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PROGRESSION OF DISEASES CAUSED BY THE OYSTER PARASITES, PERKINSUS MARINUS AND HAPLOSPORIDIUM NELSONI, IN CRASSOSTREA VIRGINICA ON CONSTRUCTED INTERTIDAL REEFS

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ABSTRACT The progression of diseases caused by the oyster parasites Perkinsus marinus and Haplosporidium nelsoni were evaluated by periodic sampling (May 1994-December 1995) of eastern oysters Crassostrea virginica on an artificial reef located in the Piankatank River, Virginia. The infections observed were recorded as a function of: (1) prevalence and intensity; (2) oyster size and age; and (3) depth below mean low water at which the host oyster was found on the reef. Only a very small number of oysters were infected with the two species of pathogens on the oyster reef during the first 11 months of life. In the second year of oyster life, epizootiological patterns of disease development followed temperature and salinity trends. Oysters at residence depths ≤45 cm below mean low water exhibited significantly (P < 0.0001) lower prevalence and intensity of infections than oysters at depths ≥90 cm. In contrast, oysters at residence depths ≥0 cm had significantly higher growth rates (P < 0.05) than those at ≤45 cm. However, size differences were not significant (P > 0.05) at the end of the study. Results from this study may be used in managing oyster fisheries on natural or artificial reefs.

KEY WORDS: Crassostrea virginica, Perkinsus marinus, Haplosporidium nelsoni, artificial reefs, disease progression, growth

INTRODUCTION

Eastern oysters Crassostrea virginica were an economic and ecological resource in the Chesapeake Bay until the early 1900s (Hargis and Haven 1999). Years of overharvesting, diseases caused by the pathogens Perkinsus marinus and Haplosporidium nelsoni, environmental degradation, and poor resource management have led to a dramatic decline in oyster populations in the Chesapeake Bay (Andrews 1988, Haskin and Andrews 1988). Today, Virginia’s oyster population is less than 1% of what it was just 35 years ago (Wessom et al. 1999). Various efforts have been initiated to rejuvenate dwindling local oyster populations. These include spreading of hatchery-reared juvenile oysters on natural oyster beds in estuaries, spreading oyster shell in an attempt to increase hard substrate for settlement of oyster larvae, and construction of artificial reefs. Rejuvenation efforts, such as spreading thin layers of shell over coastal and estuarine bottom for larval attachment have had limited success. This may be due in part to the lack of three-dimensional complexity observed in natural intertidal communities. Little is known about the colonization and ecology of C. virginica on intertidal reefs. In addition, the advantages of oyster settlement on constructed reefs are not well understood. Therefore, this study was conducted to determine: (1) whether residence depth influenced the extent or intensity of disease infection; and (2) the size and age at which oysters became infected and the depths that resulted in significant infections. Observations from the present study are of interest to individuals responsible for constructing artificial reefs and individuals who must determine when to harvest oysters to avoid excess losses.

METHODS AND MATERIALS

Perkinsus marinus Infections

Oysters were assayed for the presence of P. marinus using the Ray’s fluid thiglycollate medium technique (Ray 1954). Samples of Gill and digestive gland were incubated in the medium. Perkins (unpublished data) determined that these organs can more frequently detect the presence of the parasite when infections are very light or light than when mantle or rectal samples are used. The
intensity of infections was recorded using a modification of the Mackin scale (Mackin 1962) in which 0 = no infection, 1 = very light, 2 = light, 3 = light–moderate, 4 = moderate, 5 = moderate–heavy, and 6 = heavy.

**Haplosporidium nelsoni Infections**

Haplosporidium nelsoni was detected using histological, paraffin-embedded sections stained in hematoxylin and eosin. The scale of Burreson et al. (1988) was employed to record intensities of infections where 0 = no infection, 1 = cells were rare, 2 = fewer than two cells per field of view (40x objective), 3 = two–five cells per field of view, and 4 = more than five cells per field of view.

**Oyster Sampling**

From May 5, 1994 to December 14, 1995, the progression of diseases caused by the oyster parasites *P. marinus* and *H. nelsoni* were evaluated by periodic sampling of oysters that had naturally set on the artificial reef located in the Piankatank River, Virginia, in August 1993. The reef was constructed by the Virginia Marine Resource Commission using aged oyster shells. Details of reef construction are described elsewhere (Bartol and Mann 1997). Sampling of oysters was conducted once every 2 to 4 weeks during the study period. Oysters were obtained by hand or by using oyster tongs, depending on the depth. Six samples of 25 oysters each were obtained for each sample time at two locations on the reef. The base of the reef was 2–3 m below mean low water. Prevalence and intensity (weighted prevalence) of *P. marinus*, and *H. nelsoni* infections, oyster size and age, and depth below mean low water at which the host oyster was found on the reef were measured. The total number of reef oysters sampled was 3,908. With respect to depth, the data were analyzed according to the oyster’s residence depth on the reef: ≤45 cm and >90 cm mean low water. The observations are expressed in terms of weeks after setting. Most of the set in 1993 occurred from August 5 to August 12. To facilitate the handling of the data, August 12 was selected as the date of set.

The progression of infections in the reef-set oysters were compared with the progression through a population of adult oysters. Uninfected, adult oysters (350) were obtained from the upper James River seed beds (Horsehead rock), a region known to have low *P. marinus* and *H. nelsoni* infections in oysters (Burreson and Ragone-Calvo 1996). These adult oysters were placed in plastic mesh bags and then were placed on the Piankatank River reef near the sample sites for the reef oysters; placement was June 16, 1994, the time of the third sampling of the reef oysters. The depth of placement was about midway between the top and bottom of the reef (ca. 100 cm below mean low water). At the time of placement, a sample of 25 oysters was analyzed for the presence of the two parasites, using techniques described previously. To confirm that the parasite detection methodology was being properly applied and to check for patchiness in distribution of the parasites, 350 James River oysters from the same population used on the Piankatank River reef were placed in plastic mesh bags in the York River behind the Virginia Institute of Marine Science (VIMS), an area in which both diseases are commonly present at high levels. Treatment of the 350 oysters held at VIMS was the same as described for the 350 adult oysters deployed in the Piankatank River. Each batch of 350 oysters was sampled (25 oysters per sampling time) simultaneously with the reef oysters until none remained. In the following spring (April 14, 1995), another 350 oysters from the same James River site were placed on the reef and 350 at VIMS as in the previous year and sampled until none remained.

**Statistical Analyses**

The effects of depth and sampling time (age) of oyster on disease susceptibility (prevalence and intensity of infection) to *P. marinus* and *H. nelsoni* were examined using logistic regression analysis (Agresti 1990). The differences in growth of oysters sampled at ≤45 cm and ≥90 cm depths during different sampling times was assessed using a two-way analysis of variance (ANOVA)

**RESULTS**

**Temperature and Salinity**

Temperatures and salinities during the study period showed typical seasonal patterns, higher temperatures during summer months and lower during winter months (Fig. 1). Salinity remained fairly constant during the study period. Temperature and salinity ranges during the study period were 2–30 °C and 10–20 ppt. The conversion between oyster age and sampling dates is presented in Table 1.

**Perkinsus marinus Infections**

No *P. marinus* or *H. nelsoni* cells were found in the 25 adult oysters sampled before the deployment of the oysters at Piankatank River and VIMS. *P. marinus* prevalence (Fig. 2a) and intensity (Fig. 2b) in oysters set on the Piankatank reef indicate that infections did not appear until 14 weeks into the study when the oysters were 1 year old (Table 1). For the next 44 weeks or until the oysters became 1 year and 10 months old, the number of infected oysters ranged between 15 and 35%; in the ensuing 2 months, the prevalence rose rapidly to 100%. Oysters then exhibited high prevalence (nearly 100%) until the end of the study, when the oysters were almost 2.5 years old. The infection intensities during the plateau phase remained mostly below very light until the end of the plateau (1 year, 10 months old), rose rapidly to moderate to moderate–heavy at 2+ years old, followed by a decline to light and light–moderate at the end of the study.

**Perkinsus marinus** prevalence was significantly higher (*P* <
TABLE 1.
Time scale for sampling times used in the study. The oyster ages are estimated assuming a setting time of August 12, 1993.
NA = Not available.

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Oyster Age (weeks)</th>
<th>Sampling Time Elapsed (weeks)</th>
<th>Temperature (°C)</th>
<th>Salinity (ppt)</th>
</tr>
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<td>38</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>May 26</td>
<td>41</td>
<td>3</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>June 16</td>
<td>44</td>
<td>6</td>
<td>27.8</td>
<td>12</td>
</tr>
<tr>
<td>June 30</td>
<td>46</td>
<td>8</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>July 15</td>
<td>48</td>
<td>10</td>
<td>29.8</td>
<td>16</td>
</tr>
<tr>
<td>July 28</td>
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<td>12</td>
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<td>16</td>
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<td>16</td>
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<td>16</td>
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<td>20</td>
<td>21</td>
<td>16</td>
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<td>NA</td>
<td>NA</td>
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<td>27</td>
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<td>NA</td>
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<td>December 8</td>
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<td>31</td>
<td>11</td>
<td>16</td>
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<tr>
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<td>36</td>
<td>7</td>
<td>14</td>
</tr>
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<td>March 13</td>
<td>83</td>
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<td>14</td>
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<td>20</td>
</tr>
<tr>
<td>December 14</td>
<td>122</td>
<td>84</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

0.0001) in oysters collected from depths ≥90 cm compared to those from ≤45 cm (Fig. 2a). Prevalence significantly increased (P < 0.0001) in oysters from all depths with increasing age of oysters, indicating that continued exposure to *P. marinus* or increasing age of oysters results in increased infection. Similar results were observed when *P. marinus* infection was expressed as weighted prevalence. Although the difference in infection intensity was not as great as the prevalence, it was significantly greater at the greater depths (P < 0.01) and significantly increased in oysters from both depths with age (P < 0.0001).

*H. nelsoni* infection prevalence was the same in adult oysters held in plastic mesh bags at the York River and at the Piankatank River reef during 1994. In 1995, infection was expressed earlier at the Piankatank River and reached 100% 15 weeks before those held in the York River (Fig. 3a,b).

### Haplosporidium nelsoni Infections

Prevalence of *H. nelsoni* was markedly different from that of *P. marinus* in reef oysters (Fig. 4a). With the exception of one lightly infected, 50-week-old oyster, the onset of *H. nelsoni* infections did not occur until the oysters were over 1.5 years old, as opposed to the appearance of *P. marinus* in 1-year-old oysters. Thereafter, the infection prevalence of *H. nelsoni* rose rapidly, reaching a maximum of 45% when the oysters were 21 months old. The infections then declined precipitously to almost 0% when the oysters were over 2 years old, followed by a slight increase, which remained below 10% for the final 4 months of the study. The intensities of infections peaked at the age of nearly 2 years, which was 1 month before the prevalence peak was reached, and declined almost as rapidly as did the prevalence (Fig. 4b).

Similar to *P. marinus* infections, oysters collected from ≥90 cm depth had a significantly higher prevalence and intensity of *H. nelsoni* infections compared to those from ≤45 cm depth (P < 0.0001) (Fig. 4a,b). In addition, *H. nelsoni* prevalence and intensity increased with increasing oyster age (P < 0.0001).

*Haplosporidium nelsoni* infections (Fig. 5a,b) were nearly non-existent in the Piankatank River reef oysters during 1994; whereas, in the York River stock, infections were above a prevalence of 60% during the summer and fall of 1994.

### Growth and Size

The sampling time and the residence depth of oysters significantly influenced the growth of oysters (P < 0.001) (Fig. 6). Oysters at both depths grew with increasing age (P < 0.001) and sampling time (P 0.001). Oysters at depths ≥90 cm had significantly greater growth than oysters at depths ≤45 cm. Although the differences in size were pronounced during age 56–100 weeks, they were less pronounced during oyster ages 103–122 weeks, and insignificant (P > 0.05) at the end of the study. The rate of oyster growth, as estimated from size measurements, decreased 4 weeks
...sites. We are twice placed at the sites and assayed until the populations were depleted by sampling and natural mortalities. The disease organism data are expressed as a function of site and sampling time in the study.

Before the first P. marinus infections (10 weeks and 14 weeks into the study) (Fig. 6).

**DISCUSSION**

Interpretation of epizootiological data such as those generated in this study is confounded by many factors that dictate disease prevalence and intensity. These include temperature, salinity, water quality, density of oysters, patterns of water movement, oyster age and/or size, genetic strains, physiological condition, food availability (density and species composition of planktonic food organisms present), and numbers and levels of other parasitic species causing stress on the oysters. A further complication is the fact that the reservoir of *H. nelsoni* infective cells is unknown, and transmission of infections is not from oyster-to-oyster as with *P. marinus*. Despite these complicating factors, patterns of disease progression of both the diseases and oyster growth at different residence depths were apparent. The residence depth of the oysters relative to mean low water is of considerable interest, because the premise behind construction of artificial reefs is that the survival of oysters in the presence of *P. marinus* and *H. nelsoni* will be enhanced if they are grown in the more natural environment of an oyster shell reef off the bottom of the estuary. In fact, this study clearly indicates that residence depth of oysters significantly influences prevalence and intensity of *P. marinus* and *H. nelsoni* infections.

Oysters that are growing at ≤45 cm depth can be expected to have lower prevalences and intensities of infections of both pathogens compared to those living at ≥90 cm depth (Figs. 2a,b, and 4a,b). The prevalence and intensity of *P. marinus* in reef oysters from the current study, while agreeing with those of Mackin (1962), differ from studies by Quick and Mackin (1971) in the Atlantic and Gulf of Mexico coasts of Florida. Weighted incidences (intensities) in their study showed a decrease with increasing depth and no effect of depth on prevalences from intertidal to 3 m below mean low water. Similarly, Burrell et al. (1984) found higher prevalences and intensities of *P. marinus* in intertidal oysters than in subtidal oysters. Mackin (1962) speculated that lower infection prevalences and intensities in intertidal oysters may be because these oysters are not exposed to as many infective cells as subtidal oysters by virtue of the increased amount of time they are closed and not feeding.

Growth of oysters at both the depths (≤45 cm and ≥90 cm) decreased from age 74 weeks, and coincided with increased *P. marinus* incidence in oysters. The decrease in growth of oysters upon acquisition of *P. marinus* infections support the findings of Paynter and Burreson (1991) who observed a decrease in the growth rate of juvenile and adult oysters immediately after or just before infection. *Haplosporidium nelsoni* did not seem for more than 7 months after the change in growth rate, and it is concluded...
that *P. marinus* was responsible for the decreased growth rate. The decline in oyster sizes after the age of 2 years (65 weeks into the study) is believed to be attributable to death of the larger oysters from infections of the two pathogens.

The higher growth of oysters residing at depths ≥90 cm than those at ≤45 cm is surprising. Given the higher infection prevalence and intensities of both *P. marinus* and *H. nelsoni* in oysters at depths ≥90 cm, we would expect that these oysters would grow less because of disease pressure. The biological, chemical, and physical processes associated with the bottom waters may influence the physiological and defense responses of organisms inhabiting these areas. Organic matter near the bottom of the reef close to the sediment has been speculated (Dahlback and Gunnarsson 1981) to have contributed to the increased growth in oysters from >90 cm. However, at the end of the study, the differences in sizes of oysters from the two depths were not significant.

The present dataset is unique, because it is the first time a population of naturally set oysters of known age has been assayed *in situ* for progression of infections by *P. marinus* and *H. nelsoni* over an extended period of time. Other epizootiological studies have involved placing naturally set or hatchery set oysters of known age in containers in an endemic area or placing adult oysters of unknown ages from nonendemic or marginally endemic areas into containers in an endemic area (Ray 1953, 1954, Mackin 1962, Paynter and Burresson 1991). Placement in containers provides a greater degree of experimental control, but artificial densities of oysters can lead to results different from those in naturally set populations, where distances vary from oyster to oyster.

The prevalence and intensities of infections of *P. marinus* in reef oysters generally followed the patterns dictated by temperature and salinity trends observed in earlier studies (e.g., Soniat 1985, Andrews 1988, Burresson 1991, Burresson and Ragone-Calvo 1996, Ray 1996). *P. marinus* infections in oysters rose in the spring, peaking in October and November, and declined in the winter months into the spring. Intensities of infections were more nearly reflective of previous reports in that the peaks for the two depths were in October and November and the minima in May of the following year (Fig. 2b). Therefore, assuming that temperature and salinity values approximate those of the study period and given the infection intensities and levels, oyster mortalities from *P. marinus* can be expected to begin 13 months after setting, rising most significantly 22 months after setting.

Considering the fact that the salinity values recorded during the first year after setting did not go below 10 ppt, and most of the time were ≥16 ppt, it is reasonable to assume that infections from these two diseases did not occur before May 5, 1994, when sampling began. This assumption is based on other studies that observed when a population becomes infected, the infections do not disappear (or decline) unless the salinity decreases below 10 ppt for an extended period (Andrews and Hewitt 1957, Ragone and Burresson 1993, Burresson and Ragone Calvo 1996).

Previous studies have noted that oysters are refractory to acquiring infections in the first year of life (Ray 1953, 1954) and become increasingly more susceptible into the second year, with significant prevalence, incidence, and mortality being observed then (Andrews and Hewitt 1957). That pattern was observed in the present study (Figs. 2a,b and 4a,b). As mentioned above, the complicating factor was the low level of infection pressure from *H. nelsoni* in the first year of life at the reef. *P. marinus* infections in oysters are dose-dependent (Mackin 1962, Chu and Volety 1997), and small oysters probably filter less volumes of water to acquire enough infective cells of the parasite to initiate an infection (Burresson 1991, Burresson and Ragone Calvo 1996). Results from the current study indicate that in the first 11 months of life, only a very small number of oysters on the oyster reef become infected with
the two species of pathogens. The question that cannot be answered is whether the primary factor in encouraging or permitting a rapid increase in prevalence was temperature, oyster age, or salinity. The best answer probably is that all three played interactive roles.

Data for adult oysters imported from the upper James River seed beds, which represent a disease-susceptible population, confirmed that the two pathogens were present in the study area and in the neighboring area of the lower York River (Figs. 3a,b and 5a,b). They were used primarily to indicate presence or absence of *H. nelsoni*, because it is known to fluctuate greatly, some years being nearly absent from the lower York River region. (Andres 1988, Haskin and Andrews 1988).

It is interesting to note that *P. marinus* infection prevalence was the same in oysters held in the York River and in the Piankatan River reef during 1994, but in 1995 was expressed earlier in the Piankatan River and prevalence reached 100%, 15 weeks before those held in the York River. One would have expected that the oysters held in the York River would have shown a higher prevalence, because salinities were about 5 ppt above those of the Piankatan River and thus would have presented more favorable salinities for expression of *P. marinus*. On the other hand, in 1994, *H. nelsoni* infections were nearly nonexistent in the Piankatan River reef oysters; whereas, in the York River stock, infections were above a prevalence of 60% during the summer and fall of 1994 (Fig. 5a). These observations reconfirm the necessity of having a stock of susceptible, adult oysters present in a study where juvenile and young oysters are being observed.

The reef oysters ≥ 2 years old can reasonably be compared to the imported oysters in terms of response to the diseases. Although *P. marinus* prevalences and intensities of infections were similar in the two groups of oysters at the reef, *H. nelsoni* data suggest that the reef-set oysters were more resistant to those infections. Imported oysters at the reef reached a peak of 68% infection (Fig. 5a); whereas, the reef oysters peaked at 36 and 45%, depending upon the depth of residence (Fig. 4a). Likewise, the intensities of *H. nelsoni* infections had a mean level of 2 in imported oysters as opposed to 1.3 in the reef oysters. Therefore, some advantage seems to have been obtained for the reef-set oysters, if we can neglect minor age differences.

The data for *H. nelsoni* was somewhat surprising, because only one oyster was found to be infected in the first year of life (Fig. 4a), and the population did not otherwise begin to show infections until the oysters were over 1.5 years old. It is possible that this lag can be attributed to: (1) the oysters being young and, thus, less susceptible, as has been reported from other studies; and (2) the fact that even the susceptible, imported adult oysters did not acquire very many infections at the Piankatan River reef (Fig. 5a) in the first year of life. It was clear that *H. nelsoni* was present in strength in the nearby York River (Fig. 5a) but not in the reef area, thus illustrating the patchiness in distribution of the reservoir of infective cells, at least in that part of the Chesapeake Bay. The decline in prevalence and intensity of *H. nelsoni* in reef oysters (Fig. 5a,b) was more precipitous that has been previously reported using imported susceptible adult oysters (Haskin and Andrews 1988). In 1995, a different picture of *H. nelsoni* infection distributions in the imported, adult oysters was observed (Figs. 5a,b). The prevalences and intensities were quite similar at the two stations, with the infections appearing earlier at the York River station and lasting longer in the population. Nine more weeks of data were obtained from the York River stock, because the stock at the reef was depleted by mortalities earlier, probably caused by *P. marinus* infections (Fig. 5a,b).

Given the decreased susceptibility of oysters to *P. marinus* and *H. nelsoni* at residence depth of ≤ 45 cm compared with oysters at ≥ 90 cm, it seems that piling of shells and constructing artificial reefs is a better strategy for rejuvenating oyster stocks than spreading thin layers of oyster shells on the bottom in estuarine and coastal areas. Future studies should investigate the survival differences in oysters at different depths and the factors responsible for differences in growth rates of oysters at different depths.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


Hargis, W. J. & D. S. Haven. 1999. Chesapeake oyster reefs, their impor-
Dermato and MSX in Oysters on Artificial Reefs


