The Prevalence of Disease in the Oyster Crassotrea virginica in Rhode Island

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THE PREVALENCE OF DISEASE IN THE OYSTER CRASSOSTREA VIRGINICA
IN RHODE ISLAND

BY

RETNO ANDAMARI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
FISHERIES, AQUACULTURE, AND PATHOLOGY

UNIVERSITY OF RHODE ISLAND
1992
MASTER OF SCIENCE THESIS

OF

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1992
ABSTRACT

The disease prevalence in American oysters, *Crassostrea virginica*, from coastal areas in Rhode Island was studied. Oysters were collected by hand or tongs from the Pawcatuck River (3 stations), Narrow River (2 stations), Charlestown Pond (3 stations) and Green Hill Pond (3 stations), during July/August 1991, November 1991, March 1992, and May 1992. Of the forty oysters collected at each of the four sites, thirty were processed for histologic examination. The remaining 10 oysters of each site were used to determine the condition index.

*Haplosporidium nelsoni* was detected in four of 480 oysters (0.8%); *Sphenophrya* sp., 15 of 480 (3.1%); *Bucephalus* sp., 16 of 480 (3.3%); crustacea, one of 480 (0.4%); and basophilic inclusion bodies (possible mycoplasm-like organisms), 15 of 480 samples (3.2%). Lesions also were found in the samples including: kidney concretions, 32 of 360 (8.8%); necrosis of digestive diverticulae of gastrointestinal tract, 28 of 480 (5.8%); neoplasia of gastrointestinal tract, connective tissue, reproductive tract, and gills, 18 of 480 (3.8%); hyperplasia of digestive diverticulae, one of 480 (0.2%); ulceration of stomach epithelium, two of 480 (0.4%); cysts in the kidney, three of 360 (0.8%); atrophic adductor muscle, two of 360 (0.6%); and inflammation of kidney, gills, gastrointestinal tract, connective tissue, 447 of 480 (93.1%).

Condition index ranged from 26.52 to 197.67. Condition index of oysters from Pawcatuck River was below 75 throughout the year. In the other areas, condition index was consistently lower during the summer than during other times of the year. Lesions and parasites were found at all of the sites studied, although MSX was found only in Charlestown Pond. Disease prevalence in oysters from the Pawcatuck River was not different from other sites, but the condition index was consistently lower. These findings suggest that low condition index may not necessarily correlate with higher disease prevalence.
ACKNOWLEDGEMENTS

I would like to thank the members of my graduate committee for their help and support throughout this study. Dr. M.A. Rice my major professor, proposed the topic, reviewed the draft and provided much wisdom and guidance. Dr. R.E. Wolke helped me with the photography and reviewed the manuscript. Dr. R.C. Bullock carefully reviewed the manuscript.

Thanks are also due to Mr. A. Ganz of the Rhode Island Department of Environmental Management, Division of Fish and Wildlife, for providing the inspiration for this study and help during the sampling. I also would like to thank to Mr. G.R. Gardner for use of the equipment and facilities at the Environmental Protection Agency, Environmental Research Laboratory in Narragansett, RI, and Mr. P.P. Yevich for his invaluable assistance in interpretation of slides. Thanks to Ms. S. Polofsky, Ms. D. Borsay and Ms. S. Langloy for their technical assistance. I would also extend my thanks to Dr. S.M. Constantinides, Dr. S.J. Cobb, Dr. S.B. Saila, who provided encouragement and advice during this study and Dr. E. Sadasiv, Ms. S. MacLean, Dr. R.E. Carlson for reviewing my draft.

I am truly grateful to my husband for his love, support, and patience he has given me through my study. A special thanks to all my friends in Rhode Island, too numerous to mention, for their support and for all the good times we have had over the last two years.

Funding for my graduate studies was obtained by Overseas Training Office, National Development Planning Agency, Government of Indonesia (OTO-BAPPENAS) through the Institute of Public Administration, New York (IPA).
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INTRODUCTION

The American oyster, *Crassostrea virginica* (Gmelin, 1791) is a common inhabitant of Gulf and Atlantic coast estuaries of the United States (Galtsoff, 1964; Abbe, 1988). Historically oysters were the most valuable shellfish fisheries of Rhode Island salt ponds and estuaries (Ganz, 1973; Bean, 1990; Desbonnet and Lee, 1990). Rhode Island contributed significantly to the 26 million pounds of oysters that were produced annually in New England during the early 1900's (Matthiessen, 1970). The oyster industry declined due to pollution from sewage, coastal development, and the deposition of heavy metals in coastal areas between the 1930's and 1950's (Bean, 1990).

Environmental stress has been associated with increased incidence of infectious disease in marine animals (Sparks, 1985). Stress caused by chemical and environmental factors is also associated with increased incidence of neoplasia (Sparks, 1985). Sindermann (1990) stated that mortality is caused by the combination of an infectious agent and environmental stressors.

In recent years there has been widespread concern about the impact of the protozoan parasite *Haplosporidium nelsoni*, known as MSX (multinucleated sphere unknown) on the *Crassostrea virginica* fishery. Sindermann (1990) reported that MSX caused losses to the oyster industry that had amounted to 3 million dollars per year over a 12-year period. Total landings of oysters in the United States for 1990 was 13,242 metric tons (NOAA, 1991), down from 327,500 metric tons in 1971 prior to major MSX epizootics. Because of the major economic impact of MSX and other protozoan diseases, there has been great interest in their eradication or control. Initial concerns about MSX began when the parasite caused a 90 percent increase in oyster mortalities on planted grounds in Delaware Bay during the period 1957 - 1959 (Ford and Haskin, 1982). The *Haplosporidium* parasite has also been
responsible for mortalities on natural beds of the American oyster in Delaware and
Chesapeake Bays since early in the 1950's (Andrews and Wood, 1967; Haskin et al.,
1966; Haskin and Ford, 1982).

MSX was first found in the lower Chesapeake Bay in 1959 (Farley, 1975) and moved
progressively up the Bay into Maryland. The parasite has continued to cause extensive
damage to the oyster industry and still inhabits the waters of the Chesapeake. Couch et al.
(1966) reported MSX in oysters from the Middle Atlantic states. It has also been reported
in North Carolina, Long Island Sound (Sindermann and Rosenfield, 1967), Massachusetts
and Great South Bay of Long Island (Haskin and Ford, 1982).

Since the early epizootics, several of studies have been undertaken to assess the effects
of MSX on oyster populations. The time course of MSX infection to host mortality is quite
rapid. In the spring of 1957, approximately half of the oysters planted on the New Jersey
oyster grounds in Delaware Bay died within 6 to 7 weeks (Haskin et al., 1966). The
pattern of losses and continuing mortalities later that summer and fall indicated an infectious
disease as the cause (Andrews, 1966). Ford and Haskin (1982) estimated that at least half
of all oyster deaths in lower Delaware Bay since the early 1960's could be attributed to the
effects of *Haplosporidium nelsoni* infection. Rosenfield and Sindermann (1966) claimed
that between 1963 and 1965, MSX invaded areas of middle Chesapeake Bay formerly free
of the disease. *Haplosporidium nelsoni* occurs in water with salinity greater than 15 ppt.
According to Andrews (1966), about 50% of the oyster beds in high salinity water in
Chesapeake Bay had become unproductive during 1959 - 1963.

To interpret the status of MSX, given the known pattern of infection, Andrews (1967)
suggested following a particular oyster population through at least one growing year,
sampling live oyster for prevalence of the disease and recording standard mortality data.
There is no "best time" of the year to sample for MSX prevalence because *Haplosporidium nelsoni* kills oysters in all seasons. Infections occur during at least the five warm months of the year and have variable incubation times. Additionally, MSX may infect and kill all sizes of the American oyster (Farley, 1975).

Several studies have been conducted on the life history, pathology, and epizootiology of MSX. Haskin et al. (1966) reported that infections of MSX reduced the particle filtration rates of the oyster. The condition index of infected oysters was often found to be lowered substantially (Newell, 1985). According to Ford and Figueras (1988) and Barber et al. (1988), gamete development, fecundity, tissue lipid, protein, and glycogen of eastern oysters are all affected by the protozoan MSX. The MSX parasite appears poorly adapted to its host since infections frequently kill the oyster (Andrews, 1968; Ford, 1985).

The purpose of the present study was to determine if MSX infection and other disease exists in the oysters in Rhode Island waters. Since MSX exists in nearby states, there is a possibility of its occurrence in Rhode Island. Using histological techniques, oysters collected in Rhode Island were assessed for MSX, lesions and other disease.
MATERIALS AND METHODS

Sampling

Oysters were taken from the Pawcatuck River (3 stations), Charlestown Pond (3 stations), Green Hill Pond (3 stations), and Narrow River (2 stations in August and November 1991; 1 station in March and May 1992) in Rhode Island (Figure 1). Samples of 40 oysters from each site were obtained using tongs or by hand four times a year between July 1991 and May 1992 for determination of condition index and prevalence of disease. Data on surface water salinity and temperature were collected at each sampling station.

Laboratory Procedure

Samples collected during July/August, 1991 were brought to the laboratory within 2 hours, processed and examined. Oysters were cleaned of fouling organisms. Samples were divided as follows: from each site 10 oysters were used to determine condition index and 30 oysters for histological studies. For histological processing, oysters were removed from their shells and fixed in 10% seawater formalin (Appendix I). A cross sectional cut was made on each oyster before fixing the tissue for histology. Preserved tissue was dehydrated by alcohol series and embedded in Tissue Prep (Fisher) and six-micrometer sections were stained with hematoxylin and eosin, then mounted and examined.

Samples collected in November 1991, March and May 1992, were fixed with Helly's fixative (Appendix I). The oysters were allowed to become firm, removed from fixative, cut sagittally through the mid-line of the body up to the adductor muscle and returned to fixative for 16 hours. After fixation, the oysters were washed in running water for 24 hours then stored in 70% ethyl alcohol. The oysters were then trimmed and placed into cassettes for further processing. In trimming the oysters, the previous sagittal cut through
Figure 1. Map showing collection sites in Pawcatuck River, Narrow River, Charlestown Pond and Green Hill Pond in Rhode Island.
the middle of the body was extended through the muscle, cutting the oysters in half. If the oysters were large, each half was again sagittally cut and these halves cross sectioned so that they would fit into the cassettes. All tissues were processed by routine histopathologic methods. Slides were stained with hematoxylin and eosin. Helly's fixative has been determined to be the most suitable cytological fixative for aquatic invertebrates because it preserves the nucleoplasm of the ova nuclei, granules of the secretory cells and amebocytes better than other fixatives tested (Yevich and Barszcz, 1981).

By microscopic examination, each oyster was categorized with respect to the intensity of MSX infection as either uninfected, epithelially infected (gill only) or systemically infected (sub-epithelial general infection). Other parasites and pathologic conditions observed during this study were also recorded. The percentage of oysters infected with each parasite was calculated for each site.

Microscope slides from this study had been archived for possible future verifications. Most slides will be archived at the Marine Pathology Laboratory, East Farm, University of Rhode Island. Slides showing neoplasia will be archived at the Registry of Tumors in Lower Animals, National Museum of Natural History, Smithsonian Institution, Washington, DC.

The condition index (CI) determination followed the procedure recommended by Crosby and Gale (1990).

\[
CI = \frac{\text{dry soft tissue weight (gr)} \times 1000}{\text{internal shell cavity capacity (gr)}}
\]

Oyster shell measurements included: valve length, height, and width. Internal shell cavity capacity (grams) was determined by subtracting dry shell weight (grams) in air, from the total whole live weight (grams) in air of a cleaned oyster. Internal shell capacity is proportional to internal shell volume because oysters will trap seawater with valve closure. The dry weight of soft tissue was obtained by drying the tissue in an 80°C oven for 48
hours and determining weight (grams) of dried tissue. The condition index may also be used as an assay for monitoring various pollutants and disease (Crosby and Gale, 1990) as well as routinely monitoring the general health of oyster populations. According to Quayle (1980), oysters with high condition index (75 to 150) are in good condition, while low condition index is 75 and below.
RESULTS

The percentage of oysters with lesions and parasites from Pawcatuck River, Narrow River, Charlestown Pond, and Green Hill Pond ranged from 0.2 % to 93.1 % (Table 1). Surface water salinity ranged from 0 ppt to 30 ppt, measured temperatures ranged from $4^\circ$C to $26^\circ$C at the time of collection, and shell heights of oysters ranged from 24.6 mm to 190.0 mm (Tables 2 and 3).

Protozoan diseases

Haplosporidium nelsoni (MSX)

During the year-long study, MSX was found only in Charlestown Pond (Site 2) in November 1991 and March 1992. Percent infection of MSX was 20 (% 2 of 10) in November 1991 and 20 (% 2 of 10) in March 1992 (Tables 1, 4, and 5). The initial infections occurred in epithelia of gills and palps (Figures 2 and 3). Two advanced infections were found showing systemic disease (Table 8).

Sphenophrya sp.

The ciliate Sphenophrya sp. (Figure 4) was found in hypertrophic cells of the gill of 15 of 480 oysters examined (Table 1). The highest level of infection was seven of 120 (5.8%) in March 1992 (Table 5). Sphenophrya sp. was found at all sites (Table 1) and at all seasons of the year (Tables 4, 5, 6, 7, 8, and 9). Little or no host response to the ciliate was seen.
Table 1. Prevalence of lesions and parasites in *Crassostrea virginica* from July 1991 to May 1992 in Rhode Island (N = 480).

| Lesions                  | Pawcatuck River | Narrow River | Charlestown Pond | Green Hill Pond | Total       |
|--------------------------|-----------------|--------------|------------------|-----------------|-------------|
| **Lesions**              | *#*             | %            | *#*              | %              | *#*         | %           |
| Inflammation**           | 113/120         | 94.2         | 111/120          | 92.5           | 108/120     | 90.0        | 115/120     | 95.8         | 447/480       | 93.1         |
| Kidney concretions       | 1/90            | 1.1          | 9/90             | 10.0           | 8/90        | 8.9         | 14/90       | 15.6         | 32/360        | 8.1          |
| Necrosis***              | 4/120           | 3.3          | 1/120            | 0.8            | 11/120      | 9.2         | 12/120      | 10.0         | 28/480        | 5.8          |
| Neoplasia                | 4/120           | 3.3          | 7/120            | 5.8            | 4/120       | 3.3         | 3/120       | 2.5          | 18/480        | 3.8          |
| Hyperplasia              | 0/120           | 0.0          | 0/120            | 0.0            | 1/120       | 0.8         | 0/120       | 0.0          | 1/480         | 0.2          |
| Ulceration               | 0/120           | 0.0          | 1/120            | 0.8            | 1/120       | 0.8         | 0/120       | 0.0          | 2/480         | 0.4          |
| Cysts (Idiopathic)       | 1/90            | 1.1          | 1/90             | 1.1            | 0/90        | 0.0         | 1/90        | 1.1          | 3/360         | 0.8          |
| Atrophy Adductor Muscle  | 1/90            | 1.1          | 0/90             | 0.0            | 1/90        | 1.1         | 0/90        | 0.0          | 2/360         | 0.6          |

**Parasites**

| Parasites                | Pawcatuck River | Narrow River | Charlestown Pond | Green Hill Pond | Total       |
|--------------------------|-----------------|--------------|------------------|-----------------|-------------|
| *Haplosporidium nelsoni* | 0/120           | 0.0          | 0/120            | 0.0             | 4/120       | 3.3         | 0/120       | 0.0          | 4/480         | 0.8          |
| *Sphenophrya sp.*        | 2/120           | 1.7          | 2/120            | 1.7             | 5/120       | 4.2         | 6/120       | 5.0          | 15/480        | 3.1          |
| *Bucephalus sp.*         | 3/120           | 2.5          | 11/120           | 9.2             | 2/120       | 1.7         | 0/120       | 0.0          | 16/480        | 3.3          |
| Basophilic Inclusion     | 5/120           | 4.2          | 3/120            | 2.5             | 5/120       | 4.2         | 2/120       | 1.7          | 15/480        | 3.2          |
| Bodies                   |                 |              |                  |                 |             |             |             |               |               |               |
| Crustacea                | 1/120           | 0.8          | 0/120            | 0.0             | 0/120       | 0.0         | 0/120       | 0.0          | 1/480         | 0.2          |

* = number affected/total sample  
** Inflammation of kidney, gills, gastrointestinal tract, and connective tissue  
*** Necrosis of digestive diverticula of gastrointestinal tract
Table 2. Surface salinity (S, ppt) and temperature (T, °C) of Rhode Island waters during the sampling from July 1991 to May 1992 at each station.

| Site/station         | July/August 1991 | November 1991 | March 1992 | May 1992 |
|----------------------|------------------|---------------|------------|----------|
|                      | T    | S    | T    | S    | T    | S    | T    | S    |
| Pawcatuck River      |      |      |      |      |      |      |      |      |
| 1                    | 23   | 14   | 9    | 10   | 4    | 0    | 4    | 14   |
| 2                    | 23   | 23   | 9    | 18   | 4    | 5    | 14   | 4    |
| 3                    | 23   | 26   | 9    | 30   | 4    | 15   | 14   | 22   |
| Narrow River         |      |      |      |      |      |      |      |      |
| 1                    | 23   | 20   | 10   | 20   | 4    | 15   | 14   | 13   |
| 2                    | 25   | 20   | 10   | 20   | -    | -    | -    | -    |
| Charlestown Pond     |      |      |      |      |      |      |      |      |
| 1                    | 26   | 26   | 10   | 30   | 4    | 20   | 14   | 22   |
| 2                    | 26   | 26   | 10   | 30   | 4    | 20   | 14   | 23   |
| 3                    | 26   | 26   | 10   | 30   | 4    | 20   | 14   | 24   |
| Green Hill Pond      |      |      |      |      |      |      |      |      |
| 1                    | 26   | 25   | 10   | 30   | 4    | 20   | 14   | 24   |
| 2                    | 26   | 25   | 10   | 30   | 4    | 20   | 14   | 24   |
| 3                    | 26   | 25   | 10   | 30   | 4    | 20   | 14   | 24   |
Table 3. Valve height (mm) of *Crassostrea virginica* collected for histological observation in Rhode Island from July 1991 to May 1992. Mean and coefficient of variation of valve height (measured along longest axis) from each sampling station (n = 10).

| Site/station      | July/August 1991 | November 1991 | March 1992 | May 1992   |
|-------------------|------------------|---------------|------------|------------|
| Pawcatuck River   |                  |               |            |            |
| 1. Height (range) | 52.3 - 180.0     | 79.3 - 156.0  | 80.2 - 163.0 | 52.1 - 120.0 |
| Mean; C.V.        | 97.6 ; 37.8      | 100.9 ; 27.2  | 130.4 ; 19.6 | 89.7 ; 25.2 |
| 2. Height (range) | 51.6 - 165.0     | 59.1 - 180.0  | 62.0 - 190.0 | 79.0 - 140.0 |
| Mean; C.V.        | 114.5 ; 36.8     | 109.7 ; 38.7  | 109.0 ; 42.3 | 117.1 ; 17.5 |
| 3. Height (range) | 72.3 - 130.0     | 79.0 - 115.0  | 69.1 - 125.2 | 70.0 - 142.0 |
| Mean; C.V.        | 100.9 ; 27.2     | 94.0 ; 11.8   | 91.6 ; 22.1  | 95.2 ; 27.9 |
| Narrow River      |                  |               |            |            |
| 1. Height (range) | 24.6 - 93.5      | 49.5 - 78.0   | 54.5 - 85.4 | 53.2 - 93.7 |
| Mean; C.V.        | 66.9 ; 23.5      | 65.3 ; 15.2   | 67.5 ; 12.7  | 69.4 ; 15.9 |
| 2. Height (range) | 51.0 - 80.0      | 46.1 - 99.6   | -           | -           |
| Mean; C.V.        | 66.1 ; 13.7      | 62.2 ; 19.6   | -           | -           |
| Charlestown Pond  |                  |               |            |            |
| 1. Height (range) | 57.6 - 93.6      | 59.3 - 88.0   | 54.0 - 84.1 | 54.2 - 92.0 |
| Mean; C.V.        | 79.9 ; 15.3      | 68.1 ; 16.1   | 73.1 ; 13.3  | 65.1 ; 19.5 |
| 2. Height (range) | 63.1 - 96.2      | 53.3 - 82.3   | 58.7 - 71.0  | 50.2 - 72.2 |
| Mean; C.V.        | 75.4 ; 14.7      | 65.9 ; 15.8   | 65.1 ; 5.6   | 63.0 ; 10.8 |
| 3. Height (range) | 56.7 - 102.5     | 44.1 - 74.2   | 52.3 - 83.1  | 50.2 - 92.4 |
| Mean; C.V.        | 80.9 ; 17.6      | 60.4 ; 16.5   | 69.2 ; 13.8  | 73.7 ; 16.0 |
| Green Hill Pond   |                  |               |            |            |
| 1. Height (range) | 45.0 - 78.1      | 40.6 - 75.0   | 38.3 - 63.0  | 51.0 - 64.2 |
| Mean; C.V.        | 62.6 ; 15.9      | 51.2 ; 19.3   | 47.6 ; 18.1  | 55.2 ; 7.6  |
| 2. Height (range) | 50.7 - 108.0     | 53.1 - 90.7   | 51.2 - 67.2  | 42.1 - 63.2 |
| Mean; C.V.        | 69.9 ; 25.1      | 67.3 ; 16.8   | 59.7 ; 8.7   | 51.0 ; 13.5 |
| 3. Height (range) | 45.6 - 70.5      | 49.2 - 71.4   | 54.0 - 70.0  | 45.5 - 72.3 |
| Mean; C.V.        | 56.2 ; 14.9      | 59.8 ; 11.5   | 64.6 ; 11.6  | 64.2 ; 12.0 |

C.V. = Standard deviation x 100% 

Mean
Table 4. Lesions and parasites in *Crassostrea virginica* in Rhode Island (n = 30 from each site)

| Site            | Pawcatuck River | Narrow River | Charlestown Pond | Green Hill Pond | Total |
|-----------------|-----------------|--------------|------------------|-----------------|-------|
| Station         | 1   | 2   | 3   | 1   | 2   | 3   | 1   | 2   | 3   |-------|
| July/August 1991 |     |     |     |     |     |     |     |     |     |-------|
| Inflammation*   | 10  | 8   | 9   | 15  | 13  |     | 10  | 10  | 9   | 10   | 10   | 10   | 114  |
| Necrosis**      | 0   | 0   | 0   | 0   | 0   |     | 5   | 2   | 0   | 0    | 0    | 0    | 7    |
| *Bucephalus* sp.| 0   | 0   | 0   | 3   | 3   |     | 2   | 0   | 0   | 0    | 0    | 0    | 8    |
| November 1991   |     |     |     |     |     |     |     |     |     |     |     |     |-------|
| Inflammation*   | 10  | 9   | 9   | 15  | 15  | 10  | 10  | 10  | 10  | 9   | 10   | 10   | 117  |
| Necrosis**      | 3   | 0   | 0   | 0   | 0   |     | 0   | 0   | 4   | 5    | 6    | 1    | 19   |
| Neoplasia       | 0   | 0   | 0   | 1   | 2   |     | 1   | 0   | 1   | 0    | 0    | 0    | 3    |
| Kidney Concretions | 0  | 0   | 0   | 1   | 2   |     | 0   | 1   | 2   | 3    | 1    | 1    | 11   |
| Cysts (Idiopathic) | 1  | 0   | 0   | 0   | 0   |     | 0   | 0   | 0   | 0    | 0    | 1    | 1    |
| *Bucephalus* sp.| 1   | 0   | 0   | 1   | 0   |     | 0   | 0   | 0   | 0    | 0    | 0    | 2    |
| *Haplosporidium nelsoni* | 0  | 0   | 0   | 0   | 0   |     | 0   | 0   | 2   | 0    | 0    | 0    | 2    |
| *Sphenophrya* sp.| 0   | 0   | 0   | 0   | 0   |     | 0   | 0   | 0   | 0    | 3    | 1    | 4    |
| Basophilic inclusions bodies | 0  | 0   | 0   | 0   | 0   |     | 0   | 0   | 2   | 2    | 0    | 0    | 4    |

* Inflammation of kidney, gills, gastrointestinal tract, and connective tissue.
** Necrosis of digestive diverticula of gastrointestinal tract.
Table 5. Lesions and parasites in *Crassostrea virginica* in Rhode Island (n = 30 from each site).

| Site                      | Pawcatuck River | Narrow River | Charlestown Pond | Green Hill Pond | Total |
|---------------------------|-----------------|--------------|------------------|-----------------|-------|
|                           | Station         | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |       |
|                           | March 1992      |   |   |   |   |   |   |   |   |   |       |
| Inflammation*             |                 | 10 | 10 | 10 | 1 | 0 | 1 | 0 | 0 | 0 | 115  |
| Necrosis**                |                 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1    |
| Neoplasia                 |                 | 0  | 2  | 1  | 4  | 0  | 1  | 0  | 0  | 1  | 9    |
| Kidney Concretions        |                 | 0  | 0  | 1  | 3  | 0  | 4  | 0  | 2  | 0  | 14   |
| Cysts (Idiopathic)        |                 | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 1    |
| Atrophy Adductor Muscle   |                 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1    |
| Bucephalus sp.            |                 | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 3    |
| Sphenophyra sp.           |                 | 0  | 1  | 1  | 2  | 0  | 2  | 0  | 1  | 0  | 7    |
| Basophilic Inclusion      |                 | 1  | 1  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 4    |
|                           | May 1992        |   |   |   |   |   |   |   |   |   |       |
| Inflammation*             |                 | 10 | 9  | 9  | 24 | 7  | 9  | 7  | 10 | 8  | 8    | 101  |
| Necrosis**                |                 | 0  | 1  | 0  | 3  | 0  | 0  | 0  | 0  | 0  | 0    | 4    |
| Neoplasia                 |                 | 0  | 1  | 0  | 2  | 0  | 1  | 0  | 0  | 0  | 0    | 6    |
| Kidney Concretions        |                 | 0  | 0  | 0  | 3  | 0  | 1  | 0  | 1  | 1  | 1    | 7    |
| Cysts (Idiopathic)        |                 | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 1    |
| Atrophy Adductor Muscle   |                 | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 1    |
| Hyperplasia               |                 | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0    | 1    |
| Ulceration                |                 | 0  | 0  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 0    | 2    |
| Bucephalus sp.            |                 | 1  | 0  | 0  | 2  | 0  | 0  | 0  | 0  | 0  | 0    | 3    |
| Haplosporidium nelsoni    |                 | 0  | 0  | 0  | 0  | 0  | 2  | 0  | 0  | 0  | 0    | 2    |
| Sphenophyra sp.           |                 | 0  | 0  | 0  | 0  | 3  | 0  | 1  | 0  | 0  | 0    | 4    |
| Basophilic inclusion      |                 | 1  | 1  | 1  | 3  | 0  | 1  | 0  | 0  | 0  | 0    | 7    |
|                           |                 | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 1    |

* Inflammation of kidney, gills, gastrointestinal tract, and connective tissue.
** Necrosis of digestive diverticula of gastrointestinal tract.
Table 6. Lesions and parasites in *Crassostrea virginica* from Pawcatuck River by organ (n = 10 from each station).

| Station  | Gill  | G. I. Tract* | Connective Tissue | Kidney  | Gonad  | Total  | Total  |
|----------|-------|--------------|-------------------|---------|--------|--------|--------|
|          | 1     | 2            | 3                 | 1       | 2      | 3      | 1      |
| July 1991|       |              |                   |         |        |        |        |
| Inflammation | 0 | 1 | 0 | 0 | 0 | 10 | 8 | 9 | 0 | 0 | 0 | 9 | 10 | 8 | 10 | 28 |
| November 1991 | | | | | | | | | | | | | | | | |
| Inflammation | 1 | 1 | 0 | 1 | 2 | 0 | 10 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 12 | 9 | 33 |
| Necrosis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 |

| March 1992 | | | | | | | | |
| Inflammation | 5 | 4 | 1 | 0 | 0 | 0 | 10 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 14 | 11 | 40 |
| K. Concretions | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Neoplasia | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Bucephalus sp. | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 |
| Sphenophrya sp. | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| B.I.B*** | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 |

| May 1992 | | | | | | | | |
| Inflammation | 9 | 9 | 9 | 0 | 1 | 1 | 9 | 9 | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 19 | 19 | 57 |
| Neoplasia | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Necrosis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Atrophy A.M.** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Cyst | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Bucephalus sp. | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| B.I.B.*** | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 3 |
| Crustacean | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |

| Total | 16 | 18 | 12 | 9 | 5 | 1 | 42 | 36 | 37 | 2 | 1 | 1 | 3 | 0 | 1 | 74 | 60 | 53 | 187 |

* G. I. Tract: gastrointestinal tract
** Atrophy A.M.: atrophy adductor muscle.
*** B.I.B. Basophilic inclusion bodies gastric mucosa.
Table 7. Lesions and parasites in *Crassostrea virginica* from Narrow River by organ (n = 30).

|          | Gill | G.I. Tract* | Connective Tissue | Kidney | Gonad | Total | Total |
|----------|------|-------------|------------------|--------|-------|-------|-------|
| Station  | 1    | 2           | 1                | 2      | 1     | 2     | 1     | 2   |
|          |      |             |                  |        |       |       |       |     |
| August 1991 |      |             |                  |        |       |       |       |     |
| Inflammation | 3    | 0           | 1                | 0      | 15    | 13    | 0     | 2   |
| *Bucephalus* sp. | 0    | 0           | 1                | 1      | 2     | 2     | 0     | 3   |
| November 1991 |      |             |                  |        |       |       |       |     |
| Inflammation | 4    | 1           | 0                | 0      | 15    | 15    | 2     | 2   |
| *K. Concretions* | 0    | 0           | 0                | 0      | 1     | 2     | 0     | 3   |
| *Neoplasia* | 1    | 0           | 0                | 0      | 0     | 0     | 0     | 1   |
| *Bucephalus* sp. | 0    | 0           | 0                | 0      | 0     | 1     | 0     | 2   |
| March 1992 |      |             |                  |        |       |       |       |     |
| Inflammation | 12   | -           | 1                | -      | 29    | -     | 1     | -   |
| *K. Concretions* | 0    | 0           | -                | -      | 0     | 3     | -     | -   |
| *Neoplasia* | 2    | -           | 2                | -      | 1     | -     | 0     | -   |
| *Cysts* | 0    | 0           | -                | 0      | 1     | -     | 0     | -   |
| *Bucephalus* sp. | 2    | -           | 2                | -      | 1     | 2     | -     | 9   |
| *Sphenophrya* sp. | 2    | -           | 0                | -      | 0     | 0     | -     | 2   |
| May 1992 |      |             |                  |        |       |       |       |     |
| Inflammation | 15   | -           | 1                | -      | 24    | -     | 4     | -   |
| *K. Concretions* | 0    | 0           | 0                | -      | 0     | 3     | -     | -   |
| *Neoplasia* | 0    | 2           | 0                | -      | 0     | 0     | 2     | -   |
| *Necrosis* | 0    | 0           | 0                | -      | 1     | 0     | 0     | 1   |
| *Ulceration* | 0    | 1           | -                | 0      | 0     | 1     | 1     | -   |
| *Bucephalus* sp. | 2    | -           | 0                | -      | 0     | 0     | 1     | 5   |
| *B.I.B.* | 0    | -           | 3                | -      | 0     | 0     | 0     | 3   |
| Total     | 43   | 1           | 14               | 1      | 91    | 30    | 17    | 4   |

* G.I. Tract : gastrointestinal tract
** B.I.B. : basophilic inclusion bodies in gastric mucosa
Table 8. Lesions and parasites in *Crassostrea virginica* from Charlestown Pond by organ (n = 10 from each station).

|                          | Gill | G.I. Tract* | Connective Tissue | Kidney | Gonad | Total | Total |
|--------------------------|------|-------------|-------------------|--------|-------|-------|-------|
|                          | 1    | 2   | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| **August 1991**          |      |      |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Inflammation             | 0    | 0   | 0 | 2 | 1 |    |    | 10 | 10 | 9  | 0   | 0   | 0   | 0   | 0   | 0   | 10 | 12 | 10 | 32 |
| Necrosis                 | 0    | 0   | 5 | 2 | 0 |    |    | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 5  | 2  | 0  | 7  |
| Bucephalus sp.           | 0    | 0   | 2 | 0 | 0 |    |    | 1  | 0  | 0  | 0   | 0   | 0   | 0   | 2   | 0  | 5  | 0  | 0  | 5  |
| **November 1991**        |      |      |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Inflammation             | 8    | 7   | 10| 0 | 0 | 4  | 7   |    | 10 | 10 | 10 | 0  | 0  | 0  | 0  | 1  | 0  | 19 | 21 | 27 | 67 |
| Necrosis                 | 0    | 0   | 0 | 4 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 4  |
| Neoplasia                | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 2  |
| K. Concretions           | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 1  | 2  | 0  | 0  | 1  | 2  | 3  |
| H. nelsoni               | 0    | 2   | 0 | 0 | 2 | 0  | 0   |    | 0  | 0  | 2  | 0  | 0  | 0  | 1  | 0  | 0  | 7  | 0  | 7  |
| B.I.B.**                 | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 2  | 2  |
| **March 1992**           |      |      |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Inflammation             | 5    | 10  | 10| 0 | 0 | 0  | 0   |    | 6  | 10 | 10 | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 21 | 20 | 52 |
| Necrosis                 | 0    | 0   | 0 | 1 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| Neoplasia                | 0    | 1   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| K. Concretions           | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| Atrophy A.M.             | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 4  |
| Sphenophrya sp.          | 0    | 2   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 8  | 2  | 2  |
| B.I.B.**                 | 0    | 0   | 0 | 1 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| **May 1992**             |      |      |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Inflammation             | 4    | 9   | 8 | 0 | 1 | 1  | 7   | 9  | 9  | 7  | 0  | 1  | 0  | 0  | 0  | 0  | 11 | 19 | 17 | 47 |
| Necrosis                 | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| Neoplasia                | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| K. Concretions           | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| Ulceration               | 0    | 0   | 0 | 0 | 0 | 0  | 1   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| Hyperplasia              | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 4  |
| H. nelsoni               | 0    | 2   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 3  | 0  | 3  |
| B.I.B.**                 | 0    | 0   | 0 | 0 | 0 | 0  | 0   |    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 1  |
| **Total**                | 17   | 36  | 28| 8 | 15 | 18 | 35  | 43 | 36 | 0  | 7  | 3  | 3  | 1  | 0  | 63 | 103| 85 | 251 |

* G.I. Tract: gastrointestinal tract  
** B.I.B.: basophilic inclusion bodies in gastric mucosa  
Atrophy A. M.: atrophy adductor muscle
Table 9. Lesions and parasites in *Crassostrea virginica* from Green Hill Pond by organ (n = 10 from each station).

| Station       | Gill | G.I. Tract* | Connective Tissue | Kidney | Gonad | Total | Total |
|---------------|------|-------------|-------------------|--------|-------|-------|-------|
|               | 1    | 2           | 3                 | 1      | 2     | 3     | 1     | 2     | 3     |
| August 1991   |      |             |                   |        |       |       |       |       |       |
| Inflammation  | 1    | 2           | 1                 | 0      | 0     | 0     | 10    | 10    | 10    |
| Necrosis      |      |             |                   |        |       |       | 0     | 2     | 0     |
| K. Concretions|      |             |                   |        |       |       | 0     | 0     | 0     |
| Cysts         |      |             |                   |        |       |       | 0     | 0     | 0     |
| Sphenophrya   |      |             |                   |        |       |       | 0     | 0     | 0     |
| B.I.B.**      |      |             |                   |        |       |       | 0     | 0     | 0     |
| November 1991 |      |             |                   |        |       |       | 11    | 12    | 11    |
| Inflammation  | 9    | 10          | 9                 | 2      | 3     | 0     | 10    | 9     | 10    |
| Necrosis      | 0    | 0           | 0                 | 5      | 6     | 1     | 0     | 0     | 0     |
| K. Concretions| 0    | 0           | 0                 | 0      | 0     | 0     | 0     | 3     | 1     |
| Cysts         | 0    | 0           | 0                 | 0      | 0     | 0     | 0     | 0     | 1     |
| Sphenophrya   | 0    | 3           | 1                 | 0      | 0     | 0     | 0     | 0     | 0     |
| B.I.B.**      | 0    | 0           | 0                 | 2      | 0     | 0     | 0     | 0     | 0     |
| March 1992    |      |             |                   |        |       |       | 14    | 12    | 12    |
| Inflammation  | 4    | 1           | 2                 | 0      | 0     | 0     | 10    | 10    | 10    |
| Neoplasia     | 0    | 0           | 0                 | 0      | 1     | 0     | 0     | 0     | 0     |
| K. concretions| 0    | 0           | 0                 | 0      | 0     | 0     | 0     | 2     | 0     |
| Sphenophrya   | 1    | 0           | 0                 | 0      | 0     | 0     | 0     | 0     | 0     |
| B.I.B.**      |      |             |                   |        |       |       | 1     | 0     | 0     |
| May 1992      |      |             |                   |        |       |       | 23    | 17    | 17    |
| Inflammation  | 9    | 8           | 7                 | 1      | 1     | 0     | 10    | 8     | 8     |
| Neoplasia     | 0    | 1           | 0                 | 0      | 0     | 0     | 0     | 0     | 0     |
| K. Concretions| 0    | 0           | 0                 | 0      | 0     | 1     | 0     | 1     | 1     |
| Sphenophrya   | 1    | 0           | 0                 | 0      | 0     | 0     | 0     | 0     | 0     |
| B.I.B.**      |      |             |                   |        |       |       | 1     | 0     | 0     |
| Total         | 25   | 25          | 20                | 10     | 11    | 2     | 40    | 37    | 38    |
|               |      |             |                   |        |       |       | 9     | 5     | 9     |
|               |      |             |                   |        |       |       | 0     | 0     | 0     |

* G.I. Tract: gastrointestinal tract
** B.I.B.: basophilic inclusion bodies
Figure 2. Oyster collected November 1991 from Charlestown Pond. Plasmodia of *Haplosporidium nelsoni* (arrow) are associated with a branchitis. (H&E, 100X).

Figure 3. Higher magnification of plasmodia *Haplosporidium nelsoni* in Fig. 2. (H&E, 1000X).
Figure 4. Oyster collected March 1992 from Narrow River. Note xenomas of branchial tissue *Sphenophrya sp.* (H&E, 100X).

Figure 5. Oyster collected May 1992 from Pawcatuck River. Crustacean (sp. unknown) (arrow) located in a tubule of digestive diverticula. Note ulceration and inflammation of tubule wall. (H&E, 250X).
Metazoan Diseases

Crustacea

One of the oysters from Pawcatuck River in May 1992 was infected by one crustacean of unidentified species (Tables 5 and 6). It was located in a tubule of the digestive diverticulum and caused ulceration and inflammation of the tubule wall (Figure 5).

Trematoda

Sporocysts of the trematode *Bucephalus* sp. were found in vesicular connective tissue and the gonads of *Crassostrea virginica*. Histological sections revealed heavy infections of *Bucephalus* sp. in 13 of 480 (2.7%) oysters. Three of 480 (<1.0%) oysters were found to be lightly infected. Percent infections are listed in Table 1, 4, 5, 6, 7, and 8. Sporocysts of *Bucephalus* sp. were found in the reproductive follicles (Figure 6) and in connective tissues (Figure 7).

Bacterial Diseases

Basophilic Inclusion Bodies (BIB)

Basophilic inclusion bodies (BIB) were found in the gastrointestinal tract of *Crassostrea virginica* at all four sites in 15 of 480 oysters (Table 1). Percent infection of BIB of each station was shown in Tables 4, 5, 6, 7, 8, and 9. The basophilic bodies in the gastric mucosa is shown in Figure 8.

Lesions

Kidney Concretions and Brown Cells

Large, round, brown pigmented bodies similar to the concretions described by Rheinberger (1977) and Carmichael et al. (1979) were observed in the kidney of *Crassostrea virginica*. Concretions were located in the kidney tubule (Figure 9) and were
Figure 6. Oyster collected March 1992 from Narrow River.
Sporocysts of Bucephalus sp. in the reproductive follicle.
(Arrow). (H&E, 250X).

Figure 7. Oyster collected March 1992 from Narrow River.
Sporocysts of Bucephalus sp. in connective tissue (arrow A)
and germ balls (arrow B). (H&E, 100X).
Figure 8. Oyster collected May 1992 from Pawcatuck River. Basophilic Pigmented Bodies (BIB) in the gastric mucosa. (Arrow). (H&E, 250X).

Figure 9. Oyster collected March 1992 from Green Hill Pond. Concretions in the kidney tubule. (Arrow). (H&E, 250X).
found at all sampling sites. The percent oysters with concretions was 8.8 (32 of 360) (Tables 1, 4, 5, 6, 7, 8, and 9). Besides kidney concretions, numerous brown cells located in the connective tissue of the visceral mass were found in almost all oysters (Figure 10). The number of brown cells per oyster were categorized as light (1 - 3 cells per high magnification), moderate (4 - 6 cells per high magnification) and heavy (7 - 9 cells per high magnification). In oysters from Pawcatuck River and Narrow River, brown cells were larger, more numerous, and had darker and larger vesicles with more inclusion bodies than in those oysters from Charlestown Pond and Green Hill Pond (Table 10).

Ulceration

Only two of all the *Crassostrea virginica* examined contained ulcerative lesions (Tables 1, 5, 7, and 8). The ulceration occurred in the stomach epithelium (Figure 11). Inflammation of the submucosa and thickening of basement membrane were also found in these oysters. A causative agent could not be determined.

Necrosis

Necrotic foci were observed in the digestive tubules of the digestive diverticulum. Three forms of cell nuclei were observed: pyknotic, karyorrhectic, and karyolytic. Necrotic tissue occurred in 30 of 480 (6.2%) samples (Tables 1, 4, 5, 6, 7, 8, and 9).

Hyperplasia

Hyperplasia was observed in the tubular epithelium of the digestive diverticulum (Figure 12). It consisted of an increase in the number of basophilic epithelial cells. Only one oyster from Charlestown Pond had hyperplasia (Tables 1, 5, and 8).
Figure 10. Oyster collected May 1992 from Pawcatuck River.

Numerous “brown cells” are located in the connective tissue of the visceral mass. (H&E, 250X).

Figure 11. Oyster collected in May 1992 from Narrow River. Ulceration of the gastric epithelium. (Arrow). Note inflammation of the submucosa and thickening of basement membrane. (H&E, 100X).
Figure 12. Oyster collected in May 1992 from Charlestown Pond.
Hyperplasia of tubular epithelium (arrow). Note also
destruction of the tubules. (H&E, 250X).

Figure 13. Oyster collected in March 1992 from Pawcatuck River.
Gonadal neoplasm (germinoma), prominent large, dark,
basophilic invasive cells are undifferentiated germ cells.
(H&E, 250X).
Neoplasia

Neoplastic lesions in oysters are generally characterized by the infiltration of vesicular connective tissue and by hypertrophic, anaplastic, mitotically active, basophilic cells. Cells typically have a high nucleus to cytoplasmic ratio and pleomorphic nuclei with one or more distinct clefts. Multiple nucleoli are present in some cases (Murchelano and MacLean, 1990).

One of the oysters from Pawcatuck River in March, 1992 had germinomas (Figure 13). Basophilic, hypertrophied, neoplastic germ cells had proliferated along the walls of the gonadal follicles and ducts. Three of the oysters had adenomas in the gill (Figure 14). During this study four oysters of 120 (3.9%) from Pawcatuck River had neoplasia in March and May 1992 (Table 1, 4, 5, 6, 7, 8, and 9).

The sarcomas are characterized by the appearance of unusual cells in the connective tissue, blood vessels, and sinuses of the visceral mass, muscle and mantle tissue (Peters, 1988). One oyster from Narrow River in March 1992 was found to have an eosinophilic mass infiltrating Leydig cells (Figure 15). One oyster from this site had neoplasia in the gastrointestinal tract and in the gill in March 1992. One oyster had an adenoma in the gastrointestinal tract also in March 1992 and two oysters in May 1992. A total of seven oysters in Narrow River had neoplasms (Tables 1 and 5). One oyster from Narrow River in March 1992 had adenocarcinoma in the gills (Figure 16) and adenomatous proliferations (Figure 17). A tumor of a neural origin in the connective tissue of a gill filament was found in March 1992 (Figure 18).

Neoplasia in the gastrointestinal epithelium was found in oysters from Charlestown Pond; one in November 1991 and one in May 1992 (Tables 1, 4, 5, and 8). Adenocarcinomas in the gills and a mesenchymal of the connective tissue were also present
Figure 14. Oyster collected in May 1992 from Pawcatuck River.

Adenocarcinoma of the gill tissue. (H&E, 250X).

Figure 15. Oyster collected in March 1992 from Narrow River.

Leydig cells infiltrated by eosinophilic mass, probably of mesenchymal origin. (H&E, 100X).
Figure 16. Oyster collected in March 1992 from Narrow River.

Note adenocarcinoma of the gills. (H&E, 25X).

Figure 17. Higher magnification of the adenocarcinoma in Fig. 16.

Note proliferation of epithelium and amoebocyte infiltration of the connective tissue. Adenomatous proliferation at arrow A. (H&E, 100X).
Figure 18. Oyster collected in March 1992 from Narrow River. Neoplasm of neural origin in the gills. Note: neuron (A) and nerve fiber (B) formation in the connective tissue of the gill filament. (H&E, 100X)

Figure 19. Oyster collected in May 1992 from Pawcatuck River. Atrophy of adductor muscle bundles (A) and replacement by proliferation of collagenous connective tissue (B). (H&E, 250X).
in oysters collected from Charlestown Pond in March 1992 and November 1992 (Table 8).

Three oysters in Green Hill Pond had neoplasia, one in the gill and two in the gastrointestinal epithelium (Table 9).

During this study, 18 of 480 (3.7%) oysters had neoplasms in the gastrointestinal tract, reproductive tract, connective tissue, and gills (Tables 1, 4, 5, 6, 7, 8, and 9). Eight of these oysters had adenomas in the stomach epithelium, one had a germinoma, two had a mesenchymal origin of connective tissue and eight had branchial neoplasia (Table 10).

**Atrophy of Adductor Muscle**

One oyster collected from Pawcatuck River in May 1992 and one oyster from Charlestown Pond in March 1992 had the unusual condition adductor of muscle atrophy and replacement by proliferating collagenous connective tissue (Figure 19).

**Cyst (Idiopathic)**

Cysts were found in three kidney tubules of oysters from Pawcatuck River, Narrow River, and Green Hill Pond (Tables 1, 4, 5, 7, and 9). Figure 20 shows destruction of kidney tubular epithelium in oyster collected in May 1992 from the Pawcatuck River. Cyst formation in the tubular epithelium is also shown. Another kidney tubular epithelial cyst was found in an oyster from Narrow River collected in March 1992 (Figure 21).

**Inflammation**

Almost all the oysters had focal inflammation in the connective tissue [447 out of 480 oysters (93.1%)]. Some animals had very extensive inflammation. The inflammation in the connective tissue were categorized as very light to light and moderate to heavy (Table 10). Sixteen (3.3%) had inflammation in the kidney, 210 (43.8%) had inflammation in the gills and 28 (5.8%) had gastrointestinal tract inflammation (Table 1, 4, 5, 6, 7, 8, and 9).
Table 10. Neoplasms of *Crassostrea virginica* found from July 1991 to May 1992 (N = 480).

| Site              | Station | Time           | Neoplasms          | Organ   |
|-------------------|---------|----------------|--------------------|---------|
| Pawcatuck River   | 2       | March 1992     | Adenocarcinoma     | Gills   |
|                   | 2       | March 1992     | Adenocarcinoma     | Gills   |
|                   | 3       | March 1992     | Germinoma          | R.T.    |
|                   | 2       | May 1992       | Adenocarcinoma     | Gills   |
| Narrow River      | 1       | November 1991  | Neural origin      | Gills   |
|                   | 1       | March 1992     | Adenocarcinoma     | Gills   |
|                   | 1       | March 1992     | Adenoma            | G.I.    |
|                   | 1       | March 1992     | Mesenchymal origin | C.T.    |
|                   | 1       | March 1992     | Neural origin      | Gills   |
|                   | 1       | March 1992     | Adenoma            | G.I.    |
|                   | 1       | May 1992       | Adenoma            | G.I.    |
|                   | 1       | May 1992       | Adenoma            | G.I.    |
| Charlestown Pond  | 1       | November 1991  | Mesenchymal origin | C.T.    |
|                   | 3       | November 1991  | Adenoma            | G.I.    |
|                   | 2       | March 1992     | Adenocarcinoma     | Gills   |
|                   | 3       | May 1992       | Adenoma            | G.I.    |
| Green Hill Pond   | 2       | May 1992       | Adenoma            | G.I.    |
|                   | 2       | May 1992       | Neural origin      | Gills   |
|                   | 3       | May 1992       | Adenoma            | G.I.    |

G.I. = Gastrointestinal tract.
R.T. = Reproductive tract.
C.T. = Connective tissue.
Figure 20. Oyster collected in May 1992 from Pawcatuck River.

Necrosis of renal tubular epithelium, dilation of the tubules and thickening of basement membrane (A), cyst in the renal tubular epithelium (B), and proliferation of epithelium of renal tubules. (H&E, 100X).

Figure 21. Oyster collected in March 1992 from Narrow River.

Cyst in renal tubular epithelium. (Arrow). (H&E, 250X).
Table 11. Inflammation and "brown cells" in the connective tissue of *Crassostrea virginica* in Rhode Island (n = 120 from each site).

| SITE              | July and August 1991 | November 1991 | March 1992 | May 1992 |
|-------------------|----------------------|---------------|------------|----------|
|                   | Inflam. B.C.         | Inflam. B.C.  | Inflam. B.C. | Inflam. B.C. |
|                   | VL MH                | VL MH         | VL MH      | VL MH    |
| Pawcatuck River   |                      |               |            |          |
| 1                 | 9 1                  | 2 8           | 2 8        | 0 10     |
| 2                 | 7 1                  | 3 7           | 2 7        | 3 7      |
| 3                 | 7 2                  | 3 7           | 5 4        | 2 8      |
| Total             | 23 4                 | 8 22          | 11 19      | 5 25     |
| Narrow River      |                      |               |            |          |
| 1                 | 11 4                 | 3 12          | 8 7        | 3 12     |
| 2                 | 13 0                 | 5 10          | 10 5       | 9 6      |
| Total             | 24 4                 | 8 22          | 18 12      | 12 18    |
| Charlestown Pond  |                      |               |            |          |
| 1                 | 6 4                  | 5 5           | 9 1        | 7 3      |
| 2                 | 5 5                  | 5 5           | 6 4        | 3 7      |
| 3                 | 7 2                  | 4 6           | 9 1        | 10 0     |
| Total             | 18 11                | 14 16         | 24 6       | 20 10    |
| Green Hill Pond   |                      |               |            |          |
| 1                 | 10 0                 | 7 3           | 10 0       | 8 2      |
| 2                 | 10 0                 | 7 3           | 6 3        | 5 5      |
| 3                 | 10 0                 | 10 0          | 10 0       | 6 4      |
| Total             | 30 0                 | 24 0          | 26 3       | 19 11    |

Note: Inflam = inflammation  
B.C. = brown cells  
VL = very light to light  
MH = moderate to high
Condition Index

Condition index was determined in 150 oysters. Condition index from each site and sampling period are given in Table 12. The condition index ranged from 26.52 to 197.92. Throughout this study, condition index of oysters from the Pawcatack River remained low (below 75). In the other study areas, condition index was consistently lowest in the summer and higher in fall, winter and spring.
Table 12. Condition index, mean and coefficient of variation (C.V.) of *Crassostrea virginica* in Rhode Island from August 1991 to May 1992 (n = 10 oysters each site).

| Site               | August 1991 | November 1991 | March 1992 | May 1992     |
|--------------------|-------------|---------------|------------|--------------|
|                    |             |               |            |              |
| Pawcatuck River    |             |               |            |              |
| Range              | -           | 26.52 - 72.09 | 29.06 - 89.60 | 40.07 - 72.73 |
| Mean; C.V.         | -           | 53.88 ; 27.54 | 53.29 ; 38.73 | 53.89 ; 20.77 |
| Narrow River       |             |               |            |              |
| Range              | 47.32 - 100.30 | 77.27 - 154.09 | 63.53 - 149.43 | 68.70 - 116.55 |
| Mean; C.V.         | 66.39 ; 26.50 | 109.82 ; 23.78 | 120.61 ; 21.65 | 94.01 ; 14.38 |
| Charlestown Pond   |             |               |            |              |
| Range              | 44.28 - 140.77 | 59.54 - 197.67 | 73.97 - 143.42 | 70.98 - 137.29 |
| Mean; C.V.         | 82.39 ; 37.15 | 120.12 ; 37.15 | 105.99 ; 20.45 | 99.94 ; 18.92 |
| Green Hill Pond    |             |               |            |              |
| Range              | 51.21 - 123.39 | 45.95 - 145.31 | 44.19 - 152.68 | 54.26 - 197.92 |
| Mean; Range        | 78.63 ; 28.69 | 108.15 ; 42.95 | 83.13 ; 37.85 | 108.45 ; 43.95 |

C.V. = Standard deviation X 100%

Mean
DISCUSSION

The full life cycle of *Haplosporidium nelsoni* is not known, although patterns of infection and mortality are well documented (Andrews, 1966; Couch et al., 1966; Farley, 1967; Ford and Haskin, 1982). Studies of infection and mortality patterns along salinity gradients showed that *Haplosporidium nelsoni* can not survive in salinities below 10 ppt. Infections are generally rare and parasite development is inhibited below 15 ppt salinity. The parasite survives best in salinities above 20 ppt (Andrews, 1964, 1983; Haskin and Ford, 1982; Ford, 1985; Ford and Haskin, 1982). In this study surface salinity ranged between 0 to 30 ppt. According to Ford and Haskin (1982), the inability to transmit MSX from one oyster to another, combined with the rarity of production of spores in oysters, suggests that an alternate host must exist. Therefore a possible explanation for MSX being found in Charlestown Pond might be the presence there of an alternate host, but a previous one-year disease survey was conducted in June 1979 to May 1980, and no evidence of MSX was found (Cooper and Durfee, 1982).

*Sphenophrya* sp is a ciliate that attaches to gill epithelial cells of oysters and other bivalves (Otto et al., 1979; Murchelano and MacLean, 1990). Karyokinesis and marked hypertrophy of affected epithelial cells indicate the presence of the reproductive stages of the parasite that form xenomas in the gill. The ciliate induces focal gill lesions. The larva of *Sphenophrya* sp. is ciliated but the adult is non-ciliated. *Sphenophrya* sp. was found in all locations during March 1992 (late winter) and May 1992 (late spring). The number of these ciliates may increase under condition of stress (Murchelano and MacLean, 1990). The respiration and feeding of the oyster possibly was adversely affected by the heavy infections of these ciliates (Otto et al., 1979).

The initial infection site of *Bucephalus* sp. is the digestive tubules rather than the gonad (Cheng and Burton, 1965). Heavy infections destroy the tissues and possibly result in the
death of the oysters (Hopkins, 1957). Initial infection has the result of effectively castrating the oysters (Sparks, 1985). There is little host response to the presence of the parasite. There is neither phagocytosis nor encapsulation, but hemocyte infiltration may occur following sporocyst degeneration (Cheng and Burton, 1965). Sypek (1979) studied the trematode *Proctoeces maculatus* in *Mytilus edulis* and found it the only known example of an adult trematode eliciting a hemocytic response within a mollusc. The occurrence of lipid substances in the hemocytes was associated with the host response (Sypek, 1979). Sporocysts of the trematode *Bucephalus* sp. were found at all of the sites studied except Green Hill Pond. Gauthier et al. (1990) determined that *B. cuculus* can be found in oysters over a wide range of salinity (10 to 26 ppt). However, in this study *Bucephalus* infection was found in the Pawcatuck River at 0 ppt surface salinity. Cheng and Burton (1965) found 23 of 436 (5.3%) oysters from Charlestown Pond to be infected with this parasite. In this study, a somewhat lower rate of infection was found in Charlestown Pond 16 of 480 (3.3%). *Bucephalus* sp. may affect the recruitment of oysters by parasitic castration, causing loss of gamete production (Cheng and Burton, 1965).

The kidney of the oyster has been found to accumulate concretions of golden yellow to black granular material in the lumens of tubules when the animal is under stress (Yevich, 1980). One study showed that the accumulation of concretions did not damage the kidney (Potts, 1967), but Rheinbarger et al. (1977) showed that larger concretions can cause an inflammation of the kidney of *Mercenaria mercenaria* with a resulting loss of epithelium. The kidney concretions often consist of metals with Ca, Mg, Zn, Fe, and Pb being the major elements (Rheinbarger et al. 1977). In this study, the highest prevalence of kidney concretions was found in oysters from Green Hill Pond. The lowest rate was found in the Pawcatuck River (1/90). The kidney concretion was not found in Pawcatuck River stations
1 and 2 (low salinities) but was present in oyster from station 3. Possibly there is a correlation between kidney concretions and salinity. Kidney concretions were more prevalent in oysters from sites with higher salinity, such as Narrow River, Charlestown Pond, and Green Hill Pond. It has been suggested that kidney concretions may be indicators of environmental degradation since larger concretions are often found in more polluted areas (Rheinbarger et al., 1977).

The presence of large aggregates of yellowish bodies and larger brownish bodies (probably "brown cells") have been studied by Takatsuki in 1934. Brown cells may have functions associated with the oyster's internal defense mechanisms (Mackin 1951). The number of brown cells increase if oysters suffer disease, stress or pathological condition (Sparks, 1985). Brown cells contain lipofuscin, a complex of lipids, phospholipids and some protein (Slauson et al., 1990). Sypek (1979) analysed the pigment in mussels and found it contained lipofuscins and carotenoids. Cheng and Rifkin (1970) stated that the most important role of these cells was in the removal of degradation products and metabolic by-products of parasite infections.

Basophilic inclusion bodies (BIB) have been found in marine bivalve molluscs and electron microscopic studies showed the oyster inclusions most commonly contained mycoplasma-like organisms (Harshbarger et al., 1977). No host response was associated with BIB infections in bivalves, but the heavy infections possibly reduce the metabolic efficiency and alter the nutrients of the host (Otto et al., 1979). Six of 120 oysters in coastal Louisiana had BIB (Gauthier et al., 1990), a prevalence higher than observed in this study [15 of 480 oysters (3.2%)]. This finding is similar to those described by Barszcz et al. (1978) and Gauthier et al. (1990) of large, roundish, basophilic inclusions in the intestinal epithelium of Crassostrea virginica. Gauthier et al. (1990) also reported that in higher salinity the oyster had higher levels of parasitism.
One important finding in this study is the high incidence of neoplasia [18 of 480 oysters (3.8%)]. In two separate Chesapeake Bay studies, neoplasia was observed in 12 of 20,000 *Crassostrea virginica* (Harsbarger et al., 1979) and five out of 30,000 oysters (Farley, 1969). Several gonadal tumors were found in oysters from the Pawcatuck River at the same station # 1 as in this study (Yevich, 1992 personal communication). Similar lesions have been found by Farley (1976) and Harshbarger et al. (1979). One oyster from Narrow River in March 1992 would most likely be a tumor probably of mesenchymal origin. Newman (1972) also described an undifferentiated sarcoma in *Crassostrea virginica* from New Haven Harbor, Connecticut. Gardner et al. (1991) reported that two of 46 oysters exposed to Block Rock Harbor (Connecticut) sediments developed gill papillomas. Paquette (1992) stated that the prevalence of neoplasia in *Mya arenaria* in Rhode Island was the highest in January to March, May and October. Similarly in this study the highest prevalences of neoplasia were in March and May.

The cause of the neoplasia in *Crassostrea virginica* is not certain. Molluscan neoplasia is not a result of infection by bacteria, mycoplasma, or protozoan parasites (Oprandy, 1982; Paquette, 1992). Barry and Yevich (1975) reported that gonadal tumors in *Mya arenaria* in Maine were possibly the result of an oil spill; and Yevich and Barszcz (1976) stated that tumors in *Mya arenaria* could be related to pollution. Two types of neoplasia in *M. arenaria* were found from polluted and nonpolluted areas (Brown et al., 1977). Oprandy et al. (1981) reported that hematopoietic neoplasia in *Mya arenaria* is due to a viral agent. In several studies, a correlation was made between carcinogenic hydrocarbons in sediment and high incidence of neoplasia in bivalves (Gardner et al., 1991; Paquette, 1992). In this study, neoplasia was found in all of the sites studied. Pawcatuck River, Narrow River, and Green Hill Pond are classified as polluted on the basis of fecal coliform (Strobel et al., 1987 and Ganz, 1992). Charlestown Pond is the only one certified by RI DEM to be unpolluted and open for shellfishing.
The high prevalence of oysters with inflammation is unusual. The causes of the observed inflammation is uncertain, but it could be caused by a protozoan, bacterial or viral disease. Some oysters had very extensive inflammation in all organs. According to Farley (1992, personal communication) pollution stressors are likely cause for the inflammation in the oysters. Sparks and Morado (1988) and Slauson et al. (1990) suggest that inflammation is the host defense mechanism following tissue damage.

Condition index of oysters from the Pawcatuck River was very low during the year, but the prevalence of disease was not necessarily higher than other sites. This indicates that condition index may not be a good indicator of disease prevalence. In this site, the size of oysters were between 5 and 18 cm (mature), however there appeared to be no gonadal development. More studies are needed to investigate oyster spawning and recruitment in this area. In the other study areas, condition index was consistently lowest in the summer and higher in fall, winter, and spring. This is probably due to summer spawning.

Most of the Rhode Island oyster beds have been closed for shellfishing due to water quality degradation. All the oysters in this study were from native oysters beds. Parasitism and lesions have been found at all of the sites studied. Further studies are needed to determine the etiology of some of the diseases.
CONCLUSIONS

Histopathologic studies on oysters collected from Pawcatuck River, Narrow River, Charlestown Pond, and Green Hill Pond showed that the prevalence of MSX is very low (0.8%). This may be due to the lack of a suspected alternate host in this area and as yet uncertain environmental factors.

Many other disease conditions were found in this study, such as parasites of Sphenophrya sp. (3.1%), Bucephalus sp. (3.3%), Crustacea (0.2%), and basophilic inclusion bodies (3.2%). Some lesions also were found in these samples, such as kidney concretions (8.8%), necrosis of digestive diverticulum (5.8%), neoplasia of gastrointestinal tract, connective tissue, reproductive tract and gills (3.8%), hyperplasia of digestive diverticulum (0.2%), ulceration of stomach epithelium (0.4%), cysts in the kidney (0.8%), atrophic adductor muscle (0.6%) and inflammation of the kidney, gills, gastrointestinal tract, and connective tissue (93.1%).

Mean condition index (CI) of oysters from Pawcatuck River were below 75 throughout the year, suggesting poor condition. In other areas the mean CI was almost always almost above 75 throughout the year, despite lesions and parasitism being found at all of the sites studied. Further studies are needed to understand the ecology of the oyster populations in Rhode Island.
Appendix I

Fixatives

**Formalin (10% sea water)**

- 37 - 40% formaldehyde: 10 ml.
- Filtered ambient sea water: 90 ml.

**Helly’s Fixative**

- Zinc Chloride: 1,000 gr.
- Potassium dichromate: 500 gr.
- Distilled water: 20 l.

Add 5 ml. of 37 - 40% formaldehyde per 100 ml. of fixative at time of use.
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