THE DISTRIBUTION AND RELATIVE ABUNDANCE OF *NEMATOPSIS* SPP., AS FOUND IN *CRASSOSTREA VIRGINICA* (GMELIN) IN THE GALVESTON BAY AREA

A Thesis

by

ROGER DEAN ANDERSON

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Approved as to style and content by:

(Chairman of Committee)

(Head of Department)

(Member)

(Member)

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ABSTRACT

The Distribution and Relative Abundance of *Nematopsis* spp., as Found in *Crassostrea virginica* (Gmelin) in the Galveston Bay Area. (May 1969)

Roger D. Anderson, B.A., Saint Olaf College

Directed by: Dr. J. G. Mackin

Nematopsis ostrearum and *Nematopsis prytherchi* were found in the tissues of *Crassostrea virginica*. A third species, of unknown significance, was observed in small numbers. The two known species were found at each of the 16 sampling sites, the third species occurred at three locations. Of the 400 oysters studied, 99% were infected by *N. ostrearum*, 89% were infected by *N. prytherchi*, and 3% were infected by *N.* sp. Wide differences in levels of infection were found in the samples. This was attributed to differences in proximity of host decapods and to seasonality. Infection experiments were conducted to confirm *Eurypanopeus depressus* and *Panopeus herbstii* as hosts of *N. ostrearum*, and *Menippe mercenaria* as a host of *N. prytherchi*.

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I am indebted to Mr. Robert Hofstetter of the Texas Parks and Wildlife Department for his aid throughout this study. I would also like to thank Mr. Harry Cook of the Bureau of Commercial Fisheries, Galveston, for supplying a particular group of specimens used in this study.

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The main body of this study was carried out at the Texas A&M Marine Laboratory, Galveston, Texas. I would like to thank the entire staff of the Laboratory for their generous assistance.

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INTRODUCTION

This study was concerned with those species of Nematopsis which are found in the American oyster, Crassostrea virginica (Gmelin), in the Galveston Bay area. Though two species are widely recognized from Texas waters, they have been little studied.

It was the purpose of this investigation to observe tissue preferences in the molluscan hosts, to observe levels of infection, to note distribution in relation to salinity and other parameters, to investigate possible seasonal variations, to evaluate intensities of infection in relation to crustacean populations, and to note ecological parameters which may pertain to distribution of species.

It was the intention of this study to develop as much information bearing on such population and ecological problems as possible and to determine whether or not other, as yet unreported, species occur in the oyster host.

The citations on the following pages follow the style of The Journal of Parasitology.

REVIEW OF THE LITERATURE

The Porosporidae are classified in the subphylum Sporozoa, class Telosporea, order Eugregarinida, and suborder Cephalina (Honigberg et al., 1964). There is an absence of schizogony and the trophozoites are septate. The Cephalina consists of 14 families which are parasites of annelid and arthropod alimentary tracts (Honigberg et al., 1964).

Unlike other gregarines, the life cycle of the Porosporidae involves an alternation of hosts between decapod crustacea and various molluscs. As either naked or encapsulated sporozoites, these sporozoans will develop into typical cephaline gregarines in the stomach or mid-gut of specific crustacean hosts (Kudo, 1966). There are only two known genera in this family: Porospora Schneider, 1875, and Nematopsis Schneider, 1892. Schneider originally described the genus Porospora as spores with perforated walls found in Mytilus minimus and Trochocochlea mutabilis (Kudo, 1966). However, in a more detailed study, Schneider (1887) found that these were naked sporozoites wrapped in tight radial patterns which resembled encapsulated spores. The genus Nematopsis was later discovered in the razor clam, Solen vagina (Schneider, 1892). Schneider was accurate in his classification of Nematopsis as a

sporozoan, though he did not recognize the genus as being of a gregarine nature. The generic name refers to the nematode-like sporozoites which are coiled within the resistant spore.

The Porosporidae is then composed of two genera: Porospora, a monotypic genus, and Nematopsis which is polytypic (Kudo, 1966). Léger and Duboscq (1913a, 1913b) were the first to realize that the spores in molluscs and the gregarines in decapods were actually alternate stages in the life cycle of the same porosporid. Continued study led them to believe that they were actually working with only one genus, Porospora, rather than different genera of the same family (Léger and Duboscq, 1925). The issue was resolved by Hatt (1928) who conducted infection experiments which yielded both types of sporozoites, naked (Porospora) and encapsulated (Nematopsis).

Prytherch (1938, 1940) identified the first porosporid in America, Nematopsis ostrearum, which he found in mantle, gill and muscle tissue of the American oyster, Crassostrea virginica, and in the digestive tracts of the mud crabs, Panopeus herbstii and Eurypanopeus depressus. He reported infection of oysters ranging from Mobjack Bay, Virginia, to Lake Barre, Louisiana. After a variety of field and laboratory experiments, he suggested that Nematopsis was a factor in oyster mortality. His hypothesis created immediate interest in the Porosporidae because of unusually high oyster mortality in

periods of high temperature and salinity along the Atlantic and Gulf coasts.

In 1950, Sprague described a new species, Nematopsis prytherchi. A number of significant morphological differences led him to the conclusion that Prytherch had originally confused the large spores in the gills with the smaller spores commonly found along the mantle margin. Infection experiments showed that N. prytherchi developed in the stone crab, Menippe mercenaria, while N. ostrearum infected P. herbstii, E. depressus and Eurytium limosum (Sprague, 1950; Sprague and Orr, 1955). Studies of N. ostrearum, though not confirming Prytherch's hypothesis of oyster mortality, did reveal the widespread occurrence of these sporozoans in Atlantic and Gulf states (Landau and Galtsoff, 1951; Owen, Walters and Bregan, 1951). Feng (1957) extended the knowledge of N. ostrearum when he conducted ecological and epidemiological studies in Chesapeake Bay and its tributaries. He reported the presence of gregarines in the mud crabs Rhithropanopeus harrisii and Neopanope texana sayi. However, he questioned whether R. harrisii was a normal host. Kenk (1967) confirmed N. texana sayi as a host by means of infection experiments.

Sprague (1950) experimentally tested the pathogenicity of N. ostrearum in the host oyster with inconclusive results. Thus,

he attributed deaths of oysters to an unknown factor. This was clarified when Mackin, Owen and Collier (1950) described Dermocystidium marinum, now known as Labyrinthomyxa marina (Mackin, Owen and Collier) Mackin and Ray (1966). This organism has been convincingly shown to play a major role in the loss of condition and death of oysters (Mackin et al., 1950; Mackin, 1953; Ray, 1954a, 1954b, 1954c). It is now known that L. marina can be found at both Beaufort, North Carolina, and at Grand Isle, Louisiana, the respective study areas of Prytherch and Sprague (Mackin, 1962).

Feng (1957), working with oysters free of L. marina, conducted N. ostrearum infection experiments similar to those of Sprague (1950). He was able to produce extremely high levels of infection, far greater than any known in nature. However, his statistical analysis revealed that no direct correlation could be drawn between heavy infection and the death of oysters. Roberts (1948) examined gapers and survivors in a laboratory experiment and found no difference in N. ostrearum infection. Mackin (1962) examined thousands of oyster sections, but never found levels of infection which approached the figures reported by Prytherch (1940). Mackin found "no significant histopathologies . . . in association with the spore cysts" (1962, p. 205). He further reported lack of evidence for any toxin production by the spores, as well as no tissue reaction to Nematopsis

spores. The pathology of N. ostrearum and N. prytherchi has not been studied in detail, except for Mackin's observations (Cheng, 1967).

Most workers have immediately noted that Nematopsis spores appear to be engulfed by phagocytes. Generally referred to as cysts, the infected cells are found throughout the oyster. Prytherch (1940), Landau and Galtsoff (1951), Sprague and Orr (1955), and Feng (1957) have all noted the generally high concentrations of cysts in older oysters. However, Prytherch (1940) and Owen et al. (1951) indicated that oysters can apparently clear their tissue because they found oysters with reduced levels of infection.

Stauber (1950) and Tripp (1957) found that phagocytes can apparently digest or eliminate foreign material. Working with India ink, Stauber (1950) concluded that the oyster can apparently eliminate sporozoites by migration and elimination of sporozoite-laden phagocytes, rather than by intracellular digestion of the foreign matter. Feng (1957) was able to demonstrate this with oysters infected with N. ostrearum. He transplanted oysters with high and low levels of infection to areas of, respectively, low and high infections. Within a few months, he found that the transplanted oysters had attained levels of infection characteristic of the native

oysters of that area. Therefore, he reported a dynamic equilibrium created by elimination of cysts and reinfection by crabs.

Kruse (1966) worked out the life cycle for another American species, Nematopsis duorari. The pink shrimp, Penaeus duorarum, was found to be the crustacean host and the intermediate molluscan hosts were Aequipecten irradians, Cardita floridana, Chione cancellata and Macrocallista nimbosa. Kruse conducted a variety of infection experiments to determine how shrimp became infected so easily. Whereas the xanthid crabs are known as predators of pelecypods, the same cannot be said of the omnivorous shrimp. He found that the shrimp were infected by spores shed by host pelecypods. Aequipecten irradians was found to be the major cause of infection. Kruse observed its active nature and resulting exposure of tissue to sea water, as well as the large amounts of fecal material expelled. Microscopic examination of the mucoid fecal matter revealed cysts which had been eliminated from the host. These strings of waste material were shown to be effective means of infection, as shrimp became infected after subsequent feeding.

Stauber (1950) and Feng (1957) did not show that the discharged spores of oysters were able to infect crabs, but both indicated that this could be important.

Though the porosporids have received attention as parasites of possible economic importance, there has been a distinct lack of ecological study. The literature reflects some ecological observations pertaining to N. ostrearum, but N. prytherchi has not been studied.

Landau and Galtsoff (1951) found no definite pattern of infection, but they did note low levels of infection in correspondingly low salinity areas. Owen et al. (1951, p. 85) found "no correlation of N. ostrearum infection with salinity, dissolved oxygen, and hydrogen-ion concentration of the water." However, they did find that summer levels of intensities were considerably higher than those of the cooler months. Though not mentioned in detail, similar results were reported by Landau and Galtsoff (1951, p. 120) in oysters checked both in winter and summer months. Unfortunately, these studies were often complicated by oyster mortality and were generally limited in scope.

Feng (1957) made the first concerted effort to relate ecological factors to levels of intensity. He felt that "the death rates of oysters, the rate of discharge of spores from live oysters, and the presence of suitable crab hosts," constituted the main factors which govern the level of infection of Nematopsis (1957, p. 34).

Feng (1957) found gregarines in the four xanthid crabs which he studied in the tributaries of Chesapeake Bay. Examining E. depressus, N. texana sayi, P. herbstii and R. harrisii, he observed that the incidence of gregarines in the alimentary tract of the crabs varied directly with the size of the decapod populations. Large populations had correspondingly high levels of gregarine infection and vice versa. Since this study was in Chesapeake Bay, there was no reference to Menippe mercenaria which is not known in those waters. He found, as did Landau and Galtsoff (1951), that in approaching a fresh-water source, and thereby low salinity, the level of infection became increasingly smaller. This was presumably a result of the reduced decapod host populations.

Feng (1957) pointed to the importance of temperature in relation to the life cycle of Nematopsis. Experimental infections have indicated that temperature is important in the gregarine's development in the crab host (Prytherch, 1940; Sprague, 1950). Owen et al. (1951) reported highest levels of infection in summer months. Prytherch (1938, 1940) and Sprague (1950) conducted much of their life cycle work during the summer and refer to high temperatures and relatively short life cycles.

Suitable crab hosts, however, appear to be the most important ecological factor (Feng, 1957). Suspended trays, pilings, low

salinity waters, and similar oyster habitats with minimal decapod exposure, revealed low levels of Nematopsis infection.

Kruse (1966) redescribed the Porosporidae so that it now includes Porospora Schneider (1892) with one species, P. gigantea (van Beneden, 1869), and Nematopsis with nine species. These are N. portunidarum (Frenzel, 1885), N. maraisi (Léger and Duboscq, 1911), N. nephropis (Léger and Duboscq, 1911), N. legeri (de Beauchamp, 1910), N. ostrearum (Prytherch, 1938), N. prytherchi (Sprague, 1949), N. panopei (Ball, 1951), N. penaeus (Sprague, 1954), and N. duorari (Kruse, 1966).

Of the nine known species, only N. ostrearum has a special affinity for mantle tissue in the molluscan host. However, Sprague (1954) has observed Nematopsis spores in the mantle of Modiolus demissus and Ensis minor and Feng (1957) found spores in the mantle of Anomia simplex. The original description of the genus was based on the occurrence of Nematopsis sp. occurring in the mantle of Solen vagina (Schneider, 1892).

In C. virginica, N. ostrearum has been found concentrated in a narrow 2 mm band that parallels the mantle margin (Sprague, 1950). The cysts have been found in greatest concentrations about the adductor muscle, possibly due to the limited circulation there (Feng, 1957). Sprague (1950) found N. ostrearum to occur in the

tentacles, as well as the adductor muscle, heart, gill and labial palps. He suggested that almost any organ might be infected, though little correlation was found between infections of different organs. There is not a set number of spores per cyst, but generally there are 2 to 5.

Spores of N. prytherchi have been found in labial palps, mantle, heart, adductor muscle, and among the liver tubules (Sprague, 1950). Usually, however, they are found in the posterior blood vessels of the gills. The known species of Nematopsis all have a special affinity for the gills except for N. ostrearum. A. simplex and M. demissus reveal Nematopsis spores in the mantle, but concentrations have been similarly recorded from their gill tissue (Sprague, 1954; Feng, 1957). Sprague (1950) noted that clumps of N. prytherchi seemed to occlude the posterior portion of the gill blood vessels, possibly impairing circulation. This was apparently caused by phagocytes that were distorted by the intracellular spores. The infected blood cells appear to attach to one another and to the vessel walls, further impairing normal circulation. Apparently, both physiological and circulatory considerations are to be considered in gregarine infection (Stauber, 1950; Tripp, 1957; Feng, 1957).

MATERIALS AND METHODS

Collection and Maintenance of Hosts

Oyster and crab collections were made from early summer through the fall of 1968. Sampling was conducted at irregular intervals due to the variety of collecting methods. Artificial reefs from Galveston and East Bays were dredged for oysters and crabs with the assistance of the Texas Parks and Wildlife Department. Trinity Bay was originally mentioned as a sampling area, but was eliminated because of fresh water conditions created by heavy rainfall and an influx of fresh water from the Trinity River. Natural reefs in West Bay were hand sampled. Other collections were made throughout the Galveston Bay area wherever oysters could be procured. One dozen stone crabs, M. mercenaria, were supplied by Mr. Harry Cook of the Bureau of Commercial Fisheries, Galveston.

Decapods and oysters were held in aerated glass aquaria. Running sea water was not used in this study, but the sea water was often changed to prevent fouling. The main body of the work was conducted at the Marine Laboratory of Texas A&M University, Galveston, Texas. Some of the investigation was done at Texas A&M University, College Station, Texas.

Infection Experiments

The various crabs (E. depressus, P. herbstii and M. mercenaria) were collected in the early part of the summer from mid-Galveston Bay and from the 8 Mile Road-Sportsman's Reef in West Bay. The different species were examined for gregarine infection by squeezing abdominal contents onto a slide. Lugol's solution and neutral red were used to stain live material. Decapods with advanced stages of gregarines were considered infected. The infected crabs were placed in aerated glass aquaria. There were approximately 25 crabs in the group of E. depressus, but only 15-20 in the other two lots. This resulted because of available numbers and size of Menippe and Panopeus. Oysters were collected from the holding tank of the Lagoon Laboratory, Bureau of Commercial Fisheries. These oysters were approximately 1 year old and were found to have a relatively low level of infection. Control and experimental aquaria held 12 oysters each. The control aquaria housed only oysters, while the experimental set-up included the specific species to be tested. After 2 months, the oysters were checked for infection levels.

Separate lots of crabs which had been starved for 14 days were checked for gregarines in their alimentary tracts. Those which appeared not to be infected were fed the meat of previously infected

oysters and tissue from oysters collected in areas of heavy Nematopsis infection. Decapods were fed irregularly, but in sufficient amounts to prevent damage to oysters often housed in the same aquaria. Specific tissue, mantle or gill, was sometimes fed, rather than whole oyster meats.

Another experiment using Menippe was started on 16 July when 12 stone crabs were received from Mr. Harry Cook of the Bureau of Commercial Fisheries, Galveston. These were young crabs, their carapace measuring only 14 mm in width, but they had been raised from eggs by Cook. Their diet, after larval development, had been exclusively shrimp. Since they had never been fed oyster meat, it was expected that these decapods would be relatively free of N. prytherchi. A careful examination of the digestive tract disclosed no advanced gregarines, but there was a question as to the presence of sporozoites, possibly from the shrimp.

On successive days, the crabs were fed heavily infected gill tissue from young oysters collected at the North Jetty. These oysters were found to be infected with N. prytherchi in preliminary studies.

Field experiments, similar to those of Feng (1957), were conducted to test the hypothesis of a dynamic equilibrium being established. Oysters were collected in mid-Galveston Bay (Redfish Reef) during the summer of 1968 and moved to new locations.

Sampling stations were established at the Switchover Platform and Seabrook boat basin, both maintained by the Texas Parks and Wildlife Department. At varied intervals, 12 oysters were examined for levels of N. ostrearum infection.

Examination of Parasites

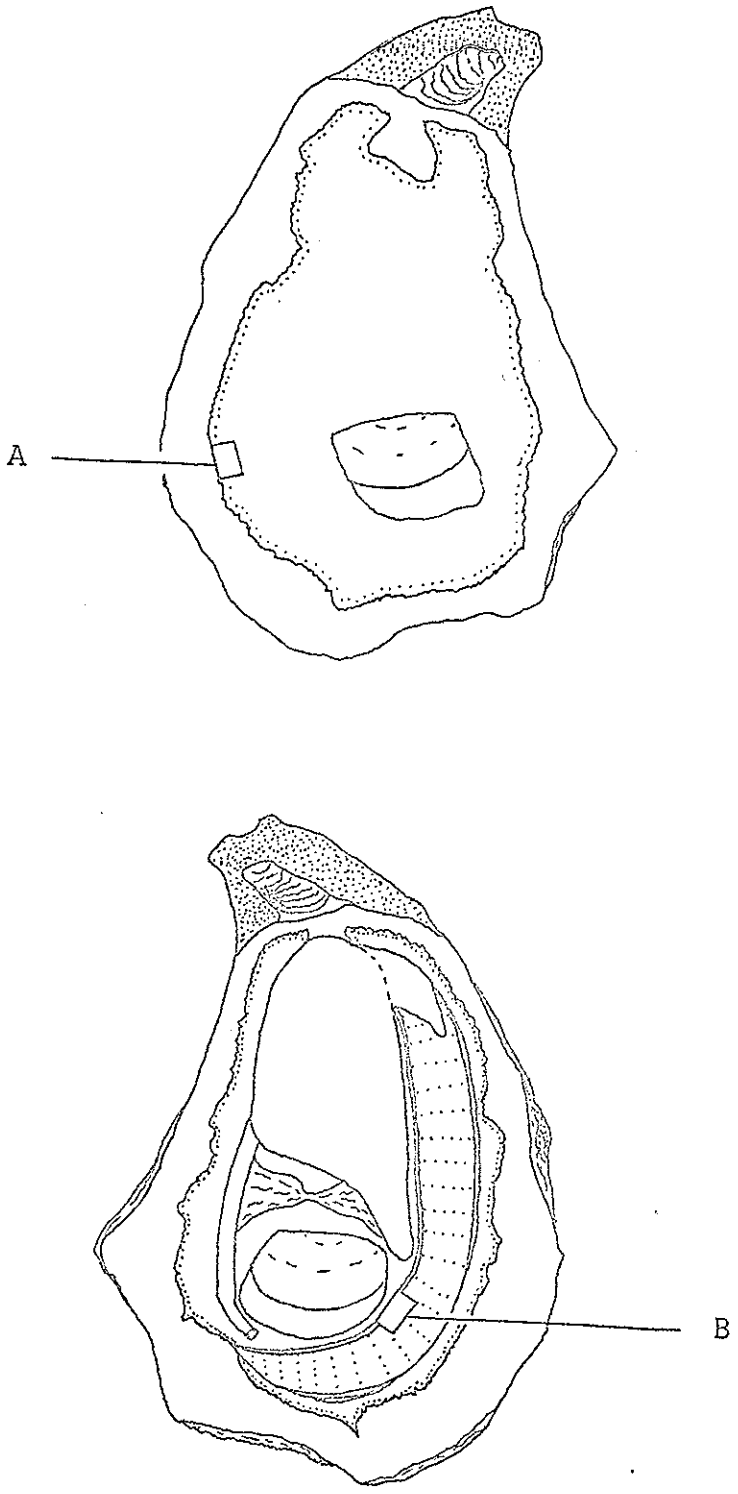
Each oyster in the study was examined for presence of Nematopsis spp. in the mantle and gill as shown in Figure 1. Section A was an 8 x 5 mm piece of mantle margin of the flat valve, while Section B was a similar section, but from the posterior portion of the right outer demibranch.

Oyster tissue was examined by using a modification of Feng's (1958) sampling technique. An 8 x 5 mm section of tissue was treated with 2 or 3 drops of 10% NaOH solution for 1 minute. Mantle tissue was removed from the flat valve of the left ventral adductor muscle region. Study of gill tissue was made by selecting tissue from the posterior portion of the right outer (lateral) demibranch adjacent to the adductor muscle. The partially digested tissue was placed between two slides and compressed until it attained an approximate size of 10 x 12 mm. Since three spore sizes were found in this study, similar tissues were examined under cover slips at high power to insure species identification. At 100X magnification, the field

FIGURE 1. Tissue sampling sites in Crassostrea virginica.

Section A - An 8 x 5 mm piece of flat valve mantle margin.

Section B - An 8 x 5 mm piece of posterior right outer demibranch.


A
B

within the cell of a Whipple ocular was 1 mm^2. Ten consecutive fields in a 2 mm band along the mantle margin or posterior demibranch were counted. The mean number of cysts per mm^2 from the 10 counts were determined and recorded.

Spores found in C. virginica fell into three size ranges. They were as follows:

1)	N. ostrearum	length:	12-16μ
		width:	9-11μ
2)	N. prytherchi	length:	15-20μ
		width:	9-11μ
3)	N. sp.	length:	10-12μ
		width:	7- 8μ

Two of the three forms listed are known species. The unidentified spores, N. sp., are tentatively considered to be a third species. Future study with this spore is intended, in order to determine its significance.

Crab hosts, which were sacrificed, were dissected and the alimentary tract was examined. Lugol's solution and neutral red were used to examine live tissue for presence of gregarines.

RESULTS

Nematopsis in Molluscan Hosts

Since there was a question as to which species of Nematopsis occur in the Galveston Bay area, preliminary studies of mollusks were made in June, 1968. Brachidontes recurvus and Mercenaria campechiensis revealed Nematopsis spores in their gills, while C. virginica was infected in both gill and mantle tissue. The spores in B. recurvus were approximately 12μ in length and 8μ wide, whereas those in M. campechiensis were much larger, being approximately 18μ long and 12μ wide (50 spore counts each). No other work was done with these two mollusks.

C. virginica was the only oyster found in the Galveston Bay area in the summer of 1968. The heavy influx of fresh water in the summer and spring eliminated Ostrea equestris (and Ostrea frons, if it ever occurred).

Three spore sizes were recognized in those oysters studied, two occurring in the gills and one in the mantle. The large spore in the gill tissue was identified as N. prytherchi, but the significance of the small spore was not established. The third spore, found in mantle tissue, was identified as N. ostrearum. The spores were phagocytized and distributed throughout the oyster. The greatest

concentrations of N. ostrearum were found about the adductor muscle, while N. prytherchi and N. sp. appeared to be randomly distributed throughout the posterior region of each demibranch.

Distribution of Nematopsis in the Galveston Bay Area

Sixteen major collections of 25 oysters per station were made (Fig. 2). N. ostrearum and N. prytherchi were noted at every station, while N. sp. was found in only three samples (Table 1). The sampling locations represented East, West and Galveston Bay. Live oysters were not found in Trinity Bay.

Percentage of the Population Infected

Each oyster in a sample was examined for infection by the three spore types. The percentage of the host population infected with Nematopsis spp. is found in Table 2. The total population of 400 oysters was 99% infected with N. ostrearum, 89% infected with N. prytherchi, and 3% infected with N. sp.

Intensity of Infection

The intensity of infection was determined by counting cysts in mantle and gill tissue. A wide range of Nematopsis intensity was noted in the varied samples. Except for three lower bay stations,

FIGURE 2. Sampling locations in the Galveston Bay area.

1 - 8 Mile Road-Sportsman's Reef

2 - Roof Tank-Lagoon Laboratory

3 - Holding Tank-Lagoon Laboratory

4 - 6 Mile Road

5 - 8 Mile Road-Gillard's Reef

6 - Triangle Reef

7 - Missing Reef

8 - Seabrook Trays

9 - North Jetty

10 - Frenchy's Reef

11 - Moody's Reef

12 - Hanna Platform

13 - Switchover Platform

14 - Spoil Island #1

15 - Spoil Island #2

16 - Redfish-Missing Reef

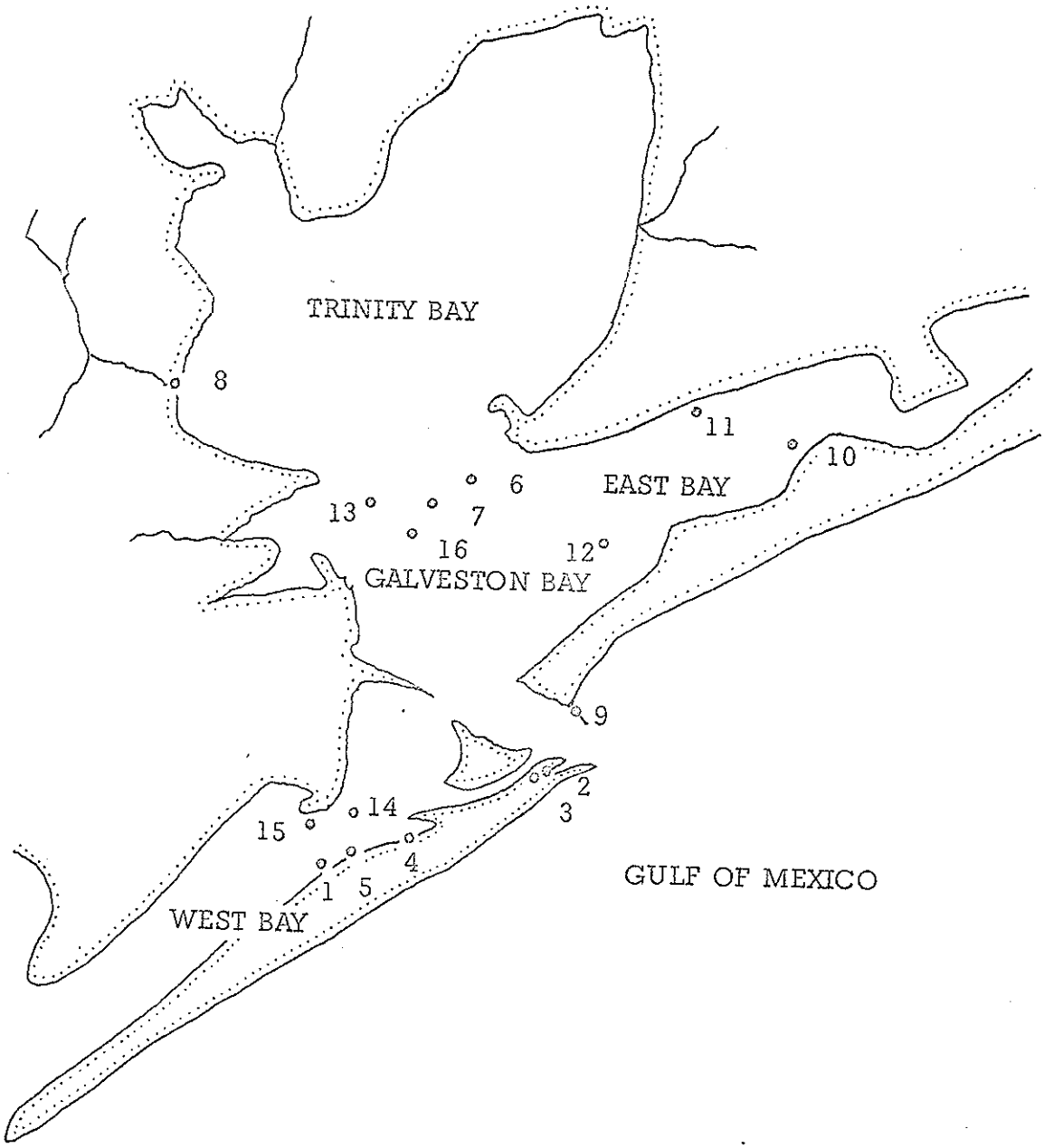

TRINITY BAY
8
11
10
6
EAST BAY
13
7
16
12
GALVESTON BAY
9
2
3
14
15
4
1
5
WEST BAY
GULF OF MEXICO

TABLE 1. Occurrence of *Nematopsis* spp. at oyster sampling stations in the Galveston Bay area.

Date of Collection	Station No.	Location	*N. ostrearum*	*N. prytherchi*	*N.* sp.
8 July	1	8 Mile Road-Sportsman's Reef	X	X	X
10 July	2	Roof Tank-Lagoon Laboratory	X	X	
10 July	3	Holding Tank-Lagoon Laboratory	X	X	X
22 July	4	6 Mile Road	X	X	
22 July	5	8 Mile Road-Gillard's Reef	X	X	
23 July	6	Triangle Reef	X	X	
23 July	7	Missing Reef	X	X	X
23 July	8	Seabrook Trays	X	X	
31 July	9	North Jetty	X	X	
1 August	10	Frenchy's Reef	X	X	
1 August	11	Moody's Reef	X	X	
1 August	12	Hanna Platform	X	X	

TABLE 1. (continued)

Date of Collection	Station No.	Location	*N. ostrearum*	*N. prytherchi*	*N. sp.*
1 August	13	Switchover Platform	X	X	
7 August	14	Spoil Island #1	X	X	
7 August	15	Spoil Island #2	X	X	
22 October	16	Redfish-Missing Reef	X	X	

TABLE 2. Percentage of oysters infected with Nematopsis spp.

Date of Collection	Location	Percent of Infection		
		N. ostrearum	N. prytherchi	N. sp.
8 July	8 Mile Road-Sportsman's Reef	100	100	28
10 July	Roof Tank-Lagoon Laboratory	100	52	0
10 July	Holding Tank-Lagoon Laboratory	100	96	24
22 July	6 Mile Road	100	96	0
22 July	8 Mile Road-Gillard's Reef	100	100	0
23 July	Triangle Reef	100	88	0
23 July	Missing Reef	100	80	4
23 July	Seabrook Trays	96	88	0
31 July	North Jetty	92	100	0
1 August	Frenchy's Reef	100	88	0
1 August	Moody's Reef	100	84	0
1 August	Hanna Platform	100	88	0

TABLE 2. (continued)

Date of Collection	Location	Percent of Infection		
		N. ostrearum	N. prytherchi	N. sp.
1 August	Switchover Platform	100	100	0
7 August	Spoil Island #1	100	100	0
7 August	Spoil Island #2	100	76	0
22 October	Redfish-Missing Reef	100	92	0
Total Percentage of Infection		99	89	3

the concentration of N. ostrearum noticeably exceeded that of N. prytherchi (Table 3). The temperature and salinity data which were collected at each station are recorded with the corresponding intensity levels. Though summer water temperatures were normal, the salinities were relatively low for the majority of samples.

Some difficulty was encountered when attempting to count cysts of N. prytherchi from the North Jetty (31 July) sample. Because of the crowding and clumping of cysts in the gill vessels, it was difficult to make an accurate count.

Discharge of Cysts by the Molluscan Host

Oysters which were collected at Redfish Reef (24 September) and moved to the Switchover Platform and Seabrook boat basin were irregularly checked for N. ostrearum intensity. Each count consisted of a 12 oyster sample.

The oysters kept at the Seabrook boat basin showed a noticeable drop in infection (Table 4). These oysters were in trays set on a mud substrate with few decapod hosts in the area. The oysters kept at the Switchover Platform showed little change between their level of infection and that of the natural reef (Redfish), both dropping in intensity levels during the cooler months. Both areas were

TABLE 3. Intensity of infection with reference to ecological parameters.

Date of Collection	Location	Sal. (‰)	Temp. (°C)	Intensity of Infection (cysts/mm^2)		
				N. ostrearum	N. prytherchi	N.sp.
8 July	8 Mile Road-Sportsman's Reef	6.6	28.4	11.4	4.6	0.5
10 July	Roof Tank-Lagoon Laboratory	6.0	28.6	0.2	0.8	-
10 July	Holding Tank-Lagoon Laboratory	6.0	26.4	0.7	1.6	0.6
22 July	6 Mile Road	6.5	29.0	8.4	3.1	-
22 July	8 Mile Road-Gillard's Reef	6.5	28.4	24.6	4.7	-
23 July	Triangle Reef	7.2	29.0	10.7	1.6	-
23 July	Missing Reef	7.8	29.0	13.6	4.2	2.2
23 July	Seabrook Trays	6.2	28.4	1.6	0.2	-
31 July	North Jetty	34.2	28.6	0.6	32.6[a]	-
1 August	Frenchy's Reef	2.8	28.8	13.6	1.2	-
1 August	Moody's Reef	2.2	29.5	21.0	1.6	-
1 August	Hanna Platform	2.2	29.8	3.6	0.8	-

TABLE 3. (continued)

Date of Collection	Location	Sal. (‰)	Temp. (°C)	Intensity of Infection (cysts/mm^2) N. ostrearum	N. prytherchi	N. sp.
1 August	Switchover Platform	7.8	30.6	17.4	4.2	-
7 August	Spoil Island #1	6.6	29.6	17.7	7.4	-
7 August	Spoil Island #2	6.6	29.6	8.6	3.6	-
22 October	Redfish-Missing Reef	17.2	26.1	12.2	3.8	-

[a]This is an approximate value, due to the difficulty in counting.

TABLE 4. Discharge of cysts by Crassostrea virginica.

Date	Seabrook Trays (cysts/mm^2)	Switchover Platform (cysts/mm^2)	Redfish Reef (cysts/mm^2)
24 September	14.1	13.6	13.8
22 October	12.2	-	-
13 December	10.6	-	-
8 January	11.1	-	12.1
6 February	4.6	-	-
6 March	3.2	8.6	9.4

exposed to relatively large populations of known hosts which are common in mid-Galveston Bay.

Infection Experiments

Oysters collected at Frenchy's and Moody's Reef were held in the laboratory and exposed to the xanthid crabs, E. depressus and P. herbstii. These oysters were known for their relatively high level of infection in nature. Other oysters collected from the same locations and at the same time, 1 August, were used in a Labyrinthomyxa spp. study. They served as controls.

At the conclusion of the experiment, all oysters in both studies were checked for levels of infection. The experimentally infected oysters showed an increase in intensity while the controls showed a slight decrease in number of cysts (Table 5a). All the oysters in this study were negative for Labyrinthomyxa spp. No significant loss of oysters was noted in either the experimental or the control group.

A second infection experiment dealing with the infection of C. virginica was also completed 24 September (Table 5b). E. depressus and P. herbstii were found to transmit N. ostrearum. P. herbstii appeared to transmit the parasite in greater numbers than the smaller E. depressus. M. mercenaria was shown to infect

TABLE 5a. Experimental infection of Crassostrea virginica.

Location	Intensity of N. ostrearum (cysts/mm^2)		
	1 August	24 September	
	All live oysters[a]	Live oysters	Oysters which died
Experimental:			
Frenchy's Reef	13.6 (16)	76.0 (12)	66.2 (4)
Moody's Reef	21.0 (13)	84.0 (10)	56.0 (3)
Control:			
Frenchy's Reef	13.6 (14)	13.0 (12)	14.5 (2)
Moody's Reef	21.0 (16)	18.6 (12)	20.1 (4)

[a]Figures in parentheses represent number of oysters examined.

TABLE 5b. Experimental infection of Crassostrea virginica.[a]

	Levels of Infection (cysts/mm^2)		
	E. depressus	P. herbstii	M. mercenaria
6 July:			
Control	0.2	0.2	0.8
Experimental	0.2	0.2	0.8
24 September:			
Control	0.2	0.2	0.7
Experimental	24.8	39.2	Enormous numbers[b]

[a] Levels of infection were determined by counting cysts in mantle tissue of oysters infected by E. depressus and P. herbstii and gill tissue of oysters infected by M. mercenaria.

[b] Too difficult to count in living tissue.

oysters with N. prytherchi. Enormous numbers of cysts occluded some of the posterior portions of gill vessels following the infection.

Crabs which had been starved and then fed infected oyster tissue were dissected and examined in mid-August. E. depressus and P. herbstii revealed advanced stages of gregarines after being fed mantle, but not gill tissue. M. mercenaria produced gymnocysts after being fed gill tissue (Table 6).

The young Menippe, which were supplied by Cook, were also infected after being fed pieces of oyster gill. The development of the gregarines in these crabs was quite slow, possibly due to the air-conditioning (approx. 25 C) in the laboratory. Therefore, crabs were sacrificed at 5-day intervals beginning 20 July, rather than daily. Advanced stages of gregarines were noted in these decapods (Table 7).

Ecological Parameters

The salinities for each sampling station in this study are recorded along with the temperature in Table 3. Representative temperatures and salinities for Galveston, East and West Bays are listed in Table 8. The low salinities for the majority of sampling stations are attributed to the influx of Trinity River water and early summer rain.

TABLE 6. Experimental infection of decapod hosts.

Tissues fed to crabs from experimentally infected oysters	Occurrence of advanced stages of gregarines					
	E. depressus		*P. herbstii*		*M. mercenaria*	
	Present	Absent	Present	Absent	Present	Absent
Mantle	15	6	14	1	0	17
Gill	0	18	0	14	16	2
Mantle and gill	12	3	9	0	13	4

TABLE 7. Experimental infection of Menippe mercenaria.

Date of Examination	Stages noted in alimentary tract			
	Sporozoites	Trophozoites	Sporadins	Gymnocysts
16 July	-	-	-	-
17 July	X	-	-	-
18 July	X	young (?)	-	-
19 July	X	X	-	-
20 July	X	X	-	-
25 July	X	X	X	-
30 July	X	X	X	-
4 August	X	X	X	X
9 August	X	X	-	-
14 August	X	X	X	X

TABLE 8. Representative temperature and salinity data for 1968.[a]

	Galveston Bay (North Redfish Reef)		East Bay (Moody's Reef)		West Bay (Mecom's Cut)	
	Temp. (°C)	Sal. (‰)	Temp. (°C)	Sal. (‰)	Temp. (°C)	Sal. (‰)
Jan.	14.3	13.9	12.5	16.7	13.1	21.1
Feb.	10.8	12.2	10.3	11.7	10.8	20.5
Mar.	15.5	14.4	17.8	17.8	16.0	24.4
Apr.	21.4	1.1	20.9	11.7	24.0	17.2
May	26.2	1.1	24.8	7.2	26.0	12.7
June	26.5	1.1	27.7	2.2	26.0	6.7
July	29.0	10.5	29.5	2.2	31.0	6.6
Aug.	29.8	15.5	29.8	5.0	28.6	27.8
Sept.	27.0	13.3	27.0	10.5	25.0	19.9
Oct.	26.1	17.2	22.0	11.1	24.0	17.8

TABLE 8. (continued)

	Galveston Bay (North Redfish Reef)		East Bay (Moody's Reef)		West Bay (Mecom's Cut)	
	Temp. (°C)	Sal. (‰)	Temp. (°C)	Sal. (‰)	Temp. (°C)	Sal. (‰)
Nov.	14.4	17.8	13.9	14.4	15.0	24.4
Dec.	12.4	19.4	13.0	12.2	13.0	25.0
Range	(10.8-29.8)	(1.1-19.4)	(10.3-29.8)	(2.2-17.8)	(10.8-31.0)	(6.6-27.8)
Mean	21.1	11.5	20.8	10.2	21.4	18.7

[a]Supplied by R. Hofstetter, Texas Parks and Wildlife Department.

Mr. Robert Hofstetter of the Texas Parks and Wildlife Department has supplied the crab data collected in Galveston and East Bay. One bushel of oysters and shell were collected from each sampling site. The different species were sorted and counted. Results of his collections are shown in Tables 9 and 10. Eurypanopeus was found at every sampling station, and was the most common decapod. E. limosum and N. texana sayi were not found in the Galveston Bay area during the summer and fall of 1968.

Collections were made at 8 Mile Road in July, August and September. One hundred crabs comprised a sample. These results are listed in Table 11. Again, E. depressus was the most common definitive host, as well as most common crab.

TABLE 9. Decapods in one-bushel oyster dredge samples from Galveston Bay.[a]

Decapods	Todd's Dump		N. Redfish	S. Redfish		Bart's Pass	
	26 Mar.	19 Nov.	24 Apr.	27 May	11 Sept.	27 Aug.	19 Nov.
Clibanarius	-	-	b	-	b	-	-
Eurypanopeus	39	17	27	40	15	68	131
Menippe	10	4	2	-	1	1	-
Panopeus	7	-	3	2	1	-	3
Petrolisthes	3	9	4	16	3	-	-
Pinnixa	-	-	-	1	-	-	-
Rhithropanopeus	-	13	-	1	-	78	9
Unidentified xanthids	-	-	-	-	-	42	-

[a]Supplied by R. Hofstetter, Texas Parks and Wildlife Department.

[b]Present, but not counted.

TABLE 10. Decapods in one-bushel oyster dredge samples from East Bay.[a]

Decapods	Moody's Reef		Frenchy's Reef		
	25 Apr.	20 Aug.	27 Mar.	20 Aug.	14 Nov.
Callinectes	-	1	-	-	-
Eurypanopeus	118	26	13	3	9
Menippe	8	-	2	-	1
Panopeus	8	2	-	-	-
Petrolisthes	2	-	-	-	-
Rhithropanopeus	1	12	2	11	9

TABLE 11. Distribution and relative abundance of genera in a 100-crab sample.

Decapods	8 Mile Road - Sportsman's Reef		
	July	August	September
Callinectes	2	3	4
Clibanarius	9	12	10
Eurypanopeus	79	69	62
Menippe	7	9	6
Panopeus	3	7	2
Petrolisthes	-	-	4
Rhithropanopeus	-	-	12

DISCUSSION

Because of the economic importance of the American oyster, C. virginica, it is easy to understand why a parasite of this mollusk would be studied. Unfortunately, the vast majority of Nematopsis work has centered around its possible role in loss of condition or death of the oyster. There is a distinct lack of literature which extends beyond the incidence and intensity of Nematopsis infection and its possible pathogenicity.

In this study, the two species known in Texas waters and a third, as yet unidentified species, were studied. It is difficult to relate the data obtained in this investigation to those collected by previous investigators. There have been varied sampling techniques, few references to seasonality, and a wide range of ecological situations. However, in reviewing the different species, reference will be made to parameters noted in this work, as well as to observations in the literature.

Review of Nematopsis ostrearum

N. ostrearum has been the most widely studied American porosporid. Spores of this species occur intracellularly in phagocytes which are scattered throughout the oyster. Though the vast majority

of the cysts occur near the adductor muscle in the mantle tissue, cysts were noted in the heart, gill, and labial palps. Sprague (1950) found resistant spores in the tentacles and adductor muscle, as well as in the already mentioned organs. Apparently, almost any organ can be infected. The circulation and physiology of the oyster appear to be closely related to the infections of different organs, but little is known in this area (Stauber, 1950; Tripp, 1957).

Landau and Galtsoff (1951) surveyed the distribution of N. ostrearum in oysters along the Atlantic and Gulf coasts. Apparently, however, they were unaware of the work of Sprague (1949, 1950) in Louisiana. In reference to Texas waters, Galtsoff (Landau and Galtsoff, 1951) found extremely heavy concentrations of spores in gill tissue, but these were not identified. It appears that these were N. prytherchi which have been observed to occur almost exclusively in the posterior portion of the demibranchs. They determined intensity of infection for their samples by examining 50 fields in each of 10 oysters. The tissue sampled was removed from the ventral posterior mantle margin. It is difficult to compare levels of intensity with any of these data.

Owen et al. (1951) counted 10 fields from 10 oysters in their investigation. They sampled tissue from below the adductor muscle on the margin of the ventral mantle. They reported 100% infection

by N. ostrearum in Louisiana and 95.1% infection in Pensacola Bay, Florida. In this study, 99% of the 400 oysters sampled were infected by N. ostrearum. There is little question as to the widespread infection of this species in Gulf waters.

Oysters appear to eliminate these parasites. Examination of fecal material revealed N. ostrearum spores. Those oysters removed from the principal decapod hosts show a drop in infection levels (Table 4). A dynamic equilibrium, as suggested by Feng (1957, 1958), appears to be established between the molluscan and decapod populations. Little is still known as to the means by which the oyster can clear its tissue. Older oysters may be more heavily infected strictly because of their increased circulation, not because of their accumulation of cysts (Feng, 1957).

Though observations were not made in this study, it appears that crab hosts may be infected by consumption of oyster fecal material, similar to shrimp infections noted by Kruse (1966). This may partially explain the role of E. depressus and P. herbstii which are not able to crush oyster shells easily.

Two of the four known decapod hosts were studied in this investigation. Both E. depressus and P. herbstii were shown to transmit N. ostrearum. Prytherch (1938, 1940) and Sprague (1949, 1950) had similar results. There were no indications that

experimental infection could produce loss of condition or death of oysters. This study was conducted in a period of low salinity and no significant oyster mortality was noted in the area. Labyrinthomyxa spp. were observed, but in small numbers in the summer of 1968.

Salinity was not observed to be a major factor in N. ostrearum distribution. This agrees with observations by Landau and Galtsoff (1951), Owen et al. (1951), and Feng (1957). However, there appears to be a seasonal variation in the levels of intensity. This is probably related to the role of temperature in the life cycle, particularly in the decapod host. Sprague (1950) found that the entire life cycle of N. ostrearum could be completed in approximately 3 weeks. Both he and Prytherch (1940) noted the rapid development of gregarines during warm summer months. In this study, no time period was noted for the complete life cycle. The cooler temperature of the laboratory appears to have slowed down the development of gregarines.

The presence of suitable crab hosts is suggested as the major factor in levels of intensity. Although only two known hosts and a third possible host, R. harrisii, were observed at the various stations, they were generally the predominant species. E. depressus was found at every station, as well as being the most common decapod. Some preliminary observations between relative number of definitive hosts and intensity of infection can be noted. Oysters

collected from the Lagoon Laboratory of the Bureau of Commercial Fisheries have very low levels of infection (Table 3). These oysters have either been brought to the holding tanks or have set there, following larval development. The tanks are exposed to running water, but very few crabs are able to invade the holding area. However, oysters with highest intensities of infection are exposed to large definitive host populations. Samples from 8 Mile Road-Sportsman's Reef and Moody's Reef reveal high levels of N. ostrearum infection and large numbers of known hosts.

Review of Nematopsis prytherchi

Originally confused with N. ostrearum, N. prytherchi was not described until Sprague (1949, 1950, 1954) developed the life cycle for this species. The decapod host, M. mercenaria, is the only known intermediate. Sprague has been the only investigator concerned with this species. Feng (1957) was unable to find N. prytherchi in Chesapeake Bay and its tributaries. He attributed this to the absence of Menippe in Chesapeake Bay waters. Therefore, there has been little study of this species beyond its description by Sprague.

Labial palps, mantle and heart tissue were found infected, along with the gills. Sprague (1950) also found the adductor muscle

and liver tubules infected. The cysts are difficult to observe in live tissue, since they are intracellular, phagocytized by other blood cells. They are not scattered about like N. ostrearum, instead they crowd together in clumps at the posterior end of gill vessels, often distorting the shape of the vessel. No pattern of distribution was noted, but study of oysters with heavier concentrations may reveal a pattern. As with N. ostrearum, both the physiology and the circulation of the oyster appear to be important in distribution of cysts in the molluscan host (Stauber, 1950; Tripp, 1957; Feng, 1957).

N. prytherchi was found at each of the 16 sampling sites, but it was not as ubiquitous as N. ostrearum. Of the 400 oysters studied, 89% were infected with this sporozoan. Only three stations revealed heavier infection by N. prytherchi than by N. ostrearum (Table 3). Each of these stations are found in the lower Galveston Bay area. Two stations are on the East Lagoon, the other is on the North Jetty. The significance here is that these stations generally have higher salinities. M. mercenaria is associated with high salinities, so these results are to be expected. The station on the North Jetty had a salinity of 34.2‰ and the correspondingly high number of crabs found amidst the rocks explains the high intensity in oyster gill tissue there (Table 3).

In 1967, Hofstetter found the salinities in Galveston Bay to be relatively high. Occurrence of Menippe during this period may explain the concentrations of N. prytherchi observed in oyster tissue.

Infection experiments confirm that M. mercenaria is a host of N. prytherchi. As in the work with N. ostrearum, the development of gregarines was slower than originally expected. Sprague (1950) worked with temperatures of 28-32 C, while this study was in an air-conditioned laboratory with an approximate temperature of 25 C. This apparently explains the slow development of gregarines in the Menippe experiment (Table 7).

Salinity appears to be important in the distribution of N. prytherchi. Unlike the euryhaline xanthids which transmit N. ostrearum, M. mercenaria is closely associated with high salinity and warm waters. It is therefore to be expected that the distribution of N. prytherchi will be more limited than the widespread occurrence of N. ostrearum.

Review of N. sp.

The unidentified spores which are found in gill tissue were first noted by Sprague (1950) in Louisiana. The spores do not fall in the size range of N. prytherchi. Sprague tentatively concluded that the

spores represented "either a distinct species (or variety) or polymorphism within N. prytherchi" (1950, p. 25). Both natural and experimental infections of Louisiana oysters revealed these smaller spores.

Only three stations revealed this third form (Table 1). No pattern could be determined, as the three sampling sites which revealed this spore were widely distributed. However, at sites where it was found, the concentrations were minimal. The only location which reveals a substantial field infection was the sample at Missing Reef in mid-Galveston Bay (Table 2).

Kruse (1966) found two different spores in the gills of pelecypods he observed in Florida. He identified the larger spore as N. duorari, but did not describe the smaller spore. He felt that the smaller spore was indeed a distinct species because it occurred in pelecypods not infected by N. duorari.

In this study, only a small number of the unidentified spores were found. These spores are similar to those observed in Brachidontes recurvus, but no conclusions were drawn.

Further Study of Nematopsis

Though nine species of Nematopsis are known, the life cycle for a number of species remains to be described. Two of the most recent species to be identified reveal only known decapod hosts. Ball (1951) described N. panopei on the basis of gregarine infection in Panopeus occidentalis and P. herbstii, while Sprague (1954) described N. penaeus from the brown shrimp, Penaeus aztecus. In the molluscan host, similar situations occur. Sprague (1950) and Kruse (1966) have observed unidentified spores in varied pelecypods.

Future studies should note the relative intensity of infection and other relative parameters. Feng (1957) was the first to point out the questionable value of any numerical index. These values are definitely relative and do not serve as true indices. Rather, they reflect general trends for particular samples. As is obvious in this study, wide differences exist between samples from various locations and seasons.

Future examination of decapod crustaceans and mollusks should reveal other species of Nematopsis. Because of the diversity of hosts, a widespread occurrence of this genus appears likely.

SUMMARY

1. Two known species of *Nematopsis* were found in the tissues of *C. virginica*. *N. ostrearum* was heavily concentrated about the adductor muscle in mantle tissue, while *N. prytherchi* was found in the posterior portion of gill vessels. A third spore, as yet unidentified, also occurred in the gills, but in small numbers.
2. Widespread levels of *Nematopsis* infection were found in the Galveston Bay area. *N. ostrearum* and *N. prytherchi* were found at every sampling site, while the third form occurred at only 3 of the 16 stations. Of the 400 oysters studied, 99% were infected by *N. ostrearum*, 89% were infected by *N. prytherchi*, and 3% were infected by *N.* sp.
3. Infection experiments were conducted. *E. depressus* and *P. herbstii* were confirmed as hosts of *N. ostrearum*, while *M. mercenaria* transmitted *N. prytherchi*.
4. Low salinities were observed at many stations, but because of the euryhaline nature of the oyster and the xanthid hosts, no effect on transmission of *N. ostrearum* was noted. *N. prytherchi* occurred in lower bay samples where higher salinities and *M. mercenaria* are noted.

5. Oysters appear to discharge cysts in sufficient quantities to reduce the level of infection during cooler winter months. Life cycles are slowed down in the decapod hosts during this period. Whether physiological considerations in the oyster are important in this seasonality is unknown.

6. Known crustacean host populations were found to significantly affect levels of infection. The proximity of sizable molluscan and crustacean populations resulted in high levels of Nematopsis infection.

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VITA

The author was born in Racine, Wisconsin, on September 1, 1945. His parents, Mr. and Mrs. Orval H. Anderson, still reside in the city of Racine, along with his younger sister, Arlo, and brother, David. After graduation from Washington Park High School in 1963, he attended Saint Olaf College, Northfield, Minnesota, where he received the Bachelor of Arts degree in May 1967. In September 1967, he entered Texas A&M University to study marine biology under the direction of Dr. J. G. Mackin. The author's permanent address is 2415 Jerome Boulevard, Racine, Wisconsin 53403.

This thesis was typed by Mrs. Margie Watson.