult Urosalpinx cinerea Say of the large variety, collected near Wachapreague, Virginia, during the summer of 1962. They were maintained in running seawater aquaria or in a recirculating aquarium until 23 January 1963. Then they were transported to the Virginia Institute of Marine Science at Gloucester Point, Virginia, where they were placed in running York River water aquaria at ambient temperature and salinity for several days or longer. Three animal samples were used in these experiments. For two months prior to the experiment, the first group (Run A) was supplied with food and maintained at a controlled temperature of ca. 20 C. The two groups of animals used in Runs B and C were maintained without food and at incoming salinity and temperature prior to the experiments.

Seven enamel trays served as the experimental containers, each containing 50 animals. Treatments were applied to each of the trays as follows:

Tray Number	Treatment
1,5	Polystream-Sevin
2,6	Polystream
3,7	Sevin
4	Control

The concentrations of Polystream and Sevin were within or near the range of treatment suggested by the United States Fish and Wildlife Service Biological Laboratory at Milford, Connecticut (Table 1). In Runs A and C concentrations of chemicals were computed assuming uniform coverage over the whole tray bottom. In Run B approximately 2/3 of the bottom of each tray was covered and concentrations were based upon this area.

After the animals were placed in the trays, the treated and untreated sand was spread over the tray bottoms.

Table 1. Concentration of chemicals

Run	Polystream	Sevin
A	3-4 $\mu l/cm^2$	100 $\mu g/cm^2$
B	7-9 $\mu l/cm^2$	200-250 $\mu g/cm^2$
C	3-4 $\mu l/cm^2$	100 $\mu g/cm^2$

Several observations were made the first day, with daily observations throughout each run. Run A was terminated at 380 hours, Run B at 168 hours, and Run C at 143 hours.

Each observation included salinity, temperature, and number of animals dead, retracted, attached or swollen. The criterion used to determine death in a gastropod was the presence of a "rotting" odor. Retracted animals included all animals that were not attached, swollen or dead and had partially or completely withdrawn into their shells. A gastropod was considered attached when the foot was extended and attached to a surface or when the animal was mobile. If the body was distended and white, and the gastropod was unable to withdraw it completely, the animal was counted as swollen. There were many cases in which one animal was included in both of the categories, "swollen" and "attached."

RESULTS

In Run A the first mortality count was made six days after the application of treatments (Table 2). In this run the number of animals killed by the Polystream-Sevin treatment was similar to the number killed by Polystream alone throughout the course of the run (Fig. 1). At the termination of Run A, 16 days, the total mortality of the Polystream-Sevin group was 77% and that of the Polystream group was 78%. In Runs B and C the total mortality was greater for those animals treated with Polystream-Sevin than for those treated with Polystream alone. There was a total mortality of 11% observed among the animals treated with Sevin alone in Run B.

The observed LD_{50} (time required to kill half the animals in a sample) of the Polystream-Sevin and Polystream groups in Run B, 3.8

and 5.4 days respectively, was less than the LD_{50}'s in Runs A and C (Fig. 1). There was a difference of 1-2 days in the LD_{50}'s of the Polystream-Sevin group and the Polystream group in Runs B and C, while the LD_{50}'s of these two groups in Run A were almost identical.

In both the Polystream-Sevin and Polystream treatments the percentage of animals retracted was high in each of the three runs (Fig. 2). This high incidence of retraction was usually first observed shortly after the application of treatments and continued throughout each run.

Most swelling occurred in the Sevin-treated groups in each of the three runs (Fig. 3). The maximum number of animals was found swollen in the Sevin-treated groups at the end of 6 or 7 hours; swollen gastropods were not found after 2 or 3 days. Swelling was also noted in the Polystream-Sevin and Polystream treatments in each of the runs and was usually still evident in both of these treatments at the termination of each run. There was a higher percentage of swelling found in the Polystream-Sevin treatments than in the Polystream treatments.

The fraction of animals attached in the Polystream-Sevin and Polystream treatments was low throughout each run. In both of these treatments in Run A and in the Polystream-Sevin treatment in Run B, the proportion of animals increased gradually until approximately one third of the surviving animals was attached.

DISCUSSION

General

The conditions under which these experiments were run, as contrasted with those obtaining in most field situations, tended to favor the pesticides against the drills. That is, the flow of dilution water through the trays was rather low compared to the large volumes moving across most natural oyster beds, and contact with the drills by the poisoned sand was maximized by the method of administration and the lack of topographic relief of the tray bottoms. Despite these facts, the treatments described here never resulted in the catastrophic mortality rates reported for field trials by Loosanoff (1962a, 1962b).

The mean terminal kill for all our "Polystream" treatments was only 72.2%; this differs from previous field studies in which 90 to "more than 99%" (Loosanoff, 1962a) were reported as "eliminated."

Table 2. Cumulative mortality

Cumulative Days	Percentage dead											
	Polystream and Sevin			Polystream			Sevin			Controls		
	Run A	Run B	Run C	Run A	Run B	Run C	Run A	Run B	Run C	Run A	Run B	Run C
0	0	0	0	0	0	0	0	0	0	0	0	0
1	-	0	0	-	0	0	-	0	0	-	0	0
2	-	0	0	-	0	2	-	0	0	-	0	0
3	-	18	27	-	17	15	-	6	0	-	0	0
4	-	54	40	-	30	31	-	8	0	-	0	0
5	-	76	59	-	45	41	-	9	0	-	0	0
6	30	85	66	30	58	50	0	10	0	0	2	0
7	56	88	-	52	74	-	0	11	-	0	2	-
8	67	-	-	66	-	-	0	-	-	0	-	-
9	69	-	-	70	-	-	0	-	-	0	-	-
10	72	-	-	74	-	-	0	-	-	0	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	77	-	-	77	-	-	2	-	-	0	-	-
14	77	-	-	77	-	-	2	-	-	0	-	-
15	77	-	-	77	-	-	2	-	-	0	-	-
16	77	-	-	78	-	-	2	-	-	0	-	-

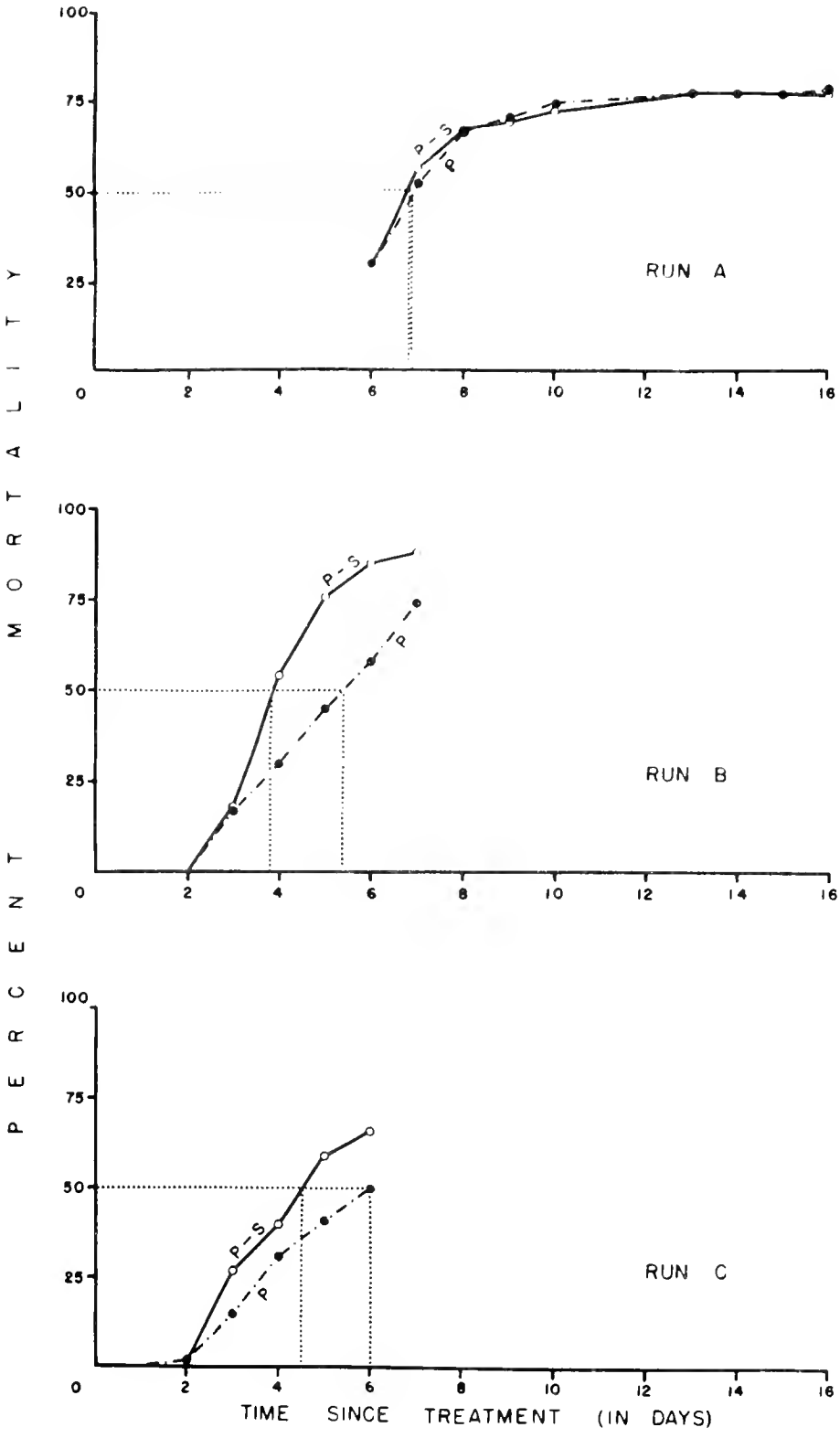

Figure 1

Cumulative mortality of *Urosalpinx cinerea* treated with Polystream and Polystream - Sevin

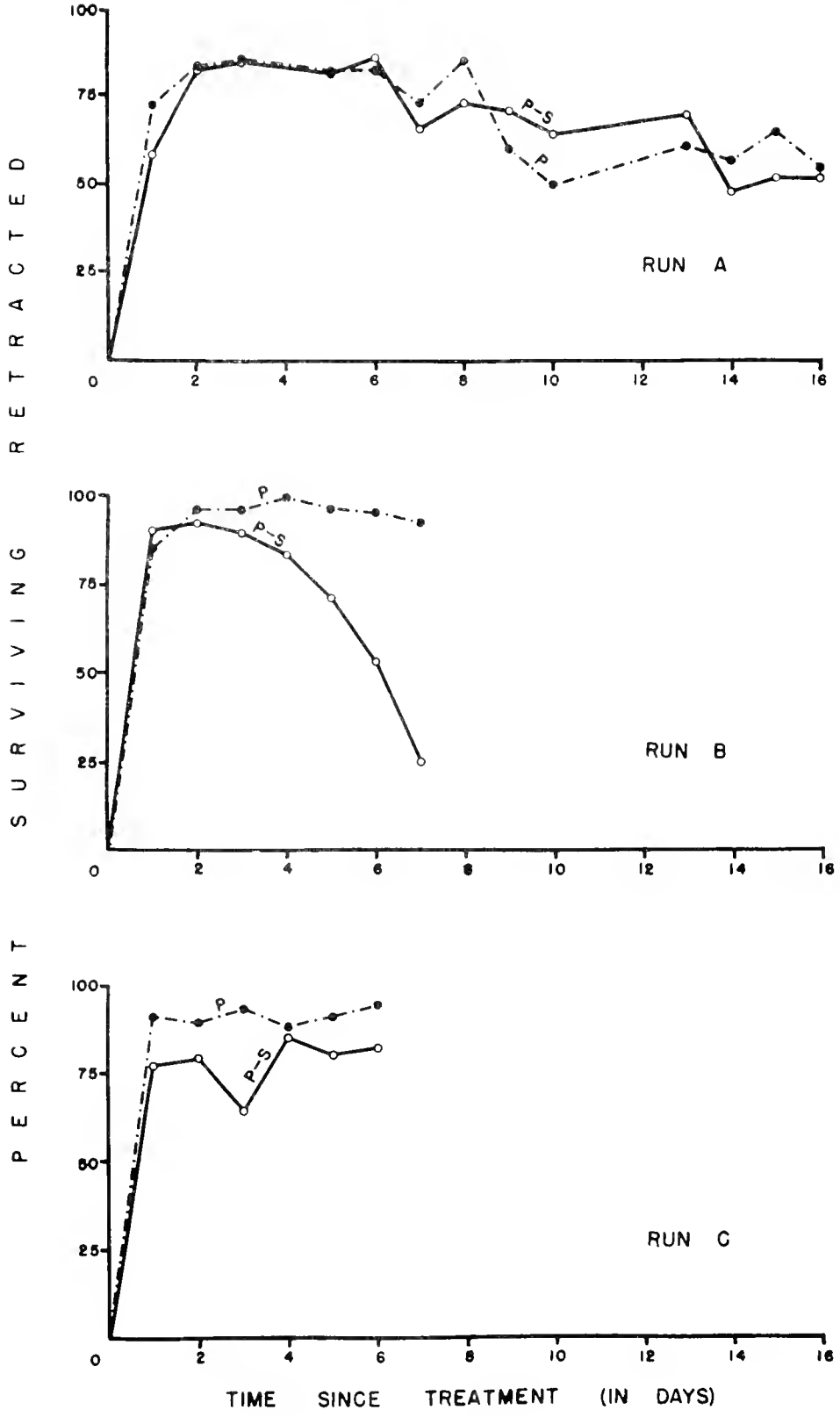

Figure 2

Retraction induced by various treatments

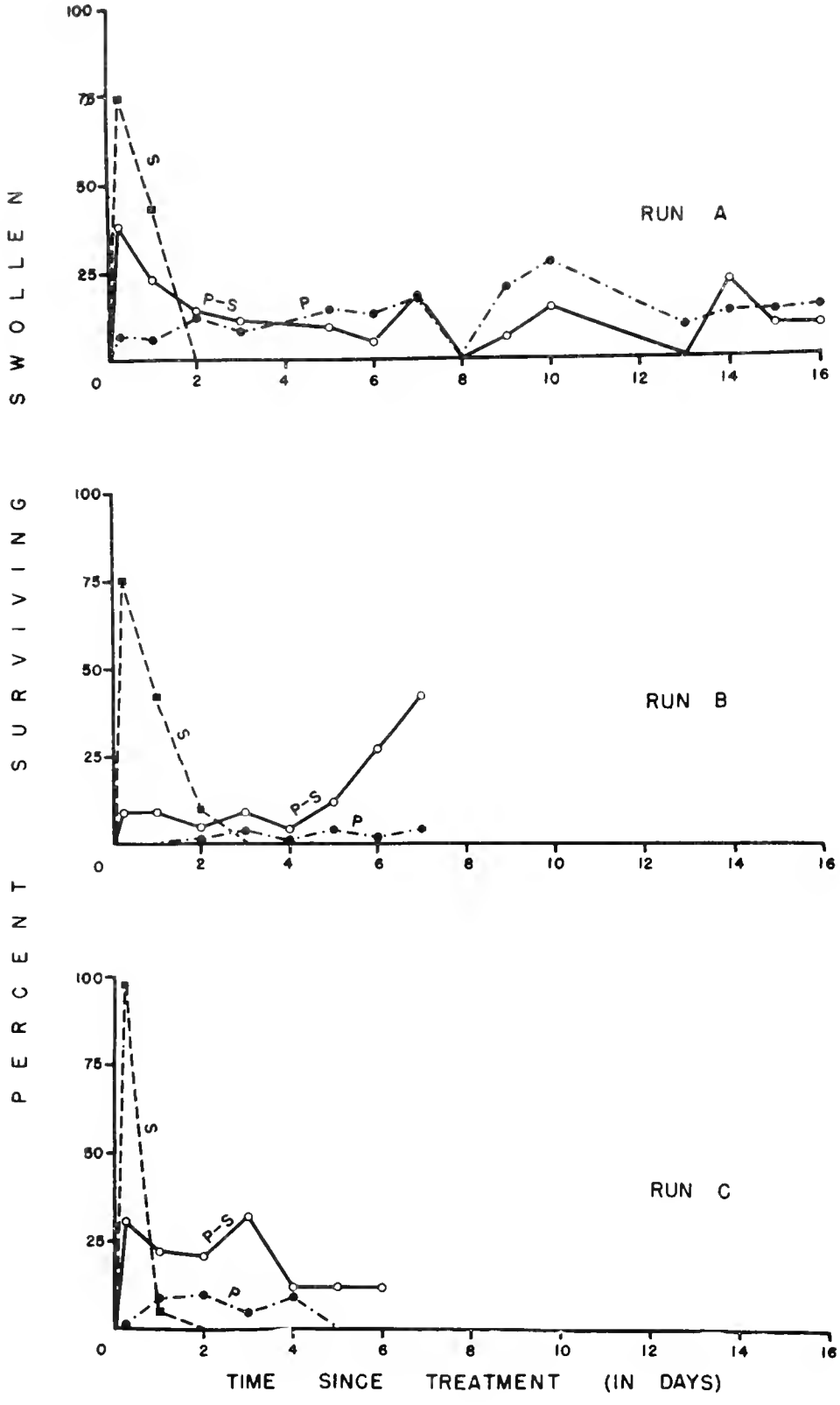

Figure 3

Swelling induced by various treatments

The differences in the mortality rates shown amongst the three runs of this experiment are not fully understood. However, it is reasonably safe to conclude that they may be the result of interaction between two factors: the condition of the drills and the concentrations of the chemicals.

It will be recalled that the test sample for Run A was conditioned and fed for two months prior to treatment; those for Runs B and C were not. Runs A and C employed the lower concentrations, Run B the higher, by a factor of about 2. On the basis of these facts, the interpretation would proceed as follows: In Run A, animals in good condition were exposed to initial concentrations comparable to those expected in the field application of the Milford Formula. The LD_{50} was obtained in about 7 days, and mortality never reached 80% even by the end of 16 days. In Run B, animals in relatively poor condition were treated with a concentration about double that of Run A. The LD_{50} was obtained in 4 to 5 days, and the maximum mortality of greater than 80% was obtained in 7 days. In Run C, animals in perhaps even poorer condition were treated with the same concentrations used in Run A, and the LD_{50} was obtained in about 4.5 to 6 days. This interpretation of our data leads to a tentative recommendation for field workers: If Polystream is to be used on an oyster bed, it might best be administered in the early spring when the drills are just emerging from their winter "hibernation" and are presumably weakened. Another investigation (Wood, unpublished) has shown that Urosalpinx is less resistant to osmotic stress in the spring than in the fall.

Polystream-Sevin or Polystream Alone?

Using the chi-square test we have determined that in Run A of our experiment it was not possible to distinguish the effects of the two treatments; however, in Runs B and C the Polystream-Sevin mixture produced slightly greater cumulative mortality on the following days:

Run B: Days 4 through 7 ($P < 0.01$)

Run C: Days 5 and 6 ($P < 0.01$)

Several investigators have reported (Carriker and Blake, 1959; Loosanoff et al., 1959) the effect of Sevin in causing drills to swell; it has been claimed further (Davis et al., 1961) that this swelling renders the gastropods easy prey to other species such as fish and sea stars. Since a primary object of most pesticides is to kill only the selected pest, and that as quickly as possible, it is, in our

opinion, a poor pesticide whose effectiveness depends upon the presence of an unaffected second party. We have also been unable to find many reports of direct observations of predation upon disabled and swollen gastropods. To the extent that this question applies to the waters of the Eastern Shore of Virginia, it would appear that the chief candidate for the job of cleaning up disabled Urosalpinx would be the blue crab, and the possibility exists that this organism would itself be rendered inoperative by the inclusion of Sevin in the treatment, at least in the early days.

Therefore it is our conclusion that in light of the failure of our experiments to indicate the absolute necessity of Sevin in this treatment, the lack of such evidence from other quarters, and finally the possibility that its inclusion might do harm to another valuable fishery (blue crab), we cannot justify the employment of Sevin in Virginia's Seaside waters. We have shown that Polystream alone kills Urosalpinx directly, in the laboratory; other investigations at the Virginia Institute of Marine Science have disclosed (Haven et al., 1964) that the Polystream-Sevin combination killed up to 85% of the benthic associates of the oyster when applied in field tests near Wachapreague, Virginia. Should it be shown, however, that Polystream does not permanently damage the bottom communities of which commercial oyster grounds are a part, this pesticide might prove to be a valuable adjunct to other modern ostreicultural practices.

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MORTALITY RATES AND THE LIFE SPAN OF THE BAY SCALLOP, *AEQUIPECTEN IRRADIANS*[1]

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ABSTRACT

When high mortalities cut down scallop populations held in the laboratory and in cages in the field, the younger specimens did not exhibit greater survival capacities than the older, even when the latter had reached what has been reported as the age of marked senescence and death. This supports more recent field observations which suggest that senescence symptoms are not pronounced. If senescense is of a lesser severity and mortalities resulting from environmental stresses play a relatively greater role, the observed variations in the life span of the scallop are accounted for more readily.

Belding (1910) stated that relatively few scallops live more than two years. He reported that scallops show a marked period of physical decline starting at the age of 18 months. Marshall (1960) also stated that the natural expectancy of the life of the scallop in the southern New England area is two years or slightly less but he encountered very high mortalities of both young and older scallops throughout the winter and early spring months and did not find the marked period of physical decline in older specimens that was so conspicuous to Belding. Sastry (1961) did not find scallops beyond an age of 19 months in his program of observations on the shoals off Alligator Harbor, Florida. Gutsell (1931), working in the Beaufort, N. C. area, was not able to determine the life span of the scallop since intense fishing mortality obscured natural conditions; however, his observations suggested the possibility of significant survival beyond two years, at least for the populations of his area of study. My field collections also suggest that third year specimens are more common in North Carolina than in New England but that there may be local and even year-class variations in life span in the latter region.

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[1] Contribution from the Graduate School of Oceanography of the University of Rhode Island. This study was supported in part by a grant, No. G-12149, from the National Science Foundation.

Reported herein are observations on the rate of survival of scallops from the Niantic River in Connecticut (Figs. 1 & 2), kept in trays of running sea water at the University of Connecticut Marine Laboratory at Noank, and comparable observations for scallops from Bogue Sound, N. C. (Fig. 3), kept in similar trays at the U. S. Bureau of Commercial Fisheries Laboratory at Beaufort. At both laboratories the running sea water was pumped continuously without filtration from a source similar to that from which the scallops were taken. Though it had been anticipated that the continuous flow of water in and out of the plastic and hard rubber holding trays might so approximate conditions of natural circulation that both growth and mortality would be normal, the growth data from trays showed stunting in the laboratory when compared with length-frequency shifts from field collections. Apparently for a variety of reasons, which may include a scarcity of benthic and epibenthic components important in the food of scallops (Davis and Marshall, 1961), the laboratory setups did not offer an adequate environment. Obviously a December pump shut-down at Noank must be suspected as the cause for heavy mortalities which followed there.

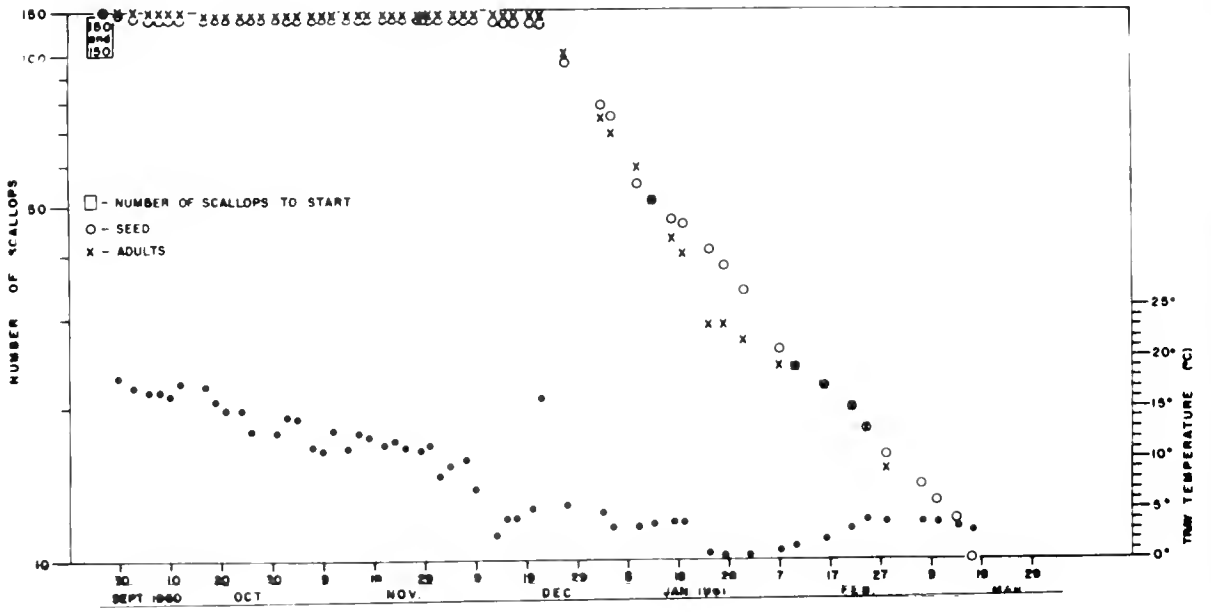

Fig. 1. Scallops surviving in trays of running sea water, University of Connecticut Marine Laboratory, Noank, Conn. 9/27/60—3/17/60.

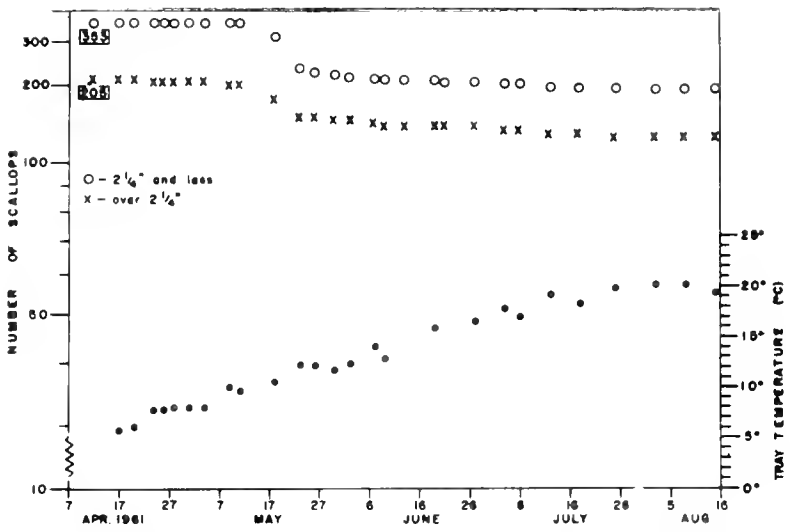

Fig. 2. Scallops surviving in trays of running sea water, University of Connecticut Marine Laboratory, Noank, Conn., 4/12/61—8/14/61.

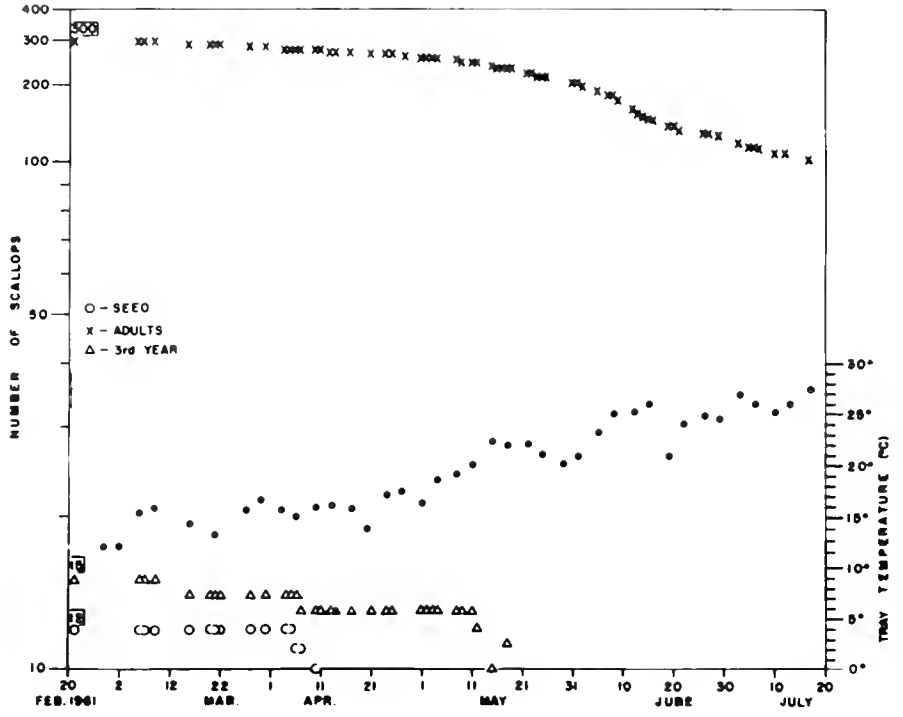

Fig. 3. Scallops surviving in trays of running sea water at the U. S. Bureau of Commercial Fisheries Laboratory, Beaufort, N. C., 2/21/61—7/17/61.

In spite of the stunted growth and eventual high mortalities in the trays, and in the caged scallops (Figs. 4, 5, and 6) previously reported by Marshall (1960), all the holding observations exhibited a similar mortality pattern in which the younger and older scallops seemed to be affected indiscriminately. For the caged specimens in the Niantic River, starfish predation seemed to strike the seed more than the adults, and the seed sample from Beaufort was too small to consider critically, yet the parallel mortality trends of the age groups is quite apparent and is very noticeable in the Noank set-ups. This may aid in the interpretation of the life span. Particularly important is the fact that this parallelism even prevailed during the late spring, the period for which Belding (1910) reported a pronounced mortality in the older scallops linked with a complex he referred to as old-age effects. Though parallel mortalities of younger and older scallops may seem to contradict Belding's emphasis on the dying off in an old-age period, it may be interpreted instead as evidence that mortalities from environmental stress, indiscriminate for age groups, may obscure the evidence of any physiological decline associated with aging. Such a masking effect may be operative in nature as well and is the probable explanation for my failure to discern senescence while following the populations closely in the field through the spring and summer of 1955 (Marshall, 1960).

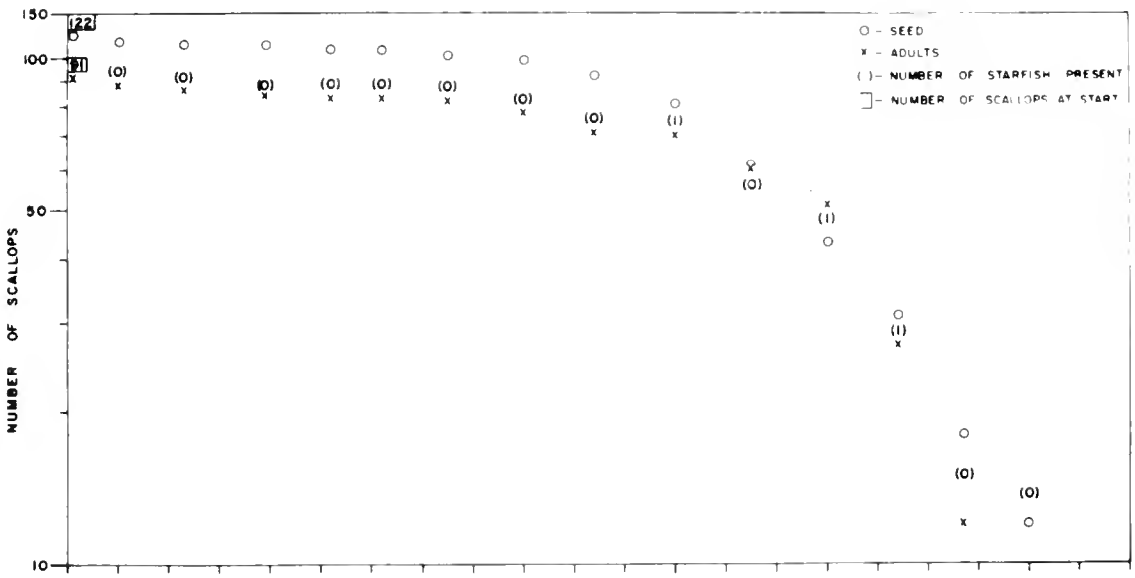

Fig. 4. Scallops surviving in a cage in the flats of the Niantic River, Conn. Average depth about 2.5 feet, 12/8/54—6/15/55.

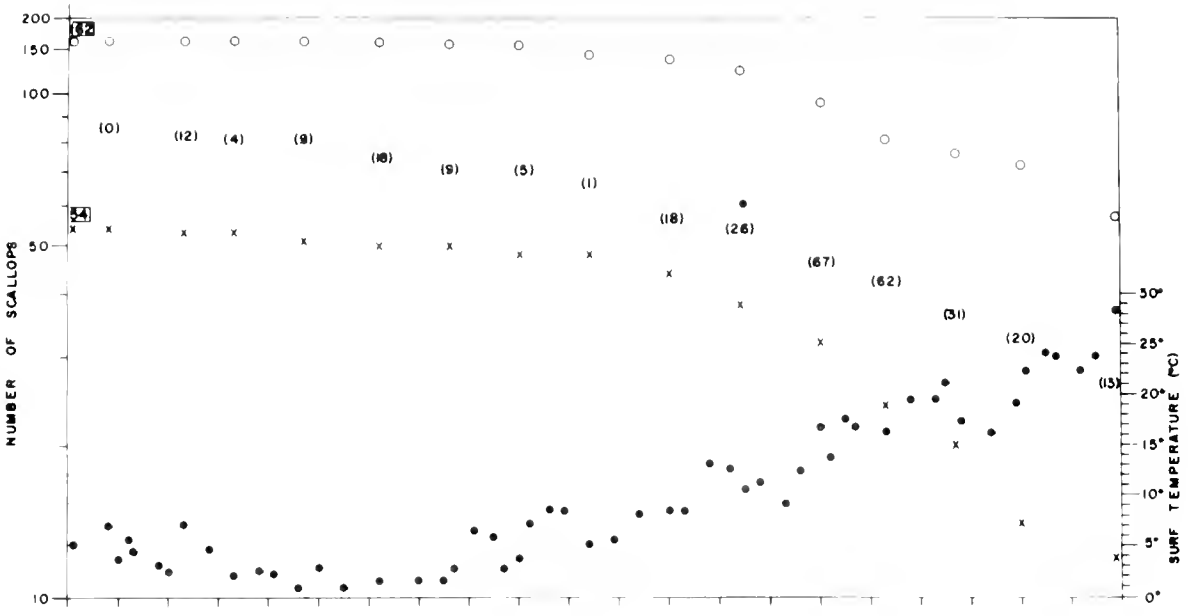

Fig. 5. Scallops surviving in a cage off Saunders Point, Niantic River, Conn. Submerged below mean low water, 12/8/54—7/4/55. For legend see Fig. 4.

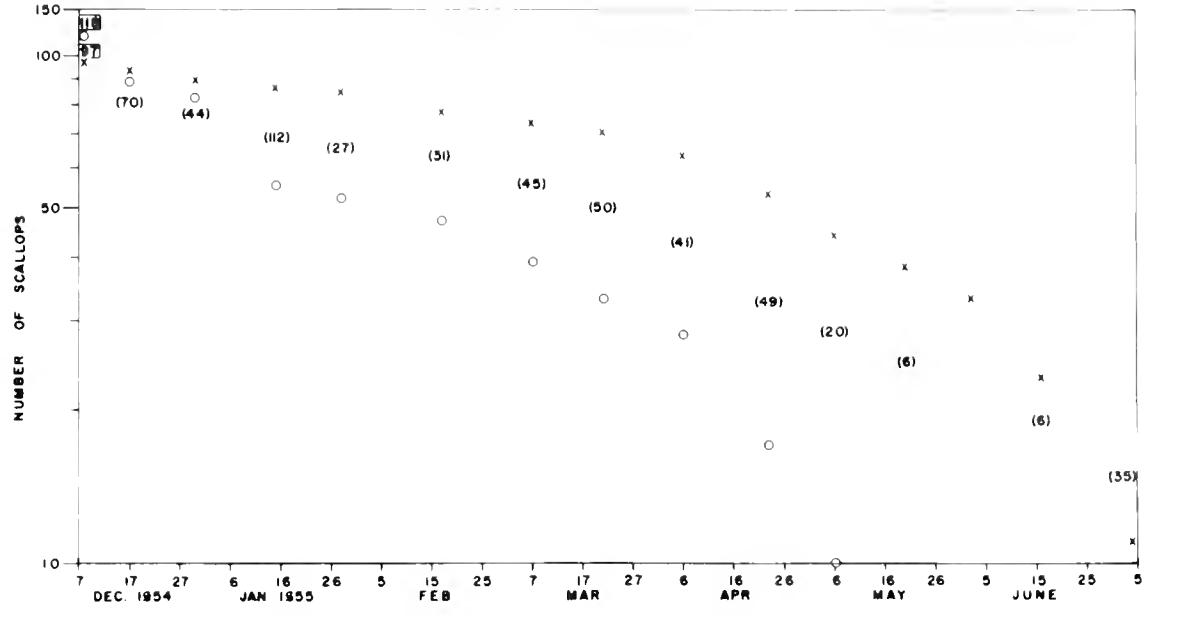

Fig. 6. Scallops surviving in a cage off Saunders Point, Niantic River, Conn. Average depth about 8 feet, 12/8/54—7/4/55. For legend see Fig. 4.

These interpretations fit better the observed discrepancies in life span. Though I continue to point to the possibility of inherent racial and even year-class differences, it is clear that varying environmental mortality rates, on a species that does not exhibit marked terminal age effects, may contribute to the observed differences in life span.

ACKNOWLEDGMENTS

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SEROLOGICAL STUDIES ON THE BAY SCALLOP, AEQUIPECTEN IRRADIANS[1]

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ABSTRACT

Serological relationships between four distant populations of the bay scallop, Aequipecten irradians, and samples of the calico scallop, Aequipecten gibbus, were studied by means of an agar gel diffusion technique and the ring precipitin test. Also the serological reactivity of these scallops with the sea scallop, Placopecten magellanicus, was investigated. Both techniques indicated a close serological similarity among the four populations of bay scallops and between the bay and the calico scallops. The serological relationship between the bay and the calico scallops was closer than the relationship of either to the sea scallop. Agar gel diffusion studies revealed some bands in common in all the samples tested.

INTRODUCTION

The taxonomic position of the members of the bay scallop, Aequipecten irradians, complex has been confused. In a recent review Abbott (1954) recognizes a northern subspecies around southern New England, a middle Atlantic population in the North Carolina area, and a southern group along the Gulf coast. Life history studies on these different subspecies near their centers of distribution show differences which seem to relate to the environment (Belding, 1910; Gutsell, 1931; Marshall, 1960; Sastry, 1961). Thus these subpopulations may not be true genetic races. Serological studies might help in determining the extent of racial difference among the populations which would then aid in evaluating the extent of environmental response.

Aequipecten gibbus, the calico scallop of the southern offshore waters, differs from Aequipecten irradians in its habitat and in minor characteristics, yet it is very closely related. Serological comparisons with this species provide a frame of references to judge

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the significance, taxonomically, of minor differences within the irradians group. The frame of reference is broadened by comparison with a more distinct species, the sea scallop, Placopecten magellanicus.

METHODS AND MATERIALS

The scallops used in this study were collected from various areas along the Gulf and Atlantic coasts of the United States as shown below:

Scientific Name	Date Received	Source
Aequipecten irradians	3/24/61	Beaufort, North Carolina
Aequipecten gibbus	3/24/61	Off North Carolina Coast
Aequipecten irradians	5/29/61	Niantic, Connecticut
Aequipecten irradians	7/13/61	Alligator Harbor, Florida
Aequipecten irradians	8/10/61	Tarpon Springs, Florida
Aequipecten irradians	5/7/62	Beaufort, North Carolina
Aequipecten irradians	7/13/62	Alligator Harbor, Florida
Aequipecten irradians	7/18/62	Niantic, Connecticut
Placopecten magellanicus	7/20/62	Off southern New England coast

Scallop specimens from Niantic were received alive; specimens from other locations consisted of only the adductor muscle which had been removed from the scallop, frozen, and shipped to this laboratory after collection.

Two methods of antigen preparation were used; both were phosphate buffered saline extracts of the scallop adductor muscle. In one method the lipid material was removed from the muscle prior to extraction.

Antisera were produced in rabbits by subcutaneous injection of a one to one mixture of Freund's complete adjuvant and the antigen once a week for three or four weeks.

Ring precipitin tests were done with serial doubling dilutions of the antigen layered over undiluted antisera in small tubes. A white line at the interface of the antigen dilution and the antiserum was a positive test. The titer was indicated by the reciprocal of the highest antigen dilution giving a detectable reaction. Each test was run in duplicate.

Agar diffusion tests were run in 1% Oxoid agar medium. Cuttings were made in the agar to provide a central well into which the antiserum was placed and six peripheral wells into which the antigens were deposited. When additional wells were needed on one plate, an extra cutting was made. Thus each well containing heterologous antigen had on either side of it a well containing homologous antigen so that cross reactions might be observed. Each test here was also done in duplicate.

DISCUSSION

In terms of the particular serological observations presented herein, the subspecies of the bay scallop as well as the calico scallop are closely related as their morphology suggests. The sea scallop, which is easily distinguished from the other two types of scallops by morphological criteria, is slightly different from the other two types of scallops serologically, although it possesses antigenic components in common with them.

These serological comparisons may be interpreted in support of the link, now recognized through shell characteristics, between Aequipecten irradians and Aequipecten gibbus. They do not support the breakdown of irradians into three readily recognized subspecies. It is possible that differences might be found among these scallops by the use of more sensitive serological techniques, i.e., adsorption tests or immunoelectrophoretic techniques. Whether or not the differences would correlate with the subspecies as now recognized remains to be seen. Meanwhile, there is good reason to focus attention on the likelihood that the bay scallop, throughout its range, may exhibit differences both in environmental responses and in genetic strains that may be highly localized and may even occur, as Marshall (1960) has suggested, in separate year classes in the same locale.

Table 1. Results of ring precipitin tests with scallop antigens prepared by method A[1] and their antisera

Antiserum	Antigen[2]			
	A.i.(N)	A.i.(B)	A.I.(AH)	A.G.(NC)
A.i. (N)	1280 1280	1280 2560	320 160	640 640
A.i. (B)	1280 1280	1280 1280	640 320	640 640
A.i. (AH)	320 160	160 160	160 160	80 160
A.g. (NC)	1280 1280	2560 2560	640 640	2560 2560

[1]Antigens prepared by method A were phosphate buffered saline extracts of the scallop muscle from which lipids were extracted.

[2]A.i. (N) = Aequipecten irradians from Niantic, Conn.
A.i. (B) = Aequipecten irradians from Beaufort, N. C.
A.i. (AH) = Aequipecten irradians from Alligator Harbor, Fla.
A.g. (NC) = Aequipecten gibbus from off the North Carolina coast.

Table 2. Results of ring precipitin tests with scallop antigens prepared by method B[1] and their antisera

Anti-serum	Antigen[2]					
	A.i.(N)	A.i.(B)	A.i.(TS)	A.i.(AH)	A.g.(NC)	P.m.(SNE)
A.i.(N)	640 640	1280 1280	1280 1280	320 320	1280 1280	160 160
A.i.(B)	1280 640	1280 2560	1280 2560	1280 640	2560 2560	640 320
A.i.(TS)	1280 1280	1280 1280	2560 2560	640 640	2560 2560	640 640
A.i.(AH)	640 640	1280 1280	2560 1280	640 640	2560 2560	320 320
A.g.(NC)	640 640	1280 1280	1280 1280	320 320	5120 2560	320 160
P.m.(SNE)	1280 2560	1280 640	1280 1280	320 640	2560 5120	1280 2560

[1]Antigens prepared by method B were phosphate buffered saline extracts of scallop adductor muscle from which lipids were not extracted.

[2]A.i.(N) = Aequipecten irradians from Niantic, Conn.
A.i.(B) = Aequipecten irradians from Beaufort, N. C.
A.i.(TS) = Aequipecten irradians from Tarpon Springs, Fla.
A.i.(AH) = Aequipecten irradians from Alligator Harbor, Fla.
A.g.(NC) = Aequipecten gibbus from off the North Carolina coast.
P.m.(SNE) = Placopecten magellanicus from off the southern New England coast.

Table 3. Results of agar diffusion studies indicating bands formed between scallop antigens prepared by method A[1] and the various antisera produced against them

Antiserum	Antigen[2] A.i.(N)	A.i.(B)	A.i.(AH)	A.g.(NC)
A.i.(N)	+[3]	C	P	C
	+	-	-	C
	+	C	P	C
	+	P	P	C
A.i.(B)	P	+	P	P
	C	+	-	C
	P	+	P	C
	C	+	-	C
A.i.(AH)	C	C	+	C
	C	-	+	P
	P	P	+	P
A.g.(NC)	C	C	P	+
	E			
	C	P	P	+
	C	C	P	+

[1]Antigens prepared by method A were phosphate buffered saline extracts of scallop adductor muscle from which liquids were extracted.

[2]A.i.(N) = Aequipecten irradians from Niantic, Conn.
A.i.(B) = Aequipecten irradians from Beaufort, N. C.
A.i.(AH) = Aequipecten irradians from Alligator Harbor, Fla.
A.g.(NC) = Aequipecten gibbus from off the North Carolina coast.

[3]Symbols: + = band present with homologous antigen and antiserum
C = band present at same location and connecting with corresponding band
P = band present at approximately same distance from central well as + band
E = extra band not present with homologous antigen
- = band not present

Table 4. Results of agar diffusion studies indicating bands formed between scallop antigens prepared by method B[1] and the various antisera produced against them

	Antigen[2]					
Antiserum	A.i.(N)	A.i.(B)	A.i.(TS)	A.i.(AH)	A.g.(NC)	P.m.(SNE)
A.i.(N)	+[3]	P	P	P	P	P
	+	P	P	P	P	P
	+	P	P E	P	P	-
A.i.(B)	-	+	-	-	-	-
	P	+	-	-	-	-
	C	+	P	P	P	-
	P	+	P	P	- E	P
	C	+	C	C	C	-
A.i.(TS)	-	P	+	P	-	-
	C	C	+	P	C	-
	P	P	+	P	P	P
	C	P	+	C	P	C
A.i.(AH)	P	C	P	+	-	P
	P	P	P	+	-	P
	P	C	C	+	P	-
	C	C	C E	+	P	-
A.g.(NC)	C	C	C	C	+	-
	C	C	P	P	+	-
	P	P	P	P	+	P
P.m.(SNE)	-	P	-	-	-	+
	C	P	P	C	P	+
	P	P	P	P	P	+

[1]Antigens prepared by method B were phosphate buffered saline extracts of the adductor muscle of the scallop from which lipids were not extracted.

[2]A.i.(N) = Aequipecten irradians from Niantic, Conn.
A.i.(B) = Aequipecten irradians from Beaufort, N. C.
A.i.(TS) = Aequipecten irradians from Tarpon Springs, Fla.
A.i.(AH) = Aequipecten irradians from Alligator Harbor, Fla.
A.g.(NC) = Aequipecten gibbus from off the North Carolina coast.
P.m.(SNE) = Placopecten magellanicus from off the southern New England coast.

[3]Symbols:
+ = band present with homologous antigen and antiserum
C = band present at same location and connecting with corresponding band
P = band present at approximately same distance from central well as + band
E = extra band not present with homologous antigen
- = band not present

ACKNOWLEDGMENTS

I wish to thank Dr. Nelson Marshall of the Graduate School of Oceanography of the University of Rhode Island for his assistance in the collection of scallop specimens and for his suggestions on the interpretation of this work with reference to the scallop classification problem. I would also like to express appreciation to Dr. Chester W. Houston for help in preparing the manuscript and for valuable assistance throughout the work.

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ASSOCIATION AFFAIRS

ANNUAL CONVENTION

The 1963 convention was held jointly with the Oyster Institute of North America and the Oyster Growers and Dealers Association on July 21-25 at the Hotel Mayflower. Washington, D. C.

The Secretary-Treasurer reported a membership of 160 (166 on 1 August 1963). Sixty-one libraries were subscribers to the Proceedings. The states of Maryland and Virginia each contributed $100 and the Oyster Institute contributed $500 toward costs of publication. Forty-eight sets of the back volumes of Convention Addresses and Proceedings on microcards were sold at $8.00 per set and 50 more sets were printed.

Dr. Victor L. Loosanoff of the U. S. Bureau of Commercial Fisheries was elected to Honorary Life Membership.

Mr. Gilbert B. Pauley of the University of Washington won the second Thurlow Nelson Award for a paper on inflammatory reactions in oysters.

The Editorial Committee consisting of Sewell H. Hopkins (Chairman), Lawrence Pomeroy, and Daniel B. Quayle was reappointed.

A new slate of officers was elected, as shown below:

President . . . . . JOHN B. GLUDE
Bureau of Commercial Fisheries,
Seattle, Wash.

Vice-President . . . . . JAY D. ANDREWS
Virginia Institute of Marine Science,
Gloucester Point, Va.

Secretary-Treasurer. . . JOHN GILMAN MACKIN
Texas A&M University,
College Station, Tex.

Members-at-Large . . . . . ALBERT K. SPARKS,
University of Washington,
Seattle, Wash.,
and DANA E. WALLACE,
Maine Dept. of Sea and Shore Fisheries,
Augusta, Me.

SPECIAL NOTICES

1. The official depository for records, back numbers of the Proceedings, and microcard sets is now the Biological Laboratory, U. S. Bureau of Commercial Fisheries, Oxford, Maryland. Mr. Arthur S. Merrill is custodian. Back volumes of the Proceedings (including those published earlier as Convention Addresses) will be sold at $4.00 each if still available. Microcard reproductions of back issues, covering a 30-year period, will be sold at $8.00 per set.

2. Beginning July 1965, dues for membership in the National Shellfisheries Association (including subscription to Proceedings) will be $6.00 per year. Dues and applications for membership should be sent to Mr. Dana E. Wallace, Department of Sea and Shore Fisheries, Vickery-Hill Building, Augusta, Maine, who will be the new Secretary-Treasurer.

3. Starting with the 1965 volume, Mr. Arthur S. Merrill, Biological Laboratory, Oxford, Maryland, will be Chairman of the Editorial Committee. He will accept manuscripts for editorial consideration at any time. Those received too late for one volume will be published in the next. The 1964 volume (Vol. 55) will be completed by the present committee of Sewell H. Hopkins (Chairman), Daniel B. Quayle, and Lawrence Pomeroy.

INFORMATION FOR CONTRIBUTORS

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

Manuscripts should be typewritten and double-spaced: original sheets are required but extra copies will facilitate reviews. Tables, numbered in arabic, should be on separate pages with the title at the top. Scientific names should be underlined. Illustrations should be reduced to a size which fits on 8 x 10 1/2-inch pages with ample margins. Glossy photographs are preferred to originals. Illustrations smaller than a page should be carefully oriented and loosely attached to plain white paper with rubber cement. Legends should be typed on separate sheets and numbered in arabic.

Authors should follow the style prescribed by Style Manual for Biological Journals, which may be purchased for $3.50 from the American Institute of Biological Sciences, 2000 P Street, NW, Washington 6, D.C. In case of a question on style that is not answered by this manual, the author should refer to the 1962 PROCEEDINGS (Volume 53) or to the present volume.

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

Reprints and covers are available at cost to authors. Master sheets will be retained for one year after publication. When proof sheets are returned to authors, information about ordering reprints will be given. The present agency from which authors may obtain reprints is Bi-City Ink, 1001 S. College Avenue, Bryan, Texas 77801.