A survey of disease in the oyster Crassostrea virginica (Gmelin, 1791) in Rhode Island coastal estuaries

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A SURVEY OF DISEASE IN THE OYSTER
*CRASSOSTREA VIRGINICA* (GMELIN, 1791) IN
RHODE ISLAND COASTAL ESTUARIES

Retno Andamari, Michael A. Rice, and Paul P. Yevich

Abstract.—The presence of potential pathogens and lesions in American oysters, *Crassostrea virginica* (Gmelin, 1791), from coastal estuaries in Rhode Island was studied. Oysters were collected by hand or tongs from three stations in the Pawcatuck River, two stations in Narrow River, three stations in Charlestown Pond, and three stations in Green Hill Pond, during July/August 1991, November 1991, March 1992, and May 1992. Oysters were processed for histologic examination and determination of condition index. MSX, *Haplosporidium nelsoni* (Haskin, Stauber et Mackin, 1966), was detected in four of 480 oysters (0.8%); *Sphenoprya* sp., 15 of 480 (3.1%); *Bucephalus* sp., 16 of 480 (3.3%); crustacea, one of 480 (0.4%); and basophilic inclusion bodies (possible mycoplasma-like organisms), 15 of 480 samples (3.2%). Lesions found in the oysters included: kidney concretions (8.8%); necrosis of the digestive diverticula (5.8%); neoplasia of the digestive tract, connective tissue, reproductive tract, or ctenidia (3.8%); hyperplasia of digestive diverticula (0.2%), ulceration of the stomach epithelium (0.4%); cysts in the kidney (0.8%); atrophic adductor muscle (0.6%); and inflammation of the kidney, ctenidia, gastrointestinal tract, or connective tissue (93.1%). Lesions and parasites were found at all of the sites studied, although MSX was found only in Charlestown Pond. Condition index—the resultant of 1,000 times the dry weight of the soft tissues divided by the water-weight volume equivalent of the mantle cavity—ranged from 26.52 to 197.67. Disease prevalence in oysters from the Pawcatuck River was not different from other sites, but the condition index values were consistently low (below 75 year around). These findings suggest that low condition index may not necessarily correlate with higher disease prevalence.

Introduction

In recent years there has been widespread concern about the impact of various parasites on the American oyster, *Crassostrea virginica* (Gmelin, 1791) fisheries along the eastern coast of North America. The economic impacts of parasites and disease have been severe. Total landings of oysters in the United States in 1990 was 13,242 metric tons (mt), down from 327,500 mt in 1971 prior to major epizootics of oyster parasites. The key parasites
of concern are *Haplosporidium nelsoni* (Haskin, Stauber et Mackin, 1966) or MSX, and *Perkinsus marinus* (Mackin, Owen et Collier, 1950), commonly called “Dermo” from its original genus designation of *Dermocystidium*. Sindermann (1990) estimated that losses to the oyster industry had been in excess of $3 million per year over a 12 year period. Dermo has been a major problem along the Gulf of Mexico coast since the early 1950s (Mackin, 1961; Gauthier et al., 1990), but has been spread northward along the Atlantic coast to Chesapeake Bay (Mann et al., 1991). MSX was found in the lower Chesapeake Bay in 1959, and spread progressively up the bay into Maryland (Farley, 1975). MSX was also found in the late 1950s in the Delaware Bay (Ford and Haskin, 1982). Since that time, MSX has progressively spread to North Carolina and Long Island Sound (Sindermann and Rosenfield, 1967) and to Massachusetts and the Great South Bay of New York (Haskin and Ford, 1982). Even though MSX has been reported in nearby waters, a 1982 study by Cooper and Durfee failed to find MSX in Rhode Island oysters. There have been no other oyster disease surveys in Rhode Island since 1982.

Several studies have been undertaken to describe the pathological effects of MSX and other diseases. Haskin et al. (1966) reported that particle filtration rates were reduced MSX infected oysters, possibly affecting their nutritional state. Barber et al. (1988) showed that sublethal MSX infections of *Crassostrea virginica* indeed resulted in reduced nutritional state and relative fecundity with subsequent lowered condition index. Condition index, a ratio of soft tissue mass to mantle cavity volume, has been suggested to be a simple means for assessing nutritional and reproductive state, and an index of general oyster health (Quayle, 1980; Newell, 1985; Crosby and Gale, 1990). The purpose of this study is to assess the incidence of parasites and disease in Rhode Island oysters, and to assess condition index as an indicator of general health.

**Materials and Methods**

Oysters were taken from the Pawcatuck River (3 stations, Charlestown Pond (3 stations), Green Hill Pond (3 stations), and Narrow River (2 stations) in Rhode Island (Fig. 1). Samples of 40 oysters were obtained quarterly between July 1991 and May 1992 from each site by hand or by tongs from a small boat. Oysters used for this study ranged from 24.6 to 190.0 mm in valve height. Surface water temperature and salinity were determined when oysters were collected.

Oysters collected in July/August 1991 were brought to the laboratory within 2 hours, and cleaned of fouling organisms. Ten of the oysters from each site were reserved for condition index determinations. The remaining 30 oysters from each site were shucked, and the soft tissues fixed in 10%
seawater formalin, but a cross sectional cut was made on each oyster before fixation. Preserved tissue was dehydrated by ethanol series and imbedded in paraffin. Afterwards, 6-μm thick sections were stained with hematoxylin and eosin prior to mounting and examination. Oysters collected in November 1991, March 1992, and May 1992 were treated similarly, but were fixed with fresh Helly's fixative (50 g zinc chloride, 25 g potassium dichromate, and 50 ml of 37% formaldehyde per liter of water). Helly's fixative has been determined to be the most suitable cytoligical 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 (ctenidium only), or systemically infected (sub-epithelial general infection). Other parasites and pathologic conditions observed during this study were recorded.

The microscope slides were archived for possible future verifications. Most
slides are at the Marine Pathology Laboratory, East Farm, University of Rhode Island. Slides showing neoplasia are housed at the Registry of Tumors in Lower Animals, Pathology Dept., George Washington University Medical Center, Washington, D.C., (accession numbers RTLA 6040 to 6066).

Morphometric analysis of oysters included determination of valve height, dry weight of soft tissues and total live weight. The condition index (CI) determination followed the recommended procedure of Crosby and Gale (1990).

\[ \text{CI} = \frac{\text{dry tissue weight (g)}}{\text{mantle cavity capacity (g)}} \times 1,000 \]

Internal shell capacity (grams) was determined by subtracting dry shell weight (grams) in air, from the total live weight (grams) in air. 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 at 80°C for 48 h. According to Quayle (1980), oysters with high condition index (>75) are in good condition, while low condition index is 75 and below.

Results

The measured water temperature in the Narrow River, Charlestown Pond, and Green Hill Pond varied between 26°C in July 1991 and 4°C in March 1992. Salinities in these sites were highest in July (20 to 26 ppt) and lowest in March (15 to 20 ppt). The average Pawcatuck River temperature and salinity extremes were 23°C and 21 ppt in July, and 4°C and 7 ppt in March.

Various types of parasites and lesions were found with frequencies of 0.2 to 93.1% in oysters from the four collection sites (Table 1). Specific parasites and lesions found include the following:

Protozoan parasites

*Haplosporidium nelsoni* (MSX). During the year-long study, MSX was only found in Charlestown Pond in November 1991 and March 1992 (Fig. 2). Of four oysters found with infections, two were initial epithelial infection, and two were systemic.

*Sphenophrya* sp. The ciliate *Sphenophrya* sp. was found in hypertrophic cells of the ctenidium of 15 of 480 oysters examined (Table 1). The highest level of infection was seven of 120 (5.8%) in March 1992. *Sphenophrya* sp. was found at all sites and at all seasons of the year. Little or no host response to the ciliate was seen.

Metazoan parasites

Crustacea. One of the oysters from Pawcatuck River was infected by a crustacean of unidentified species (Table 1). It was located in a tubule of
Table 1. Prevalence of lesions and parasites in *Crassostrea virginica* from July 1991 to May 1992 in Rhode Island (N = 480).

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Pawcatuck River</th>
<th>Narrow River</th>
<th>Charlestown Pond</th>
<th>Green Hill Pond</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>%</td>
<td>#</td>
<td>%</td>
<td>#</td>
</tr>
<tr>
<td>Inflammation**</td>
<td>113/120</td>
<td>94.2</td>
<td>111/120</td>
<td>92.5</td>
<td>108/120</td>
</tr>
<tr>
<td>Kidney concretions</td>
<td>1/90</td>
<td>1.1</td>
<td>9/90</td>
<td>10.0</td>
<td>8/90</td>
</tr>
<tr>
<td>Necrosis***</td>
<td>4/120</td>
<td>3.3</td>
<td>1/120</td>
<td>0.8</td>
<td>11/120</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>4/120</td>
<td>3.3</td>
<td>7/120</td>
<td>5.8</td>
<td>4/120</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>0/120</td>
<td>0.0</td>
<td>0/120</td>
<td>0.0</td>
<td>1/120</td>
</tr>
<tr>
<td>Ulceration</td>
<td>0/120</td>
<td>0.0</td>
<td>1/120</td>
<td>0.8</td>
<td>1/120</td>
</tr>
<tr>
<td>Cysts (Idiopathic)</td>
<td>1/90</td>
<td>1.1</td>
<td>1/90</td>
<td>1.1</td>
<td>0/90</td>
</tr>
<tr>
<td>Atrophy Adductor Muscle</td>
<td>1/90</td>
<td>1.1</td>
<td>0/90</td>
<td>0.0</td>
<td>1/90</td>
</tr>
<tr>
<td>Parasites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haplosporida* nelsoni</td>
<td>0/120</td>
<td>0.0</td>
<td>0/120</td>
<td>0.0</td>
<td>4/120</td>
</tr>
<tr>
<td>Sphenophyra sp.</td>
<td>2/120</td>
<td>1.7</td>
<td>2/120</td>
<td>1.7</td>
<td>5/120</td>
</tr>
<tr>
<td>Bucephalus sp.</td>
<td>3/120</td>
<td>2.5</td>
<td>11/120</td>
<td>9.2</td>
<td>2/120</td>
</tr>
<tr>
<td>Basophilic Inclusion Bodies</td>
<td>5/120</td>
<td>4.2</td>
<td>3/120</td>
<td>2.5</td>
<td>5/120</td>
</tr>
<tr>
<td>Crustacea</td>
<td>1/120</td>
<td>0.8</td>
<td>0/120</td>
<td>0.0</td>
<td>0/120</td>
</tr>
</tbody>
</table>

* # = number affected/total sample.
** Inflammation of kidney, gills, gastrointestinal tract, and connective tissue.
*** Necrosis of digestive diverticula of gastrointestinal tract.
a digestive diverticulum and caused ulceration and inflammation of the tubule wall (Fig. 3).

Trematoda. Sporocysts of the trematode *Bucephalus* sp. were found in vesicular connective tissue and gonads of *Crassostrea virginica*. Histological sections revealed heavy infections of *Bucephalus* sp. in 13 of 480 (2.7%) oysters (Table 1). Three of the 480 (<1.0%) oysters were lightly infected. Sporocysts of *Bucephalus* sp. were found in reproductive follicles and gonadal connective tissue (Fig. 4).

**Bacterial infection**

Basophilic inclusion bodies (BIB). Basophilic inclusion bodies, similar to mycoplasma organisms, were found in the gastrointestinal tract of *Crassostrea virginica*. BIB in the gastric mucosa is shown in Figure 5.

**Lesions**

Kidney concretions and brown cells. Large, round, brown pigmented bodies similar to the concretions described by Rheinbarger *et al.* (1977) and Carmichael *et al.* (1979) were observed in the kidney tubules of *Crassostrea*
virginica (Figure 6). Concretions were found in oysters from all four sites, with overall incidence of 8.8% (Table 1). In addition to kidney concretions, numerous brown cells located in the connective tissue of the visceral mass were found in almost all oysters. In oysters from the Pawcatuck River and Narrow River, brown cells were larger, more numerous, and had darker and larger vesicles with more inclusion bodies than those oysters from Charlestown Pond or Green Hill Pond.

Ulceration. Only two of all the oysters examined had ulcerative lesions (Table 1). The ulcerations occurred in the stomach epithelium. Inflammation of the submucosa and thickening of the basement membrane were also found in these oysters. An etiologic agent could not be determined.

Necrosis. Necrotic foci were observed in the tubular epitheli of digestive diverticula. Three forms of cell nuclei were observed: pycnotic, karyorrhetic, and karyolytic. Necrotic tissue occurred in 6.2% of the samples (Table 1).

Hyperplasia. Hyperplasia was observed in the tubular epithelium of the digestive diverticulum, consisting of increased numbers of basophilic epithelial cells. Only one oyster from Charlestown Pond had hyperplasia (Table 1).
Neoplasia. Neoplastic lesions in oysters are generally characterized by the infiltration of vesicular connective tissue 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 nuclei are present in some cases (Murchelano and MacLean, 1990). Sarcomas in molluscan tissue are typically characterized by the appearance of unusual cells in connective tissue, hemolymph vessels and sinuses of the visceral mass, muscle tissue, and mantle tissue (Peters, 1988). During this study, 3.7% of the oysters had neoplasms in the gastrointestinal tract, gonads, connective tissue or ctenidia (Table 1). Eight of these oysters had adenomas of the stomach epithelia, one had a germinoma, two had mesenchymal origin in connective tissue, and eight had branchial neoplasia.

Four oysters from the Pawcatuck River had neoplastic lesions (Table 1). One oyster in March 1992 had germinomas. Basophilic, hypertrophied, neoplastic germ cells had proliferated along the walls of the gonadal follicles and ducts. The other three oysters from the Pawcatuck River had adenomas in the ctenidia (Fig. 7).
A total of seven oysters from Narrow River had neoplasms (Table 1). One oyster was found to have an eosinophilic mass infiltrating the Leydig cells. Four oysters had neoplasia in the gastrointestinal tract and two oysters had neoplasms in the ctenidia. A tumor of neural origin was found in the connective tissue of the ctenidium of another oyster (Fig. 8). Neoplasia in the gastrointestinal epithelium was found in oysters from Charlestown Pond; one in November 1991 and one in May 1992 (Table 1). Adenocarcinomas in the gills and connective tissue were also present in oysters collected from Charlestown Pond in March 1992 and November 1992. Three oysters from Green Hill Pond had neoplasia, one in the ctenidium and two in the gastrointestinal epithelium.

Atrophy of adductor muscle. One oyster collected from the Pawcatuck River in May 1992 and one oyster from Charlestown Pond in March 1992 had the unusual condition of adductor muscle atrophy and replacement by proliferating collagenous connective tissue (Fig. 9).

Cysts (Idiopathic). Cysts in the renal tubules were found in all of the sites except for Charlestown Pond (Table 1).

Inflammation. Almost all the oysters (93.1%) had focal inflammation in the
Figure 6. Concretion in a kidney tubule of *Crassostrea virginica* from Green Hill Pond (250× magnification).

connective tissue (Table 1). Sixteen oysters (3.3%) had inflammation in the kidney, 210 (43.8%) had inflammation in the ctenidia, and 28 (5.8%) had gastrointestinal tract inflammation.

Condition index was determined in 150 oysters. The condition index values ranged from 26.52 to 197.92 (Table 2). Throughout the study, condition index values of oysters from Pawcatuck River remained low (below 75). In the other study areas, condition index values were consistently lowest in the summer, and higher in fall, winter and spring.

Discussion

This study provides evidence that *Haplosporidium nelsoni* can be found in Rhode Island coastal waters, but it was found in one single location, Charlestown Pond, and the incidence was very low. Studies of infection and mortality patterns along salinity gradients showed that MSX can not survive in salinities below 10 ppt. The parasite survives best in salinities above 20 ppt with infection rates reduced and parasite development inhibited below 15 ppt (Haskin and Ford, 1982). The oyster population in the Pawcatuck River may be less prone to MSX infection than the other sites studied because
Figure 7. Adenocarcinoma of the branchial tissue of *Crassostrea virginica* collected from the Pawcatuck River (250 × magnification).

Table 2. 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).

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Pawcatuck River</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>—</td>
<td>26.52–72.09</td>
<td>29.06–89.60</td>
<td>40.07–72.73</td>
</tr>
<tr>
<td>Mean; C.V.</td>
<td>—</td>
<td>53.88; 27.54</td>
<td>53.29; 38.73</td>
<td>53.89; 20.77</td>
</tr>
<tr>
<td>Narrow River</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>47.32–100.30</td>
<td>77.27–154.09</td>
<td>63.53–149.43</td>
<td>68.70–116.55</td>
</tr>
<tr>
<td>Mean; C.V.</td>
<td>66.39; 26.50</td>
<td>109.82; 23.78</td>
<td>120.61; 21.65</td>
<td>94.01; 14.38</td>
</tr>
<tr>
<td>Charlestown Pond</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>44.28–140.77</td>
<td>59.54–197.67</td>
<td>73.97–143.42</td>
<td>70.98–137.29</td>
</tr>
<tr>
<td>Mean; C.V.</td>
<td>82.39; 37.15</td>
<td>120.12; 37.15</td>
<td>105.99; 20.45</td>
<td>99.94; 18.92</td>
</tr>
<tr>
<td>Green Hill Pond</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>51.21–123.39</td>
<td>45.95–145.31</td>
<td>44.19–152.68</td>
<td>54.26–197.92</td>
</tr>
<tr>
<td>Mean; C.V.</td>
<td>78.63; 28.69</td>
<td>108.15; 42.95</td>
<td>83.13; 37.85</td>
<td>108.45; 43.95</td>
</tr>
</tbody>
</table>

C.V. = \( \frac{\text{Standard deviation} \times 100}{\text{Mean}} \).
salinity profile data show that the Pawcatuck River experiences lowered salinity associated with spring freshets to levels below the tolerance of the parasite.

In addition to MSX, other parasites were found in Rhode Island oysters. *Sphenophrya* sp. was found in all locations during March 1992 and May 1992. The number of these ciliates infecting a host is believed to increase under conditions of stress (Murchelano and MacLean, 1990). The respiration and feeding of oysters can be adversely affected by heavy *Sphenophrya* sp. infections (Otto et al., 1979). Infections of the trematode *Bucephalus* sp. were generally found in the gonad tissues; however, initial infection is generally believed to be in the digestive tubules (Cheng and Burton, 1965). Heavy infections of *Bucephalus* destroy tissues and result in oyster death (Hopkins, 1957), but lighter infections can result in parasitic castration (Sparks, 1985). Thus *Bucephalus* sp. infections may affect oyster recruitment by reducing reproductive output.

A number of lesions found in the Rhode Island oysters are associated with responses to environmental stressors. Concretions form in the lumens of kidney tubules of stressed bivalves (Yevich, 1980). Rheinberger et al. (1977) showed that large concretions can cause inflammation or loss of epithelial
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tissue in the kidney. They also suggested that concretions may be indicators of environmental degradation since larger concretions are often found in polluted areas. Brown cells, containing lipofuscin, increase if oysters suffer disease, stress, or pathological condition (Sparks, 1985). A likely role of brown cells is the removal of degradation products and metabolic by-products associated with parasite infections or environmental stressors (Cheng and Rifkin, 1970). The high prevalence of oysters with inflammation is unusual. Some oysters had very extensive inflammation in all organs. The causes of the observed inflammation are uncertain, but Sparks and Morado (1988) and Slauson et al. (1990) suggest that inflammation is a host defense mechanism following tissue damage.

Basophilic inclusion bodies (BIB) have been found in marine bivalves and electron microscopic studies have shown that oyster inclusions most commonly contained mycoplasma-like organisms (Harshbarger et al., 1977). Similar to our results, Gauthier et al. (1990) showed a 5% incidence of BIB infections in Louisiana oysters. They also reported that in higher salinities, oysters had higher levels of infection. Our data do not clearly show the same trend; lower salinity waters of the Pawcatuck River had infection levels comparable to the other higher salinity sites.
One important finding of this study is the high incidence of neoplasia (3.8%). In two separate Chesapeake Bay studies, neoplasia was observed in 12 of 20,000 Crassostrea virginica (Harshbarger et al., 1979) and five out of 30,000 oysters (Farley, 1969). The cause of neoplasia in C. virginica is uncertain, however it is not likely to result from infections by bacteria, mycoplasma, or protozoan parasites. Barry and Yevich (1975) reported that gonadal tumors in Mya arenaria in Maine were possibly the result of an oil spill. A more recent study has shown a strong correlation between carcinogenic hydrocarbons in sediments and neoplasia in bivalves (Gardner et al., 1991). Another cause of neoplasia may be by a viral infection. Oprandy et al. (1981) reported that hematopoietic neoplasia in M. arenaria is due to a viral agent. Recently, reverse transcriptase activity was demonstrated in viral particles that cause the hematopoietic neoplasia, suggesting that the virus belongs to the family of retroviruses (Medina et al., 1993).

Finally, condition index values of oysters from the Pawcatuck River were very low throughout the year, but the prevalence of disease was not much different than the other study sites. This suggests that condition index values alone may not be a good indicator of disease prevalence. The Pawcatuck River oysters had valve heights between 50 and 180 mm, suggesting a mature size, but there appeared to be little gonadal development. Further studies in this area are recommended to elucidate the reasons for the surprisingly low condition index values and arrested gonad development.

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