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DEVELOPMENT OF DISEASE CAUSED BY THE PARASITE, *PERKINSUS MARINUS* AND DEFENSE-RELATED HEMOLYMPH FACTORS IN THREE POPULATIONS OF OYSTERS FROM THE CHESAPEAKE BAY, USA

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ABSTRACT The development of infection caused by the protozoan parasite, *Perkinsus marinus* (Dermo) and some specific potential defense-related cellular and humoral components in oysters collected from three geographic areas, Deepwater Shoal of James River (DW), Wachapreague (WP), and Mobjack Bay (MJ) were examined over time. Oysters were maintained in estuarine water (salinity = 20 ppt) or in water at a salinity similar to the ambient salinity of the collection sites. Oysters were sampled at the initiation of the experiment (day 0), day 35, and day 100 to determine defense-related parameters and disease prevalence and intensity. All populations experienced a significant increase in *P. marinus* infection prevalence and intensity from the initiation of the experiment to the termination of the study. Oyster mortality differed between oyster populations. None of the DW oysters perished while cumulative mortalities for WP at 32 ppt and 20 ppt and MJ oysters were respectively, 23, 25, and 35%. The experimental oyster populations demonstrated significant differences with respect to cellular and humoral defense-related variables. As the study progressed, the mean number of total hemocytes declined in the WP and MJ populations and increased in the DW population. The percentage of granulocytes in DW oysters was consistently higher than other populations. DW oysters also had the highest concentrations of protein and lysozyme. This pattern persisted throughout the experimental period. Oyster condition index significantly decreased during the course of the study in all populations except the DW oysters at 10 ppt. Results suggest that the increase of hemocyte number and higher percentage of granulocytes, and lysozyme concentration in DW oysters may have contributed to the high (100%) survival rate of this population. Salinity may have affected disease development. Disease prevalence and intensity tended to be lower in the WP oysters maintained at low salinity than those maintained at high salinity. In the DW population, unexpectedly, oysters maintained at 20 ppt had lower infection prevalence and intensity than oysters maintained at 10 ppt. Salinity induced, to some extent, changes in certain hemolymph components: lysozyme concentration tended to be higher in oysters maintained at low salinity than those maintained at high salinity. Increase in percentage of granulocytes was also observed in WP oysters after transferring to a salinity lower than ambient salinity.

KEY WORDS: oyster disease, hemolymph factors, *Perkinsus marinus*

INTRODUCTION

Disease-induced mortality in eastern oysters (*Crassotrea virginica*) caused by two parasites, *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) is one of the factors contributing to the decline in oyster harvest in the Chesapeake Bay, U.S.A. Previously, disease pressure from *H. nelsoni* has been more intense on oysters than that from *P. marinus*. Because of its current expanded distribution and increase in abundance in waters of the Chesapeake Bay, *P. marinus* is now considered more significant than *H. nelsoni* as an oyster pathogen (Andrew 1988, Burreson 1989). It has been well documented that prevalence and intensity of *P. marinus* infections in oysters are related to milieu salinity (e.g., Soniat 1985, Soniat and Gauthier 1989, Crosby and Roberts 1990, Gauthier et al. 1990, Paynter and Burreson 1991). Significant growth reduction due to *P. marinus* infection in oysters raised in habitats of different salinity in the Chesapeake Bay has been reported by Paynter and Burreson (1991).

Hemocyte activities and lysozyme concentrations of eastern oysters have been reported to change seasonally (Fisher et al. 1989, Feng and Canzonier 1970, Chu and La Peyre 1989) and to be affected by salinity (Fisher 1988, Chu and La Peyre 1989, Chu et al. In review). Increased salinity suppressed hemocyte spreading and locomotion. Hemolymph lysozyme concentration in oysters was negatively correlated with salinity in oysters (Chu et al. In review).

The purpose of this study was to compare the development of disease caused by *P. marinus* in oysters collected from three different salinity habitats of the lower Chesapeake Bay and to determine if any changes occurred in some measurable cellular and humoral components in these oysters during the course of disease development.

METHOD AND MATERIALS

Experiment

To encompass the natural salinity range of oysters in the lower Chesapeake Bay, oysters were collected from 3 locations: a low salinity site, Deep Water Shoal of James River (DW, ambient temperature = 22.5°C, salinity = 10 ppt), a high salinity site, Burtons Bay, Wachapreague (WP, ambient temperature = 19.5°C, salinity = 32 ppt), and a moderate salinity site, Mobjack Bay (MJ, ambient temperature = 20.0°C, salinity = 20 ppt), in early October 1990. Oysters were cleaned of fouling organisms and a hemolymph sample was withdrawn from 30 oysters from each population to measure initial total hemocyte count (TC), percent of granulocytes (PG) and protein and lysozyme concentrations. Oysters were then sacrificed to determine initial condition index (CI = dry meat weight/dry shell weight $\times 100$, Lucas and Beninger 1985) and to examine for *P. marinus* infection (Ray 1952, 1966).

Sixty oysters from each population were maintained in 250 l static fiber-glass tanks at 22 ± 1°C and at conditions indicated below. Oysters from MJ (N = 60) were maintained in filtered (1μ filter) estuarine water (York River water, YRW, salinity = 20 ppt). Oysters from DW and WP were each divided into 2 groups

(60 oysters/group/tank); one group of the oysters was maintained in filtered YRW; the other group was maintained in water adjusted to ambient salinity (i.e. 10 ppt for DW oysters, 32 ppt for WP oysters). Oysters were fed daily with an algal diet (a mixture of *Pavlova lutheri, Isochrysis galbana* and Tahitian *Isochrysis galbana*). Water was changed every other day. The experiment was terminated in the middle of January, 1991 (100 days after experiment initiation). Thirty five days after the initiation of the experiment and at the end of the experiment, subsamples of oysters (N = 20 oysters, 35 days after initiation, N = 30 oysters at the end of the experiment) from each group were sampled for TC, PG, protein, lysozyme and CI measurement and *P. marinus* diagnosis.

Total and Differential Counts and Preparation of Sera

Hemolymph from individual oysters was withdrawn with a syringe from the adductor muscle sinus through notches in the shell and placed in micro test tubes in an ice bath. Total and differential (number of granulocytes and agranulocytes) hemocyte counts were obtained on each hemolymph sample using a hemocytometer. Differential counts were expressed as percentage of granulocytes (PG = 100 × number of granulocytes/total hemocytes). To determine protein and lysozyme concentrations in oyster serum (cell-free hemolymph), serum of each hemolymph sample was separated from hemocytes through centrifugation (400 × g at 4°C for 10 min). Serum was withdrawn and stored in a freezer (−20°C) for subsequent protein and lysozyme measurement.

Protein and Lysozyme Measurements

Lysozyme concentration was determined spectrophotometrically according to the method of Shugar (1952) and Chu and La Peyre (1989). Cell-free oyster serum (0.1 ml) was added to 1.4 ml of the bacterial (*Micrococcus lysodeikticus*) suspension and the decrease in the absorbance was recorded at 450 nm on a Schimadzu UV 600 spectrophotometer for 2 minutes. All measurements were duplicated and were taken at room temperature (21 ± 1°C). Recorded lysozyme activities were converted to lysozyme concentration using egg white lysozyme as a standard. Standard curves at different salinities were constructed by dissolving egg white lysozyme in a balanced salt solution of appropriate salinities (i.e. 10, 20, and 32 ppt), assuming that reactivity of oyster lysozyme and egg white lysozyme were similar if assayed in buffer of the same salinity.

Serum protein was measured by the method of Lowry et al. (1951) using bovine albumin as a standard. Ten µl of a cell-free hemolymph sample from individual oysters was used for the serum protein measurement.

Perkinsus Assay

The thioglycollate assay described by Ray (1952, 1966) was used for *P. marinus* diagnosis. Rectal tissue was removed from each oyster and incubated in thioglycollate medium for 4–5 days. Intensity of infection was ranked from 0 (negative) to 5 (heavily infected) based on the relative number of stained *P. marinus* hypnospores contained in the tissue smear.

Statistical Analysis

One factor analysis of variance (ANOVA) and Student-Newman-Keuls test were used to compare total hemocyte counts (TC) and percentage of granulocytes (PG), protein (P) and lysozyme concentrations, condition index, and prevalence and intensity of *P. marinus* infection between population groups and between different salinity treatments of the same population (i.e. DW and WP oysters). Data were Log_{10} or Arcsine transformed whenever data showed a large variance. Differences were considered statistically significant at $P \leq 0.05$. Linear correlation (Pearson correlation analysis) was calculated between the measured variables, condition index, serum protein and lysozyme concentrations, and *P. marinus* infection intensity.

RESULTS

The infection prevalence and intensity of oysters sampled from DW, WP, and MJ populations on day 0, day 35, and day 100 are shown in Figure 1. At the beginning of the experiment, prevalences in DW, WP, and MJ oyster samples (N = 30/population)

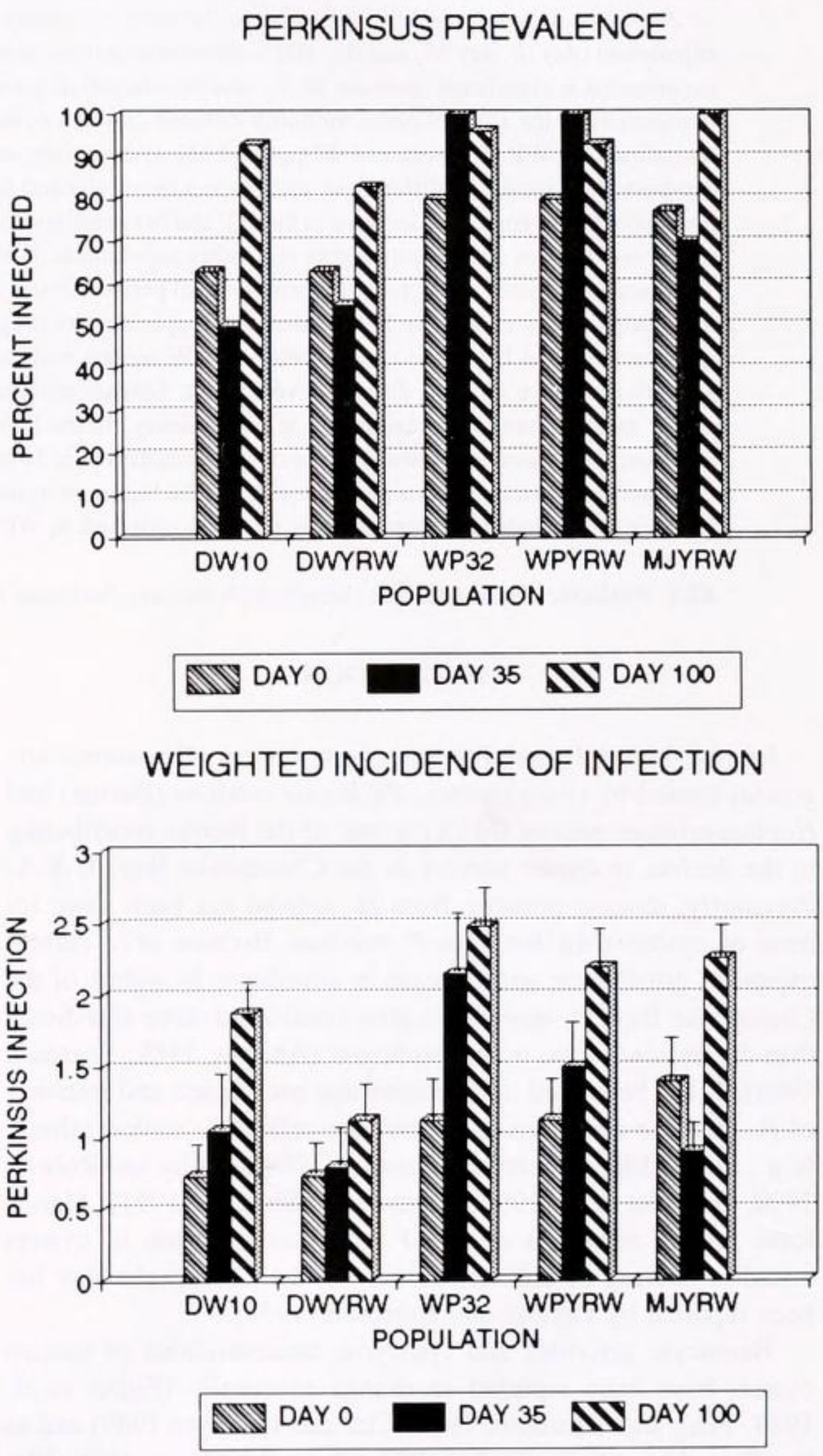

Figure 1. *Perkinsus marinus* prevalence and weighted incidence in DW (Deep Water Shoal, James River), WP (Burtons Bay, Wachapreague) and MJ (Mobjack Bay) oysters at day 0 (N = 30), day 35 (N = 20) and day 100 (N = 30). DW10 = DW oysters at 10 ppt water, DWYRW = DW oysters in York River Water, WPYRW = WP oysters in York River Water, WP32 = WP oysters at 32 ppt water, MJYRW = MJ oysters in York River Water.

were 63, 80, and 70%, respectively (Fig. 1). Infection intensities expressed as weighted incidence (WI = sum of disease code numbers/number of oysters) in WP and MJ oysters were significantly higher than in DW oysters (Fig. 1). *P. marinus* prevalence in oysters sampled on day 35 (N = 20/group) were 50% in DW at 10 ppt (DW10), 55% in DW at YRW (20 ppt, DW20), 70% in MJ, 100% in WP at 32 ppt (WP32) and at YRW (20 ppt, WP20). Weighted incidence increased in both WP and DW populations and decreased in the MJ oysters at day 35. At the termination of the experiment, prevalence in oyster samples (N = 30/group) were 93, 83, 96, 93 and 100% in DW10, DW20, WP32, WP20 and MJ populations respectively. All population groups experienced a significant increase in *P. marinus* infection prevalence and intensity from the initiation to the termination of the experiment, a period of 100 days. Generally, as in the beginning of the experiment, at the end of the experiment, DW oysters maintained relatively lower *P. marinus* weighted incidence than WP and MJ oysters. At all sampling dates, DW20 oysters had significantly lower ($P < 0.05$) weighed incidence than all other groups of oysters. The DW oysters maintained in YRW (20 ppt) had lower prevalence and weighted incidence than those maintained at 10 ppt. Only four DW20 oysters developed moderate to advanced (level 3–5) infections. Disease prevalence did not appear to differ in WP20 and WP32 oysters, but disease intensity was lower in the former than the latter at both day 35 and day 100.

Oyster mortality differed among populations (Fig. 2). During the course of the study, none of the DW oysters perished. Cumulative mortalities in WP at 32 ppt, WP at 20 ppt, and MJ groups were 23, 25, and 35%, respectively.

At the initiation of the experiment, mean TC was significantly higher in WP and MJ oysters than in DW oysters (Fig. 3). However, mean PG was much higher ($P < 0.05$) in the DW oysters than in the other oyster populations. As the study progressed, the mean TC declined in the WP and MJ groups and increased in the DW20 group. In the DW20, WP20 and MJ groups, the final mean TC differed significantly from the mean TC at day 0. No significant change in mean TC was observed over time in the DW10 and WP32 oysters. A trend of increasing TC with time was noted in the DW groups, although differences were not statistically significant. Generally, DW oysters had the highest PG over the course of the study. Generally, in both WP and DW oysters, no significant difference was observed in both TC and PG between salinity treatments.

Serum protein and lysozyme concentrations differed among the three oyster populations (Fig. 4). Concentrations of lysozyme and protein were significantly lower ($P < 0.05$) in the WP and MJ than in DW populations on day 0 (Fig. 4). No significant difference in lysozyme or protein concentration was observed between MJ and WP oysters. This pattern persisted throughout the experimental period; DW oysters had the highest ($P < 0.05$) concentration

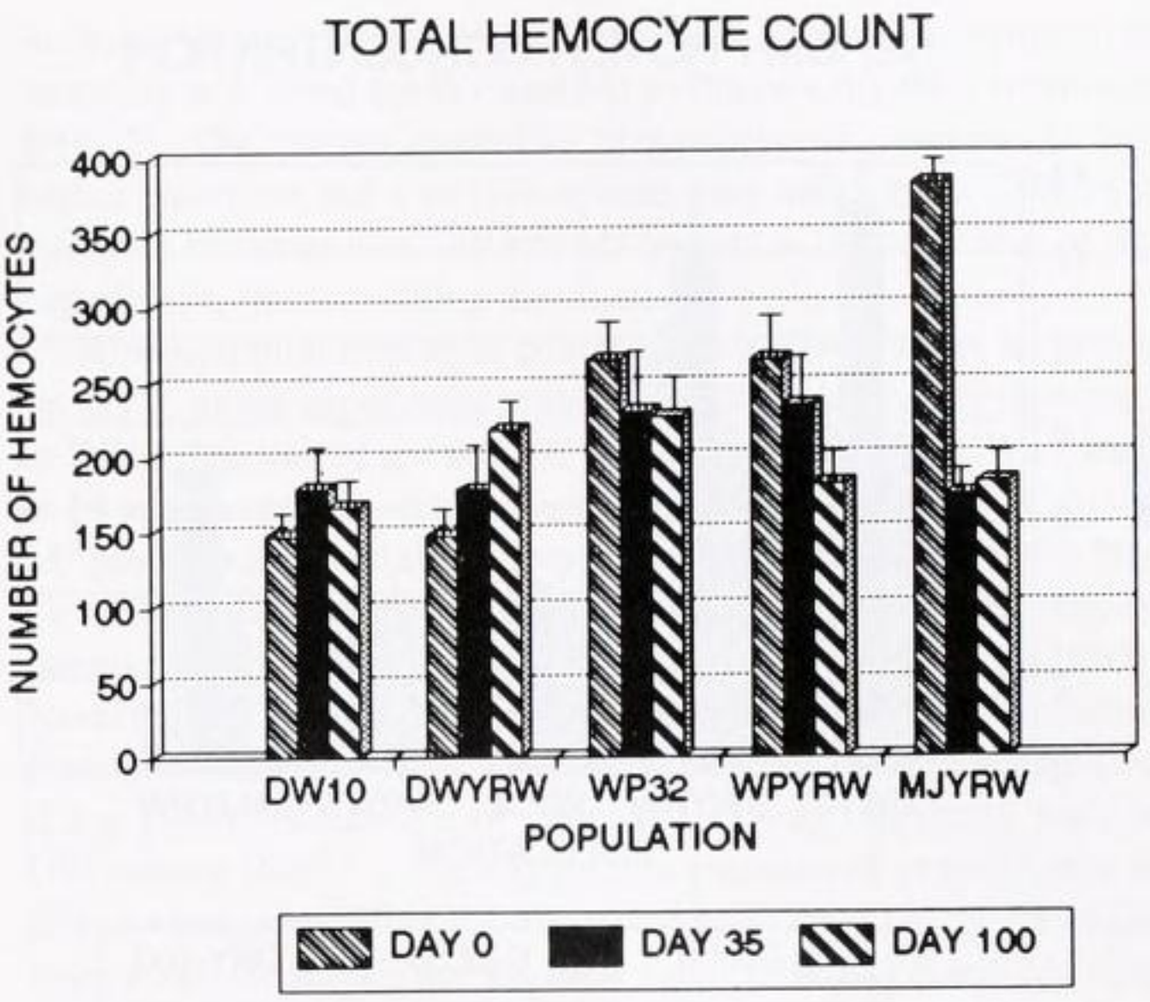

Figure 3. Mean total hemocyte counts and percentage of granulocytes (±SE) in DW (Deep Water Shoal, James River), WP (Burtons Bay, Wachapreague) and MJ (Mobjack Bay) oysters at day 0 (N = 30), day 35 (N = 20) and day 100 (N = 30). DW10 = DW oysters at 10 ppt water, DWYRW = DW oysters in York River Water, WPYRW = WP oysters in York River water, WP32 = WP oysters at 32 ppt water, MJYRW = MJ oysters at 20 ppt in York River water.

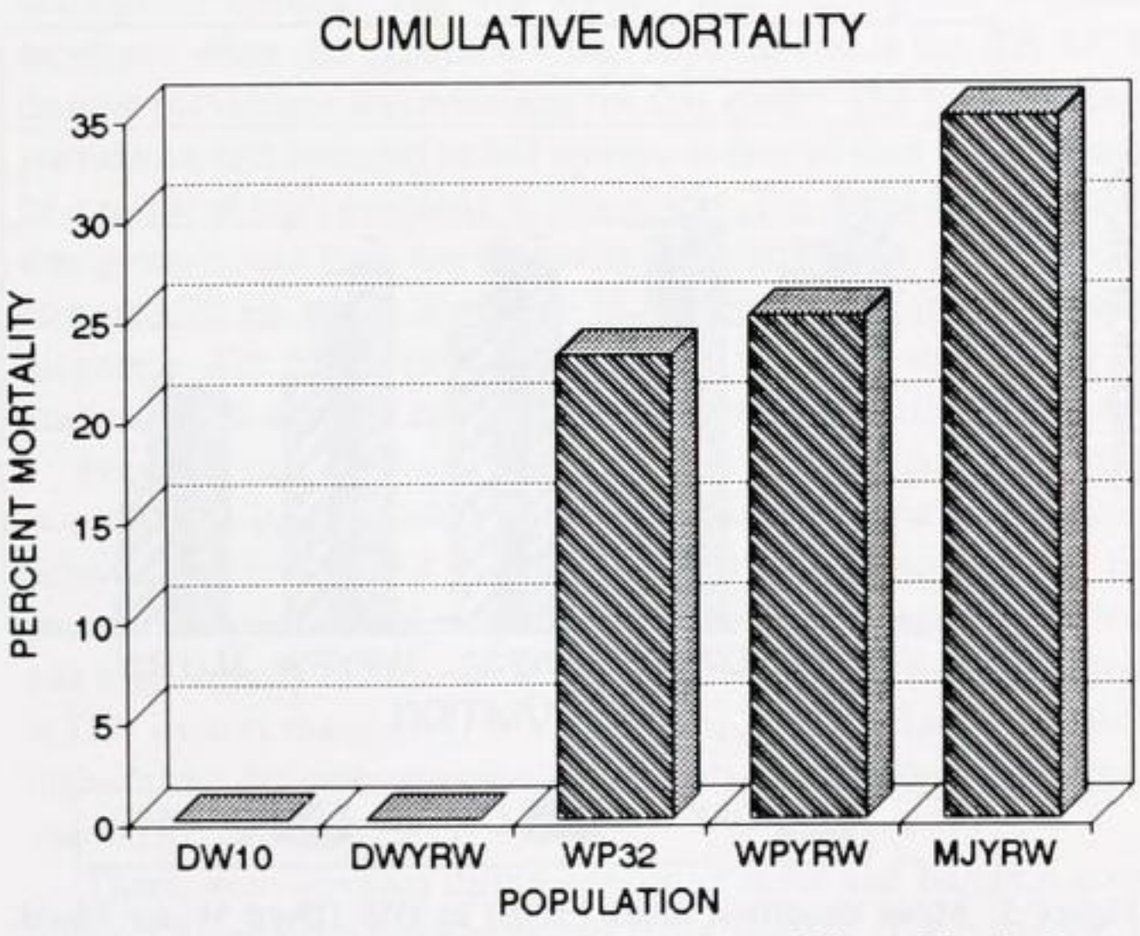

Figure 2. Cumulative mortality of DW (Deep Water Shoal, James River), WP (Burtons Bay, Wachapreague) and MJ (Mobjack Bay) oysters at the termination of the experiment. DW10 = DW oysters at 10 ppt water, DWYRW = DW oysters in York River Water, WPYRW = WP oysters in York River Water, WP32 = WP oysters at 32 ppt water, MJYRW = MJ oysters at 20 ppt in York River water.

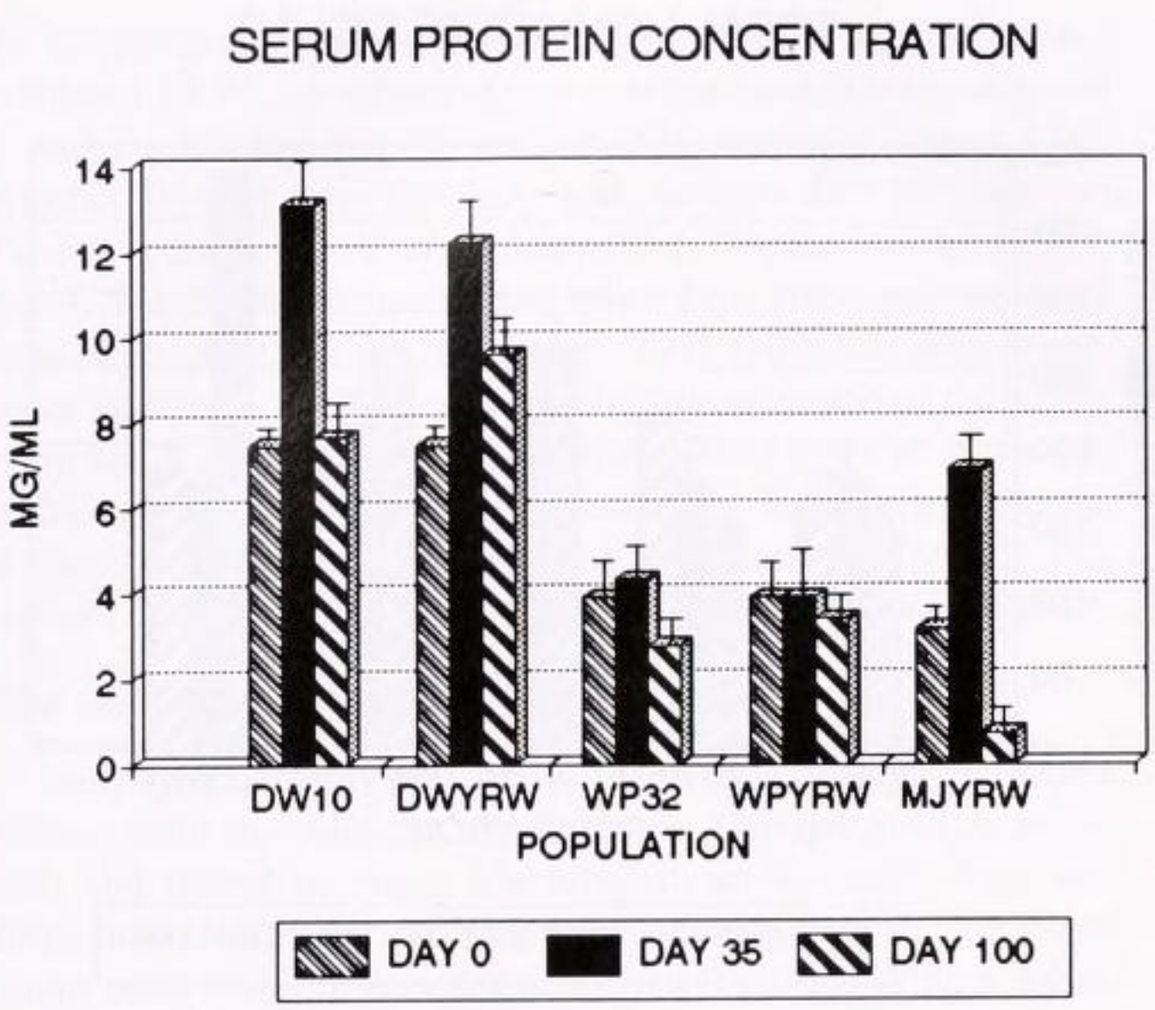

Figure 4. Mean hemolymph protein and lysozyme concentrations (±SE) in DW (Deep Water Shoal, James River), WP (Burtons Bay, Wachapreague) and MJ (Mobjack Bay) oysters at day 0 (N = 30), day 35 (N = 20) and day 100 (N = 30). DW10 = DW oysters at 10 ppt water, DWYRW = DW oysters in York River Water, WPYRW = WP oysters in York River water, WP32 = WP oysters at 32 ppt water, MJYRW = MJ oysters at 20 ppt in York River water.

of protein and lysozyme on all sample dates. The concentrations of these two serum components fluctuated between sample dates. Within the DW populations, oysters sampled at day 35 had a significantly higher protein concentration than those sampled at day 0 and day 100; but lysozyme concentration in DW oysters did not change significantly through time. Protein concentration in MJ oysters also peaked at day 35 and differed significantly from both initial and final sample concentrations. Both lysozyme and protein concentrations in MJ oysters declined from day 35 to day 100; MJ oysters sampled at day 100 had the lowest protein and lysozyme concentrations. Protein and lysozyme concentration in the WP population did not differ significantly over time. WP oysters did not differ from MJ oysters in protein and lysozyme concentration except at day 35. At day 35, MJ oysters had significantly higher protein concentrations than WP oysters. Although insignificant statistically, lysozyme concentrations tended to increase in WP20 oysters and to decrease in DW20 oysters. The mean protein concentrations in DW20 oysters was higher ($P < 0.05$) than DW10 oysters. The lysozyme concentrations in DW10, MJ, and WP32 oysters sampled at the termination of the experiment were negatively correlated with infection intensity.

Oyster condition, as indicated by condition index (Fig. 5), was significantly lower at the end than the beginning of the experiment in all population groups except the DW10 group. When Pearson correlation analysis was performed on data pooled from each group, it revealed that the condition index of DW10, WP32, WP20 and MJ oysters was negatively correlated with *P. marinus* infection intensity; condition index of DW20, WP32, WP20, and MJ oysters were positively correlated with serum protein concentrations.

DISCUSSION

Oysters from the upper James River, in areas such as Horsehead and Deep Water Shoal, are quite vulnerable to both *P. marinus* and *H. nelsoni* (Andrews 1984, Ford and Haskin 1987, Andrews 1988, Barber et al. 1991, Burreson 1992) but have remained relatively disease free because of prevailing low salinity (Andrews 1988, Burreson 1989, 1990, 1991). Mobjack Bay of the lower York river is an endemic area for both *P. marinus* and MSX. Progeny from survivors of the 1960 MSX epizootics in Mobjack Bay were shown to be less susceptible to MSX than seed oysters from the James River (Andrews 1971, Andrews 1984). Until 1990, oysters from Wachapreague had a low incidence of *P. marinus* and low mortality caused by *P. marinus* (Burreson 1990, 1991). The three oyster populations under investigation may be genetically different. However, they displayed a similar response to *P. marinus*. Almost all oysters (83 to 100%) from each popu-

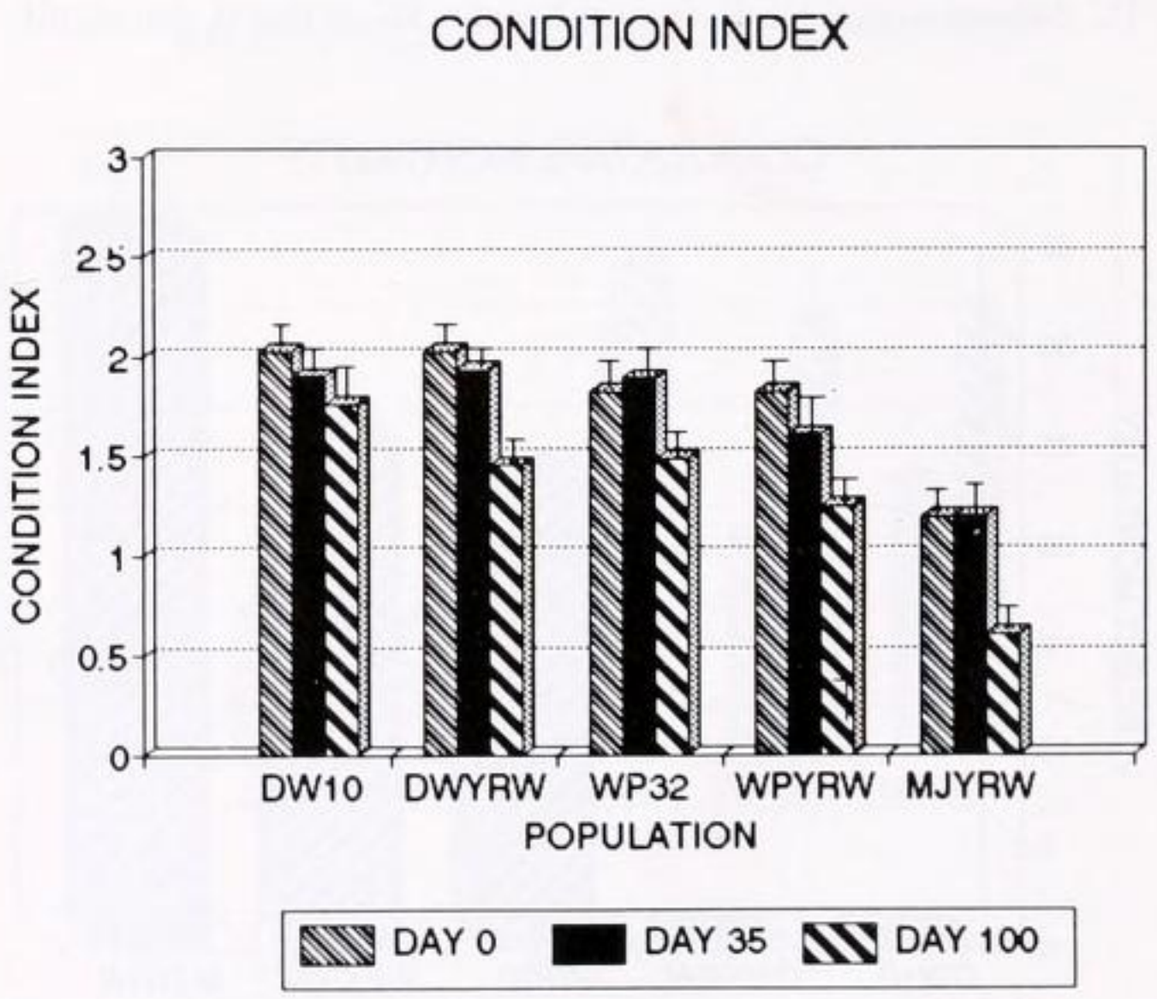

Figure 5. Mean condition index (±SE) in DW (Deep Water Shoal, James River), WP (Burtons Bay, Wachapreague) and MJ (Mobjack Bay) oysters at day 0 (N = 30), day 35 (N = 20) and day 100 (N = 30). DW10 = DW oysters at 10 ppt water, DWYRW = DW oysters in York River Water, WPYRW = WP oysters in York River water, WP 32 = WP oysters at 32 ppt water, MJYRW = MJ oysters in York River Water.

lation were infected by the parasite when the experiment was terminated (Fig. 1). Oysters from Mobjack Bay were found to be less susceptible to *H. nelsoni* than oysters from James River (Andrews 1984), but results of the present study indicates that they are equally susceptible to *P. marinus* as are oysters from the James River.

Results of the present study confirm that *P. marinus* can endure low salinity (Chu and Greene 1989, Ragone 1991, Burreson 1990, 1991). At the beginning of the experiment, oysters from Deep Water Shoal of James River began with lower disease prevalence (63% at day 0 and 50% at day 35) and intensity than both WP and MJ oysters. The increased disease prevalence in DW oysters at ambient salinity (10 ppt) at the end of the experiment is, apparently, a result of disease transmission between infected and uninfected oysters maintained in the same tank. Thus, it is clear that low salinity did not restrict disease transmission among oysters. Under continuous disease pressure, salinity of 10 ppt did not inhibit the progress of disease development. It has been shown that *in vitro*, only salinities lower than 6 ppt restrained *P. marinus* sporulation from prezoosporangia (Perkins 1966, Chu and Greene 1989).

Oysters from Deep Water Shoal of the James River (DW oysters) and WP oysters may have responded differently to the low salinity treatment. It is surprising to note that DW oysters maintained at ambient salinity (10 ppt) had higher *P. marinus* weighted incidence than those DW oysters at 20 ppt, whereas placing WP oysters at a salinity (20 ppt) lower than ambient salinity (32 ppt) reduced, relatively, the weighted incidence in these oysters. It is not known whether this difference is based on genetic dissimilarities between DW and WP oysters or whether it was an artifact. Further study is needed to verify this result. Restraint of disease progress has been noted in DW oysters infected by *P. marinus* maintained at low salinity (<12 ppt) (Ragone 1991, Chu unpublished results).

High disease prevalence and relatively higher disease intensity in WP and MJ oysters at the beginning of the experiment (Fig. 1) may account for the high cumulative mortality in these two populations of oysters. The WP oysters had the highest weighted incidence when the experiment was initiated and at day 35, 100% disease prevalence was recorded for this group. The lower disease prevalence and intensity in MJ oysters at day 35 than at day 0 may be a result of high mortality in this group. The deceased oysters of this group could have been heavily infected by the parasite. Unfortunately, no tissue was able to be recovered for *P. marinus* diagnosis. The lower disease prevalence in DW oysters at day 35 than at day 0 was unexpected. These results are unexplained.

Previous studies (Ray 1954, Andrews and Hewatt 1957, Scott et al. 1985, Ragone 1991) have demonstrated that low salinity delayed and/or reduced mortality induced by *P. marinus*. In the present study, differing mortalities were not observed between low and high salinity treatments in WP oysters and no deaths occurred in DW oysters maintained at a salinity of 20 ppt (which was much higher than the ambient salinity, 10 ppt) even though prevalence was 63%, initially.

There were obvious differences in cellular and humoral components among the three populations of oysters. The variation in these components which presumably reflect different genetic and habitat backgrounds of the oysters, may account for the different survival rates among these oysters. The DW oysters started not only with higher differential hemocyte counts, serum protein and lysozyme concentrations, but also with higher condition index. No mortality occurred in DW oysters while 23 to 35% cumulative mortality was noted for WP and MJ oysters during the experiment (Fig. 2). The greater quantities of hemolymph components and higher condition index of DW oysters may indicate that DW oysters were healthier than WP and MJ oysters at the beginning of the experiment, thus surviving the disease.

Although total hemocyte counts (TC) in DW oysters were low on day 0, as the experiment progressed, TC significantly increased in DW oysters at 20 ppt and TC tended to increase in DW oysters at 10 ppt. In contrast, there was a significant decrease of TC in the MJ group, which had the highest cumulative mortality (35%, Fig. 2). It has been suggested that the increase of hemocyte number (hemocytosis) in oysters is a response to parasitism (Ling 1990). Nevertheless, greater hemocytosis was observed in MSX-resistant than in MSX-susceptible oysters when infected by *H. nelsoni* (Ling 1990). Percentage of granulocytes was inherently high in DW oysters (Fig. 3). The higher concentration of granulocytes in DW oysters is probably habitat-associated. The DW oysters were from a habitat with lower ambient salinity than WP and MJ oysters. Fewer granulocytes were found in oysters from high salinity than from low salinity locations (Fisher and Newell 1986). However, it is interesting to note that the DW oysters which were transferred to 20 ppt also maintained a similar level of PG as those at 10 ppt. The increase of TC and relatively high PG in DW oysters may have provided a physiological advantage (increased disease tolerance).

Exposing oysters infected with *P. marinus* to low salinity significantly increased their lysozyme concentrations and a positive correlation was found between lysozyme concentration and the survivorship of the oysters (La Peyre et al. 1990, Ragone 1991). Lysozyme concentration in DW oysters was persistently higher than in MJ and WP oysters and as infection become more intensified at the end of the experiment, the level of lysozyme in these oysters remained high. In contrast, the lysozyme concentration in WP oysters stayed low and a significant decrease ($P = 0.003$) in lysozyme level was found in MJ oysters when the experiment was terminated.

It is known that salinity affects lysozyme activity in oysters (La Peyre and Chu unpublished results). Lysozyme concentration decreases with increased salinity (Chu et al. In review). The higher lysozyme concentrations in WP oysters at 20 ppt than the WP oysters at ambient salinity is a result of salinity effect. The same explanation can be applied to the decrease of lysozyme concentration in DW oysters maintained at a salinity higher than ambient salinity.

It has been well-documented that the parasites, *H. nelsoni* and *P. marinus*, induce significant changes in growth, reproduction, and certain physiological functions of the oyster (e.g. Feng and Canzonier 1970, Newell 1985, Ford 1986, Barber et al. 1988, Ford 1988, Ford and Figeras 1988, Chu and La Peyre 1989, Ling 1990, Chintala and Fisher 1991, Barber et al.1991, Burreson 1991, Paynter and Burreson 1991). Generally, parasitism depressed growth and feeding rate, reduced tissue and hemolymph protein, and impaired gonadal development at the gametogenesis stage. Decline in condition index was found in all groups of oysters at the end of this experiment, although the decrease of condition index observed in the DW oysters at 10 ppt was statistically insignificant. The observation that hemolymph protein concentrations were higher on day 35 than on day 0 in DW and MJ oysters was unusual. A possible interpretation is that the observed higher protein concentrations in DW and MJ oysters at day 35 resulted

from the relatively lower disease prevalence in DW and MJ oysters at day 35 (50–55% for DW oysters and 70% for MJ oysters) than day 0 (63% for DW oysters and 77% for MJ oysters). Although the difference was statistically insignificant, the protein contents in uninfected oysters in these 2 groups were found to be slightly higher than in infected oysters at day 35. Moreover, disease weighted incidence (WI) was lower in MJ oysters (0.9) at day 35 than at day 0 and WI remained relatively low in DW oysters at day 35 (0.8–1.1), thus depletion of hemolymph protein was not observed. It has been noted that hemolymph protein reduction occurred only in oysters heavily infected by the parasite *H. nelsoni* (Ling 1990). However, decrease of protein took place in all oysters at day 100, particularly in the MJ groups which had the highest cumulative mortality.

In summary, based on the infection prevalence and intensity at the end of the experiment, the three populations of oysters under investigation showed a similar response to *P. marinus*. Since no mortality was observed for the DW oysters, the higher hemolymph cellular and humoral components and condition index of these oysters may be an indication of better physiological fitness, which provides them with greater tolerance of infection and prolonged survival. Salinity induced changes in certain hemolymph factors (i.e. lysozyme and PG). Salinity may have affected disease development, but results between populations were inconsistent.

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LITERATURE CITED

Andrews, J. D. 1971. Production of disease-resistant oysters. Completion Report, submitted to Commercial Fisheries Research and Development Act, NMFS, NOAA, Washington, D.C.

Andrews, J. D. 1984. Epizootiology of diseases of oysters (*Crassostrea virginica*) and parasites of associated organisms in eastern North America. *Helgol. Meeresunters*. 37:149–166.

Andrews, J. D. 1988. Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. *Amer. Fish. Soc. Sp. Publ*. 18:206–224.

Andrew, J. D. & W. G. Hewatt. 1957. Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of the Chesapeake Bay. *Ecol. Monogr*. 27:1–25.

Barber, J. B., S. E. Ford & H. H. Haskin. 1988. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism: II. Tissue biochemical composition. *Comp. Biochem. Physiol*. 91A:603–608.

Barber, J. B., S. E. Ford & D. T. J. Littlewood. 1991. A physiological comparison of resistant and susceptible oysters *Crassostrea virginica* (Gmelin) exposed to the endoparasite *Haplosporidium nelsoni* (Haskin, Stauber & Mackin). *J. Exp. Mar. Bio. Ecol*. 146:101–112.

Burreson, E. M. 1989. Prevalence of the major oyster diseases of Virginia waters—1988. A summary of the annual monitoring program. Marine Resource report, 89-1, Virginia Institute of Marine Science, Gloucester Point, Virginia.

Burreson, E. M. 1990. Status of the major oyster diseases in Virginia–1989. A summary of the annual monitoring program. Marine Resource Report 90-1 Virginia Institute of Marine Science, Gloucester Point, Virginia.

Burreson, E. M. 1991. Status of the major oyster diseases in Virginia–1990. A summary of the annual monitoring program. Marine Resource Report 91-1, Virginia Institute of Marine Science, Gloucester Point, Virginia.

Burreson, E. M. 1992. Status of the major oyster diseases in Virginia–1991. A summary of the annual monitoring program. Marine Resource Report 92-1, Virginia Institute of Marine Science, Gloucester Point, Virginia.

Chintala, M. M. & W. S. Fisher. 1991. Disease incidence and potential mechanisms of defense for MSX resistant and -susceptible eastern oysters held in the Chesapeake Bay. *J. Shellfish Res*. 10:439–443.

Chu, F.-L. E. & K. H. Greene. 1989. Effect of temperature and salinity on in vitro culture of the oyster pathogen, *Perkinsus marinus* (Apicomplexa: Perkinsea). *J. Invert. Patho*. 53:260–268.

Chu, F.-L. E. & J. F. La Peyre. 1989. Effect of environmental factors and parasitism on hemolymph lysozyme and protein of American oyster (*Crassostrea virginica*). *J. Invert. Patho*. 54:224–232.

Chu, F.-L. E., J. F. La Peyre & C. Burreson. In review. *Perkinsus marinus* infection and potential defense-related activities in eastern oysters, *Crassostrea virginica:* salinity effects. *J. Invert. Patho*.

Crosby, M. P. & C. G. Roberts. 1990. Seasonal infection intensity cycle of the parasite *Perkinsus marinus* and an absence of *Haplosporidium spp*) oysters from a South Carolina salt marsh. *Dis. Aquat. Organ*. 9:149–155.

Feng, S. Y. & W. J. Canzonier. 1970. Humoral response in the American oyster (*Crassostrea virginica*) infected with *Bucephalus sp*. and *Minchinia nelsoni*. *Amer. Fish. Soc. Sp. Publ*. 5:497–510.

Fisher, W. S. & R. E. I. Newell. 1986. Salinity effects on the activity of granular hemocytes of American oysters, *Crassostrea virginica*. *Biol. Bull*. 170:122–134.

Fisher, W. S. 1988. Environmental influence on bivalve hemocyte function. *Amer. Fish. Soc. Spe. Pub*. 18:225–237.

Fisher, W. S., M. M. Chintala & M. A. Moline. 1989. Annual variation of estuarine and oceanic oyster *Crassostrea virginica* Gmelin hemocyte capacity. *J. Exp. Mar. Biol. Ecol*. 127:105–120.

Ford, S. E. 1986. Effect of repeated hemolymph sampling on growth, mortality, hemolymph protein and parasitism of oysters, *Crassostrea virginica*. *Comp. Biochem. Physiol*. 85A:465–470.

Ford, S. E. 1988. Host-parasite interactions in eastern oysters selected for resistance to *Haplosporidium nelsoni* (MSX) disease: survival mechanisms against a natural pathogen. *Amer. Fish. Sp. Publ*. 18:206–224.

Ford S. E. & H. H. Haskin. 1987. Infection and mortality patterns in strains of oysters *Crassostrea virginica* selected for resistance to the parasite *Haplosporidium nelsoni* (MSX). *J. Parasit*. 73:368–376.

Ford, S. E. & A. J. Figueras. 1988. Effects of sublethal infection by the parasite *Haplosporidium nelsoni* (MSX) on gametogenesis, spawning, and sex ratios of oysters in Delaware Bay, USA. *Diseases of Aquatic Organisms* 4:121–133.

Gauthier, J. D., T. M. Soniat & J. S. Rogers. 1990. A parasitological survey of oysters along salinity gradients in coastal Louisiana. *J. Wor. Aquat. Soc*. 21(2):105–115.

La Peyre, J. F., F.-L. E. Chu & L. M. Ragone. 1989. The effect of

salinity and *Perkinsus marinus* infection on some immunological parameters of the oyster *Crassostrea virginica*. *J. Shellfish Res*. 8:469.

Ling, W.-J. 1990. Cellular and humoral responses of resistant and susceptible oysters, *Crassostrea virginica*, to the infection of *Haplosporidium nelsoni* (MSX). M. S. Thesis. Univ. of Conn. 102 pp.

Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 193:265–275.

Lucas, A. P. & G. Beninger. 1985. The use of physiological condition index in marine bivalve aquaculture. *Aquaculture* 44:187–200.

Newell, R. I. E. 1985. Physiological effects of the MSX parasite *Haplosporidium nelsoni:* (Haskin, Stanber and Mackin) on the American oyster *Crassostrea virginica* (Gmelin). *J. Shellfish Res*. 5:91–95.

Paynter, K. T. & E. M. Burreson. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica:* II. Disease development and impact on the growth rate at different salinities. *J. Shellfish Res*. 10:425–431.

Perkins, F. O. 1966. Life history studies of *Dermocystidium marinum* an oyster pathogen. Dissertation, Florida State University. 273 pp.

Ragone, L. M. 1991. The effect of low salinity on established infections of *Perkinsus marinus* (Apicomplexa; Perkinsasica) in the eastern oyster, *Crassostrea virginica*. M. S. Thesis, College of William and Mary, 52 pp.

Ray, S. M. 1952. A culture technique for the diagnosis of infections with *Dermocystidium marinum* Mackin, Owen and Collier in oysters. *Science* 116:360–361.

Ray, S. M. 1954. Biological Studies of *Dermocystidium marinum*, a fungus parasite of oysters. Rice Institute pamphlet, 114 pp. (Monograph in Biological Special Series Issue).

Ray, S. M. 1966. A review of a culture method for detecting *Dermocystidium marinum* with suggested modifications and precautions. *Proc. Natl. Shellfish Assoc*. 54:55–80.

Scott, G. I., D. P. Middaugh & T. I. Sammons. 1985. Interactions of chlorine-produced oxidants (CPO) and salinity in affecting lethal and sublethal effects in the eastern or American oyster, *Crassostrea virginica* (Gmelin), infected with the protistan parasite, *Perkinsus marinus*, In: Marine Pollution and Physiology: Recent advances. Vernberg, F. F., F. P. Thurgerg, A. Calabrese, and W. B. Vernberg (eds). pp 351–376. University of South Carolina Press.

Shugar, D. 1952. The measurement of lysozyme activity and ultra-violet inactivation of lysozyme. *Biochimica et Biophysica Acta* 8:302–309.

Soniat, T. M. 1985. Changes in levels of infection of oysters infected by *Perkinsus marinus*, with special reference to the interaction of temperature and salinity upon parasitism. *Northeast Gulf Sci*. 7(2):171–174.

Soniat, T. M. & J. Gauthier. 1989. The prevalence and intensity of *Perkinsus marinus*, from the mid northern Gulf of Mexico, with comments on the relationship of the oyster parasite to temperature and salinity. *Tulane Stud. in Zool. and Bot*. 27:21–.