1991

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Journal of Shellfish Research, Vol. 10, No. 2, 417–423, 1991.

EFFECTS OF *PERKINSUS MARINUS* INFECTION IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*: I. SUSCEPTIBILITY OF NATIVE AND MSX-RESISTANT STOCKS

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ABSTRACT A selective breeding program was implemented to attempt to decrease the disease susceptibility of the eastern oyster, *Crassostrea virginica*, to *Perkinsus marinus*. Six oyster strains were spawned and the progeny exposed to *Haplosporidium nelsoni* (MSX) and *P. marinus* in the lower Chesapeake Bay. Three strains, a Delaware Bay MSX-resistant strain, a Delaware Bay native strain, and a Mobjack Bay native strain (lower Chesapeake Bay) were exposed for three years (1988–90); three other strains, a separate Delaware Bay MSX-resistant strain, a lower James River native strain (lower Chesapeake Bay) and a susceptible control strain, were exposed for two years (1989–90). During the study period, *P. marinus* abundance was high and increased each year; *H. nelsoni* abundance was low and decreased each year. Both strains of MSX-resistant oysters developed by Rutgers University were highly susceptible to *P. marinus*. Cumulative mortality at the end of the study was 99% for both strains and growth virtually stopped after acquisition of *P. marinus*. Mean shell height did not reach market size in either MSX-resistant strain. All native strains (Delaware Bay, Mobjack Bay and James River) had about 80% cumulative mortality, mainly from *P. marinus*, but the Mobjack Bay strain also experienced moderate mortality from *H. nelsoni*. However, these strains continued growing and survivors reached market size during the study period. The MSX-resistant strains offer little immediate benefit in a selective breeding program for the Chesapeake Bay oyster industry because of their high susceptibility to *P. marinus* and poor growth; however, they may be valuable, especially in crosses with native strains, during periods of *H. nelsoni* resurgence and *P. marinus* decline. The three native strains performed better than the resistant strains and will be utilized, both as direct lines and as intraspecific hybrids, in a continuing selective breeding program to decrease the disease susceptibility of *C. virginica* stocks.

KEY WORDS: oysters, disease, Chesapeake Bay, *Haplosporidium*, growth, mortality

INTRODUCTION

The oyster industry in Virginia has been in serious decline since 1960. Between 1932 and 1960 oyster landings in Virginia actually increased, reaching a peak of 4.0 million bushels in 1959 (Hargis and Haven 1988). This increase was primarily the result of a dramatic increase in landings from the private sector of the industry; landings from public beds continued to decline gradually during the period and accounted for only about 20% of the total harvest by the late 1950s. Beginning in 1960, the well documented MSX epizootic, caused by the protozoan *Haplosporidium nelsoni* (Haskin, Stauber and Mackin), caused large scale mortality and eventually resulted in abandonment of traditional leased beds in high salinity portions of the lower Chesapeake Bay (Haskin and Andrews 1988). As a result, oyster landings from the private sector declined precipitously during the 1960s and early 1970s and by 1974 landings from private and public beds were approximately equal (Hargis and Haven 1988). During the 1970s and early 1980s, landings were relatively stable, although very low compared with pre-1960 levels. Unfortunately, successive drought years from 1985 through 1988 caused a resurgence of *H. nelsoni* in Chesapeake Bay (Burreson and Andrews 1988, Haskin and Andrews 1988) and also an unprecedented intensification and spread of another protozoan pathogen, *Perkinsus marinus* (Mackin, Owen and Collier) to all oyster beds in Virginia (Andrews 1988, Burreson and Andrews 1988). High oyster mortality from combined effects of the two diseases during this period resulted in successive record low landings each year from 1988 through 1990 (Virginia Marine Resources Commission, landings records).

Although many factors, including overharvesting, are contributing to the continuing decline of the Virginia oyster industry (Hargis and Haven 1988), it is clear that disease-induced mortality directly, or reduced planting in the private sector because of the fear of high losses, were primarily responsible for the rapid decline in landings during the 1960s and again in the 1980s. Therefore, it seems unlikely that the industry can be rehabilitated rapidly unless oysters can be developed that are less susceptible to disease. One approach to developing resistant oysters is through a selective breeding program in which surviving oysters from disease endemic areas are selected and bred over successive generations in an attempt to decrease disease-induced mortality. This approach has been used successfully to increase the survival of oysters exposed to *H. nelsoni* (MSX) (Andrews 1968, Haskin and Ford 1979, Ford and Haskin 1987, Ford 1988, Haskin and Andrews 1988). Between 1960 and 1985, *H. nelsoni* was responsible for most of the disease-induced mortality in Chesapeake Bay oysters. However, since 1985, *P. marinus* has gradually replaced *H. nelsoni* as the most important oyster pathogen in Chesapeake Bay (Burreson and Andrews 1988, Andrews 1988) and little effort has been devoted to developing oysters with decreased susceptibility to this pathogen. The purpose of this paper is to compare the susceptibility to *P. marinus* of oysters bred for decreased susceptibility to *H. nelsoni* and of surviving oysters from various disease endemic areas in the lower Chesapeake Bay. Results will be used to choose broodstock for a continuing selective breeding program to increase survival of oysters exposed to both local diseases.

METHODS

Hatchery-reared broods from six stocks of oysters were utilized in this study:

1. MSX-selected A: Delaware Bay native strain selected through six generations in Delaware Bay for resistance to *H. nelsoni*. These oysters demonstrated significantly greater

survival than unselected control oysters (Ford and Haskin 1987) when exposed to *H. nelsoni*. Broodstock (Rutgers University BXF) was provided by Drs. H. Haskin and S. Ford.

2. Delaware Bay natives: native oysters from the lower seed area in Delaware Bay provided by Drs. H. Haskin and S. Ford, Rutgers University.
3. Mobjack Bay natives: oysters from a population on Pultz Bar in Mobjack Bay, VA that has suffered annual exposure to both *H. nelsoni* and *P. marinus* since 1959.
4. MSX-selected B: upper James River native strain selected through five generations in Delaware Bay for resistance to *H. nelsoni*. These oysters demonstrated significantly greater survival than unselected control oysters (Ford and Haskin 1987) when exposed only to *H. nelsoni* in Delaware Bay. Broodstock (Rutgers University AVA2A) was provided by Drs. H. Haskin and S. Ford.
5. Lower James River natives: oysters from a population on Nansemond Ridge in the lower James River that has suffered annual exposure to both *H. nelsoni* and *P. marinus* since 1960 and severe exposure to *P. marinus* since 1985.
6. Susceptible controls: oysters from Horsehead Rock, until 1988 a low salinity sanctuary from disease in the upper James River. Oysters from this location have historically exhibited high disease susceptibility to both *H. nelsoni* and *P. marinus* and are used routinely in the oyster disease monitoring programs conducted by the Virginia Institute of Marine Science (VIMS) and Rutgers University.

Broodstock from all stocks were conditioned at 22°C in the VIMS hatchery. Spawning was induced by raising the water temperature to 30°C; all spawnings used at least 10 individuals. If elevated temperature did not induce spawning, a male from the broodstock group was stripped and sperm added near the incurrent region of each oyster by pipet. When oysters spawned, they were identified as to sex and placed in separate containers to collect sperm and eggs; eggs from the spawning trough were collected on a 20 μm sieve and added to the egg container. Eggs were fertilized by the addition of sperm. Larvae were reared in 400 gallon conical tanks, set on minicultch and hardened in upwellers. When spat were large enough to be transfered from upwellers to flumes they were placed in small 6.0 mm mesh bags and held in Nestier trays in seawater flumes until deployed for field challenge. Because of oyster and hatchery availability, not all stocks were spawned at the same time. Stocks 1–3 (above) were spawned in November, 1987 and oysters were approximately 6 mo old when placed in the York River for exposure to diseases on 1 May 1988. Stocks 1–3 were monitored in the river until September 1990. Stocks 4–6 (above) were spawned in April, 1988 and were approximately 1 yr old when disease challenge was initiated on 1 May 1989. Stocks 4–6 were monitored in the river until December 1990. Spat from all strains were singled ($n \geq 1000$) and placed into labeled nylon 6.0 mm mesh bags that were held in 0.6 × 1.2 m legged oyster trays. The tray frames were covered with 2.5 cm plastic mesh to exclude large predators. Trays were suspended from a pier at VIMS in the lower York River, an endemic area for both *H. nelsoni* and *P. marinus*.

For comparison with experimental strains, susceptible oysters (2 to 3 inches shell height) from the routine VIMS oyster disease monitoring trays were used to assess the annual prevalence and intensity of both *H. nelsoni* and *P. marinus*. These trays were identical in construction to those mentioned above and contained 500 oysters collected in late April each year from either Horsehead Rock or Deepwater Shoal in the upper James River. Oysters from these beds have only rarely been exposed to *H. nelsoni* and *P. marinus* and are highly susceptible to both pathogens. The trays are typically deployed on 1 May each year and removed on 1 December and provide a long term record of annual disease severity.

Live and dead oysters in trays were counted every two weeks from 15 May until 1 December. Weekly counts were usually made during periods of high mortality. Samples of 25 oysters for disease diagnosis were removed periodically from each tray; samples were usually taken in May, July or August and September or October. *Perkinsus marinus* was diagnosed in all samples by thioglycollate culture (Ray 1952) of mantle, gill and rectal tissue; *H. nelsoni* was diagnosed by routine paraffin histology of oysters preserved in Davidson's AFA. A subsample of 100 oysters from each group was measured for total shell height in May of each year and also in July and late September of the second and third years.

Differences in shell height among the various oyster strains were analyzed by one-way ANOVA with subsequent Scheffé multiple comparison tests. Differences in parasite prevalence, intensity and oyster mortality between groups were analyzed by chi-square contingency tables with continuity correction. For prevalence comparisons, the contingency table columns were numbers of infected and uninfected oysters; for intensity comparisons one column was the sum of the number of heavy and moderate infections and the other column was the number of light infections. For mortality comparisons the columns were the number of live and dead oysters to that date. Table rows were the various strains of oysters. All statistical tests were run on an Apple Macintosh II using Statview II.

RESULTS

Prevalence and intensity of *H. nelsoni* (MSX) and *P. marinus*, oyster growth, and cumulative oyster mortality for experimental oyster stocks 1–3 are shown in Figure 1. There was no difference in growth among the three stocks during the first year (Fig. 1C). However, cumulative mortality was significantly greater ($P < 0.01$) in the Mobjack Bay stock than either the MSX-selected A or the Delaware Bay native stock from December 1988 through May 1990. The higher mortality in the Mobjack Bay stock appears to be the result of significantly higher ($P < 0.01$) MSX prevalence in this stock (Fig. 1A) in August and September 1988. No *P. marinus* was observed in any of these three stocks during 1988 (Fig. 1B) although prevalence in market size control oysters reached 68% in August (Table 1) indicating that *P. marinus* was abundant during the period. Prevalence of MSX was also greater in the market size control oysters (Table 1) than in any of the experimental stocks 1–3 during 1988 and 1989 (Fig. 1A).

During 1989, the second year of exposure, experimental stocks 1–3 all became infected with *P. marinus* (Fig. 1B). Prevalence of *P. marinus* was significantly greater ($P < 0.01$) in the MSX-selected A stock than in the other two stocks during August and October 1989. The prevalence of MSX gradually declined during this period and MSX was absent in October 1989 samples of experimental stocks (Fig. 1A) even though prevalence and intensity of MSX was high in market size control oysters (Table 1). The high prevalence of *P. marinus* in the MSX-selected A stock re-

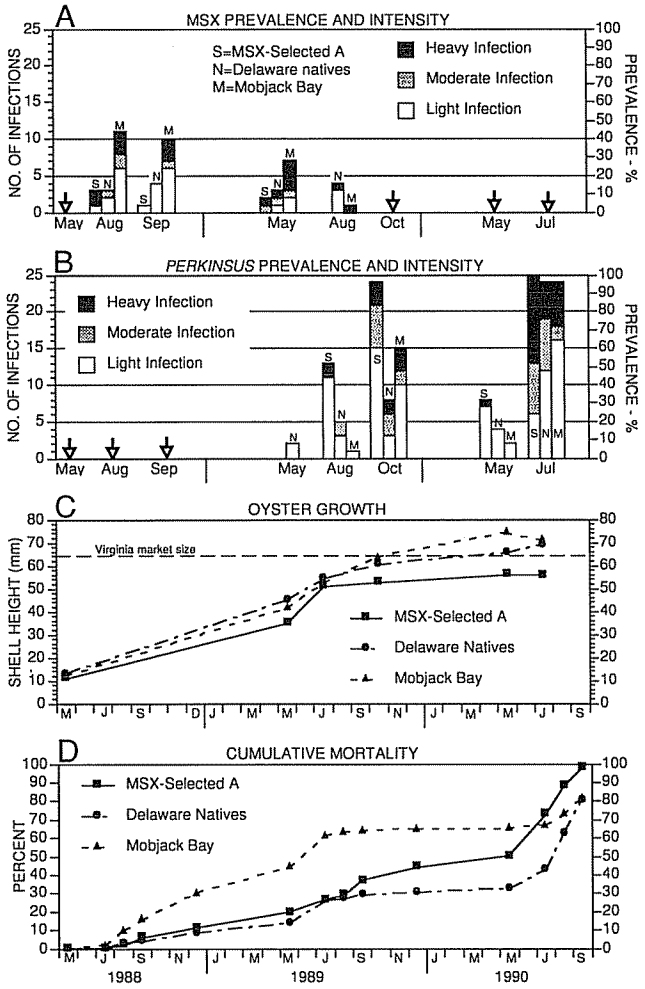

Figure 1. Results from oyster stocks deployed in May 1988. In A and B total bar height is the number of infected oysters (left axis) and prevalence (right axis) in a sample of 25 oysters. Arrows indicate samples examined, but no infections found. Oyster stock designations listed in A also apply to B. Time line shown in D applies to all figures.

sulted in increased cumulative mortality (Fig. 1D) and greatly decreased growth (Fig. 1C, Table 2). Although the Mobjack Bay native stock had higher prevalence of *P. marinus* than the Delaware Bay native stock (Fig. 1B), the Mobjack Bay stock grew faster (Fig. 1C, Table 2) than the Delaware Bay native stock between July 1989 and May 1990. In May 1990 shell heights (Fig. 1C) and cumulative mortalities (Fig. 1D) were significantly different ($P < 0.01$) between all experimental stocks 1–3. In July 1990 all experimental stocks had high prevalence of *P. marinus*, but the number of heavy and moderate infections was significantly greater ($P < 0.01$) in the MSX-selected A stock (Fig. 1B) than in the other two stocks. The high prevalence and intensity of *P. marinus* resulted in greatly increased mortality in all three stocks during summer of 1990, but especially in the MSX-selected A and the Delaware Bay native stock (Fig. 1D). When the experiment was terminated in September 1990, the cumulative mortality in the MSX-selected A stock was significantly greater ($P < 0.01$) than in the other two stocks. Mean shell height decreased during summer 1990 in the Mobjack Bay stock and the MSX-selected A stock (Fig. 1C, Table 2), apparently as a result of selective mortality of large oysters. Mean shell height for the MSX-selected A stock never reached market size (Fig. 1C). No MSX was observed in the experimental stocks during 1990 and only one infection was found in the market size control oysters (Table 1).

Prevalence and intensity of *H. nelsoni* (MSX) and *P. marinus*, oyster growth and cumulative oyster mortality for experimental stocks 4–6 are shown in Figure 2. These three stocks were approximately one year old when placed in the York River and, thus, were larger than stocks 1–3 when first exposed to disease challenge (Figs. 1C, 2C; Table 2). The lower James River stock and the susceptible control stock apparently acquired MSX in the holding flume because both stocks had a low prevalence of MSX when field challenge was initiated (Fig. 2A). The lower James River stock was not infected with MSX in subsequent samples, but prevalence remained low in the susceptible control stock. No MSX was found in the MSX-selected B stock during 1989 and only 2 infected oysters were found during May 1990, although both had heavy infections (Fig. 2A). The MSX infections observed in experimental stocks during May 1990 were probably acquired during fall 1989 because no MSX infections were found during May or July 1990 in market size control oysters (Table 1) or during July or September in the experimental stocks, suggesting that typical early summer MSX infections did not occur during 1990. All three stocks acquired *P. marinus* by August 1989, but both prevalence and intensity were significantly lower ($P < 0.05$) in the lower James River stock than in the other two stocks (Fig. 2B). The MSX-selected B stock seemed to be most affected by the *P. marinus* infections; shell height of the MSX-selected B stock was significantly lower ($P < 0.05$) than the other two stocks in both July and October 1989 (Fig. 2C) and cumulative mortality of the MSX-selected B stock was significantly greater ($P < 0.05$) by December 1989 (Fig. 2D). During 1990, both prevalence and intensity of *P. marinus* were high in July and September with no significant differences among the various stocks (Fig. 2B). Cumulative mortality of the MSX-selected B stock was significantly greater ($P < 0.01$) than the other two stocks at each sampling date during 1990 (Fig. 2D). By December 1990 the cumulative mortality of the lower James River stock was also significantly lower ($P < 0.01$) than the susceptible control stock. Mean shell height of the MSX-selected B stock did not reach market size and was significantly lower ($P < 0.05$) than the other two stocks in July 1990 (Fig. 2C, Table 2). There were insufficient oysters remaining in September 1990 for disease diagnosis or growth measurements in the MSX-selected B stock.

DISCUSSION

Interpretation of the results was confounded by decreasing prevalence of *H. nelsoni* and increasing prevalence of *P. marinus* over the three year study period based on prevalence of both pathogens in the large control oysters. In addition, the six oyster stocks assessed were spawned and exposed at two different times and, thus, were two different ages at the initiation of disease challenge. Nevertheless, some important conclusions can be reached about the value of using each of these stocks in a continuing selective breeding program to decrease the disease susceptibility of *C. virginica*.

Susceptibility to H. nelsoni

The two stocks selected for decreased susceptibility to *H. nelsoni* in Delaware Bay (MSX-selected A and B stocks) and the

Virginia market size of 64 mm during the study period and the size of both stocks was significantly lower than any other stock at the end of the study. The growth of the MSX-selected A stock virtually stopped after the first infections of *P. marinus* were observed in August 1989. The average shell height of all other stocks reached Virginia market size. It is interesting that the Delaware Bay native stock grew almost as well as the Mobjack Bay stock since Mobjack Bay oysters have a long history of annual exposure to *P. marinus* and the pathogen has only rarely been present in Delaware Bay.

The retardation of oyster growth by *P. marinus* was first reported by Menzel and Hopkins (1955) and later by Andrews (1961). More recently, Paynter and Burreson (1991) demonstrated rapid reduction in growth in oysters selected over 18 generations for fast growth (Paynter and Dimichele 1990) even when the prevalence and intensity of *P. marinus* were relatively low. The results of the present study and those of Paynter and Burreson (1991) suggest that the growth of highly inbred oysters is more affected by *P. marinus* than is the growth of native stocks, whether or not they have a history of exposure to *P. marinus*.

Mortality

Mortality was high in all groups, but mortality in both MSX-selected stocks was significantly greater than in the other four stocks. Based on the relative prevalence and intensity of the two pathogens, these MSX-selected stocks experienced low mortality from *H. nelsoni,* but very high mortality from *P. marinus,* especially during the second year of infection. The Mobjack Bay stock appeared to have the lowest mortality from *P. marinus*, but the moderate mortality in this stock from *H. nelsoni* resulted in a total mortality similar to the Delaware Bay native stock. The lower James River stock had lower mortality from *P. marinus* than the susceptible control stock, but there was no significant challenge from *H. nelsoni* during 1990, so the susceptibility of the lower James River stock to that pathogen is unclear.

On the basis of this study, under conditions of relatively low *H. nelsoni* abundance and relatively high *P. marinus* abundance, it does not appear that the MSX-resistant oyster stocks developed by Rutgers University offer any hope for immediate rejuvenation of the Chesapeake Bay oyster industry. Only two strains were tested, but these resistant oysters seem to be extremely susceptible to *P. marinus;* mortality is very high and growth, after infection, is very low. Growth is important because this study again demonstrates that high mortality from *P. marinus* does not occur until the second summer of infection and, thus, oysters that reach market size in less than two years may avoid significant disease-induced mortality. For example, if hatchery-reared spat were deployed to grow-out areas in the fall and reached market size in 18 months they would experience only one summer of disease exposure. If these oysters had also been selected for decreased susceptibility to both *H. nelsoni* and *P. marinus,* mortality might be relatively low prior to harvest. Acceptable growth and the lowest mortality occurred in the Delaware Bay native stock, the Mobjack Bay stock and the lower James River stock. These stocks, including both direct lines and intraspecific hybridization, will be used in a continuing selective breeding program.

Although the MSX-resistant oyster stocks appear to offer no immediate benefit to the Chesapeake Bay oyster industry, their value could be substantial in the future if *H. nelsoni* returns to its previously dominant abundance and *P. marinus* abates. Intraspecific hybridization between an MSX-resistant stock and a native stock selected for rapid growth and *P. marinus* resistance could yield a strain with decreased susceptibility to both diseases.

ACKNOWLEDGMENTS

This study could not have been completed without the expert assistance of Juanita Walker, who performed most of the disease diagnoses, and Judith Meyers who coordinated sampling and tray cleaning and maintenance. Mike Castagna provided hatchery time and hatchery manager Ken Kurkowski and his staff performed the spawnings and maintained spat through the hardening phase. Others who assisted in various aspects include Dr. Bruce Barber, Beth McGovern, Lisa Ragone, Gustavo Calvo and Chris Maclauchlin. Special thanks to Drs. Hal Haskin and Susan Ford, Rutgers University, for providing broodstock from their MSX-resistant stocks and from Delaware Bay native oysters. The manuscript was improved by suggestions from Drs. W. J. Hargis, Jr., J. D. Andrews and K. T. Paynter, Jr. This research was supported in part by the Virginia Sea Grant College Program under grant number NA-86AA-D-SG042. Virginia Institute of Marine Science contribution number 1684.

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