ETIOLOGICAL STUDIES ON OYSTER MORTALITY

I. *Nematopsis ostrearum* Prytherch 1940 (Sporozoa : Porosporidae)[1]

By

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ABSTRACT

Oysters from the major producing area of Louisiana were found to be 100% infected with the gregarine parasite *Nematopsis ostrearum;* those from a check station in Pencacola Bay, Florida, were found to be 95.1% infected. In Louisiana, the population of *N. ostrearum* was found to be higher in the area east of the Mississippi River. *N. ostrearum* infections in oysters increased significantly during summer months in areas of relatively low oyster mortality. It was concluded on indirect and negative evidence that *N. ostrearum* was not a controlling factor responsible for the condition of oysters or for their extensive mortalities in Louisiana waters.

INTRODUCTION

During extensive investigations on causes of oyster mortalities in Louisiana coastal waters, the authors studied the intensity and distribution of *Nematopsis ostrearum* Prytherch in its secondary host, the oyster. This phase of our etiological study is concerned only with the parasite *N. ostrearum.*

Both Prytherch (1938) and Kudo (1946) have described the life cycle of the oyster parasite which Prytherch later (1940) described as a new species, *N. ostrearum*, and which we consider to be the species observed in this study. Sprague (1949), recognizing two species, described *N. prytherchi*, which occurs simultaneously with *N. ostrearum* in the secondary host but which is concentrated in the gills. With this latter species we are not concerned in this study.

Systematic studies were made on oysters from selected locations in Louisiana and Florida. The incidence and degree of infection were determined and the results were compared with mortality and with the Index of Condition determinations.

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MATERIALS AND METHODS

Twenty-one stations were established in Louisiana (Fig. 1) and one at Pensacola, Florida.[3] The stations were sampled at regular intervals in 1947 and 1948 and sporadic checks were made in 1949. Because of the extent of the territory, an amphibious plane was used to transport a standard sample of 25 oysters from the collection point to the laboratory for immediate analysis and examination.

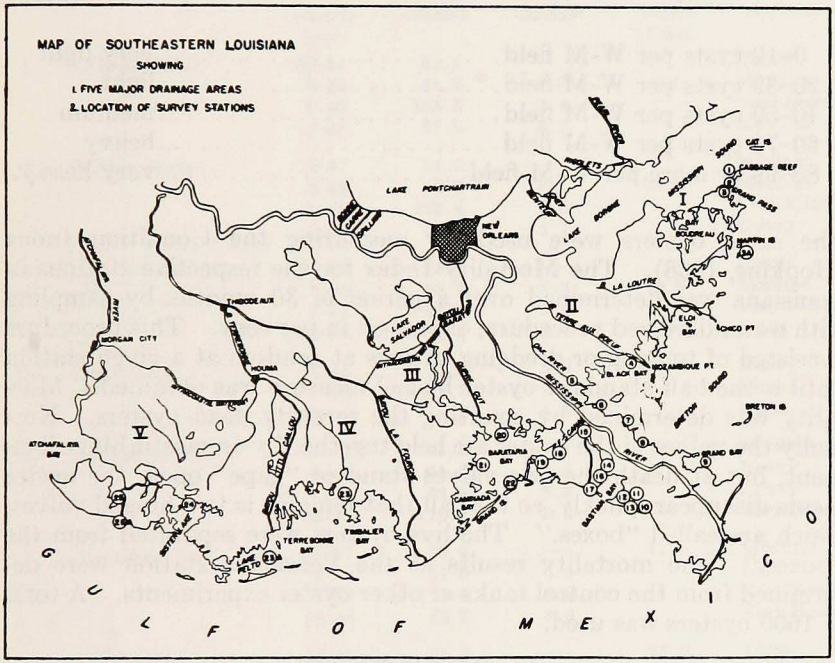

Figure 1. Outline Map showing the location of stations and the five major drainage areas in the eastern oyster-producing region of Louisiana.

The technique employed for the microscopic examination of the tissue was as follows. A square centimeter of mantle tissue, ventrally adjacent to the adductor muscle, was dissected from each of ten oysters, and each sample tissue was then individually mounted in a 20% NaOH solution between two firmly compressed microscope slides. After each tissue was partially digested (10 minutes), it was studied under the low power of a compound microscope equipped with a Whipple Ocular Micrometer. By use of the mechanical stage, ten microscope fields

[3] Laboratory of the U. S. Fish and Wildlife Service, where space was made available to the State of Louisiana for the purpose of conducting independent investigations.

were examined and the number of cysts included in the micrometer field were counted and recorded. At least ten oysters were thus examined, one sample having been taken from each. The results were averaged to give the intensity of infection.

The following arbitrary values were selected on the basis of accumulated experience in order to facilitate an understanding of the comparative degree of infection that was found to be present in Louisiana and Florida oysters:

0–19 cysts per W-M field	very light
20–39 cysts per W-M field	light
40–59 cysts per W-M field	medium
60–79 cysts per W-M field	heavy
80–99 or more per W-M field	very heavy.

The same oysters were used for measuring the Condition Index (Hopkins, 1938). The Mortality Index for the respective stations in Louisiana was determined over a period of 30 months by sampling with a standardized procedure, expressed in per cent. This procedure consisted of tonging or dredging oysters at random at a given station until a one-half standard oyster bushel measure was obtained. Mortality was determined by counting the recently dead oysters. Normally the valves of the oyster are held together by an elastic hinge ligament, but at death the two valves stand or "gape" open, the oyster meats disappear quickly, so that all that remains is two hinged valves, which are called "boxes." The live oysters were separated from the "boxes." The mortality results at the Pensacola station were determined from the control tanks of other oyster experiments. A total of 1600 oysters was used.

RESULTS

The microscopic examination of 723 live oysters from 30 locations in Louisiana coastal waters showed a 100% infection; no dead oysters were examined. In Florida, 260 live oysters were examined, 95.1% being infected; of 495 dead oysters examined, 94.2% had been infected.

The results of the Louisiana examinations, the Condition Index determinations, and the mortality percentages are summarized in Table I. The results of the Florida investigations are summarized in Table II. For clarity in discussion, the results in Table I are arranged and summarized according to the natural drainage areas of the oyster-growing regions (Fig. 1, Table III). Table IV contains the results from the studies of all Louisiana stations.

Our data show no correlation of *N. ostrearum* infection with salinity, dissolved oxygen, and hydrogen-ion concentration of the water. These factors were carefully checked from the tank experiments conducted at the Pensacola Laboratory.

TABLE I.—Infection of Louisiana Oysters with *Nematopsis ostrearum*, Compared with the Indices of Condition and Mortality

Station Number	*Location*	*Date of Sampling (month-year)*	*Av. No. Cysts per Sample*	*Condition Index per Sample*	*Mortality Index per Station (Total)*	*Comparative Intensity of Infection*
1	Cabbage Reef	10–47	61.1	—		Heavy
		3–48	41.2	8.6	5.5	Medium
		7–48	125.5	6.4		Very Heavy
		12–48	57.9	5.7		Medium
2	Bayou Pierre	10–47	51.2	—		Medium
		3–48	42.0	8.1	30.5	Medium
		7–48	122.4	7.0		Very Heavy
		12–48	66.7	4.7		Heavy
3	Grand Pass	10–47	75.1	—		Heavy
		3–48	48.6	9.6	9.5	Medium
		7–48	98.7	6.3		Very Heavy
		12–48	31.9	4.8		Light
4	Black Bay	3–48	75.2	3.8	5.8	Heavy
		7–48	69.7	7.8		Heavy
		12–48	32.6	4.9		Light
6	American Bay	10–47	41.2	—	6.6	Medium
		3–48	64.5	5.5		Heavy
		7–48	63.6	8.2		Heavy
		12–48	116.1	3.3		Very Heavy
7	California Bay	10–47	40.6	—	11.5	Medium
		3–48	31.0	4.4		Light
		7–48	123.8	8.3		Very Heavy
		12–48	83.7	3.9		Very Heavy
8	Quarantine Bay	10–47	31.0	—	25.2	Light
		3–48	36.8	5.7		Light
		7–48	79.6	6.9		Heavy
		12–48	33.3	3.9		Light
9	Grand Bay	10–47	17.2	—	43.5	Very Light
		3–48	15.7	9.5		Very Light
		7–48	165.1	8.4		Very Heavy
		12–48	30.1	6.3		Light
10	Sandy Point	10–47	42.1	—	21.8	Medium
		3–48	52.0	10.1		Medium
		7–48	41.0	3.3		Medium
		12–48	32.2	7.4		Light
11	Skipjack Bay	10–47	18.0	—	39.0	Very Light
		3–48	—	12.2		
		7–48	21.1	4.1		Light
		12–48	21.3	7.7		Light

TABLE I—*Continued.*

Station Number	*Location*	*Date of Sampling (month-year)*	*Av. No. Cysts per Sample*	*Condition Index per Sample*	*Mortality Index per Station (Total)*	*Comparative Intensity of Infection*
13	Bayou Scofield	10–47	21.1	—		Light
		3–48	24.2	12.2	42.0	Light
		7–48	42.5	5.8		Medium
		12–48	45.1	8.9		Medium
16	Bastian Bay	10–47	30.5	—	80.1	Light
		3–48	29.6	12.8		Light
		7–48	24.1	6.0		Light
		12–48	28.7	10.6		Light
18	Rattlesnake Bayou	10–47	20.7	—	32.8	Light
		3–48	—	—		
		7–48	31.2	6.4		Light
		12–48	22.7	6.5		Light
20	St. Mary's Point	10–47	—	—		
		3–48	31.8	7.2	23.3	Light
		7–48	36.2	4.0		Light
		12–48	54.9	4.3		Medium
21	Bassa-Bassa Bay	10–47	—	—		
		3–48	17.9	8.8	36.0	Very Light
		7–48	25.3	6.2		Light
		12–48	17.8	9.0		Very Light
22	Lower Barataria	10–47	26.7	—	58.1	Light
		3–48	33.4	9.6		Light
		7–48	52.6	9.2		Medium
		12–48	88.6	7.1		Very Heavy
23	Lake Felicity	10–47	—	—		
		3–48	43.2	9.1	22.6	Medium
		7–48	58.0	4.7		Medium
		12–48	15.0	5.8		Very Light
23A	Lake Pelto	10–47	32.5	—		Light
		3–48	34.1	7.4	—	Light
		7–48	44.8	4.9		Medium
		12–48	15.4	6.4		Very Light
24	Sister Lake	10–47	18.2	—	8.4	Very Light
		3–48	13.3	5.5		Very Light
		7–48	71.3	5.0		Heavy
		12.48	10.4	5.3		Very Light
25	Four League Bay	10.47	—	—		
		3–48	22.5	7.4		Light
		7–48	33.5	4.8	—	Light
		12–48	21.6	5.5		Light
25A	Oyster Bayou	10–47	—	—		
		3–48	8.8	7.6	9.6	Very Light
		7–48	42.6	6.7		Medium
		12–48	38.1	5.5		Light

TABLE II.—Infections of Florida Oysters with *Nematopsis ostrearum* Compared with Condition Index and Mortality from Controlled Experiments at the Pensacola Laboratory (20 Oysters Used Each Month).

Month and Year	*Average Number of Cysts*	*Index of Condition*	*Mortality Monthly Av.*	*Intensity of Infection*
4–48	19.4	5.14	——	Light
5–48	——	6.18	3.5	——
6–48	10.5	4.26	16.8	Very light
7–48	41.2	3.71	30.4	Medium
8–48	64.1	3.21	21.7	Heavy
9–48	26.0	2.48	13.7	Light
10–48	29.4	2.61	17.3	Light
11–48	46.3	2.48	29.1	Medium
12–48	——	——	17.8	——
1–49	112.3	2.34	8.0	Very Heavy
2–49	21.1	2.95	3.4	Light
3–49	22.8	4.04	2.2	Light
4–49	25.9	4.98	2.2	Light

TABLE III.—Compilation of Results by Natural Drainage Areas of Louisiana; Data from Table I.

Area	*Month and Year*	*Av. No. Cysts per Sample*	*Index of Condition*	*Intensity of Infection*
I	10–47	62.5	——	Heavy
	3–48	43.9	8.8	Medium
	7–48	115.5	6.6	Very Heavy
	12–48	52.2	5.0	Medium
II	10–47	32.5	——	Light
	3–48	44.6	5.8	Medium
	7–48	100.3	7.9	Very Heavy
	12–48	59.1	4.5	Medium
III	10–47	26.5	——	Light
	3–48	31.5	10.4	Light
	7–48	34.2	5.6	Light
	12–48	38.9	7.7	Light
IV	10–47	32.5	——	Light
	3–48	38.6	8.2	Light
	7–48	51.4	4.8	Medium
	12–48	15.2	6.1	Very Light
V	10–47	18.2	——	Very Light
	3–48	14.9	6.8	Very Light
	7–48	49.1	5.5	Medium
	12–48	23.4	5.5	Light

TABLE IV.—Total Mortality and Infection Results from 30 Louisiana Stations Grouped by Natural Drainage Areas.

Area	*Total Oysters Examined*	*Infection*	*Mortality*	*Intensity*
I	171	58.5	15.2	Medium
II	160	56.3	18.5	Medium
III	217	30.9	41.6	Light
IV	75	29.3	22.6	Light
V	100	26.7	9.0	Light

DISCUSSION

Largely because of Prytherch's work, *N. ostrearum* was suspected of being one of the possible causes of poor condition and mortality of oysters. However, our data do not support the application of Prytherch's view to Louisiana oysters.

The condition or quality of oyster meats is determined by several factors, one of the most important being seasonal differences in temperature and the reproductive cycle. This Condition Index is expressed by the equation: $\text{C. I.} = \frac{100 \times \text{dry wt. (gr.)}}{\text{Vol. of Shell Cavity (cc)}}$.

On recovery from spawning, oysters in Area II (Table III) attained their peak of condition in July 1948 when the intensity of encysted *N. ostrearum* spores attained a simultaneous maximum. However, this was not the case in Areas I, III, IV, and V, where the condition of the oysters followed the usual seasonal pattern (C. I. high in winter and spring, low in summer) and where the intensity of *N. ostrearum* still reached its peak in July, except in Area III. Oysters from stations 7, 10, 16, 18, 22, 24, 25, and 25A showed good condition at the maximum of infection (Table I).

At Pensacola, the oysters examined came from among those held in concrete tanks. On examination of Table II, it will be seen that the same lack of correspondence between the intensity of *N. ostrearum* and Index of Condition prevails.

There have been two known cases where *N. ostrearum* has been reported as a causative agent for oyster mortalities (Prytherch, 1940; Southern Reporter, 1944). The inference has been drawn that heavy infection results in the physical impairment of the ostia or vascular system or of both, this resulting in debilitation and finally death. From our observations, occasionally there are extremely heavy infections that are detrimental to the health of the organism. However, because Areas I and II, both low mortality areas, show a significantly higher infection than does Area III, a high mortality area, it appears logical to conclude that the extensive mortalities can not be attributed to this organism (Table IV). Station 16 (Table I) clearly illustrates this point. Of the 295 dead oyster meats recovered from the experimental tanks at the Pensacola Laboratory, only four oysters had an abnormally high incidence of infection. The remaining oysters averaged 8.2 cysts per field, with a maximum of 132 and a minimum of zero. From these results it is apparent that there are other factors that cause the differential mortality.

Sprague (personal communication) has stated that "experimentally produced infections incomparably more intense than anything seen in

nature, using the two [*Nematopsis*] species separately, do not show conclusively that either one is particularly harmful to the oyster." Engle (1946) stated that no correlation could be found between distribution and intensity of infection of *Nematopsis* with poor condition and mortality in oysters of upper Chesapeake Bay.

The fact that histolysis surrounding spores was not observed in any infected tissues is additional evidence that eliminates *N. ostrearum* as a factor responsible for poor quality or mortality of oysters.

The spores found in the tissues are inactive, i. e., they do not reproduce within the body of the oyster. The outer wall of the cyst is laid down as a result of the tissue reaction of the host organism. There is no evidence of a continued irritation response. This, of course, does not eliminate the potential pathogenic effect which might result from migrating larvae before phagocytosis occurs.

It is significant, however, that the intensity of infection increases during the summer months. In contrast to Area III, Areas I, II, IV, and V of Louisiana and the Pensacola Station illustrate this seasonal variation. No positive reasons for the variation can be offered other than ecological differences from one area to another. One could postulate variation in intensity as a function of distribution of the definitive host and concentrations of gymnospores in the water, but there are no data available for this.

The intensity of infection is generally considered to be a function of the age of the oyster, the older oysters generally having the larger number of cysts. However, some individuals of obvious old age were comparatively free of the parasite, although they were found in a community of high incidence of infestation. This fact, plus the seasonal fluctuation in infections, warrants our calling attention to the possibility that the oyster resists the infection or is able to eliminate the encysted spores, or both.

CONCLUSIONS

1. Based on representative samples over a period of two years (1947–1948), oysters from the major producing area of Louisiana were found to be 100% infected with the gregarine parasite *Nematopsis ostrearum*. Oysters from Pensacola Bay, Florida, were found to be 95.1% infected.

2. In Louisiana, the population of *N. ostrearum* was found to be higher in the area known as the "Louisiana Marsh," which is located east of the Mississippi River.

3. *N. ostrearum* infections in oysters increased significantly during the summer months in the lower mortality areas of Louisiana and at Pensacola, Florida.

4. *N. ostrearum* is not the controlling factor which is responsible for the Index of Condition of oysters or for the extensive mortalities of Louisiana oysters.

References

ENGLE, J. B.
1946. Commercial aspects of the Upper Cheasapeake bars in light of recent mortalities. 3rd Ann. Rep. Maryland Bd. Nat. Res.: 134–140.

HOPKINS, A. E.
1938. In Higgins, Rep. U. S. Comm. Fish. (1937), Appendix I: 1–70.

KUDO, R. R.
1946. Protozoology. 3rd Ed. Ch. C. Thomas, Springfield, Illinois. 778 pp.

PRYTHERCH, H. F.
1938. Life cycle of a sporozoan parasite of the oyster. Science, *88:* 451–452.
1940. The life cycle and morphology of *Nematopsis ostrearum* sp. nov., a gregarine parasite of the mud crab and oyster. J. Morph., *66:* 39–65.

SOUTHERN REPORTER
1944. West Pub. Co. Doucet vs Texas Co. 17 So. 2d: 340–349.

SPRAGUE, VICTOR.
1949. Species of Nematopsis in *Ostrea virginica.* J. Parasit., *35* (6), Sec. 2: 42 (abstract).