1958

Observations on distribution and elimination of spores of Nematopsis ostrearum in oysters

Sung Yen Feng
Virginia Fisheries Laboratory

OBSERVATIONS ON DISTRIBUTION AND ELIMINATION OF SPORES OF *NEMATOPSIS OSTREARUM* IN OYSTERS*

Sung Yen Feng**

Virginia Fisheries Laboratory
Gloucester Point, Virginia

Abstract

A non-random distribution of cysts of *Nematopsis* along the mantle margin of oysters was discovered. Sampling sites must be carefully chosen for comparative studies. When oysters with high and low initial infections of *Nematopsis* were transplanted into areas of low and high infestation respectively, the transplants attained the characteristic level of infections of native oysters in that area. Presumably a dynamic equilibrium of elimination and reinfection of the parasite was reached. Spores discharged from living oysters may infect crabs. -- S. Y. Feng.

INTRODUCTION

Nematopsis ostrearum, a sporozoan parasite of oysters, was first described by Prytherch (1938). Sprague (1950) emended the description. The known definitive hosts for *N. ostrearum* are three species of xanthid crabs, *Panopeus herbstii*, *Eurypanopeus depressus*, and *Eurytium limosum*, according to Prytherch (1940) and Sprague (1950). The most commonly recognized stages in crabs are mobile sporadins or gregarines and spherical gametocysts which produce gymnospores. Only vegetative spores or sporozites are found in oysters. These spores occur most abundantly in mantle tissues, but also in adductor muscle, heart, gills, labial palps, and perhaps other organs (Prytherch 1940, Sprague and Orr 1955). In this paper the peculiar distribution of cysts in the mantle margin and evidence of discharge of spores by oysters are investigated.

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*Contributions from the Virginia Fisheries Laboratory, No. 88. Part of a thesis submitted in partial fulfillment for the degree of Master of Arts, College of William and Mary, September, 1957.

**Author's current address is Department of Zoology, Rutgers University, New Brunswick, N. J.

Reprinted from PROCEEDINGS OF THE NATIONAL SHELLFISHERIES ASSOCIATION, Volume 48, 1958

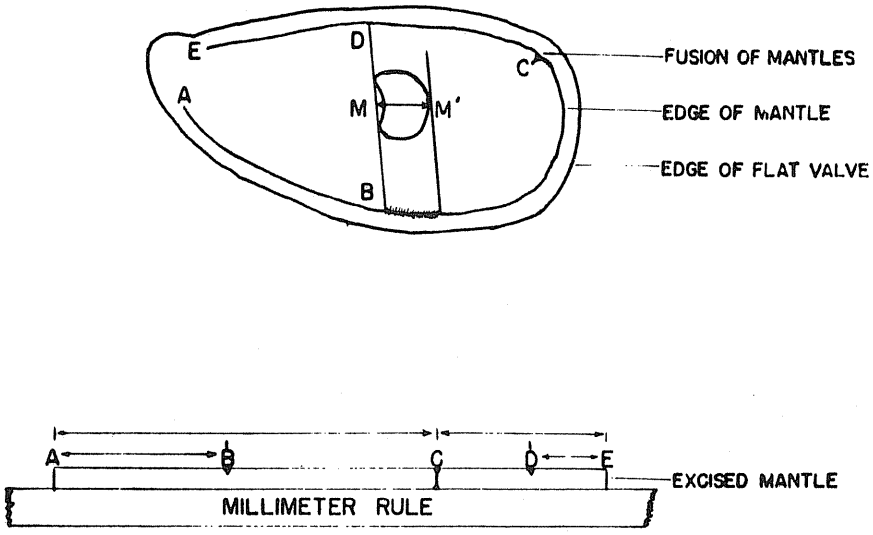

Fig. 1. To determine distribution of cysts, the edge of the mantle was excised, measured, and subdivisions designated by letters. In three oysters, cysts were counted in a continuous band on the entire excised strip (Fig. 2) but in later groups carefully spaced samples were taken. In subsequent discussions and figures, data are arranged in sequence A to C (ventral mantle) and C to E (dorsal mantle). The shaded area beneath the adductor muscle was selected as a sampling site for other studies.

RESULTS

Distribution of Nematopsis ostrearum Cysts in Mantles of Oysters

Sprague discovered that in mantles of oysters, N. ostrearum cysts occurred predominantly in a band two mm wide, near and parallel to the margin. He also reported uniform distribution of cysts within the band, but this conclusion presumably was based upon examination of short sections of the mantle margin. In June and August, 1956, the author counted cysts in the band along the entire mantle margins of three oysters. A strip of tissue five mm wide was excised from the margin of the mantle, and lengths of ventral (A to C) and dorsal (C to E) mantles were measured as shown in Figure 1. The counts, from 305 to 393 microscopic fields of one mm^2 each, were grouped by zones, each representing 10 per cent of the mantle margin (Figure 2). Great variations in counts were noted but typically there were many cysts opposite the adductor muscle and few cysts at the anterior and posterior ends of the mantles.

The burden of counting the entire mantle margin in heavily-infected oysters led to refinements of technique and the choice of oysters from an area of relatively light infections for further study of the pattern of distribution of cysts. On October 4, 1956, 20 oysters were collected from Hoghouse Bar in the Rappahannock River and opened by removing their cupped valves. In each oyster, incisions were made in the mantle of the flat valve at B and D to mark the position of the adductor muscle (Figure 1); the strip of tissue was excised, and the subdivisions were measured. Instead of counting cysts in the whole margin, the strips were divided into 10 equal sections. An 8 by 5 mm sample of tissue was taken from the center of each section and treated with 10 per cent KOH solution for one minute. The partially-digested tissue was placed between two slides and compressed until it attained a length of 10 to 12 mm. The preparation was examined at 100 X magnification under a microscope equipped with an ocular Whipple cell. The intensity of infection in each section was determined from the mean number of cysts per mm^2 calculated from the count in 10 fields. Each point in Figure 3 represents the mean value for 20 oysters.

Counts of 20 oysters confirmed the pattern observed in the first three oysters (Figure 3). The lowest numbers of cysts were observed at the anterior (oral) ends of the dorsal and ventral mantles. The number increased rapidly from the anterior ends of both mantles towards the adductor muscle. The greatest concentration of cysts occurred immediately above the adductor muscle in the dorsal mantle and slightly posterior to it in the ventral mantle. A decrease in the number of cysts was found posteriorly in the region of the pallio-branchial fusion.

This general pattern is usually shown by individual oysters including those with low counts but seems to be accentuated in those

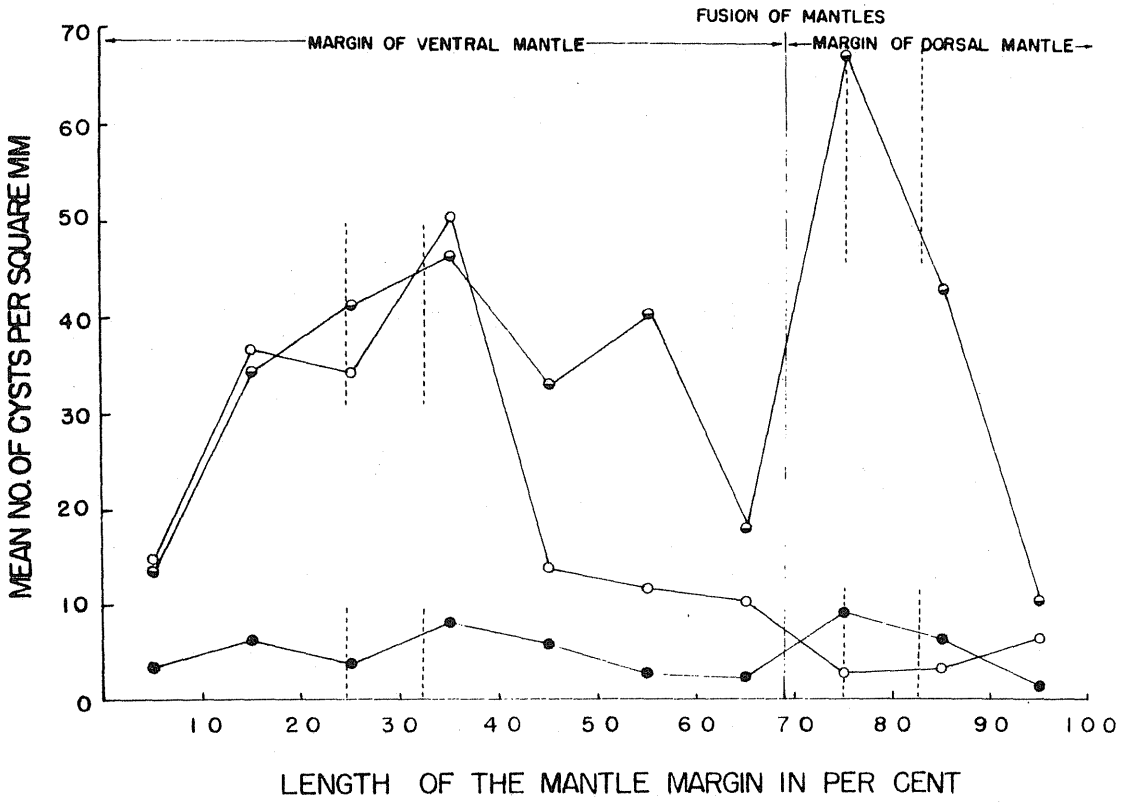

Fig. 2. Variation in individual oysters in distribution of Nematopsis ostrearum along margin of flat-valve mantle. Each line represents counts made along the entire mantle margin of a single oyster. Areas enclosed by the two sets of broken lines mark position of adductor muscle.

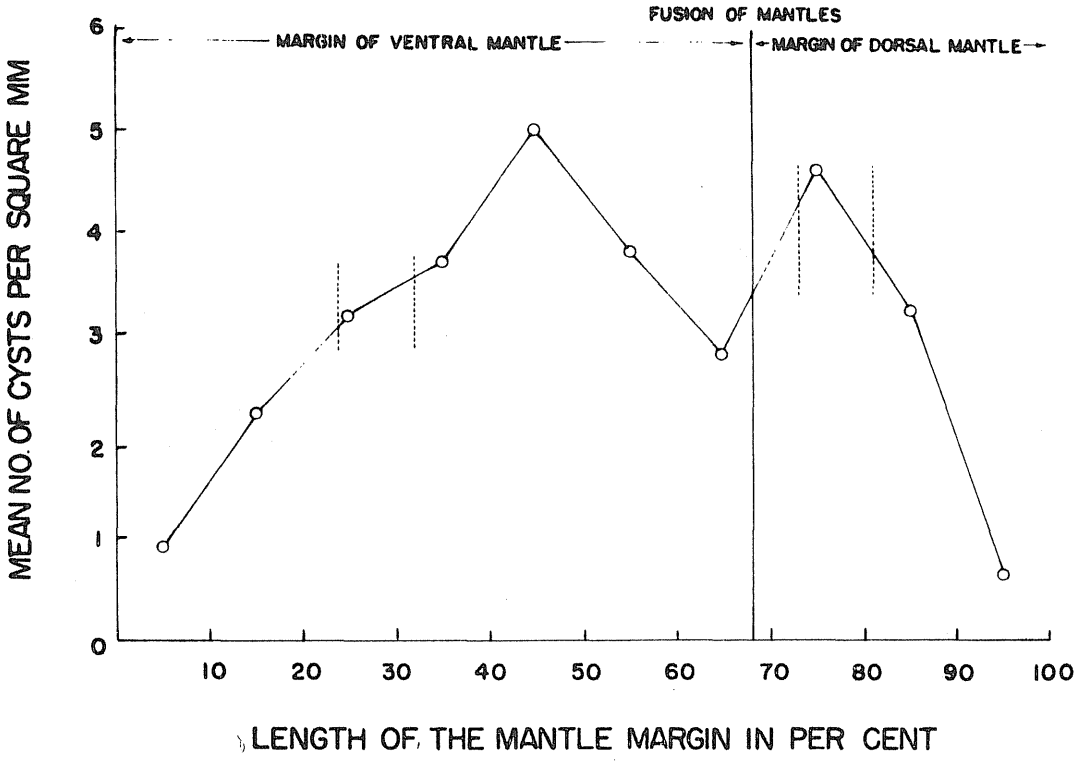

Fig. 3. Mean distribution of Nematopsis ostrearum in margin of flat-valve mantle of oysters, October 1956. The mean count of 20 oysters is plotted. The area enclosed by the two sets of broken lines marks position of the adductor muscle.

with high counts. In any one oyster the highest concentration of spores may occur in either the dorsal or ventral mantles.

Elimination or Disappearance of N. ostrearum Cysts from Mantle Margins of Oysters

Controversial opinions concerning the nature of Nematopsis infections in oysters have been found in the literature. Prytherch (1940), Lanadau and Galtsoff (1951), and Sprague and Orr (1955) note that spores were more abundant in older oysters and, therefore, believe that Nematopsis was cumulative in oysters. The occurrence of spores in walled cysts probably encouraged this viewpoint. Owen et al. (1951) were of the opinion that oysters eliminate spores because some old oysters comparatively free of spores were encountered. Prytherch (1940) estimated that the sporozoites suffered a better than 50 per cent mortality from phagocytosis before they reached the final stage in oysters. Stauber (1950) noted, "It is possible that in addition to the mortality mentioned above, there is an important sporozite loss due not to intracellular digestion by phagocytes but to emigration and elimination of the sporozoite-laden phagocytes" (p. 239). In the present studies, field experiments were carried out to determine whether oysters did retain spores once they were encysted in the tissues.

Experiment I

On May 2, 1956, three stations, representing the full range of salinities in the oyster-growing areas of the James River-Hampton Roads system, were selected for holding oysters in trays. The lower station was Darling's Watchhouse (J4)* in the relatively high-salinity (20 parts per thousand) waters of Hampton Roads; the intermediate station was Miles' Watchhouse (J12) with salinities of 16 ppt; and the upper station at Deep Water Shoal (J24) had mean salinities of about 7 ppt. Two groups of oysters were collected for the experiment -- one from J4 where infections of Nematopsis were moderately heavy (21 cysts per mm^2) and the other from J 24 where infections averaged 0.1 cysts per mm^2. At each of the three stations, oysters from the two sources were placed in separate compartments of a tray which was set on the bottom. On September 4, 1956, a sample of 25 oysters was examined from each lot at the three stations.

After a period of four months, oysters with high initial infections showed slight increases in number of cysts at J4 and J12, and a significant decrease at J24(Figure 4). Oysters with low initial infections had a marked increase in number of cysts at the lower stations, but no increase at J24.

*J4 indicates distance in nautical miles above the mouth of the James River.

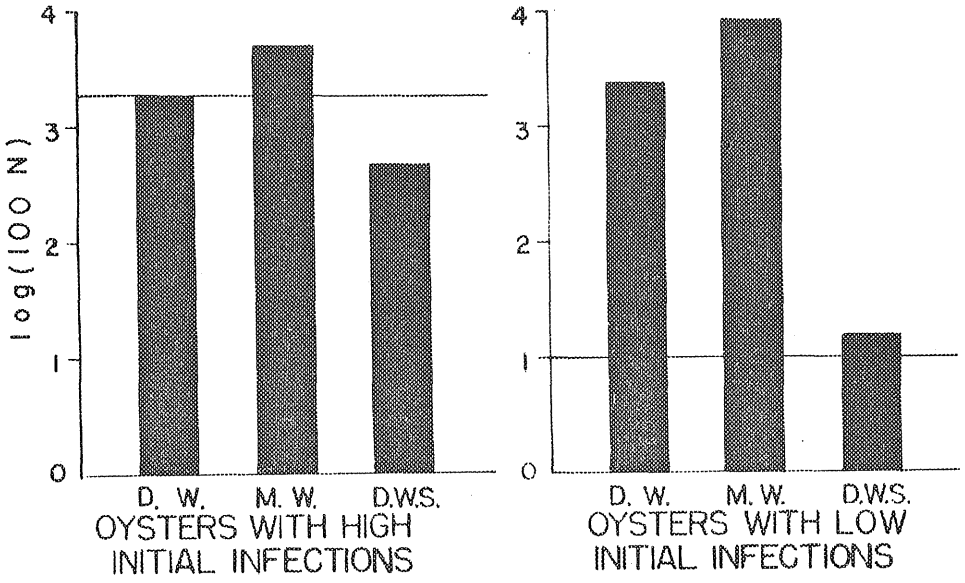

Fig. 4. Changes in level of Nematopsis infections when oysters were moved to areas characterized by lower and higher intensities of parasites. At each station (D.W., M.W., and D.W.S. representing Darling's Watchhouse, Miles Watchhouse, and Deep Water Shoal Lighthouse respectively), oysters with low and high initial infections (represented by horizontal lines) were placed in opposite ends of a tray on the bottom. The levels of infections four months later (September 4) are denoted by vertical bars. N is the mean number of cysts per mm^2.

At each station it appears that a characteristic level of infection was reached in a relatively short time. If cysts do accumulate in tissues, the numbers in oysters at J4 and J12 should have increased as much in heavily-infected individuals as in lightly-infected ones. At J24, oysters with heavy initial infections showed a decline in number of cysts from 21 to 6 cysts per mm^2, and this can be explained only by removal of cysts from the mantle. This decrease occurred during the warm summer season when spores are probably most abundant. Little is known of seasonal variations in infections and there is a possibility that spores may be redistributed in the oyster. However, this experiment strongly suggests that oysters can destroy spores or remove them from their tissues. Unfortunately the trays were lost after September and subsequent changes could not be followed.

Experiment II

The hypothesis that oysters eliminate or discharge cysts of Nematopsis is further supported by observations made on oysters in suspended trays. On June 1, 1955, oysters collected from Hoghouse Bar in the Rappahannock River, an area of relatively low level of infection, were placed in Trays 56, 57 and 59 suspended at Gloucester Point in the York River. These oysters had been fully acclimated during an 8- to 12-month period at Gloucester Point where both salinities and level of Nematopsis infections in oysters on natural grounds are high. On January 6, and June 5, 1956, respectively, Trays 56 and 59 were moved to the Fleet Pier near J24 in the James River. In this low-salinity area the level of Nematopsis infections in oysters is low. Tray 57 was retained at Gloucester Point and Tray 67 containing native Deep Water Shoal (J24) oysters was suspended at the Fleet Pier in the James; these two trays served as control groups for high- and low-incidence areas. On September 23 and October 27, 1956, samples of 25 oysters from each tray were examined and the results are given in Table 1.

The mean number of cysts in oysters decreased significantly in all trays with the exception of Tray 57 which was retained in a high-intensity area (Table 1 χ^2 values). Since all trays were suspended off the bottom, most mud crabs were kept out and this may explain the relatively low intensity of Nematopsis infections at Gloucester Point. It must be remembered also that the experimental groups were moved to the low-salinity area several months before these examinations were made. The experiment was designed for purposes other than Nematopsis studies.

Level of Nematopsis Infections and Distribution of Mud Crabs

Surveys of James, York and Rappahannock Rivers revealed that species of crabs known to be hosts for Nematopsis were scarce in low-salinity (under 15 ppt) waters. Low incidence of Nematopsis at J24

Table 1. Decrease in number of spores of N. ostrearum in oysters suspended in trays between September 23 and October 27, 1956.

Source of oysters		Tray no.	New location	No. of cysts[1] per mm^2		χ^2	d.f.	P
				Sep.23	Oct.27			
High-intensity area (Gloucester Point)	Experimental	56	James River Fleet Pier	5.7	2.9	24.109	2	0.001
		59		10.7	2.1	19.474	2	0.001
	Control	57	Remained at Gloucester Point	5.2	4.6	9.384	5	0.10
Low-intensity area (Deep Water Shoals)	Control	67	Remained at James River Fleet Pier near Deep Water Shoal	0.14[2]	0.07	5.997	1	0.02

[1] Average number of cysts in samples of 25 oysters.

[2] Based upon 24 oysters.

can probably be attributed to paucity of mud crabs and low death rate among oysters. The high-level of infection at J4 may have been accentuated by abundance of mud crabs around the watchhouse which had unusually favorable habitat niches. Furthermore, the death rate of oysters--from causes other than Nematopsis--is high at J4.

DISCUSSION

The non-random distribution of cysts in the oyster mantle reveals that standard counting techniques and sampling methods are essential; it also suggests that samples must be taken from various parts of the mantle margin in order to estimate accurately the total number of cysts in a particular oyster. For most purposes, however, it should not be necessary to make counts from numerous parts of the mantle, for by sampling carefully in a chosen site, comparisons between oysters and locations can be made. The shaded area of the left ventral mantle (Figure 1) where infections approach a maximum, is suggested as a sampling site.

The question arises: What are the causes of this peculiar distribution? Hopkins (1934, 1936) first discovered the accessory heart in oysters, observed much surging back and forth of blood in the circumpallial arteries, and advanced the theory that the circumpallial arteries received both arterial and venous blood. Stauber (unpublished data, 1957) has clearly demonstrated the pattern of blood circulation in the mantle margin by injection of green vegetable dye into the ventricle, which supplies the anterior circumpallial network, and red dye into the accessory heart which feeds the posterior circumpallial arteries at the fusion of left and right mantles; the two colors were found to meet in dorsal and ventral mantles in areas where the highest counts of cysts were observed. This suggests that distribution of N. ostrearum cysts in the mantle is probably associated with the pattern of circulation. It is possible that cysts tend to accumulate where blood flow is slowest. There are indications, however, that localization of cysts can not be explained, solely on the basis of mechanical transport; the special affinity of cysts for gill tissue in N. prytherchi and for mantle tissue in N. ostrearum suggests specific physiological requirements.

Seasonal patterns of infection, about which much more information is needed, are now of great importance. For example, if oysters are continually discharging spores which reinfect crabs, there is no need for an oyster to die to complete the cycle, and the incidence and intensities of infections may not be as closely related to the pattern of mortalities of oysters as previously suspected. Furthermore, the whole cycle may be speeded up considerably. It is already evident that Nematopsis infections are more common and the number of spores produced by crabs is much greater than had been assumed under the theory of accumulation of spores in the oysters. Stauber (1950, p. 239) conjectured that, "unless the hypertrophy of the sporozoite-

infected phagocyte restricts amoeboid activity, it is possible that elimination of developed spores in this fashion may constitute the more normal route of infection for crab hosts". The finding of substantial Nematopsis infections in James River, where deaths of oysters are few, tends to support this supposition. Viability of discharged spores could be easily tested by placing starved mud crabs (free of gregarines) in closed aquaria with live oysters heavily-infected with Nematopsis. If crabs become infected, it would prove that some spores are discharged; if not, the problem of disposition of spores would remain unsettled.

The exact mechanisms by which oysters remove spores is still a mystery; however, Stauber (1950) and Tripp (1957) have demonstrated the rates and methods by which india ink and metabolizable vertebrate red blood cells are digested or eliminated by phagocytes. Presumably Nematopsis is eliminated by similar activities. Probably temperature plays an important role in regulating the rate.

Many phases of the ecology of this parasite are still unsolved, though it now appears that cysts, which have been reported to be non-toxic, are even less harmful to oysters than was previously supposed--as indicated by the ability to discharge them and thereby prevent massive accumulations over long periods of time.

SUMMARY AND CONCLUSIONS

Distribution of Nematopsis ostrearum along mantle margins of oysters was non-random. Lowest numbers of cysts were observed at the anterior ends of dorsal and ventral mantles. Numbers increased posteriorly toward the adductor muscle. The greatest concentration of cysts occurred immediately above the adductor muscle in the dorsal mantle and slightly posterior to it in the ventral mantle. A decrease in number of cysts was found posteriorly in the region of pallio-branchial fusion. Techniques for estimating number of cysts in the mantle margin of oysters and the possible significance of this unusual distribution of cysts are discussed. A standard sampling site on the mantle is suggested for estimating levels of infection.

Evidence for the hypothesis that oysters eliminate or discharge encysted spores of Nematopsis ostrearum has been presented. It is possible that spores discharged from living oysters may infect crabs. Seasonal patterns of infection, mechanisms of discharge of spores, and toxicity of this parasite to oysters have not yet been adequately studied.

Literature Cited

Feng, Sung Yen. 1957. Ecological and epidemiological studies of Nematopsis ostrearum, a sporozoan parasite of the oyster Crassostrea virginica, in lower Chesapeake Bay and its tributaries. Unpublished Thesis for M.A., College of William and Mary, Williamsburg, Va.

Hopkins, A. E. 1934. Accessory heart in the oyster, Ostrea gigas. Biol. Bull. 67: 346-335.

_____. 1936. Pulsating blood vessels in the oyster. Sci. 83: 581.

Landau, H. and Paul S. Galtsoff. 1951. Distribution of Nematopsis infection on the oyster grounds of the Chesapeake Bay and in other waters of the Atlantic and Gulf States. Texas Jour. Sci. 1(3): 115-130.

Owen, H. M., L. L. Walters, and L. A. Bregan. 1951. Etiological studies on oyster mortality I. Nematopsis ostrearum Prytherch, 1940 (Sporozoa: Porosporidae). Jour. Mar. Res. 10: 82-90.

Prytherch, H. F. 1938. Life cycle of a sporozoan parasite of the oyster. Sci. 88: 451-452.

_____. 1940. The life cycle and morphology of Nematopsis ostrearum sp. nov., a gregarine parasite of the mud crabs and oysters. Jour. Morph. 66: 39-65.

Snedecor, George W. 1956. Statistical Methods. Fifth ed. The Iowa State College Press. Ames, Iowa. 534 pp.

Sprague, V. 1950. Studies on Nematopsis prytherchi Sprague and N. ostrearum Prytherch, emended. Texas A & M Research Foundation. 59 pp., (Mimeo.).

Sprague, V. and P. E. Orr, Jr. 1955. Nematopsis ostrearum and N. prytherchi (Eugregarinina: Porosporidae) with reference to the host-parasite relations. Jour. Parasit. 41: 89-104.

Stauber, Leslie A. 1950. The fate of india ink injected intracardially into the oyster, Ostrea virginica Gmelin. Biol. Bull. 98: 227-241.

_____. 1957. Unpublished data on the circulatory system of the oyster. Dept. of Zoology, Rutgers Univ.

Tripp, M. R. 1957. Disposal by oysters of intracardially injected red blood cells of vertebrates. Proc. Natl. Shellfish. Assoc. 48:143-147.