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Evidence of a naturally occurring inhibitor possibly limiting Dermocystidium marinum in Virginia

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ABSTRACT

Dermocystidium marinum, a fungus parasite common in oysters on the eastern shore of Chesapeake Bay, suddenly disappeared at the beginning of the marsh and bay system of the outer Virginia coast. No. D. marinum was found on the outer coast (Seaside) although temperatures and salinities seem to be optimal. Introductions of populations of infected oysters seem to lose the disease, but individuals do not. What seems to be a water derived inhibitor was found in a few water samples collected at Wachapreague on the Seaside. Comparable inhibition was not found in Chesapeake Bay water. The inhibitor is difficult to demonstrate and its nature is uncertain. It is suggested that the inhibitor may keep the disease from invading Virginia's outer bays.

INTRODUCTION

The parasitic oyster fungus, Dermocystidium marinum Mackin, Owen, and Collier, 1950 is a widely distributed organism in Chesapeake Bay. It is absent only from the fresher upper bay and rivers and from the Seaside of the Delmarva peninsula from Cape Charles to Cape Henolopen. This absence is unusual since these outer bays have temperatures and salinities optimal for development of the disease.

Dermocystidium marinum is clearly not native to the Seaside region of Virginia but no one has satisfactorily explained its absence. The distribution of D. marinum on the Eastern Shore of Virginia (Fig. 1) is here discussed and experimental data is presented showing a possible cause of its absence.

I wish especially to thank Dr. J. D. Andrews, who discovered its absence, for a great amount of assistance and data. Mr. W. T. Davis did most of the culture work and Mrs. Bonnie Hill most of the laboratory experiments on inhibition. These studies were conducted at the Eastern Shore Laboratory of the Virginia Institute of Marine Science.

D. marinum infections were determined by Ray's (1952) thioglycollate culture method.

DISTRIBUTION OF D MARINUM ON THE EASTERN SHORE

Andrews and Hewatt (1957) found D. marinum practically throughout lower Chesapeake Bay. They found none in the upper James and Potomac

Rivers and in the upper bay above the Patuxent River. This absence supports Mackin's (1956) conclusion that it is scarce in lower salinities. They also found none on the Virginia Seaside from Hog Island to Chincoteague Bays and they were surprised by this absence. It is apparent that such an ubiquitous and common organism would also be found on the Seaside were it not for some ecological factor.

In 1959-61 during June through December 485 native oysters from the Seaside of Virginia were tested without finding any Dermocystidium (Table 1). It is safe to conclude that the fungus is not native to this region.

In 1961 the precise distribution around the lower tip of the peninsula (Fig. 1) was determined. Infections had been previously found be common in all creeks sampled on the eastern shore of Chesapeake Bay from Messongo to Plantation (Table 2). Below Plantation Creek there are no natural creeks nor reefs, but a few oysters grow intertidally on piers off sandy beaches of lower Chesapeake Bay. Infections were common in these native intertidal oysters from the ferry terminal pier at Kiptopeke Beach (Table 2). Oysters taken from piling farther south at Fishermen's Island were 8 % infected, but others from the area were negative. These are considered bay oysters since they were covered with heavy sets of barnacles and mussels, Mytilus edulis, both which were absent from the Seaside but common in the lower bay.

Fishermen's Island is apparently the farthest south and west D. marinum exists on the eastern shore of Chesapeake Bay. But it

is rare there compared to infections in oysters taken during similar periods from the remainder of the eastern shore of the bay (Table 2). The weighted incidence of Dermocystidium from recovered dying oysters from Messongo to Cherrystone Creeks in 1959 and 1960 was over 4.0, comparable to other areas with Dermocystidium mortalities (Ray, 1954; Andrews and Hewatt, 1957).

In addition to a complete absence on the Seaside from Wise Point to Wachapreague in live oysters, there was none found in oysters dying in trays. A sample of oysters from Seaside marshes at Wise Point contained none, even though they grew only 1500 yards from infected Fishermen's Island oysters. D. marinum easily traverses comparable distances in Chesapeake Bay. Both the Cape Charles Jetty and the Kiptopeke Beach ferry terminal where oysters are infected are farther removed from other oysters. Mackin (1962) concluded that it could traverse long distances of open water.

INTRODUCTIONS OF D. MARINUM

Oystermen have inadvertently introduced the disease into the Seaside from Chesapeake Bay. There are many probable cases, but only two are documented.

One group of South Carolina oysters was 12 % infected when introduced in February, 1960. This same group was examined in July and September, 1960 and no infections were found (Table 3). A tray of 250 of these oysters had no mortality from July 19 through September

17, which included the peak of Dermocystidium mortality in Chesapeake Bay.

A group of a few bushels of Occahannock Creek oysters were experimentally planted by local oystermen in Hog Island Bay in March, 1961. These oysters were carried through their first summer in Occahannock Creek where the disease is common. Although a control group from the same population of oysters left in Occahannock Creek was 32 % infected on September 8, 1961, no infections were found in those transplanted to Hog Island Bay (Table 3).

Several experimental plantings of infected oysters were made in late 1961, and one accidentally in February, 1961 (Table 3). These introductions are inadequate to show the ultimate fate of the disease, but they do show that the fungus is not immediately destroyed in oysters already parasitized. The data suggest that new infections do not occur. The oysters originally from Mobjack Bay contained only 2 moderate infections after spending the summer in Bradfords Bay. In areas where D. marinum is continually infecting oysters light cases always predominate over moderate and heavy (Andrews and Hewatt, 1957; Andrews, personal communication).

OBSERVATIONS ON INHIBITION

Several series of water samples collected at Wachapreague were exposed soon after collection to tissue moderately to heavily infected with Dermocystidium. Ten cc of sample water with infected tissue and 5,000 units of a penicillin-streptomycin mixture were kept in test

tubes for three days. The tissue was then cultured in thioglycollate medium for 48 to 72 hours.

Most samples caused no inhibition (inhibition being negative for D. marinum) and exposure of tissue to 50 control samples of Chesapeake Bay water from Kiptopeke Beach always resulted in moderate to heavy ratings of D. marinum infection. A continuous series of samples of Wachapreague water collected through two tidal cycles on December 27-28, 1961 and a shorter series collected in late January, 1962 caused no inhibition.

Inhibition was demonstrated from some samples taken on December 20, 1961, January 11 and 15-16, 1962 (Fig. 2). The differences observed after exposure to different water varied from heavy ratings usually found at high tide to negative usually found at low tide. My findings generally confirm those of Ray (1954) and others that moderate to heavy infections are about evenly distributed throughout the body. Failure of a given piece of tissue to show any enlarged D. marinum cells is suspect, especially since controls were never negative.

A few showed dead cells (interpreted from lack of stain and small size) around the periphery of cultured tissue but normal development deep in the tissue. Although this could be due to nutritional differences, it is reasonable that the inhibitor would be more affective (at lower concentrations) against free cells in the water than those parasitizing tissue.

Inhibition was observed from water samples held at temperatures of 2-4 C and 19-25 C. Water was collected during temperatures of -1.5 to 6 C, but most samples were then held in temperatures over 20.

DISCUSSION

Presumably the inhibition observed in tissue cultures could also be responsible for inhibition in natural waters, thereby limiting the distribution of *D. marinum* to lower Chesapeake Bay. The observed could not be caused by a growth stimulant because growth occurs normally in cultures with distilled water and NaCl.

Salinity is definitely not involved. Normal development occurred in tissue exposed to Chesapeake Bay water evaporated to 38.5 °/oo. Normal cultures were found in various salinities with no general correlation, except with the immediate series of samples (Fig. 2). No inhibition was found in salinities over 30 °/oo, whereas it was present in salinities as low as 24 °/oo. Mackin (1956) reported normal development in cultures of 50 °/oo. Ray (1954) obtained aquarium infections in salinities of about 10 to 28 °/oo, the total range he studied.

Considerable differences have been reported on the affect of salinity in laboratory experiments (Ray, 1954; Mackin, 1956) and field data (Mackin, 1956; Dawson, 1955; Andrews and Hewatt, 1957). (See also the discussion by Johnson and Sparrow, 1961:232-6). These differences could be explained on the basis of a limiting factor

correlated with salinity within a given bay but varying between bay systems with comparable salinities. This agrees with previous observations which led to the theory of amount of flushing controlling the disease (Mackin, 1956, 1962; Andrews and Hewatt, 1957). This factor might be common in low salinities in most bays (but varying the salinity at which it appears) and in high salinities on the Virginia Seaside.

Temperature, shown to be important to D. marinum development, could not be involved. There is little available temperature data but the general impression that lower Chesapeake Bay waters have cooler summer temperatures than Seaside water is strengthened by the distribution of Mytilus edulis. Wells and Gray (1960) found that this mussel was killed by temperatures over 26 C in North Carolina. M. edulis was common in lower Chesapeake Bay during 1961; some set on Seaside oysters but none survived the summer as they did in the lower bay. Temperatures over 27 were often recorded in Seaside bays in July, August, and early September. But shallow waters in lower Chesapeake Bay seem to be more under the influence of colder ocean waters than are either the bayside or Seaside shallows which probably follow air temperatures more closely. Also shallow water and intertidal oysters are subject to approximately the same minimums on both sides of the peninsula. Although temperatures seem to favor the fungus in Seaside water, it is absent there, but common in less favorable lower Chesapeake Bay.

Due to the observations on inhibition, and the sharp limiting of D. marinum at the southern tip of the Eastern Shore of Virginia, it

is suggested that the fungus would likely infect Seaside areas were it not for some fungicide or fungistatic agent derived from these waters and absent from lower Chesapeake Bay. The correlation with salinity suggests that it is derived from either the land or the marsh, probably the marsh.

LITERATURE CITED

Andrews, J. D. and W. G. Hewatt. 1957. Oyster mortality studies in Virginia. II. The fungus disease caused by Dermocystidium marinum in oysters of Chesapeake Bay. Ecol. Monogr. 27:1-26.

Dawson, C. E. 1955. Observations on the incidence of Dermocystidium marinum infection in oysters of Apalachicola Bay, Florida. Tex. J. Sci. 7(1):47-56.

Johnson, T. W., Jr. and F. K. Sparrow Jr. 1961. Fungi in oceans and estuaries. J. Cramer, Weinheim, Germany:668pp.

Mackin, J. G. 1956. Dermocystidium marinum and salinity. Proc. Nat'l Shellfish. Assoc. 46:116-128.

_____ 1962. Oyster disease caused by Dermocystidium marinum and other microorganisms in Louisiana. Publ. Inst. Mar. Sci. Univ. Tex. 7:132-229.

Ray, S. M. 1952. A culture technique for the diagnosis of infection with Dermocystidium marinum. Conv. Add. Natl. Shellfish. Assoc: 9-13.

_____ 1954. Biological studies of Dermocystidium marinum, a fungus parasite of oysters. Rice Inst. Pamp. Spec. Issue, Nov: 1-114.

Wells, H. W. and I. E. Gray. 1960. The seasonal occurrence of Mytilus edulis on the Carolina coast as a result of transport around Cape Hatteras. Biol Bull. 119(3):550-559.

Table 1. Samples of live native oysters from the Virginia Seaside examined for Dermocystidium marinum, 1959-61. All samples were negative based on 25 oysters except the last two of 17 and 18.

LOCALITY	DATE
Swash Bay	25 June 59, 25 Aug 59, 3 Oct 59, 30 Dec 59
Bradfords Bay	9 June 60, 6 July 60
Hog Island Bay	17 June 59, 15 July 59, 25 Aug 59, 26 Oct 59, 19 July 60, 17 Sept 60
Outlet Bay	16 Dec 59
Cobb Bay	16 June 59, 13 Aug 59, 25 Aug 59, 10 Nov 59 16 Dec 59,
Magotha Bay	6 Sept 61
Wise Point (Marsh)	6 Sept 61

Table 2. Distribution and incidence of *Dermocystidium marinum* in old native oysters on bayside of the Eastern Shore of Virginia. Samples taken between June and December (inclusive) and are based on 25 per collection date.

Location	Number Sampled	Per Cent infected
Messongo Creek	100	25
Chesconessex Creek	50	61
Nandua Creek	25	12
Occahannock Creek	100	20
Hungars Creek	75	47
Gulf	225	36
Cherrystone Creek	75	47
Cape Charles Jetty	25	44
Plantation Creek	50	42
Kiptopeke Beach	50	40
Wise Point (Bay)	25	0
Fishermen's Island	25	8

Table 3. Introductions of Dermocystidium into Seaside bays.

DATE OF INTRODUCTION	LOCALITY	ORIGIN	INCIDENCE OF INFECTION WHEN INTRODUCED	INCIDENCE OF INFECTION AFTER	NUMBER SAMPLED
19 Feb 60	Hog Island Bay	South Carolina	12 %		25
17 Sept 60				0	25
Mar 61	Hog Island Bay	Occahannock Creek	? (probably light)		
8 Sept 61				0	50
Feb 61	Bradfords Bay	Mobjack Bay, VA.	?		
5 Oct 61				9 %	21
27 Sept 61	Wachapreague	Occahannock Creek	16		25
11 Oct 61				25	12
6 Nov 61				25	8
26 Sept 61	Wachapreague	Cape Charles Jetty	44	33	25
11 Oct 61				33	12
31 Oct 61				8	12

Fig. 1. Eastern Shore of Virginia. Seaside refers to the system of bays and marshes from Magotha to Chincoteague. Bayside refers to the eastern shore of Chesapeake Bay including the tidal creeks from Messongo to Plantation.

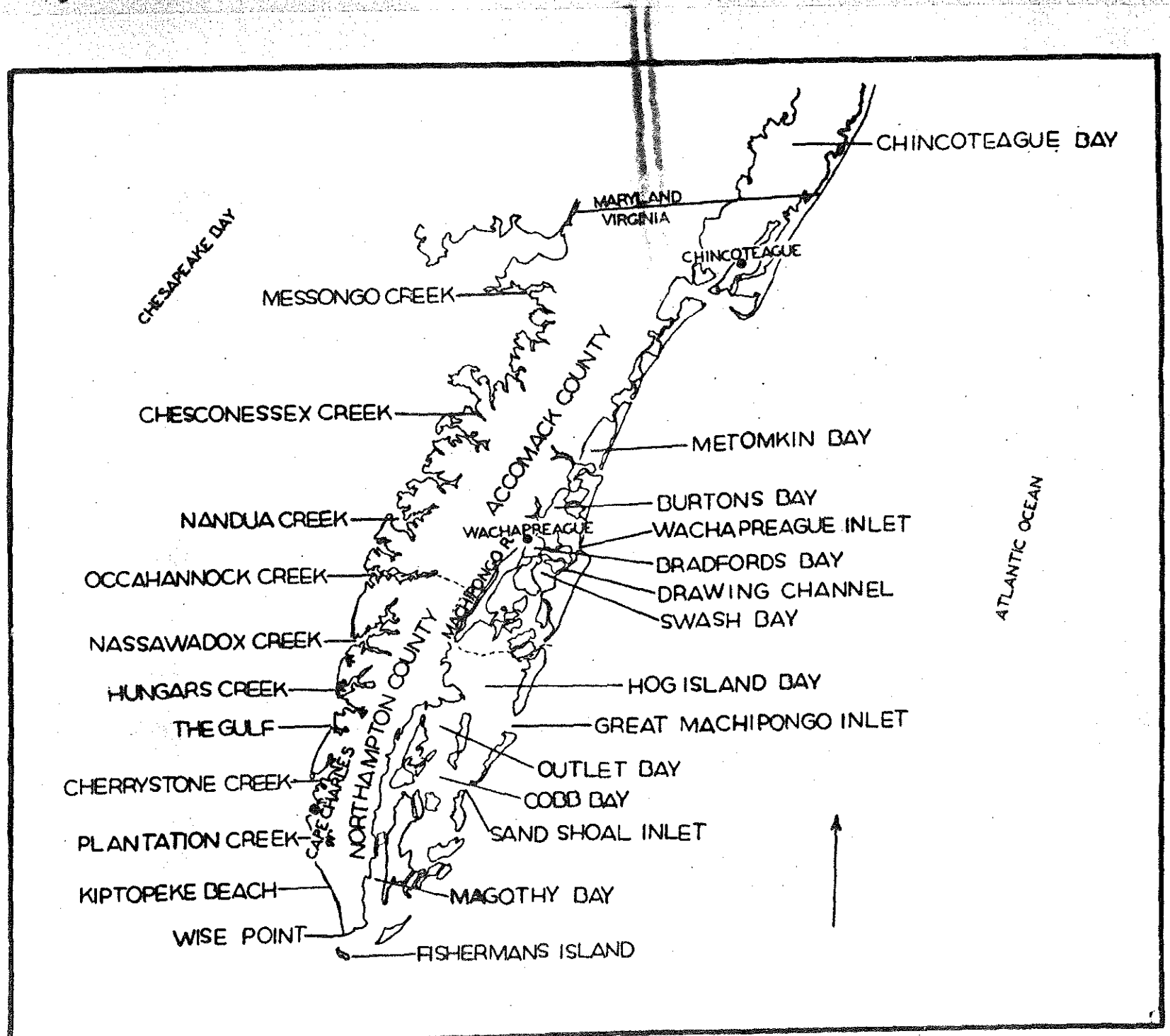
CHINCOTEAGUE BAY
MARYLAND
VIRGINIA
CHESAPEAKE BAY
CHINCOTEAGUE
MESSONGO CREEK
ACCOMACK COUNTY
CHESCONESSEX CREEK
METOMKIN BAY
BURTONS BAY
NANDUA CREEK
WACHAPREAGUE
WACHAPREAGUE INLET
MACHIPONGO R.
BRADFORDS BAY
OCCAHANNOCK CREEK
DRAWING CHANNEL
SWASH BAY
ATLANTIC OCEAN
NASSAWADOX CREEK
NORTHAMPTON COUNTY
HUNGARS CREEK
HOG ISLAND BAY
THE GULF
GREAT MACHIPONGO INLET
OUTLET BAY
CHERRYSTONE CREEK
CAPE CHARLES
CODB BAY
SAND SHOAL INLET
PLANTATION CREEK
KIPTOPEKE BEACH
MAGOTHY BAY
WISE POINT
FISHERMANS ISLAND

Fig. 2. Variance of infection of tissue exposed to two continuous series of Seaside water at Wachapreague. Question marks indicate damaged sample

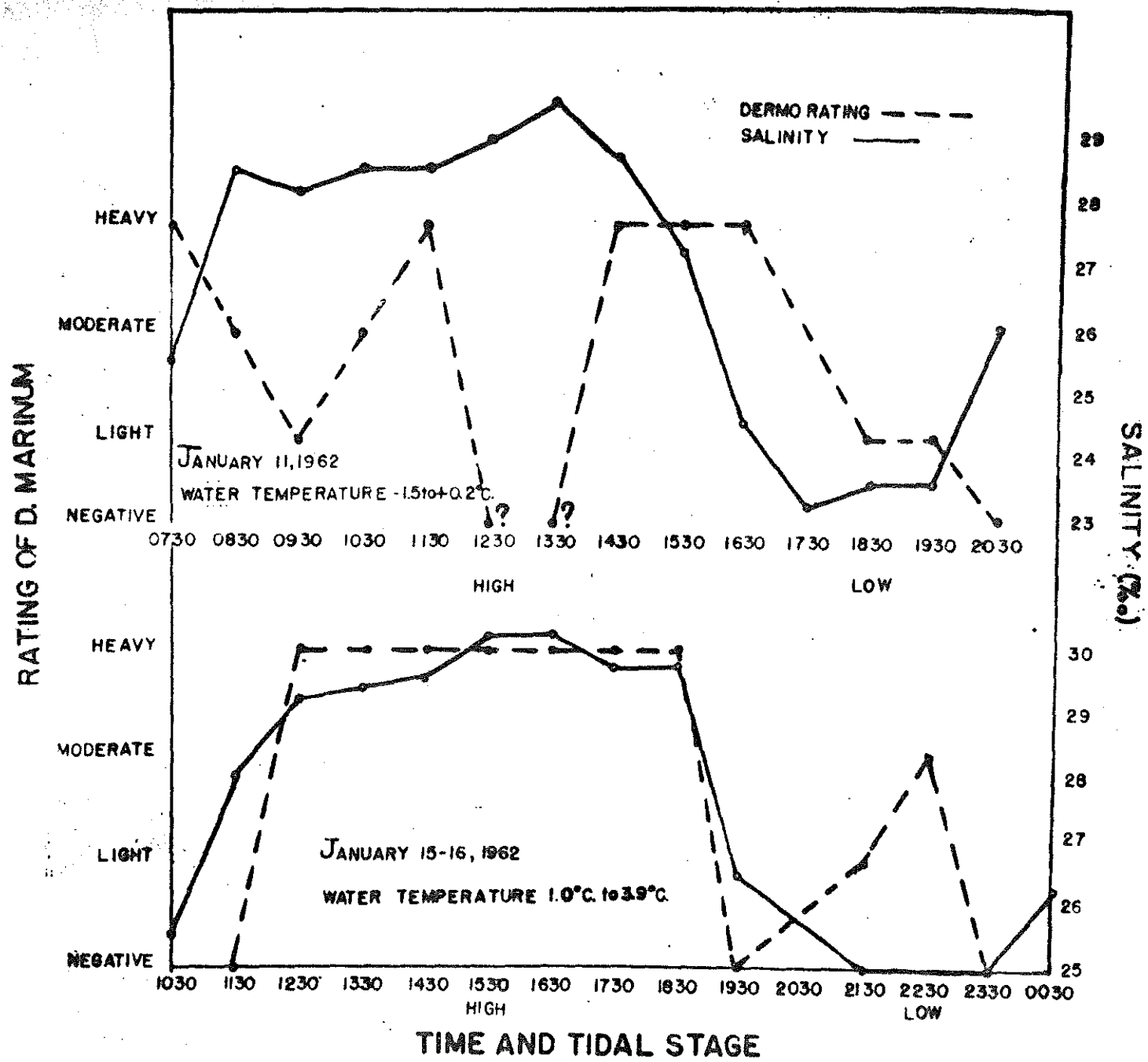
DERMO RATING
SALINITY
HEAVY
MODERATE
LIGHT
NEGATIVE
JANUARY 11, 1962
WATER TEMPERATURE -1.5 to +0.2°C.
0730 0830 0930 1030 1130 1230 1330 1430 1530 1630 1730 1830 1930 2030
HIGH
LOW
HEAVY
MODERATE
LIGHT
NEGATIVE
JANUARY 15-16, 1962
WATER TEMPERATURE 1.0°C. to 3.9°C.
1030 1130 1230 1330 1430 1530 1630 1730 1830 1930 2030 2130 2230 2330 0030
HIGH
LOW
RATING OF D. MARINUM
SALINITY (‰)
29 28 27 26 25 24 23
30 29 28 27 26 25
TIME AND TIDAL STAGE

Fig 2

THE JOHNS HOPKINS UNIVERSITY · BALTIMORE 18, MARYLAND

CHESAPEAKE BAY INSTITUTE

23 April 1962

Mr. Hoese
Virginia Fisheries Laboratory
Wachapregue, Virginia

Dear Mr. Hoese:

The results of the copper analyses are as follows:

Bottle No.	Copper µg/liter
1	34
2	50
3	20
4	30

The values are rather high for estuarine waters, and I wonder if you know the source of the copper. Our work on the distribution of copper in the Chesapeake Bay is just started so that I am unable to give a good comparison with your values.

Sincerely yours,

James H. Carpenter

James H. Carpenter
Research Associate

JHC:mc

How about that!
Mike