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EFFECTS OF PERKINSUS MARINUS INFECTION IN THE EASTERN OYSTER, CRASSOSTREA VIRGINICA: II. DISEASE DEVELOPMENT AND IMPACT ON GROWTH RATE AT DIFFERENT SALINITIES

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ABSTRACT In order to assess the impact of Perkinsus marinus infection on oyster growth and mortality, oysters were raised in floating rafts at six sites around Chesapeake Bay. The sites were comprised of two low salinity sites (8–10%), two moderate salinity (12–15%) sites and two high salinity sites (16–20%). Oyster growth was monitored biweekly along with various water qualities including temperature and salinity. Condition index was measured monthly and disease diagnosis was performed bimonthly. Oyster growth was initially greatest at the high salinity sites but was subsequently retarded by Perkinsus infection at both the moderate and high salinity sites (where the parasite was more prevalent). Comparison of pre-infection and post-infection growth rates between sites showed that the reduction in growth rate was mitigated by lower salinity. Condition index was not related to salinity or site but was significantly reduced by P. marinus infection. Reduction in condition, however, was not associated with increased mortality. Mortality was also less related to salinity or temperature than it was to infection history (previous infection). Groups which incurred high infection prevalences and intensities exhibited low mortality during their first year, but suffered high mortality during the following year. The results are discussed in relation to management and aquacultural practices and their relation to genetics and selective breeding of disease resistant oysters.

KEY WORDS: oyster, growth, Perkinsus, disease, mortality, Chesapeake Bay

INTRODUCTION

Once considered the most abundant source of oysters in the world, the Chesapeake Bay has lost most of its oyster population to the combined effects of disease and overharvesting. In the last 100 years, the existing population has been reduced by an estimated 99%. The subsequent loss of biofiltration normally provided by the oysters has been cited as the principal cause of the historical increase in phytoplankton biomass in Chesapeake Bay which has caused widespread eutrophicaton problems including hypoxia and anoxia (Newell 1988). The effects of two diseases (MSX and Dermo) caused by two parasitic protozoans (Haplosporidium nelsoni and Perkinsus marinus, respectively) combined with continued intense harvesting pressure over the last three decades have depleted the natural population to this critically low level (Hargis and Haven 1988). Infections of P. marinus have been documented in Chesapeake Bay oyster populations since the early 1950s (Andrews 1988), and the continued susceptibility of the population to the disease has puzzled scientists over the last two decades. Several oyster strains tolerant of other protozoan infections have naturally evolved (Sindermann 1977) or been developed by breeding programs (Ford and Haskin 1987), so the potential for the development of disease resistance is present in the species. The lack of development of resistance or tolerance to H. nelsoni or P. marinus by the oyster population in the Bay suggests that unselected susceptible oysters living in low salinity areas are the major contributors to annual spawn and recruitment and/or that additional pressures may have compromised the species ability to adapt to disease pressure. Pollution has been shown to affect oyster immune systems (Anderson 1988) and may have insuluted the oyster population further. However, continued intense harvest pressure on the natural stocks has probably limited the oyster population's ability to develop resistance to the diseases, especially if sublethal effects of infection have caused resistant animals to be harvested before susceptible animals.

Significant sublethal effects of H. nelsoni have been demonstrated by a number of studies. These effects include reduction in clearance rates and condition index (Newell 1985) and reduction in other physiological parameters (Barber et al. 1988a, 1988b, Ford and Figueras 1988) including fecundity. However, few studies have examined the sublethal effects of P. marinus infection. If significant sublethal effects occurred which resulted in resistant individuals being harvested in disproportionate numbers compared to susceptible animals, then resistance would most likely not evolve or would be slow to evolve. The inhibition of growth in susceptible oysters might cause such a disproportionate harvest and, since the effect is sublethal, would lead to higher contributions by susceptible animals in the next years spawn and recruitment.

The decline of the natural population of oysters in Chesapeake Bay has caused an increased interest in oyster aquaculture in the region. Oyster culture techniques other than traditional remote setting methods have begun to receive more attention. Intensive oyster cultivation in suspended culture has been shown to promote rapid oyster growth (Paynter and DiMichele 1990); however,
Menzel and Hopkins (1955) and Andrews (1961) have shown that *P. marinus* infection greatly reduces oyster growth and may compromise the advantages of suspended culture in Chesapeake Bay.

While the previous studies (Menzel and Hopkins 1955, Andrews 1961) showed clearly that growth reduction was associated with *P. marinus* infection, genetic differences among individuals was not assessed and parasite diagnoses were performed only at the end of the experiment so time of parasite acquisition was unknown. In order to make a more complete assessment of the effects of *P. marinus* on the growth and mortality of whole oyster populations, we initiated a study utilizing large numbers of genetically equivalent oysters raised at different salinities in Chesapeake Bay. Test growouts at several sites that represented different salinity regimes and parasite abundance were established to assess the possible sublethal effects of infection on growth and condition of oysters since infection intensities and disease related mortalities caused by *H. nelsoni* and *P. marinus* have been related to salinity (Ford 1985, Soniat 1985, Burresson and Andrews 1988, Gauthier et al. 1990). Oyster growth, condition and infection status were monitored regularly at all sites. *H. nelsoni* was not detected in any oysters during the two year study; *P. marinus* prevalence, however, was high at several sites. Infection severely inhibited growth in oysters at high and moderate salinities. Mortality during the second year of infection was determined more by history of infection in the first year than by immediate infection intensity.

**MATERIALS AND METHODS**

Oyster spat were introduced to six sites representing different salinities typical of Chesapeake Bay in order to assess the relative impact of *P. marinus* infection on growth and condition in the oysters. Cultchless oysters were produced from selectively inbred broodstock (see Paynter and DiMichele 1990, Brown and Paynter 1991) using traditional hatchery methods. Depending on the experiment, oysters from 10 to 25 mm in shell height were introduced in floating rafts within 2 days at all sites. Rafts were constructed of wooden frames with polyethylene mesh (1.9 cm) folded into a rectangular box which hung below the wooden frame and was stapled to the wooden frame along the edges. The resulting mesh box was 91 cm long × 61 cm wide × 20 cm deep. A 90 cm × 60 cm panel of extruded styrofoam was placed underneath the wooden frame to keep the raft afloat. A polyethylene mesh cage inserted into the raft was used to hold small animals until they were large enough to be held on the 1.9 cm mesh. Approximately 1000 oysters were initially placed in a single tray. If a tray became crowded the group was split into another tray.

The Maryland (MD) sites were comprised of two low salinity sites (8–12‰; Wye River and Deep Cove Creek) and two moderate salinity sites (12–15‰; Worlds End Creek and Slaughter Creek). The Virginia (VA) sites (VIMS and Mobjack Bay) had salinities of 16–20‰ and were denoted as high salinity sites in this study. Typically a site was a shallow tidal creek, well protected from weather and boat traffic. The sites were removed from any point source pollution such as marinas and sewage outlets. Horizontal water flow as judged by casual observation was low at all sites except VIMS. Many of these factors are known to affect bivalve growth, especially water flow, and their effects have been neutralized by the selection of sites with similar characteristics. The VIMS site, which had greater horizontal water flow and was exposed to more open-water conditions (greater wave action), however, showed no differences in growth rate compared to the site of similar salinity in Mobjack Bay.

Growth as shell height was measured every two weeks. Twenty five to fifty oysters were removed from a tray *en masse*, measured to the nearest mm with a ruler and returned to the tray. Length, total weight, shell weight, wet tissue weight, and dry tissue weight were measured monthly throughout the study period in five animals from each tray at each site. Condition indices were calculated from that data as:

$$CI = \frac{\text{dry wt (g)}}{(\text{total wt (g)} - \text{shell wt (g)})}$$

Bimonthly, 25 animals from each site were removed for parasite diagnosis. Diagnosis of *P. marinus* was by thioglycollate culture of rectal, gill and mantle tissue samples (Ray 1952). Based on Ray (1954) and Mackin (1962), infection intensities were rated and, for calculation of weighted incidence (WI), assigned numerical values as follows: negative = 0, light = 1, moderate = 3, and heavy = 5. WI was calculated as the average value of infection intensity for a sample of twenty five oysters. Diagnosis of *H. nelsoni* was by routine paraffin histology of tissue fixed in Davidson’s AFA.

Animals were first introduced to all sites in late July 1989. Additional animals from the same spawn were maintained in floating rafts at the Wye River site where parasite prevalence was zero. Growth, infection status, and condition index were measured through November 1989. Because the initiation of the experiment was in late summer of 1989 and because the experimental trays were lost at the moderate salinity sites over the winter, a second set of oysters was introduced from the Wye River stock to all sites in May 1990 and were monitored until November 1990. This effort provided a more complete assessment of disease onset and its effects.

In an extension of the original growout experiments, additional introductions were made from the Wye River stock to the Mobjack Bay site which exhibited high *P. marinus* prevalences. At that site, where the initial introduction (Group A) was in late July 1989, a second introduction of *P. marinus* free animals (Group B) from the same spawn was made in early September 1989. Additionally, a third group of oysters (Group C) from that spawn was introduced in early May 1990. The subsequent introductions were made to compare the relative infection rates and disease impacts for introductions at different times of the year.

Earlier studies (Paynter and DiMichele 1990, Paynter and Malonee 1991, Paynter, unpublished observations) have shown that oyster shell height increases at a constant rate under the culture conditions described above throughout the growing season. Growth rate, measured as increase in shell height, does not decrease as the animals grow, even when the group triples its original size during a single growing season. Linear regression offers a highly precise estimate of growth rate in a given oyster group at a given site. Therefore, data were treated using linear and polynomial regression to estimate pre- and post-infection growth rates. Analysis of variance (ANOVA) was used to distinguish among salinity, seasonal (monthly) and parasite contributions to the variance of condition indices. ANOVA, regressions and comparisons of β coefficients generated by regression were conducted according to Sokal and Rohlf (1981).

**RESULTS**

*Haplosporidium nelsoni* (MSX) was not detected in any oysters during the two year study described here. *Perkinsus marinus* was the only pathogen detected during the entire study. For clarity of
presentation, data from three (Mobjack Bay, Worlds End Creek, and Wye River) of the six sites will be presented. Trays were lost at Slaughter Creek during the winter months, and data from the VIMS and Deep Cove sites were essentially identical to the Mobjack and Wye River sites respectively.

Infection by *P. marinus* occurred in experimental groups raised at moderate and high salinities, but not at low salinity sites. In August 1990 at both moderate and high salinities, infection prevalences and intensities were equal and relatively low (Fig. 1). However, during the following months both prevalence and intensity of infection increased at high salinities while infection at moderate salinities remained unchanged (Fig. 1).

Growth as shell height was constant with respect to time at all sites in uninfected oyster populations (Fig. 1). Linear regression offered a highly precise estimate of growth rate. Oyster growth rates were higher at high salinity sites compared to growth at low salinity sites (*P* < 0.05; Table 1). The regression of pre-infection growth at moderate salinities was less precise due to the paucity of data collected before infection at both sites. Average oyster growth was severely inhibited as soon as a population became infected, even when the intensity of infection was very low (Fig. 1, Fig. 2, Fig. 3). This effect was quantified by linear regression of pre- and post-infection growth curves (Table 1). This analysis showed that growth rates at high and moderate salinities were significantly reduced after *P. marinus* infection (*P* < 0.001; Fig. 1C; Table 1). Oysters grown at low salinity did not become infected and continued to grow at a constant rate throughout the growing season (Fig. 1A).

In the oyster groups sequentially introduced at Mobjack Bay during 1989, Group A was infected within 45 days of introduction, and growth in that group appeared to be reduced (Fig. 2) as a result. While linear regression over the entire time period yielded a highly significant relationship (*r*² = .94), treating the data as having a distinct break in the growth curve associated with the detection of disease yielded even greater significance (*r*² > .98 for both lines). Group B was infected in October 1989 but infection was not detected in November 1989. Growth in Group B did not appear to be affected by infection in October, however winter temperatures stopped growth before enough data could be collected to make a comparison of pre- and post-infection growth. No unusual mortality occurred in 1989 in any of the groups at any sites.

No infections were detected in May 1990 in groups that had become infected during 1989. Latent infection or misdiagnosis seems unlikely because the oysters grew well until infection was detected, and only then did the oysters exhibit a marked reduction in growth rate (Fig. 3, Table 2). In the two groups held over from 1989 at Mobjack Bay (Groups A & B), significant mortalities occurred only in the group that had been infected and exhibited reduced growth in 1989 (Group A; Figs. 2 & 3). When Group B became infected and had equivalent intensity of infection as Group A, mortality was comparatively low (Fig. 3). Group A was diagnosed with 20% heavy infections in November 1990 while Group B had only 8% heavy infections.

Three factor ANOVA was used to differentiate among the effects of site, month, and infection status on condition index (CI). CI was not different among sites in uninfected or infected oyster groups. However, CI was closely related to month and was significantly lower in infected groups compared to uninfected groups (Fig. 4) regardless of site. Condition was not significantly different between Mobjack groups A & B during any months even though A had significantly greater mortality than B.

The effects of infection were related to salinity in two ways: 1) *P. marinus* was not detected at low salinities (Fig. 1A), and 2) the intensity of infection and subsequent reduction in growth rate was not as great at moderate salinities (Fig. 1B) as at high salinities (Fig. 1C). Growth rates decreased from approximately 14 mm/...
TABLE 1.

Effect of *P. marinus* infection on oyster growth rates at different salinities.

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>Pre-infection</th>
<th>Post-infection</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (16-20%)</td>
<td>14.34 (.39)</td>
<td>2.85 (.39)</td>
<td>80</td>
</tr>
<tr>
<td>Moderate (12-15%)</td>
<td>9.45 (1.7)</td>
<td>3.84 (.45)</td>
<td>60</td>
</tr>
<tr>
<td>Low (8-10%)</td>
<td>7.86 (.33)</td>
<td>N.I.</td>
<td>N.I.</td>
</tr>
</tbody>
</table>

Growth rates were calculated by performing linear regression on shell height over time and are presented in mm/month. All regressions were highly significant (P < .005) (r² > 0.95 pre-infection). Numbers in parentheses are standard errors of the estimate (S.E.E.). Infections at high and moderate salinities were associated with significantly reduced growth rates (P < 0.05). Pre-infection growth rates were significantly higher at the high salinity sites compared to the low salinity sites (P < 0.05). N.I. = not infected.

... month to nearly 2.85 mm/month at the high salinity site after infection occurred. At the moderate salinity site the pre-infection growth rate was 9 mm/month and declined to approximately 3 mm/month (Table 1). Time to infection after introduction of the 1990 groups ranged from as early as 45 days to as long as 120 days and was quite variable between sites and salinities. For instance, infection was first detected at the Slaughter Creek site (a moderate salinity site) within 45 days of introduction while infection at the VIMS site occurred within 75 days and at Mobjack Bay within 120 days. Time to infection of the larger animals (Groups A & B) at

**DISCUSSION**

This study confirms the observations made by Menzel and Hopkins (1955), Ray et al. (1953), and J. D. Andrews (1961) over three decades ago: infection by *P. marinus* results in the severe retardation of growth in oysters. However, those studies were conducted on individual oysters which prevented the assessment of infection status in the animals except at the end of the experiment or when an oyster died. Therefore, the time of initial infection, the relationship of infection intensity to growth reduction, or the effect of infection on large populations of oysters could not be addressed.

Interestingly, however, the measurement of growth in the present and related studies (Paynter and DiMichele 1990, Paynter and Mallonee 1991), which used shell height as a measure of growth...
Effects of Perkinsus on Oyster Growth

Size distribution of oysters raised at the Mobjack Bay site.

<table>
<thead>
<tr>
<th>Date</th>
<th>Group</th>
<th>Mean Ht</th>
<th>Variance</th>
<th>S.E.M.</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/31/90</td>
<td>A</td>
<td>61.97</td>
<td>79.76</td>
<td>0.30</td>
<td>45</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>60.32</td>
<td>128.04</td>
<td>0.26</td>
<td>40</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>34.00</td>
<td>19.60</td>
<td>0.12</td>
<td>25</td>
<td>45</td>
</tr>
<tr>
<td>7/4/90</td>
<td>A</td>
<td>69.43</td>
<td>56.17</td>
<td>0.33</td>
<td>55</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>70.83</td>
<td>148.06</td>
<td>0.33</td>
<td>54</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>46.29</td>
<td>40.61</td>
<td>0.30</td>
<td>38</td>
<td>57</td>
</tr>
<tr>
<td>8/29/90</td>
<td>A</td>
<td>73.55</td>
<td>46.07</td>
<td>0.31</td>
<td>62</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>72.95</td>
<td>78.05</td>
<td>0.42</td>
<td>54</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>66.04</td>
<td>36.22</td>
<td>0.25</td>
<td>56</td>
<td>78</td>
</tr>
<tr>
<td>9/26/90</td>
<td>A</td>
<td>67.94</td>
<td>45.40</td>
<td>0.22</td>
<td>58</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>72.48</td>
<td>84.18</td>
<td>0.34</td>
<td>59</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>68.47</td>
<td>98.00</td>
<td>0.31</td>
<td>45</td>
<td>85</td>
</tr>
</tbody>
</table>

Oysters were sampled as described in text.

and subsampled large groups of genetically equivalent oysters, has yielded results remarkably similar to Andrews underwater weighing technique, and has shown that oysters grow at a relatively constant rate throughout the warm season (Menzel and Hopkins 1955, Andrews 1961).

The characterization of the effects of P. marinus on large numbers of animals and the influence of salinity on those effects is important to the developing aquaculture industry. However, few studies have focused on sublethal or physiological effects of protozoan parasitism in bivalves (see Newell and Barber 1988). Barber et al. (1988a, 1988b) showed that condition and fecundity were reduced by infection of Haplosporidium nelsoni (MSX). Newell (1985) showed that P. marinus infection was not tested in that study, and the immunological and physiological characteristics of oysters with respect to disease tolerance are more robust at lower salinities (Fisher and Newell 1986).

The reduction in growth rate after P. marinus infection was essentially immediate within a tray at a given site (Figs. 2 & 3). Once infection occurred, the entire population within the tray appeared to be affected. For instance, growth in group A (Fig. 2) during 1989 was reduced when the detected prevalence was low (=12%) with low intensity through November. Oysters grown at moderate salinity showed over a 60% reduction in growth rate (Table 1) but incurred only a 30 to 40% infection prevalence throughout the season (Fig. 1). The lack of an increase in size variation or range within infected trays (Table 2), which would be expected if only a small percentage of the animals stopped growing, also suggested that most of the animals within the tray had stopped growing. The data in Table 2 show that the mean size of oysters did not significantly increase after infection (detected on 7/4/90 for Groups A & B) and that the variance of the samples decreased greatly (from 148 to 84 for Group B). In addition, the range of sizes did not change indicating that very few, if any, oysters continued to grow.

During part of the study, disease diagnosis was conducted on oysters whose size and wet tissue weight were also measured. Within a sample for a given tray, no relationship between size and infection was found. When data were pooled from all trays exposed for the same period at a given site, a weak but significant positive correlation was discovered (P < 0.05; data not shown). The larger animals tended to acquire the disease sooner and more intensely. This relationship was also evident in Fig. 3 which shows that the smaller group (C) did not become infected until nearly 40 days after groups A or B. Although disease was not detected in any groups in May 1990, latent infections which did not affect growth could have been present in Groups A & B and led to earlier infections in those groups. Crosby and Roberts (1990) found no relationship between size and infection, however, our findings support the generally accepted theory that small oysters or spat are less likely to become infected due to the limited volume of water they filter compared to large animals. However, in this study all oysters within a tray appeared to be affected even before 100% prevalence could be detected. This observation could be the result of disease development and subsequent reduction of growth rate, but not the time to infection or the effect of infection on condition index. These results suggest that the concentration and/or virulence of infective P. marinus life stages is higher at higher salinities (Chu and Greene 1989, Chu and La Peyre 1991), and/or that the immunological and physiological characteristics of oysters with respect to disease tolerance are more robust at lower salinities (Fisher and Newell 1986).

Figure 4. Mean condition indices (CI) of infected and uninfected oysters during 1990 in all groups at all sites by month. Bars represent ±2 S.E.M.
Salinity was positively correlated with growth rate of oysters in the present study. Unfortunately, the increase in growth rate was compromised by the increased prevalence and effects of *P. marinus* at higher salinities. It is therefore important to understand the relationship between infection, its effects, and salinity. This study has shown that infection rate, measured as time to infection, was not related to salinity but probably more to the number of infective stages present in the water. Quick and Mackin (1971) found little relationship between salinity and infection. Sionit (1985) and Ragone and Burreson (1990), on the other hand, found a significant correlation between salinity and infection. The findings of the present study are compatible with both sets of studies. While little difference in initial infections was found between high and moderate salinities, no infections occurred at low salinities. In relative terms, the impact of infection appeared positively correlated with salinity. Growth in oysters infected at moderate salinities was reduced by an average of 60% compared to a reduction of 80% at higher salinities. Unfortunately, mortality at moderate salinities could not be quantitated due to the loss of experimental trays at these sites during the second year of study. However, the absolute values of the growth rates of infected oysters were not different between salinities indicating that the relative differences were a function of pre-infection growth rate and that the growth rates of all infected populations, regardless to salinity, declined to the same low (approximately 3 mm/month) level.

Finally, the selectively inbred population employed in this study and the MSX-resistant strains employed in a closely related study (Burreson et al. 1990, Burreson 1991) were both more affected by *P. marinus* infection than either Mobjack Bay native or Delaware Bay native animals. This suggests that the results of the present study may not be representative of other oyster populations, but also indicates that a genetic component of disease resistance exists in the species which might be enhanced by selective breeding. Significant evidence supports the opinion that genetically distinct populations of *C. virginica* exist along the Atlantic and Gulf coasts (King and Gray 1990, Reeb and Avise 1990, Brown and Paynter 1991). It is possible that the genetic differences defined by these studies are reflective of physiological differences which may be important to identify. For instance, the southern ‘race’ of oysters as identified by Reeb and Avise (1990) has probably been exposed to *P. marinus* for many more generations than the northern race. It is entirely possible that the southern oyster population would exhibit more tolerance to infection than the Chesapeake Bay native populations. Transplantation experiments to test this and related hypotheses are currently underway.

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**LITERATURE CITED**

EFFECTS OF PERKINUS ON OYSTER GROWTH


