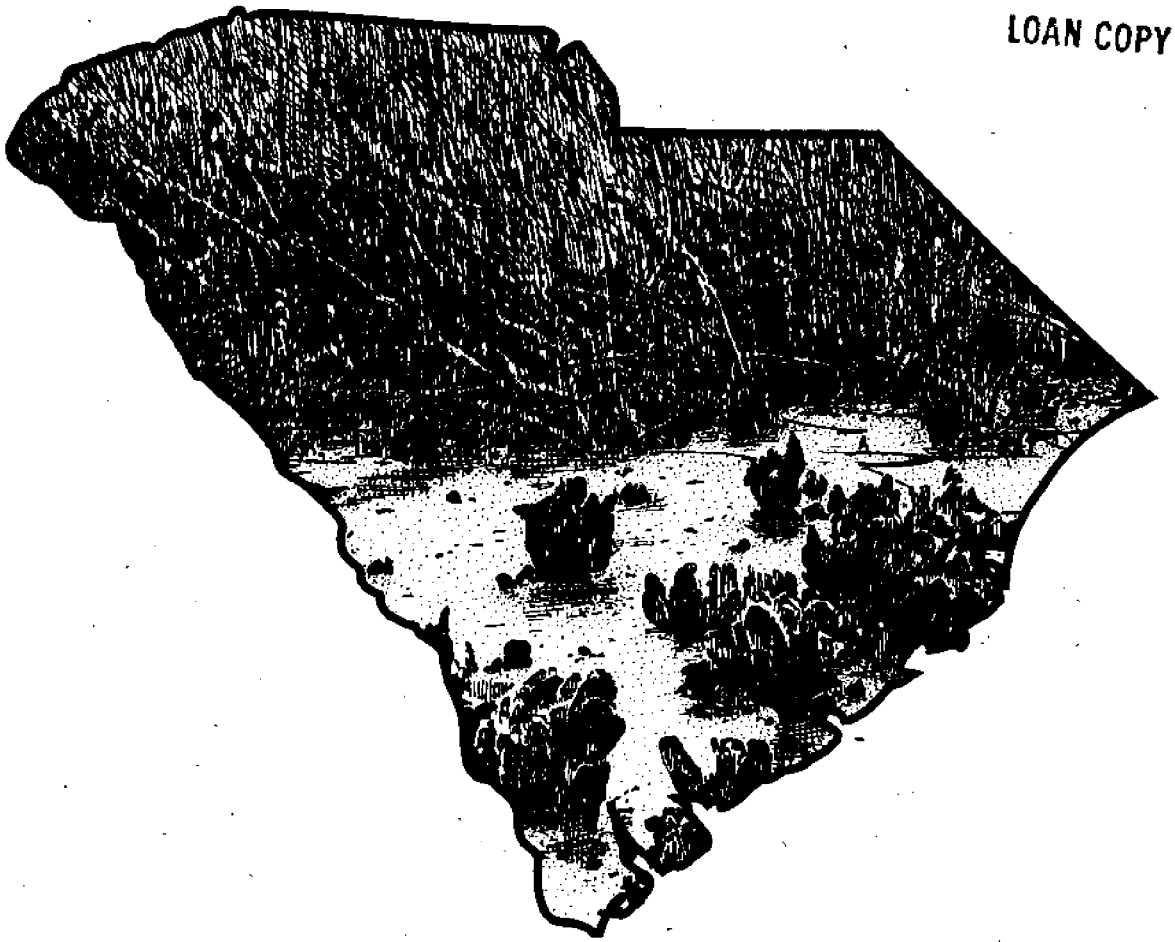
A Report on the Protozoan Pathogens *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) in South Carolina Shellfish Populations



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Prepared By
M.Y. Bobo, D.L. Richardson, L.D. Coen and V.G. Burrell

South Carolina Department of Natural Resources
Marine Resources Division
Marine Resources Research Institute
P.O. Box 12559, Charleston, South Carolina 29422-2559

A REPORT ON THE PROTOZOAN PATHOGENS *PERKINSUS MARINUS* (DERMO) AND *HAPLOSPORIDIUM NELSONI* (MSX) IN SOUTH CAROLINA SHELLFISH POPULATIONS, WITH AN OVERVIEW OF THESE SHELLFISH PATHOGENS

PREPARED BY

M. Yvonne Bobo

Donnia L. Richardson

Loren D. Coen

Victor G. Burrell

SOUTH CAROLINA DEPARTMENT OF NATURAL RESOURCES
MARINE RESOURCES DIVISION
MARINE RESOURCES RESEARCH INSTITUTE
P.O. BOX 12559
CHARLESTON, SOUTH CAROLINA 29422-2559

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EXECUTIVE SUMMARY

Human activities and natural phenomena have significantly affected the distribution and abundance of oysters in U.S. waters. In many areas, oyster production has declined drastically due to many interrelated causes including, over-harvesting, natural diseases, physical disturbance, nutrient enrichment through runoff, alteration of natural flow regimes and salinity patterns and removal of appropriate habitats for new recruits, to name just a few. Over 95% of South Carolina's oysters grow intertidally, making their 'habit' very different from subtidal oysters more typical of the Chesapeake and Delaware Bays. Since 1972, SCDNR's Marine Resources Research Institute (MRRI) has been documenting the occurrence of the oyster parasite *Perkinsus marinus* (commonly called "Dermo") in South Carolina oysters. More recently, our work with another oyster parasite *Haplosporidium nelsoni* (commonly called "MSX") has increased our concerns, regarding current and future fishery declines. Here we: (1) review the history of these two oyster parasites and resulting diseases; (2) briefly review past and present sampling/monitoring programs in other states; (3) summarize the state of our knowledge (1972 to present) of these two important and widespread shellfish parasites in South Carolina; (4) discuss management implications; and finally (5) recommend future disease research/ monitoring directions.

Between 1972 and 1996, over 21,000 oysters from over 60 sites around South Carolina were examined for *Perkinsus marinus*. *P. marinus* (Dermo) was present in all South Carolina oyster populations examined. When all data were combined, several patterns emerged. First, seasonal patterns of infection follow those observed in Gulf Coast populations, rather than those from the northeast. Prevalence and intensity levels were greatest during late summer and fall and, unlike populations in the northeast, the parasite was present in oyster samples throughout the year. *Perkinsus marinus* is known to respond to fluctuations in both water temperature and salinity, with elevated levels often significantly enhancing *P. marinus* prevalence and infection intensities. In South Carolina, most oyster populations inhabit estuaries with year round salinities typically between 20-35 ppt. This intertidal existence exposes them to a microenvironment whose winter and summer daily temperature fluctuations often exceed 20°C or more, with extended summer exposures approaching 54°C or 129° F. However, *P. marinus* does not appear to produce the high mortalities reported in the northeast, although during extended periods of reduced rainfall and/or above average temperatures (e.g., low tide at midday), the additional stress of *Perkinsus marinus* may initiate some localized oyster die-offs (for example in 1986, V. Burrell pers. oberservation).

Second, there was an obvious rarity of samples (a sample consisting of 25 or more oysters) with infection intensities (or weighted incidences) averaging 3.0 or greater and an absence of systemically high infections exceeding 4.0 (Quick and Mackin scale, 0 - 6). Over the 24 year period covered by this report, only 5% (or 42/831) of all composite oyster samples exceeded weighted incidence levels of 3.0. These results differ significantly from those observed in the northeast, where *Perkinsus marinus* intensities often exceed 4.0 and may even reach 5.0 or greater.

A third and potentially significant pattern also emerged. When these data are examined by decade and month across all sites, of the 345 samples collected from 1972 to 1979, none exceeded a *Perkinsus marinus* weighted incidence threshold of 3.0. However, from 1980 to 1989, when 230 samples were collected, 30 (or 13%) of these samples exceeded an intensity of 3.0, with peak intensities occurring from June to October. Finally, from 1990 to 1996, 256 samples were collected, of which 12 (or 4.7%) of these exceeded 3.0, with peak intensities occurring from July-November. From these trends, it appears that elevated (> 3.0) *Perkinsus marinus* infections greatly increased during the 1980s and remained elevated in the 1990s.

This pattern was also evident when one examined the temporal occurrence of the 42 composite oyster samples observed with mean intensities greater than 3.0, regardless of the samples size from that period (i.e. 345, 230 and 256). From 1972 to 1979, no mean intensities above 3.0 were observed. Then from 1980 to 1989, 71% (30 of the above 42) of all intensities greater than 3.0 occurred. Finally, from 1990 to 1996, 29% (12 of the 42) of all elevated intensities were observed. Care must be exercised, however, in drawing any definitive interpretations, as sample sizes,

spatial scales, associated site attributes (salinities, development, etc.) and inclusive sampling periods (7-10 years) have varied considerably. We simply have had no monitoring sites to compare that were sampled, even sporadically, over these three decades.

In South Carolina, we are just beginning to gain an understanding of *Haplosporidium nelsoni* (MSX). Since 1994, 1,924 individual oysters were examined from 21 sites. Of these, approximately 8% (or 150 individuals) of the oysters examined were infected with *H. nelsoni*, with the parasite present in oysters from 52% (or 11 of 21) of the sampling stations included here. Disease intensity, among individually infected animals varied from light to heavy. Of the 150 individuals with MSX infections, 9% of the individuals were infected from the Grice-Charleston Harbor station, 3% were infected from the 1994 South Carolina Summer Oyster Study sites and 8% (Inlet Creek) and nearly 16% (Toler's Cove Marina) from the Oyster Reef Ecosystem Project sites. To date, no observed mortalities have been documented in South Carolina due to MSX. Comparing other states in the southeast, in North Carolina, 31% of the sites examined detected MSX. In Georgia, MSX was not noted in oyster samples from 1966 and 1968, but was first observed in January 1986. *H. nelsoni* appears to be sensitive to low salinities, with the parasite disappearing in oysters after only about 10 days at salinities of 10 ppt or less. In South Carolina, most sites examined rarely, if ever, experience salinities this low. In fact, nearly all South Atlantic estuaries experience significantly fluctuating, but generally high salinities at many temporal scales.

The Shellfish Research Section is currently conducting a one year monitoring study across the state and has an ongoing long-term research program to understand seasonal patterns and effects of *P. marinus* and *H. nelsoni* on oyster populations, including potential implications for managing this critical fishery.

GLOSSARY

(After Fuxa and Tanada 1987, Ewart and Ford 1993, Woo 1995)

Enzootic: a disease that is usually low in prevalence and constantly present in an animal population (equivalent to endemic in humans).

Epidemiology/Epizootiology: the study of diseases in animal populations.

Epizootic: a disease that is rapidly spreading throughout an animal population (equivalent to epidemic in humans).

Infection: the presence of an infectious or foreign organism in tissues of a host.

Parasite: an organism living on or in another host organism to its advantage and the disadvantage of the host.

Patent: levels of infection that are spreading.

Pathogen: any disease causing organism.

Prevalence: the percentage of a population with a particular characteristic (e.g. disease) at a particular time.

Protozoan: a single celled organism often free living, but sometimes parasitic, such as *Perkinsus marinus* and *Haplosporidium nelsoni* that cause the diseases Dermo and MSX.

Resistance: the relative ability of an organism to avoid infection or to withstand its effects.

RFTM: Ray's modified fluid thioglycollate medium. This special medium is used to detect *P. marinus*, which causes the parasite to enlarge and stain blue-black with Lugol's iodine.

Subpatent: levels of infection that are undetectable.

Systemic: throughout the body, typically involving multiple tissues.

Vector: any agent (living or inanimate) that acts as an intermediate carrier or alternative host for a pathogenic organism and transmits it to a susceptible host.

Virulence: the capacity of a parasite to cause disease in an animal; the damage may be modified by the defense mechanism of the host.

INTRODUCTION

Throughout its extensive geographic range, the American oyster, *Crassostrea virginica* (Gmelin) is unique in its ecological role in that it forms living subtidal and intertidal habitats in the estuary. These habitats in turn support a host of other associated organisms generally not found in the surrounding sand or mud (Dame 1972, 1979, Bahr and Lanier 1981, Klemanowicz 1985, Stanley and Sellers 1986, Zimmerman et al. 1989, Luckenbach et al. 1995). Oysters also can have important direct and indirect effects, through their tremendous processing capacity as filter feeders, removing sediments and affecting hydrodynamic flow (e.g., Haven et al. 1978, Dame et al. 1984, 1993, Heck 1987, Newell 1988, Dame and Libbes 1993). Recent studies in Chesapeake Bay further support the notion that oyster-dominated ecosystems are critical in sustaining overall ecosystem production and natural functioning (Heck 1987, Newell 1988, Ulanowicz and Tuttle 1992, Gerritsen et al. 1994, Rothschild et al. 1994).

In the southeastern United States (portions of North Carolina, South Carolina, Georgia, and Florida), oyster reefs are a conspicuous feature of the intertidal zone in most estuaries (SCDNR estimates its areal extent to exceed 3,500 acres). Much remains to be studied about how these extensive intertidal oyster habitats (Dame 1979, Bahr and Lanier 1981, Coen et al. 1997) contribute to the broader functioning of the inshore ecosystems in which they occur (cf. Zimmerman et al. 1989 for the Gulf of Mexico). In South Carolina, over 95% of the oysters grow intertidally (Lunz 1950, Maggioni and Burrell 1982, W. Anderson, pers. comm.). They are often adjacent to emergent vegetation, with tides greater than 1-2 m (see Monbet 1992), making them very different from extensively studied subtidal oyster reefs, for example in Chesapeake Bay.

Human activities, in concert with natural phenomena, have greatly affected the distribution and abundance of oysters in the United States. In many areas, oyster production has declined significantly in recent years due to many interrelated causes including: (1) diseases; (2) physical disturbance; (3) over-harvesting; (4) nutrient enrichment through run-off; (5) natural predators; (6) alteration of natural flow regimes and salinity patterns; (7) removal of appropriate habitats for new recruits; (8) oyster cannery closings (e.g., Haven et al. 1978, Officer et al. 1978, 1982, Maggioni and Burrell 1982, Stanley and Sellers 1986, Newell 1988, Anonymous 1989a, Rothschild et al. 1994, W. D. Anderson SCDNR pers. comm.); and (9) foreign competition (Maggioni and Burrell, 1982). Today, there is essentially no oyster production in Delaware Bay and production from Maryland has drastically decreased. Virginia, once the leading producer of oysters in the United States, now harvests less than 10,000 bu (public bed, 1995), compared with more than 3 million bu in 1960. In fact from 1992 to 1993, more oysters were harvested in South Carolina than in North Carolina, Georgia and Virginia combined, with nearly 100,000 bu reported (Figure 1). Similar major declines have also been observed in the southeast, with North Carolina recently reporting 100 year lows (Frankenberg et al. 1995). A Blue Ribbon panel there recently concluded (Frankenberg et al. 1995) that over 95% of the state's natural oyster populations were no longer available to harvesting, due either to reduced water or habitat quality, diseases (Dermo and MSX) and over-harvesting.

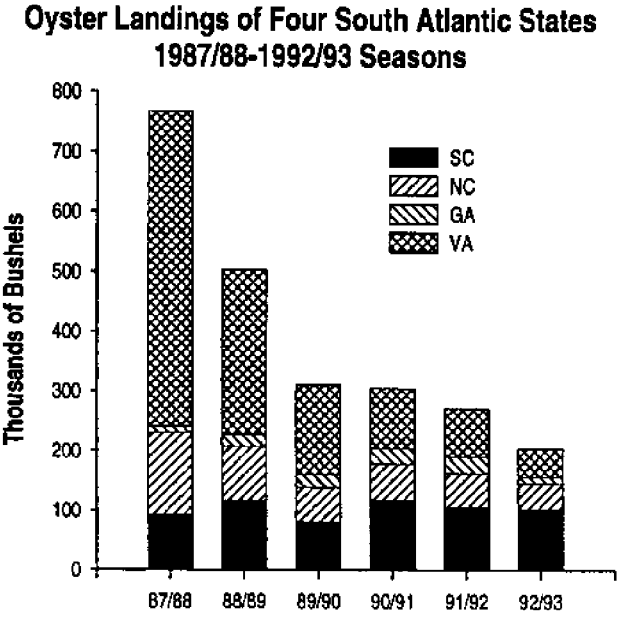

Figure 1. Comparison of oyster harvests for selected eastern states.

The oyster pathogen *Perkinsus marinus* (Mackin, Owen, and Collier), commonly called "Dermo", is widely distributed in oyster populations from the Gulf of Mexico to Maine (Mackin 1962, Andrews 1979,

1988, Burreson et al. 1994, Ford 1996). *Haplosporidium nelsoni* (Haskin, Stauber & Mackin), generally called "MSX" has been reported from the east coast of Florida to Maine (Andrews 1976, 1979, Ford and Haskin 1982, Ewart and Ford 1993). In the northeast and Gulf of Mexico, significant oyster mortalities have been attributed to these pathogens (e.g., Quick and Mackin 1971, Hofstetter 1977, Ray 1987, Haskin and Andrews 1988, Sindermann 1990, Wilson et al. 1990, Burreson 1991). *P. marinus* (Dermo) has been shown to affect the physiological condition of the oyster (Crosby and Roberts 1990, Gauthier et al. 1990, Paynter and Burreson 1991) and to significantly reduce growth rates (Ray et al. 1953, Menzel and Hopkins 1955, Andrews 1961, Burreson 1991, Paynter and Burreson 1991). It has been suggested that *P. marinus* virulence may be correlated with higher salinities (Chu and Greene 1989, Chu and La Peyre 1991), variation in races (Bushek et al. 1996a,b) or alternatively, that tolerance of oysters to *P. marinus* may be greater at lower salinities (Fisher and Newell 1986). In general, disease susceptibility increases with age, size and/or duration of exposure (Sindermann 1990). Interestingly, the presence of *H. nelsoni* had not been noticed in the mid-Atlantic states before the epizootic of 1957 (Haskin et al. 1966, Ford and Haskin 1982). There is some evidence, however, that the pathogen was present before that, but did not cause significant observed mortalities (Andrews 1968, Sindermann 1990). Factors which would have led from an enzootic to an epizootic situation are unknown. Despite more than 30 years of research on MSX and its causal agent *H. nelsoni*, the complete life cycle, and therefore its mode of transmission are unknown (Burreson 1988, Haskin and Andrews 1988).

Brief History of *Perkinsus marinus* (Dermo)

Perkinsus marinus (Dermo) was first described in the Gulf of Mexico nearly fifty years ago (Mackin et al. 1950) when mass mortalities of oysters were first observed. *Perkinsus marinus* has since been reported from the east coast of the United States from Maine to Florida and along the Gulf of Mexico, as far south as Tabasco, Mexico (Burreson et al. 1994a, Ford 1996, Soniat 1996). The oyster parasite was initially called *Dermocystidium marinum* because it was thought to be a fungus. Since then, it has been moved several times to several other taxonomic groups (Sprague 1954, Mackin and Ray 1966). The discovery by electron microscopy that *D. marinum* produced biflagellated zoospores (Perkins and Menzel 1966) with organelles called "apical complexes" (Perkins 1976) resulted in its being reclassified as the genus *Perkinsus* in the class Perkinsea, within the protozoan phylum Apicomplexa (Levine 1978). The disease, however, is still commonly called "Dermo."

The common diagnostic technique for the identification of a *Perkinsus marinus* infection is the thioglycollate (RFTM) test developed by S. M. Ray (1952, 1954a). This growth medium causes the parasite to enlarge and develop walls that stain blue-black when Lugol's iodine solution is added. Many developmental stages in the life cycle of *P. marinus* have been described (Mackin and Boswell 1956, Perkins 1976, 1988), although the details of its biology remain unclear (Andrews 1988). It is commonly believed that dead and disintegrating oysters release the infective stages into the surrounding water column where they infect other oysters and repeat the cycle (Ewart and Ford 1993). Invasion is thought to take place through the epithelium of the digestive system, although infections have also been detected in both gill and mantle tissues. In heavily infected oysters, normal gonadal development may be inhibited, the infected oysters may become severely emaciated (Ray et al. 1953, Ray 1954b), and growth is retarded (Menzel and Hopkins 1955). Mortality occurs when the parasite causes extensive tissue lysis (Mackin 1951).

The two most important environmental factors regulating the life cycle of *P. marinus* are temperature and salinity (Hewatt and Andrews 1956, Mackin and Boswell 1956, Quick and Mackin 1971). In the mid-Atlantic region of the United States, this pathogen causes a warm season disease since it proliferates and spreads most rapidly at temperatures above 25°C, with a few cases of low intensity persisting through the winter at water temperatures of 0-5°C (Andrews 1988). Generally, infections and associated mortalities rise during the warm months and decline during colder periods. The parasite, however, is capable of overwintering and has been cultured in RFTM even after being frozen (Ewart and Ford 1993). Cold water temperatures during winter months do not guarantee the elimination of *P. marinus* from oysters (Chu and Green 1989). Andrews (1965, 1967) found that the parasite readily proliferated only at temperatures above 25°C

and over-wintered as subpatent (undetectable) infections. The parasite can also survive salinities less than 5 ppt; however, salinities above 12 ppt are usually required for a full epizootic. In more southern waters, the interplay of this disease with environmental factors is less clear (Craig et al. 1989, Gauthier et al. 1990, Powell et al. 1992).

Although a host of scavengers associated with oyster beds feed on dead oysters, perhaps dispersing *P. marinus*, most natural infections are believed to be caused by *P. marinus* released from disintegrating oyster tissue. *Boonea impressa*, an ectoparasitic gastropod, may also act as a vector in the transmission of *P. marinus* between live oysters by feeding on oyster hemolymph. *Boonea* can increase the infection intensity of oysters already infected with *P. marinus*, and also initiate new infections in the oysters on which it feeds (White et al. 1987).

Brief History of *Haplosporidium nelsoni* (MSX)

The disease "MSX" caused by *Haplosporidium nelsoni* is found from Maine to Florida's Atlantic coast (Ewart and Ford 1993). This protozoan parasite was originally given the acronym "MSX" for Multinucleated Sphere with unknown affinity ("X"). Haskin et al. (1966) originally named the plasmodial stage of the parasite as *Minchinia nelsoni*. Sprague (1970, as cited in Sprague 1978) later suggested that the absence of tails on the spores determined *Haplosporidium* from *Minchinia* and therefore, it was renamed *Haplosporidium nelsoni*. *H. nelsoni* was first recognized as the cause of oyster mortalities in lower Delaware Bay in 1957 and in lower Chesapeake Bay beginning in 1959 (Mackin 1960, Haskin 1961, Engle and Rosenfield 1963, Andrews 1964). In each of these affected areas, mortalities exceeded 95% for several years. Of late, there is evidence that *H. nelsoni* may have been introduced to the east coast via the importation of *Crassostrea gigas* (Burreson 1996).

Much of the biology of *H. nelsoni* remains unknown. The earliest *H. nelsoni* infection is found in the epithelia of the oysters gills and palps (Farley 1965, 1968). This has led to the conclusion that the infective stage is water-borne and can be easily spread (Haskin and Andrews 1988). Neither the infective stage nor the mode of transmission, however, has ever been identified and the parasite is commonly present in oysters as a multinucleated cell (=plasmodium), probably entering the blood stream after lodging in the gill. Mortality occurs after plasmodia become abundant in all tissues, but the manner in which *H. nelsoni* causes death is not completely understood. Farley (1965, 1968) attributed the death of oysters from *H. nelsoni* to the combined action of seasonal, environmental or physiological stresses on oysters weakened by the disease. Ewart and Ford (1993) suggested that overwhelming numbers of *H. nelsoni* cells damage tissues and interfere with normal functions, such as respiration and feeding, eventually causing death. The means by which *H. nelsoni* disease is transmitted is also not known. Experimental transmission of *H. nelsoni* between oysters via spores has been unsuccessful (Andrews 1982, Burreson 1988). Many investigators (Ford and Haskin 1982, Andrews 1984a, 1984b, Burreson 1988) have speculated that an alternate or intermediate host may be involved in the life cycle. Proximity to other oysters might not be a significant factor in Delaware Bay (Haskin and Andrews 1982).

Studies of *H. nelsoni* infection and mortality patterns in Chesapeake and Delaware Bays and in the James River have suggested that infections are rare at salinities below 10 ppt, intensifying above 15 - 20 ppt (Andrews 1964, 1983, Haskin and Ford 1982, Ford 1985, Ford and Haskin 1988). Temperature is also a factor influencing the activity and distribution of *H. nelsoni*. Ford and Haskin (1982) noted that below 5°C, the parasite is inactive, between 5 and 20 °C, the parasite multiplies faster than the host can contain it and above 20°C, resistant oysters can inhibit parasite multiplication or eliminate it from tissues.

Review of Oyster Diseases in the Southeast

Oyster pathogens have been studied intensively from oyster populations in the northeast (Chesapeake and Delaware Bays) and the Gulf of Mexico, where mass mortalities have been attributed to both *Perkinsus marinus* and *Haplosporidium nelsoni* (Ford and Haskin 1982, Haskin and Andrews 1988, Sindermann 1990, Wilson et al. 1990). However, relatively little is known about these oyster diseases in the southeast. Although *P. marinus* seems endemic to North Carolina, South Carolina and Georgia (Burrell et al. 1984, Crosby and Roberts 1990, Lewis et al. 1992), it apparently does not cause mass mortalities like those

which have decimated Chesapeake and Delaware Bay oyster populations (Sindermann, 1990). Recently (1985-1987) major die-offs have been observed in Georgia and South Carolina, presumably due to elevated salinities, record high temperatures and potentially enhanced *P. marinus* infections (W. Anderson pers. comm., Lewis et al. 1992). We know that successive dry years during 1985-1987 may have also caused severe losses, most of which have been attributed to *P. marinus*. In Virginia, few oysters are currently left on public or private beds for harvest or broodstock (Andrews 1988). *Perkinsus marinus* is considered to be the etiological agent responsible for the mortalities observed in Georgia during the same period (Lewis et al. 1992), with similar conclusions made in North Carolina (Frankenberg et al. 1995).

For the southeast (Georgia, South Carolina, North Carolina) as discussed above, our understanding of these diseases and their epizootiology is at a very early stage of development, with much of the historical knowledge derived from studies on subtidal oysters from either the Gulf of Mexico or the northeast. In South Carolina oysters are nearly all intertidal (over 95% from Lunz 1950, Maggioni and Burrell 1982, W. Anderson pers. comm.), often adjacent to emergent vegetation in tidal creeks, with tides generally >1-2 m and elevated salinities and temperatures during exposure. Hence, disease epizootiology in the southeast may be very different from that observed for subtidal populations.

Crosby and Roberts (1990) studied *P. marinus* in oyster populations from North Inlet, South Carolina. They found that the seasonal patterns of infections in South Carolina were similar to those found in other areas. O'Beirn et al. (1994, 1996a) also found increasing *P. marinus* infections in the spring followed by peak levels in late summer/fall months. Burrell et al. (1984) similarly found that the highest prevalence levels occurred in late summer and early fall in both intertidal and subtidal oyster populations sampled from Cape Romain. However, whereas infections seem to disappear during the winter months in the northeast, further south, light to moderate infections often persist all year (Andrews and Hewatt 1957, Quick and Mackin 1971, Burrell et al. 1984, Crosby and Roberts 1990, O'Beirn et al. 1994, 1996a). Lewis et al. (1992) found that *Perkinsus marinus* intensity in Georgia resembled that of Quick and Mackin's (1971) observations for Florida. Because of the preponderance of intertidal oysters in South Carolina and Georgia, some differences may be expected. Burrell et al. (1984) compared subtidal and intertidal *P. marinus* infection intensity in native populations at two sites in South Carolina and found little difference in infection levels between tidal levels within either site (see data included here also). Similar results were observed by O'Beirn et al. (1994) in oysters planted subtidally and intertidally in Georgia.

Only recently have *Haplosporidium nelsoni* (MSX) infections been reported in the southeast (Haskin and Andrews 1988, Lewis et al. 1992, Morrison et al. 1992, Dougherty et al. 1993, Bobo et al. 1996) so few generalizations are available. Preliminary examinations in North Inlet, near Georgetown, South Carolina did not detect the parasite (Crosby and Roberts 1990). And to date, no mass mortalities among oysters have been attributed to *H. nelsoni* south of Cape Fear, North Carolina.

Overview of Monitoring in Other States

Gulf Coast - Past EPA status and trends monitoring programs included 49 sites along the Gulf of Mexico between January and March. Low *P. marinus* median infection intensities were typically observed since sampling was done during the winter when infection intensities are normally low (see Craig et al. 1989).

Virginia - Sampling is conducted at four stations that represent the only locations in Virginia with sufficient oysters for monthly monitoring; these are also the major sources of seed oysters for private planters. Two of these four stations are monitored for *H. nelsoni*. Spring and fall monitoring is conducted at selected sites throughout Virginia. The samples are collected in conjunction with ongoing stock assessment surveys. The Fall survey is especially important because both pathogens are near maximum abundance at this time and the samples provide a good indication of the severity of the diseases during the year. Only a subsample of the sites is chosen for *H. nelsoni* diagnosis (Burreson and Calvo 1994).

Maryland - This fall monitoring program consists of 43 sites representing a compromise among three factors: (1) the spring-summer spat set must grow to a size to be visually identified; (2) *P. marinus* and *H.*

nelsoni generally have exerted their effects on the population (mortality) during the preceding summer and; (3) although the oyster harvest season begins before the time of sampling, early fall sampling minimizes the effects of harvest, given the other constraints on the survey. A subset of sites is chosen for *H. nelsoni* diagnosis using both blood histocytology (major methodology) and tissue histopathology (Smith and Jordan, 1992).

North Carolina - There is no defined disease monitoring program at this time. Monitoring of diseases is conducted during their seed planting and repletion program. Primarily through current enhanced research efforts (M. Marshall pers. comm.).

Objectives of Report

1. To review current knowledge of *Perkinsus marinus* in South Carolina oyster populations;

2. To discuss epizootiology of *Perkinsus marinus* (Dermo), especially with reference to water temperature and salinity;

3. To review current knowledge of *Haplosporidium nelsoni* (MSX) in South Carolina oyster populations;

4. To discuss these results, in conjunction with those observed previously in other states and finally;

5. To summarize the potential implications of oyster diseases to management of the state's oyster resources, including relevant recommendations and current and future research directions.

METHODS

Between 1972 and 1996, over 21,000 oysters were examined for the presence of the protozoan *Perkinsus marinus* (Dermo). Samples were intermittently collected from over 60 intertidal and/or subtidal sites throughout the state. From 1994 to 1996, 1,924 oysters were examined for *Haplosporidium nelsoni* (MSX) infection from 21 sites. Hydrographic data were collected coincidentally with sampling, in most cases, including water temperature and salinity measurements. More recently, temperature (both intertidal and subtidal), salinity, dissolved oxygen, pH and depth, have been measured using environmental dataloggers (from Hydrolab and Onset). Oysters were collected by hand at low tide from the intertidal zone or dredged (subtidal).

Oyster sample size per collection was generally 25 oysters. Shell abnormalities (Table 1) were noted before each oyster was scrubbed clean. Individual shell height (maximum anterior-posterior length) was measured to the nearest millimeter using vernier calipers. Each oyster was opened aseptically and general physiological condition (Howard and Smith 1983) and/or other abnormalities noted.

Perkinsus marinus (Dermo) Evaluation

Perkinsus marinus was diagnosed by Ray's fluid thioglycollate medium culture method (RFTM, Ray 1966). Rectal tissue and/or approximately 3-4 mm of gill and mantle tissue of each oyster were incubated in RFTM inoculated with penicillin and streptomycin (after 1985 chloromycetin and mycostatin were used). Tissues were incubated at room temperature for a period of 3-7 days, stained with Lugol's iodine and then examined. An infection level was scored for each oyster as a disease code number (Quick and Mackin 1971) ranging from 0 (absence of hypnospores) to 6 (heavily infected) (reviewed here in Table 2). Prevalence (the percent infected) and mean infection intensity (or weighted incidence) for each sample ($n \geq 25$) were then calculated. Weighted incidence (WI) was determined after Ray (1954b) and Mackin (1962) as follows:

WI=sum of disease code numbers (or infection intensity)/ number of oysters examined

Choi et al. (1989) have demonstrated that this scale is essentially a log of the number of parasites per gram weight. Similarly, Bushek et al. (1994) have shown that weighted incidence accurately reflects the average infection intensity in an oyster population.

Haplosporidium nelsoni (MSX) Evaluation

Diagnosis of *Haplosporidium nelsoni* (MSX) was determined by routine paraffin histopathological techniques (Preece 1972). One or two transverse tissue

Table 1. Shell abnormalities typically associated with oysters in SC (after Howard and Smith 1983).

Mantle recession	Heavy fouling of the inside shell margin indicates prolonged mantle recession.
Shell pustules	Raised yellow-brown conchiolin deposits on the nacreous surface of the shell. May contain creamy yellow fluid.
Shell blisters	Frequently found on the inside of the shells near the adductor muscle. Blister cavities contain mud or sea water.
Polydora sp. (mud worm)	Settles on the inner surface of the shell and builds a U-shaped mud tube with both orifices external. The deposit is soon covered by a layer of conchiolin - forming a shell blister.
Cliona sp. (boring sponge)	Small round holes on the surface of mollusk shells. Dark pigmented pustules form opposite the holes in the shell.
Drill cases/drill holes	Tough, greenish leathery capsules in which oyster drill eggs are deposited. Small symmetrical holes in molluscan shells can be attributed to oyster drills.
Calcareous malformations	These abnormalities are pathological and are associated with the disturbance of calcium metabolism which manifests itself in an over calcification of selected parts of the organism.

cross sections, approximately 4 mm thick, including gill, mantle, digestive diverticula, and gonadal tissue were dissected and fixed in Davidson's fixative (formalin, 95% ethyl alcohol, glacial acetic acid). After routine tissue processing involving dehydration, clearing and infiltration, tissues were embedded in paraffin and 5-7 mm sections were cut from each oyster tissue using a rotary microtome. These tissue sections were stained with hematoxylin and counterstained with eosin. Slides were examined for *H. nelsoni* to determine prevalence and intensity (Table 3).

Study Sites

The coastal region of South Carolina was arbitrarily divided into three areas (Figure 2) representing the northern, central and southern regions (see also Orlando 1994 for additional information):

Region A (Sites 1-9) represents the area from Murrell's Inlet to Bull Bay. This area includes the Winyah Bay estuary which is a small coastal plain system occupying 78 km (NOAA 1990) and contains numerous marshes, shoals, and interior islands (Blood and Vernberg 1992, DeVoe 1992). The North and South Santee Rivers are also in this region.

Region B (Sites 10-46) comprises those areas from Venning Creek to the Ashepoo River. An area included in this region is the Charleston Harbor estuary which is a coastal plain, drowned river valley system occupying 96 km (Mathews et al. 1981, NOAA 1990). The Ashley, Cooper and Wando Rivers are also included in this region.

Region C (Sites 47-62) is the area from St. Helena Sound to Skull Creek. St. Helena Sound estuary is a drowned river valley/bar-built system containing numerous marsh islands and tidal creeks (Hopkins 1956, Mathews et al. 1980, Stapor 1984, Bearden et al. 1985). Also, included in this region are the Coosaw and Colleton Rivers.

Table 2. Evaluation of *Perkinsus marinus* (Dermo) infection intensities (after Quick and Mackin 1962).

Intensity Description	Number Code	Cell Concentration	Appearance Microscopic/Macroscopic
Negative	0	None	No cells/tissue orange-brown
Very Light	1	1-10/sample	Cells very scattered
Light	2	11-100/sample	Cells in most fields, but sometimes concentrated in specific areas
Light Medium	3	99-1,000/sample	Cells common, beginning to make up a significant portion of each field
Medium	4	31-300/5mm field	Cells present everywhere
Medium Heavy	5	301-1,000/5mm field	All fields are darkened by cells, tissue often bluish
Heavy	6	1,000 and up/5mm field (commomly to 3,000/5mm field)	All fields are black, tissue difficult to see

15x wide field oculars and 4X objective gives 5mm field.

Table 3. Explanation of codes assigned for *Haplosporidium nelsoni* (MSX) infection intensity and category (after Ford and Figueras 1988, Burreson 1994).

H	=	Number of Heavy Infections (>5 plasmodia/ 400x field)
M	=	Number of Moderate Infections (2-5 plasmodia/ 400x field)
L	=	Number of Light Infections (<2 plasmodia/ 400x field)
G	=	Plasmodia Confined to Gill Epithelial
LS	=	Rare to Light Systemic Infections
HS	=	Heavy Systemic Infections

RESULTS

Overview of *Perkinsus marinus* in South Carolina

Between 1972 and 1996, over 21,000 oysters were examined for *Perkinsus marinus* infection from 62 sites along South Carolina's coast. This monitoring program demonstrated that *Perkinsus marinus* was present (measured as prevalence) at all of these sites (see Tables 4 & 5 and Figures 2 & 3 for overview). In South Carolina, *P. marinus* infections have tended to more closely follow those observed in Gulf Coast oyster populations, rather than those typically observed in the northeast (i.e. Chesapeake and Delaware Bays). We have observed that infections fluctuate both temporally and spatially in estuaries and that winter prevalence levels rarely approach zero. As in the Gulf of Mexico (Mackin and Hopkins 1962, Andrews and Ray 1988), South Carolina's coastal region is subject to

Figure 2. Map of South Carolina sites sampled for diseases. The coast was arbitrarily divided into three areas representing northern (region A), central (region B) and southern (region C) regions.

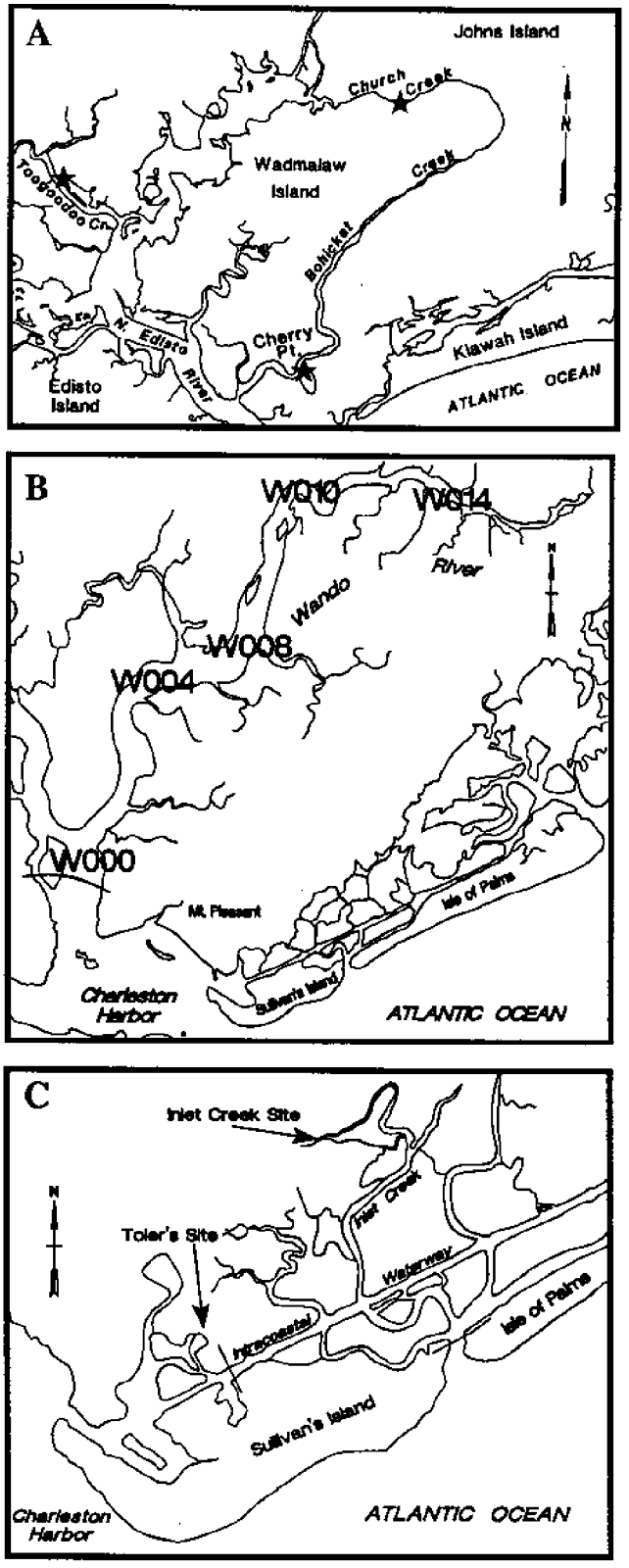

Figure 3. Detailed maps of oyster disease sites near Charleston, SC: (A) Toogoodoo Creek (site #36), Church Creek (#32) and Cherry Point (#35); (B) all stations in the Wando River (#17); (C) Toler's Cove Marina (#16) and Inlet Creek (#15).

Table 4. Numerical listing of field sites sampled for *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) in oysters between 1972 and 1996.

1.	MURRELL'S INLET	33.	KIAWAH CREEK ⊙ , ●
2.	LITCHFIELD	34.	LONG ISLAND
3.	WINYAH BAY ⊙, O	*35.	CHERRY POINT ⊙ , O
4.	NORTH SANTEE	*36.	TOOGOODOO CREEK ⊙ , O
5.	SOUTH SANTEE	37.	LEADENWAH CREEK ⊙ , ●
6.	ALLIGATOR CREEK	38.	NORTH EDISTO INLET
7.	CASINO CREEK	39.	TOM POINT CREEK
8.	CAPE ROMAIN ⊙ , ●	40.	FRAMPTON INLET
9.	BULL BAY ⊙ , O	41.	SCOTT CREEK
10.	VENNING CREEK	42.	ST. PIERRE'S CREEK
11.	PRICE'S INLET	43.	FISHING CREEK
12.	CAPERS INLET	44.	BAILEY CREEK
13.	LONG CREEK	45.	SOUTH EDISTO INLET
14.	SWINTON CREEK	46.	ASHEPOO RIVER ⊙ , ●
*15.	INLET CREEK ⊙ , ●	47.	ST. HELENA SOUND
*16.	TOLER'S COVE MARINA ⊙ , ●	48.	SOUTH WIMBEE CREEK
*17.	WANDO RIVER ⊙ , O	49.	FRIPP INLET ⊙ , O
18.	ALSTON CREEK ⊙ , O	50.	STORY RIVER
19.	SHEM CREEK ⊙ , O	51.	COOSAW AT BRICKYARD PT.
20.	NOISETTE CREEK ⊙ , ●	52.	WARSAW CREEK ⊙ , O
21.	SHIPYARD CREEK ⊙ , ●	53.	DISTANT ISLAND CREEK
22.	DIESEL CREEK ⊙ , O	54.	CHOWAN CREEK ⊙ , O
22a.	PLUM ISLAND CREEK ⊙ , ●	55.	McCALLY'S CREEK
23.	KOPPERS CREEK ⊙ , ●	56.	JENKIN'S CREEK ⊙ , O
24.	CHARLESTON HARBOR/MRRI-NMFS/GRICE ⊙ , ●	57.	PORT ROYAL SOUND
25.	METCALF CREEK-STONO/CHAS. HARBOR ⊙ , O	58.	HAZARD CREEK
26.	CLARK SOUND ⊙ , ●	59.	COLLETON RIVER ⊙ , O
27.	SECCESSIONVILLE CREEK	60.	CHECHESEE CREEK
28.	LIGHTHOUSE CREEK ⊙ , ●	61.	MACKAY CREEK ⊙ , O
29.	FOLLY CREEK ⊙ , ●	62.	SKULL CREEK ⊙ , ●
30.	STONO INLET		
31.	WALLACE CREEK ⊙ , O		
*32.	CHURCH CREEK		

*See Figure 3

⊙ Sites Sampled For MSX; ● Sites Positive For MSX; O Sites Negative For MSX

wide annual and seasonal variations in rainfall, with corresponding salinity fluctuations. There are few oyster growing areas with salinities low enough (typically < 6 ppt) to preclude the occurence of *Perkinsus marinus* (Ragone and Burreson 1993). The prevalence (% infected) and weighted incidence (mean infection intensity) of *P. marinus* varied with location, but generally, highest prevalence and weighted incidence levels occurred during the summer and early fall (see Figures 4-11).

Another major difference between our findings and those observed previously for the northeast was the rarity or lack of elevated (hence referred to here as those infections averaging 3.0 or greater on the Quick and Mackin scale which ranges from 0-6) *Perkinsus marinus* infection intensities. When analyzed overall, of the 831 mean values (each based on

Table 5. Alphabetical listing of oyster disease sampling sites.

6.	ALLIGATOR CREEK	20.	NOISETTE CREEK ⊙ , ●
18.	ALSTON CREEK ⊙ , O	38.	NORTH EDISTO INLET
46.	ASHEPOO RIVER ⊙ , ●	4.	NORTH SANTEE
44.	BAILEY CREEK	22a.	PLUM ISLAND
9.	BULL BAY ⊙ , O	57.	PORT ROYAL SOUND
8.	CAPE ROMAIN ⊙ , ●	11.	PRICE'S INLET
12.	CAPERS INLET	42.	ST. PIERRE'S CREEK
7.	CASINO CREEK	47.	ST. HELENA SOUND
24.	CHARLESTON HARBOR/MRRI-NMFS/GRICE ⊙ , ●	41.	SCOTT CREEK
60.	CHECHESEE CREEK	19.	SHEM CREEK ⊙ , O
*35.	CHERRY POINT ⊙ , O	21.	SHIPYARD CREEK ⊙ , ●
*32.	CHURCH CREEK	62.	SKULL CREEK ⊙ , ●
26.	CLARK SOUND ⊙ , ●	45.	SOUTH EDISTO INLET
59.	COLLETON RIVER ⊙ , O	48.	SOUTH WIMBEE CREEK
51.	COOSAW AT BRICKYARD POINT	5.	SOUTH SANTEE
54.	CHOWAN CREEK ⊙ , O	30.	STONO INLET
22.	DIESEL CREEK ⊙ , O	50.	STORY RIVER
53.	DISTANT ISLAND CREEK	27.	SECCESSIONVILLE CREEK
43.	FISHING CREEK	14.	SWINTON CREEK
29.	FOLLY CREEK	*16.	TOLER'S COVE MARINA ⊙ , ●
40.	FRAMPTON INLET	39.	TOM POINT CREEK
49.	FRIPP INLET ⊙ , O	*36.	TOOGOODOO CREEK ⊙ , O
58.	HAZARD CREEK	10.	VENNING CREEK
*15.	INLET CREEK ⊙ , ●	31.	WALLACE CREEK ⊙ , O
56.	JENKINS CREEK ⊙ , O	*17.	WANDO RIVER ⊙ , O
33.	KIAWAH CREEK ⊙ , ●	52.	WARSAW CREEK ⊙ , O
23.	KOPPERS CREEK ⊙ , ●	3.	WINYAH BAY ⊙ , O
37.	LEADENWAH CREEK ⊙ , ●		
28.	LIGHTHOUSE CREEK		
2.	LITCHFIELD		
13.	LONG CREEK		
34.	LONG ISLAND		
61.	MACKAY CREEK ⊙ , O		
55.	McCALLY'S CREEK		
25.	METCALF CREEK-STONO/CHAS. HARBOR ⊙ , O		
1.	MURRELL'S INLET		

*See Figure 3.

⊙ Sites Sampled For MSX; ● Sites Positive For MSX; O Sites Negative For MSX

a sample size of at least 25 oysters) calculated for the period between 1972 and 1996, only 5% (or 42 of 831 samples) exceeded an infection intensity threshold of 3.0 or greater. This result differs greatly from observations made in the northeast (i.e. Chesapeake and Delaware Bays), where significant *Perkinsus*-related mortalities have been documented. In those areas, disease-related oyster die-offs are associated with systemic *Perkinsus* infections, with mean intensities or weighted incidences often exceeding 4.0-5.0 (Bushek et al. 1994, Ford and Tripp 1996).

Examining the above 24 year dataset by decade and month across all sites, of the 345 samples taken

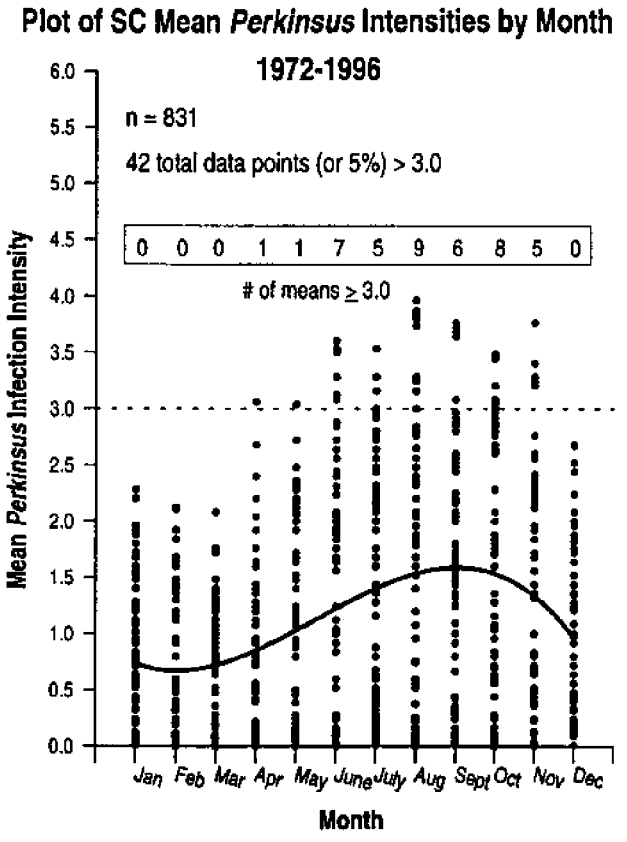

Figure 4. Summary plot of all *Perkinsus marinus* intensity values (n=831) from 1972-1996 by month, across all years and sites. Each sample mean based on 25 or more oysters. Third order regression and mean intensities ≥3.00 above line.

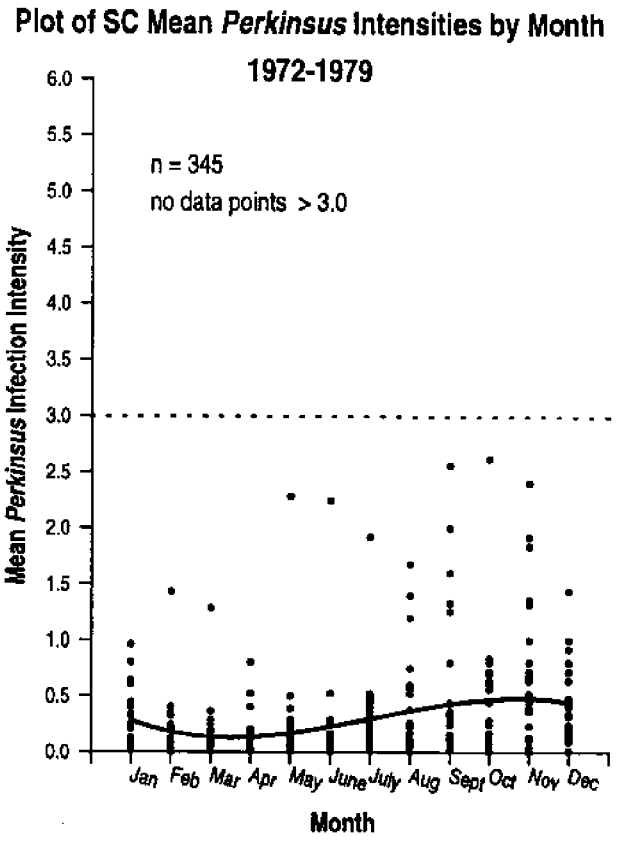

Figure 5. Summary of plot of all mean *Perkinsus marinus* intensity values (n=345) from 1972-1979. (See Figure 4 for details).

from 1972 to 1979, no (0%) mean values exceeded the above *Perkinsus* infection intensity threshold and a regression through these mean values never exceeded an intensity of 0.5 (Figures 5 & 9). From 1980 to 1989, when 230 samples were collected, 13% (or 30 of 230 samples) of these samples now exceeded 3.0, with peak intensities occurring from June to October. Finally, from 1990 to 1996, 256 samples were collected, of which 4.7% (or 12 of 256) of these exceeded 3.0, with peak intensities now occurring from July-November.

Focussing only on the 42 values exceeding 3.0 from 1972 to 1996, there were no mean intensities above 3.0 during the 1970s (Figures 5 & 9). Then from 1980 to 1989, 71% (or 30 of the above 42) of all mean intensities over 3.0 were observed (Figures 6 & 10). Lastly, from 1990 to 1996, the remaining 29% (or 12 of the 42) of the elevated intensities were observed (Figures 7 & 11). Comparing these three decades of observations, it appears that *Perkinsus* intensities have risen significantly during the 1980s versus the 1970s (compare Figures 5 & 9 and 6 & 10). It appears therefore, that elevated *Perkinsus* infections increased significantly in the 1980s, since sampling in South Carolina was initiated and have remained elevated throughout the 1990s (Figures 7 & 11).

Geographical Distributions

The coastal region of South Carolina was divided into three areas designated as the Northern (Area A), Central (Area B), and Southern (Area C) regions (Figure 2).

Northern Region of South Carolina-Area A

The northern most region (Figure 2, Sites 1 - 9) consisted of nine oyster beds located in areas from Murrell's Inlet (Area 1) to Bull Bay (Area 9). The oyster pathogen *Perkinsus marinus* was detected in oyster populations at Cape Romain, North Santee, South Santee, Bull Bay and Alligator Creek (Figures

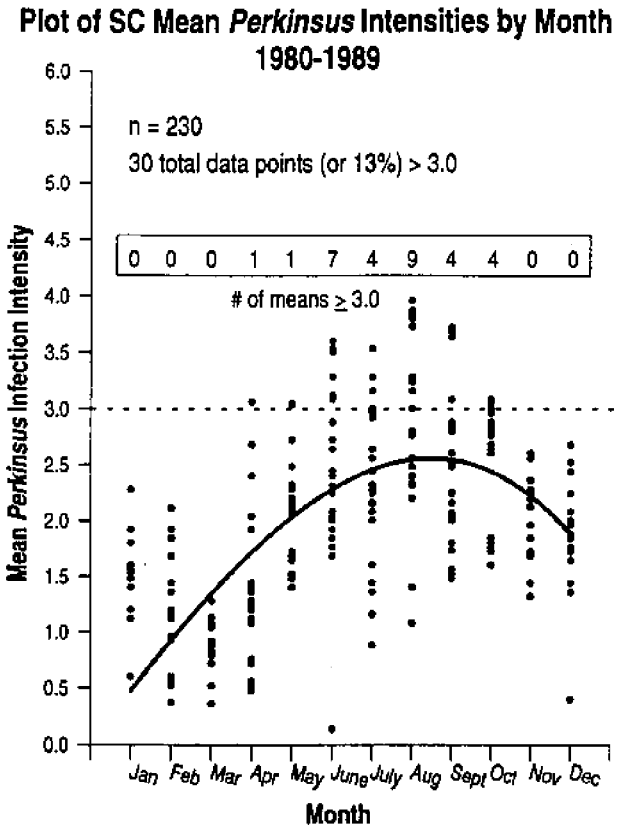

Figure 6. Summary of plot of all mean *Perkinsus marinus* intensity values (n=230) from 1980-1989. (See Figure 4 for details).

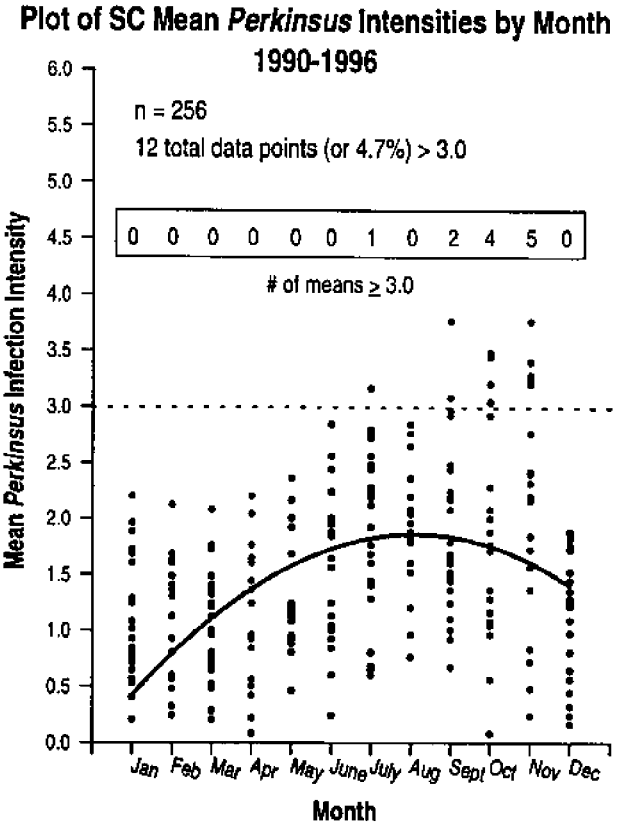

Figure 7. Summary of plot of all mean *Perkinsus marinus* intensity values (n=256) from 1990-1996. (See Figure 4 for details).

A-1 - A-5) sampled between 1972 and 1979. Infection intensities (<1.00) and prevalences (<30%) were generally low at all of these sites, except for Alligator Creek, sampled between 1972 and 1974, where infection intensity levels were approximately 2.0 and the prevalences were >60% (Figure A-5). Examination of oysters from Area A in 1994 revealed that *Perkinsus marinus* occurred in oysters from all of the sites sampled. Infection intensity levels were generally >1.5 and at some sites as high as 3.0. Prevalence levels were generally > 90%.

Charleston Harbor Region of South Carolina-Area B

Thirty-six oyster beds were examined for the presence of *Perkinsus marinus* in the central region of South Carolina (Figures 2 & 3, Sites 10 - 46). This area consisted of samples taken from Venning Creek southward to Ashepoo River. Several of the sites were sampled over an extended period and the results are discussed later (See Overview of *P. marinus* by Site section). *P. marinus* prevalences levels ranged from 0.0 to 100%. *P. marinus* infection intensity and prevalence levels appeared to be low in the 1970s and early 1980s (<1.00 and < 50%), except at Fishing Creek where infections reached 2.63 and 80% in May 1974 (Figure A-6). In the mid 1980s and 1990s prevalence levels almost always exceeded 50% and usually were > 80%. *Perkinsus marinus* infection intensity levels varied with the time of sampling but, generally were >1.5 occurring in 12 of the 18 samples (or 67%). Mean infection intensity levels >3.0 were observed in only 3 of the 18 samples (or 17%, Table A-1).

Southern Region of South Carolina-Area C

A similar pattern to that observed in the Charleston Harbor Region (Area B) occurred in the southern portion of the state (Figure 2, Sites 47 - 62). Low intensity and prevalence levels (<1.0 & < 50%) were detected in the 1970s and early 1980s. Oysters examined in the late 1980s and 1990s revealed *P. marinus* infection intensities usually >2.0 and prevalences typically >50% with levels of 100% common (Table A-2).

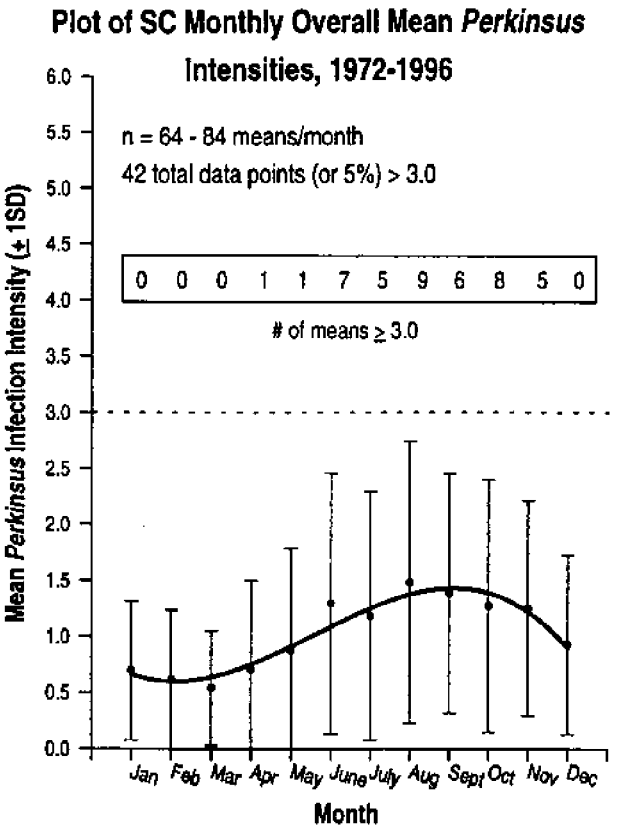

Figure 8. Summary of plot of grand means by month of mean *Perkinsus marinus* intensity values (n=64-84, for each month presented in Figure 4) from 1972-1996 across all sites. (See Figure 4 for details).

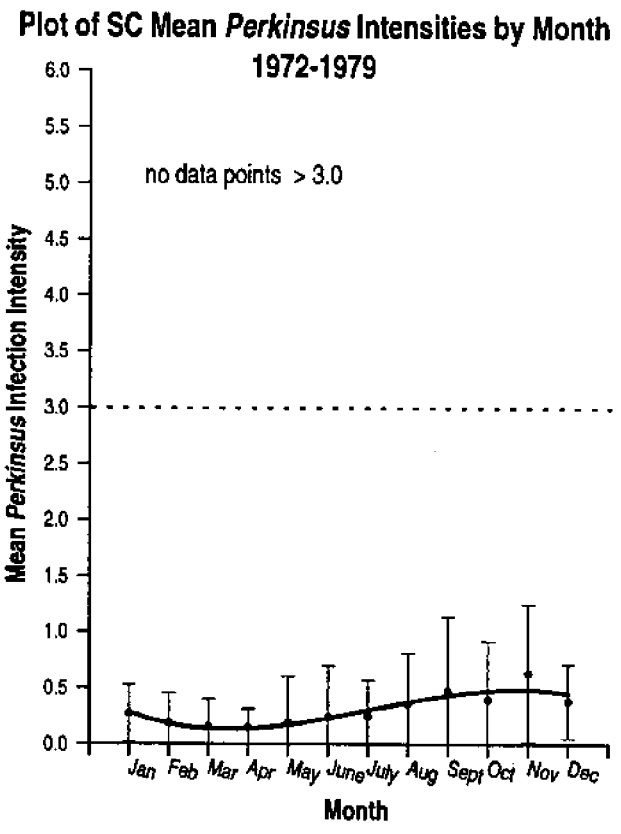

Figure 9. Summary of plot of grand means by month of mean *Perkinsus marinus* intensity values from 1972-1979 across all sites. (See Figure 4 for details).

Perkinsus marinus Observations by Site (Extended Sampling)

Toler's Cove and Inlet Creek (Sites 15 & 16)

As part of a long-term intertidal oyster ecosystem study (Coen et al. 1995, 1997, Wenner et al. 1996), native oysters were collected from two experimental reef sites (Figures 2 & 3) from September 1994 to January 1996. One developed site located at Toler's Cove Marina, the other, at a fairly pristine tidal creek system (Inlet Creek). Examination of these oysters revealed *P. marinus* prevalences of nearly 100% during summer, early fall, and late spring. A summer peak was detected at the developed site (WI = 2.56), and at the Inlet Creek site (WI = 2.84). Mean infection intensities never exceeded 3.0, even in the hot summer months. Toler's Cove Marina and Inlet Creek intensities remained fairly constant, despite the observed variable prevalence levels (Figure 12). Long-term environmental monitoring data (Figures A-9 and A-10), collected concurrently with oyster samples at these sites, demonstrated that subtidal salinities remained consistently high, but fluctuated between 20-35 ppt (as sampled every 48 min).

Toogoodoo River and Cherry Point (Sites 35 & 36)

Oysters were sampled monthly for *Perkinsus marinus* between July 1986 and December 1991 at sites 35 and 36. Generally, in the North Edisto River system the highest weighted incidence levels occurred in the summer and early fall.

Toogoodoo High Intertidal Zone

Oysters sampled monthly from the high intertidal zone of Toogoodoo Creek (Figures 2 & 3, Site 36), sampled from July 1986 to December 1991 (52 sampled months), had prevalence levels ranging from 25 to 100% (see Figure 13). In 45 of the 52 (87%) samples, however, *Perkinsus marinus* prevalence typically exceeded 80%. The highest weighted incidence

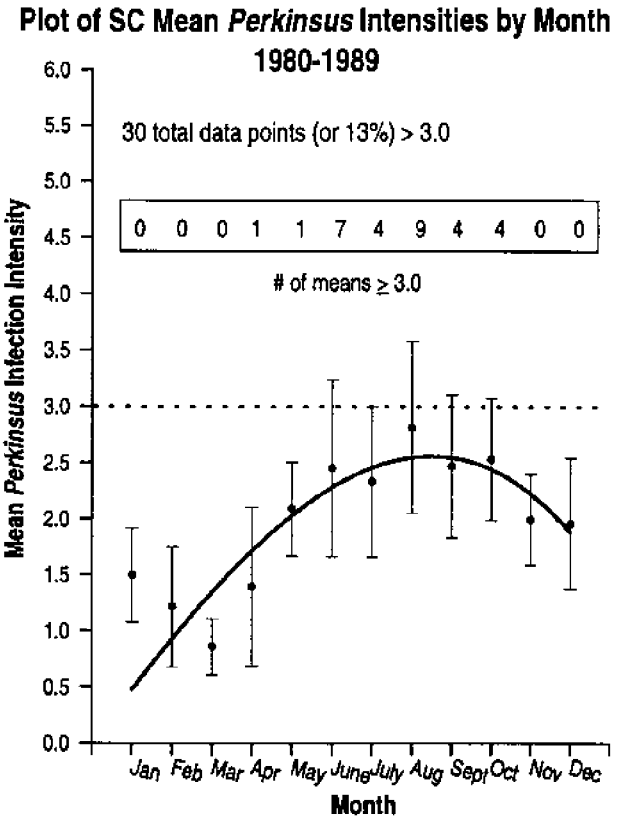

Figure 10. Summary of plot of grand means by month of mean *Perkinsus marinus* intensity values from 1980-1989 across all sites. (See Figure 4 for details).

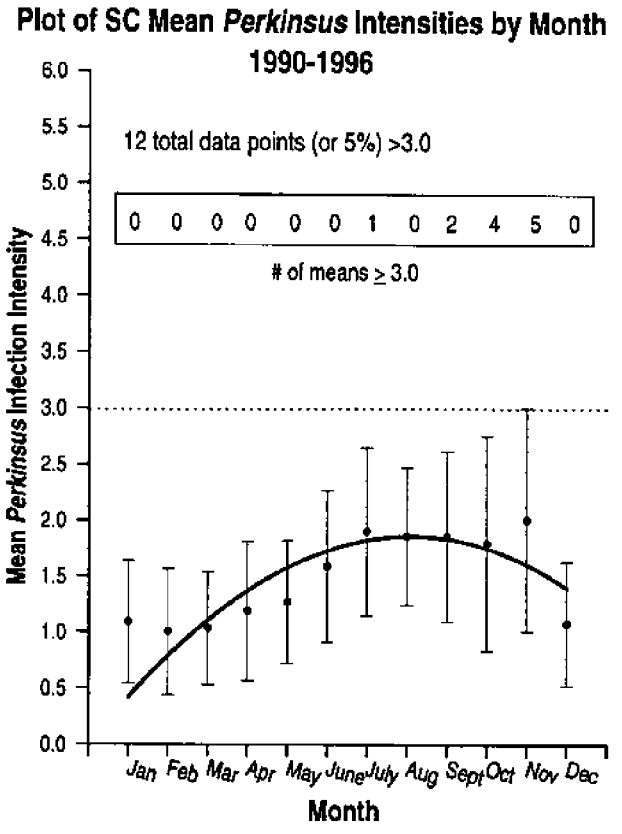

Figure 11. Summary of plot of grand means by month of mean *Perkinsus marinus* intensity values from 1990-1996 across all sites. (See Figure 4 for details).

(3.76) over the five year period was detected in September 1991. The lowest weighted incidence level of 0.40 was observed in December 1989. Weighted incidence levels ≥ 2.00 occurred in 23 of the 52 (44%) samples (see Figure 13). Salinity and water temperature (taken at the time of sampling) ranged from 8-32 ppt and 13.5-31°C (Figure A-7).

Toogoodoo Low Intertidal Zone

Oysters examined from the low intertidal zone of Toogoodoo Creek (Figures 2 & 3, Site 36), sampled monthly from July 1986 to December 1991 (52 months), had prevalences ranging from 28-100% (Figure 13). Prevalence levels ≥ 80% were observed in 44 of the 52 (or 85%) samples. The highest weighted incidence recorded over the nearly five year period occurred in September 1987 (3.64), with 100% of the oysters infected. The lowest weighted incidence occurred in March 1989 (0.36) when only 28% of the oysters were infected. Weighted incidence levels ≥2.00 occurred in 23 of the 52 (44%) samples (Figure 13). Hydrographic data taken at the time of collections were the same as for the high intertidal zone.

Cherry Point High Intertidal Zone

From 1986-1991 (54 sampled months), *P. marinus* prevalence levels ranged from 40-100% in the high intertidal zone of Cherry Point (see Figure 14). Prevalence levels ≥ 80% were observed in 48 of the 54 (or 89%) samples. The highest weighted incidence (3.44) over the nearly five year period was observed in October 1990 with a 100% prevalence. The lowest weighted incidence (0.56) and prevalence (40%) levels were observed in April 1988. Weighted incidence levels ≥ 2.0 were observed in 28 of the 54 (or 52%) samples and occurred generally in the summer and early fall. Salinity and water temperatures recorded at the time of sampling ranged from 16-35 ppt and 8-32°C (Figure A-8).

Cherry Point Low Intertidal Zone

Oysters examined from the low intertidal zone of Cherry Point (Figures 2 & 3, Site 35) between July 1986 and December 1991 (54 sampled months) had prevalence levels ranging from 40-100% (see Figure 14). Prevalence levels ≥ 80% were observed in 50 of the 54 (93%) samples. In November 1991, the high-

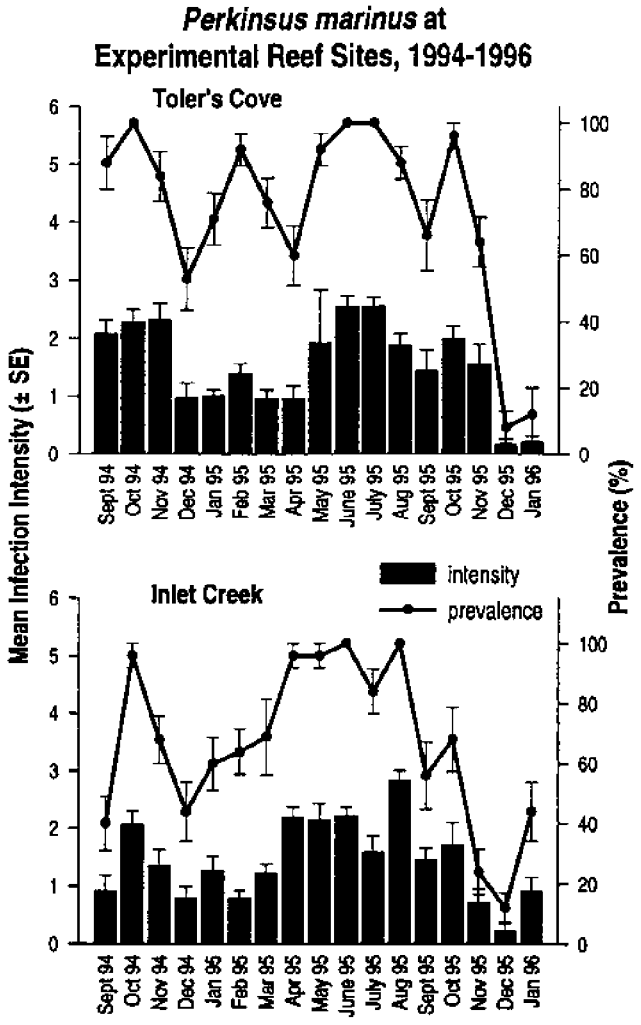

Figure 12. Prevalence and weighted incidence levels of *Perkinsus marinus* (Dermo) in native oysters from Toler's Cove Marina, site #16 (developed site) and Inlet Creek, site #15 (reference or control site) (n=25 oysters/site, 5 replicate samples).

est weighted incidence level (3.76) during the nearly five year period was observed with 100% of the sample population infected. The lowest weighted incidence (0.72) and prevalence levels (40%) were observed in April 1987. Weighted incidence levels ≥ 2.0 were observed in 31 of the 54 (or 57%) samples (Figure 14). Physical environmental data, taken at the time of sampling, were the same as recorded for the Cherry Point high intertidal zone.

Differences between the high and low intertidal samples from Toogoodoo and Cherry Point were examined by the Mann-Whitney U test. Results indicated no significant difference in mean infection intensities between the high and low intertidal oysters at each site ($P > 0.05$).

Wando River (Site 17)

Relatively low weighted incidence (<1.0) and prevalence (< 40%) levels of *P. marinus* infections were observed during most of the sampling times at each of the five stations sampled from the Wando River from 1973 to 1977. Generally, weighted incidence levels ≥ 1.0 occurred during the summer and fall (Figures 15 & 16). Oysters were collected from subtidal populations in the Wando River. This is one of the few subtidal populations existing in South Carolina. Sampling sites from the Wando River were established with increasing numerical designations moving upriver (see Figure 3).

W000

Oysters sampled from the mouth of the Wando River, near Remley's Point Landing, (Figure 3) from 1973 to 1977 revealed the presence of *P. marinus*. Prevalence levels ranged from 0% (May 1975) to 63% (November 1974). The highest weighted incidence level (1.20) occurred in July 1976. Weighted incidence levels <1.00 were observed in 46 of the 48 (or 96%) sampling times. Prevalence levels ≥ 40% were observed in only 5 of the 48 (or 10%) sampling times (Figure 15). Water temperatures ranged from 8-30°C, with salinities from 5-19 ppt.

W004

At station W004, the junction of Nowell Creek, *P. marinus* weighted incidence (1.52) and prevalence (60%) levels were highest in September 1976. Weighted incidence levels <1.00 were observed 23 of the 26 (or 88%) sampling times. Prevalence levels ≥40% were observed in only 5 of the 26 (or 19%) sampling times (Figure 15). Salinities and water temperatures ranged from 7-18 ppt and 7-32°C.

W008

The highest mean infection intensity level (WI) in the oysters examined from the Wando River during this sampling period (1973 to 1977) occurred at station W008, located just below Deyten's Shipyard. The weighted incidence ranged from 0.0 to 2.40. The highest observed prevalence (68%) and weighted incidence (2.4) levels occurred in November 1973. Weighted incidence levels <1.0 were observed in 48 of the 50 (or 96%) sampling times. Prevalence levels >40%

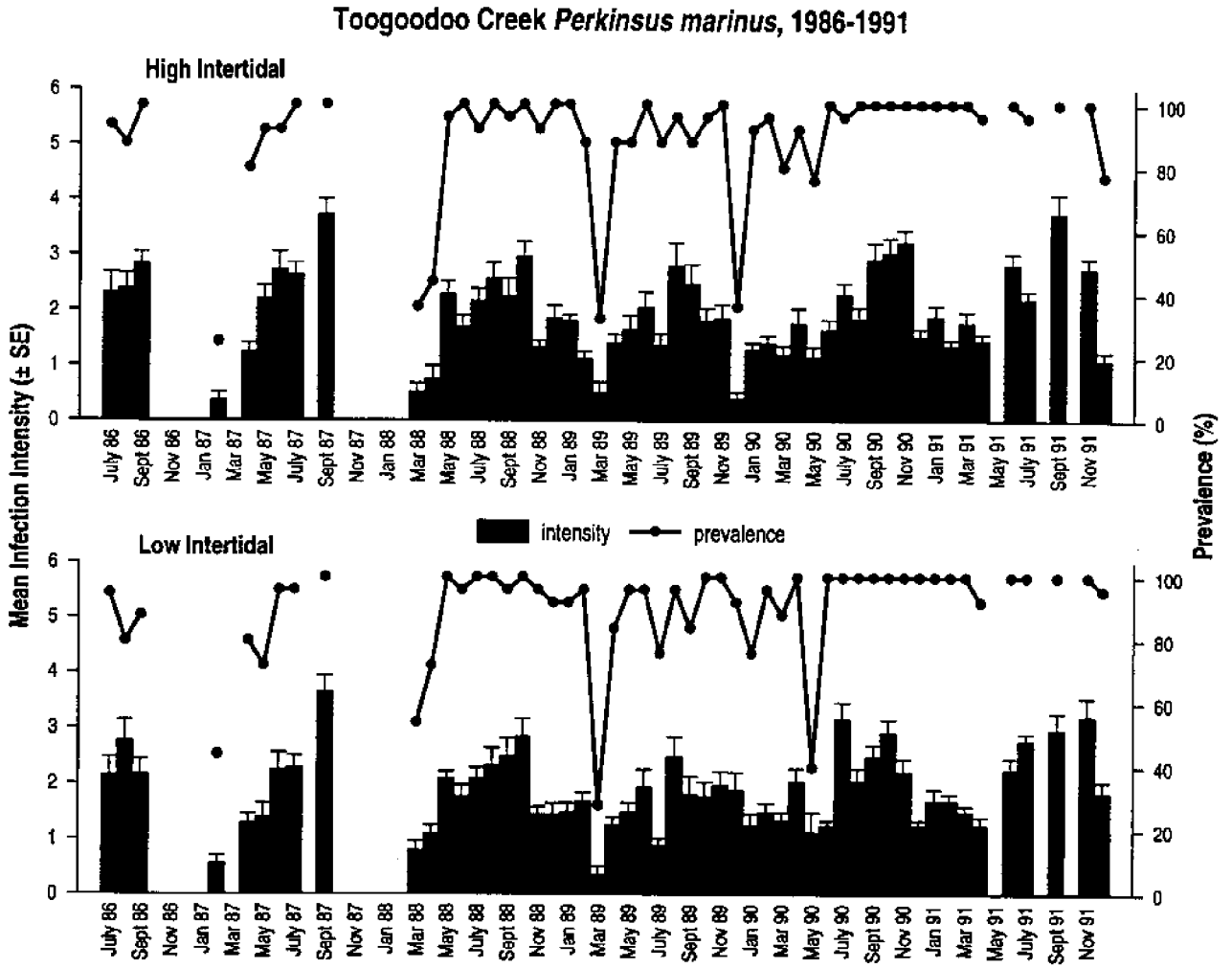

Figure 13. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oysters from the high and low intertidal zones at Toogoodoo Creek, site #36 (n=25 oysters/month).

were observed in only 6 of the 50 (or 12%) sampling times (Figure 15). Observed salinities and water temperatures ranged from 7 to 19 ppt and 7 to 32°C.

W010

At station W010, near the Highway 41 Bridge (upstream), the highest weighted incidence (1.92) and prevalence (60%) levels were observed in November 1973. During the sampling period between 1973 and 1977, 48 of the 50 (or 96%) sampling times had weighted incidence levels <1.0. Prevalence levels >40% were observed in only 5 of the 50 (or 10%) sampling times (Figure 16). Water temperatures ranged from 8 to 32°C, with salinities from 6 to 19 ppt.

W014

At station W014, near the Paradise Boat Landing, weighted incidence and prevalence levels were the highest recorded (2.08 and 72% respectively) in November 1973. During the other 44 sampling times, the weighted incidence was never >1.00. Prevalence levels > 40% were only observed in 2 of the 45 (or 4%) sampling times (Figure 16). Observed water temperatures ranged from 7 to 31°C, with salinities from 5 to 19 ppt.

Differences in infection intensity among the five stations at Wando River were significant, ($P = 0.002$, Kruskal-Wallis), with the disease being most intense at W008. The lowest mean infection intensity occurred at the upriver station, W014, near Paradise Boat Landing. During 1973, oysters were infected at a significantly higher level than during the following four years, with a notable decline in intensity during 1977. Seasonal variation in infection intensity was also ap-

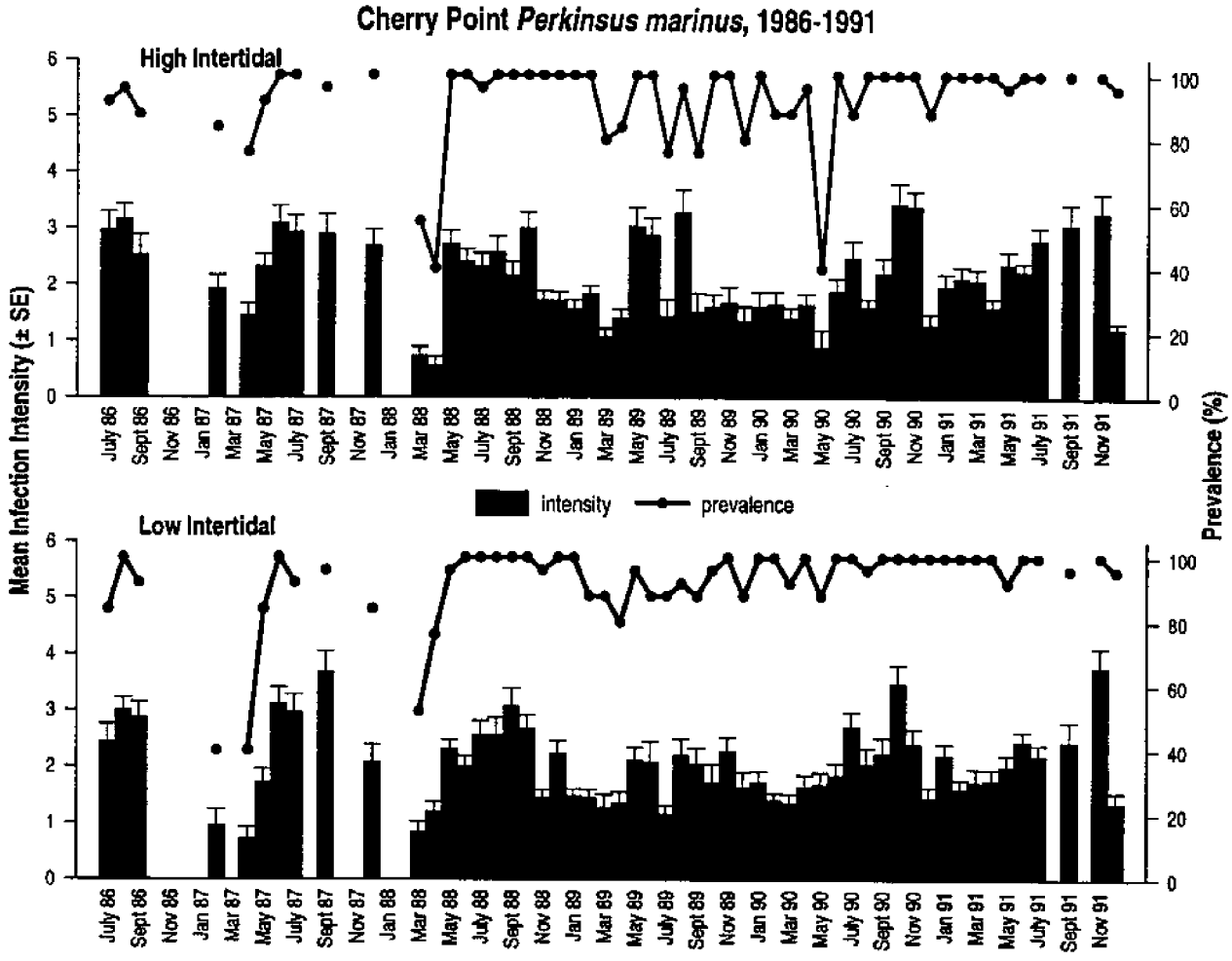

Figure 14. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oysters from the high and low intertidal zones at Cherry Point, site #35 (n=25 oysters/month).

parent, with the disease most prevalent during the months of August through December (P<0.001, Kruskal-Wallis test).

Cherry Point and Church Creek (Sites 35 & 32)

Oysters were sampled monthly from the high and low intertidal zones from Cherry Point and Church Creek during the period March 1993 to February 1995. *P. marinus* was present throughout the study. Highest infection intensity and prevalence levels occurred in summer and fall months (Figures 17 & 18).

Cherry Point High and Low Intertidal

The weighted incidence and prevalence levels ranged from 0.08 to 2.65 and 8% to 100% at the high intertidal zone. Oysters sampled from the low intertidal zone had weighted incidence values from 0.28 to 2.24. *P. marinus* was observed in 28-100% of the oysters examined over the nearly two year period. Surface water temperatures ranged from 9-35°C, with salinities from 21-36 ppt (Figure A-8).

Oysters sampled from the high intertidal zone had prevalence levels >80%, 11 of the 24 (or 46%) sampling times. Weighted incidence levels <2.0 were observed in 23 of the 25 (or 96%) sampling times (Figure 17).

Oysters sampled from the low intertidal zone had prevalence levels > 80%, 5 of the 24 (or 21%) sampling times. Weighted incidence levels <2.0 were observed in 21 of the 24 (or 87%) sampling times (Figure 17).

Church Creek High and Low Intertidal

Oysters collected from the high intertidal zone of Church Creek had *P. marinus* in 8 -100% of the oys-

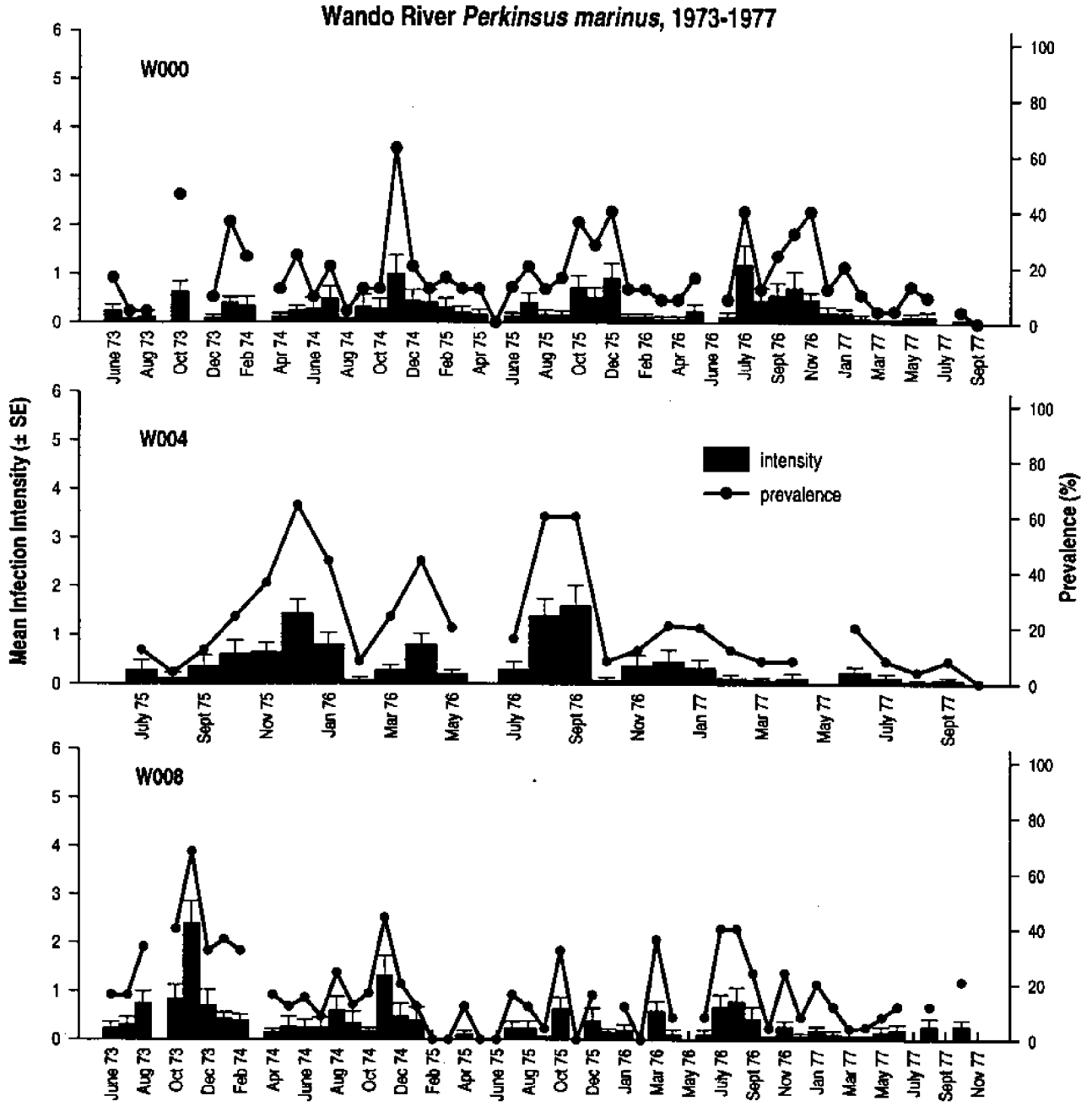

Figure 15. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oysters from the Wando River, site #17 (n=25 oysters/month).

ters examined; infection intensity levels ranged from 0.08 - 2.17. Prevalence levels from the low intertidal zone ranged from 20-100% and weighted incidence levels ranged from 0.2 - 2.76. Observed salinities were from 8 - 32 ppt; temperatures from 8 - 31°C (Figure A-7).

Oysters sampled from the high intertidal zone had prevalence levels > 80% 6 of the 24 (or 25%) sampling times. Weighted incidence levels <2.00 were observed in 23 of the 24 (or 96%) sampling times (Figure 18).

Oysters sampled from the low intertidal zone had prevalence levels > 80%, 8 of the 24 (or 33%) sampling times. Weighted incidence levels <2.0 were observed in 21 of the 24 (or 87%) samples (Figure 18).

Disease data collected on oysters from the high and low intertidal zones at Cherry Point and Church

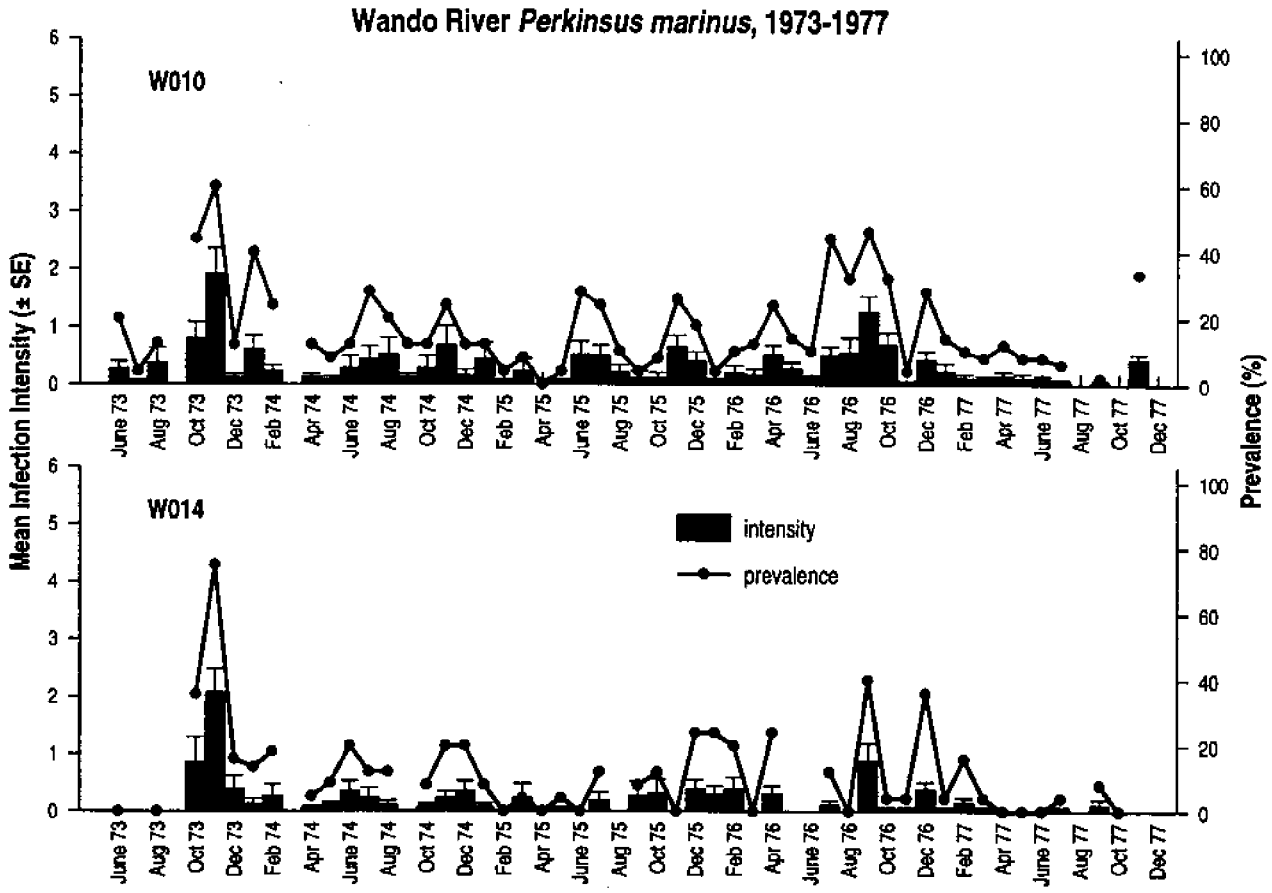

Figure 16. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oysters from the Wando River, site #17 (n=25 oysters/month).

Creek for 24 months detected significant differences ($P < 0.05$, Mann-Whitney U-test) in weighted incidence levels (mean infection intensity) among the four stations and for the two years of sampling (see Figure 19). Differences between the high and low intertidal zones at Church Creek were significant (see statistics, Figure 19) in 7 of the 24 (or 29%) sampling dates (July, August and November 1993, March, July, August and October 1994). Significant differences were also detected 7 of 24 (or 29%) sampling dates at Cherry Point, during August and November 1993, February, April, August and October 1994 and February 1995. After pooling the data from the high and low zones from each site, significant differences were detected between sites ($P< 0.05$, Mann-Whitney U-test) for April, May, June and July 1993 and April and November 1994.

Charleston Harbor-Grice Marine Laboratory (Site 24)

Perkinsus marinus was present in Charleston Harbor (Grice Marine Laboratory, adjacent to Fort Johnson) where oysters were examined monthly (Figure 20) between June 1994 and February 1996. Prevalence levels ranged from 22% (February 1996) to 100% (August 1995) and weighted incidence levels ranged from 0.32 (February 1996) to 3.20 (October 1995) (Figure 20). Water samples taken at the time of sampling found salinities ranging from 15 to 25 ppt and water temperatures ranging from 12 to 31°C.

Lighthouse Creek (Site 28)

Monthly oyster samples were collected from Lighthouse Creek from January 1987 to October 1988. *Perkinsus marinus* was present in all months sampled. Prevalence levels ranged from 40 to 100%. High prevalence levels (>80%) were present in 17 of the 22 (or 77%) months sampled. Weighted incidence

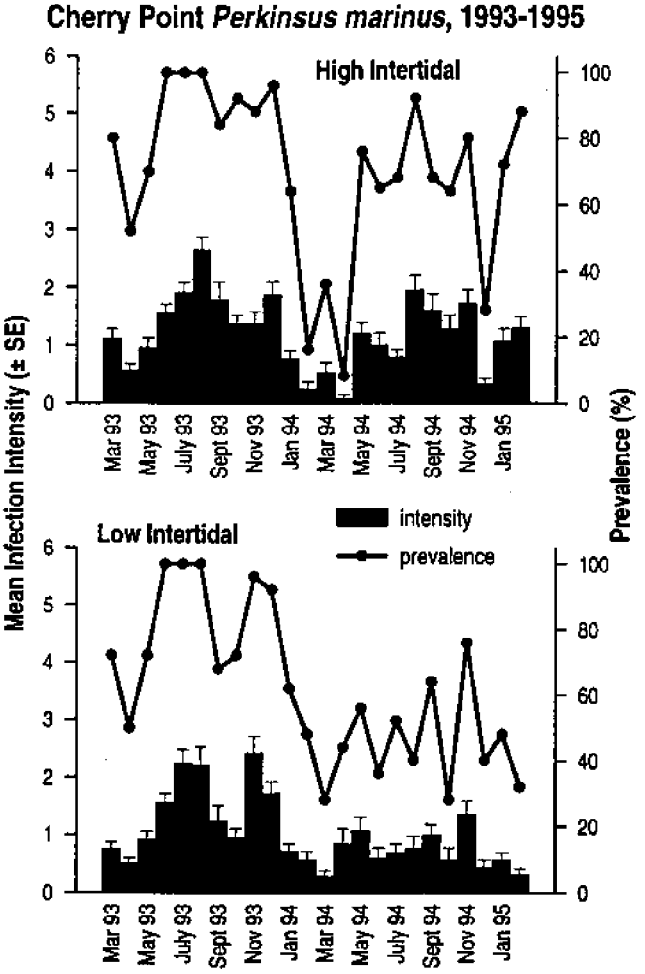

Figure 17. Prevalence (%) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oysters from the high and low intertidal zones at Cherry Point, site #35 (n=25 oysters/month).

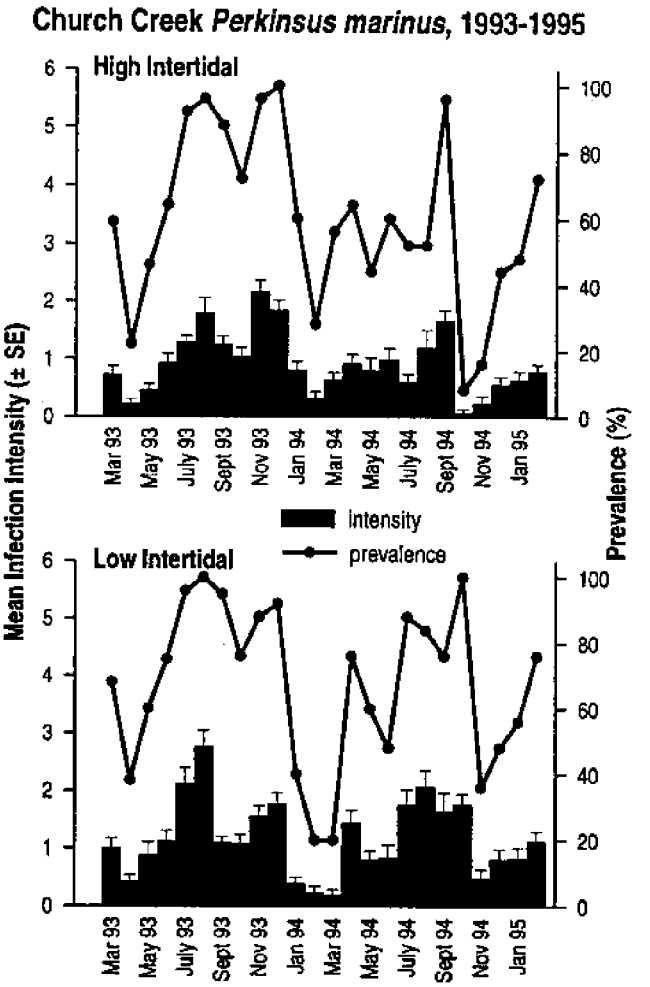

Figure 18. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oysters from the high and low intertidal zones at Church Creek, site #32 (n=25 oysters/month).

levels ranged from 0.52 to 2.92. Moderate infections (weighted incidence, 1.92 - 2.92) were observed in 12 of the 22 (or 55%) samples (Figure 21).

Folly Creek (Site 29)

Native oysters were transplanted from Lighthouse Creek to Folly Creek (Figure 2) in 1986 and 1987, as part of a project to assess the effect of mechanical harvester transplanting of oysters to determine growth and survival (see Klemanowicz 1985, Burrell et al. 1989). They were examined monthly between 1987 and 1988 to determine *Perkinsus marinus* infection intensity and prevalence (see Figures 22-23).

Folly Creek High Intertidal

Oysters transplanted to the high intertidal zone at Folly Creek had prevalence levels ranging from 67 - 100%, with 13 of the 16 (or 80%) samples having *P. marinus* in 100% of the oysters examined (Figure 22). The highest weighted incidence was observed in August 1987 (3.80). However, weighted incidence levels >3.00 were observed in 50% (or 8 of 16) of the monthly samples examined. The lowest weighted incidence (0.93) and prevalence (67%) levels were observed in February 1988 (see Figure 22).

Folly Creek Low Intertidal

Oysters transplanted to the low intertidal zone had prevalences ranging from 47-100%. Ten of the 16 (or 63%) sampling times had *P. marinus* in 100% of oysters examined (Figure 22). The highest weighted incidence (3.87) occurred in August 1987. Weighted incidence levels >3.0 were only observed in 19% of

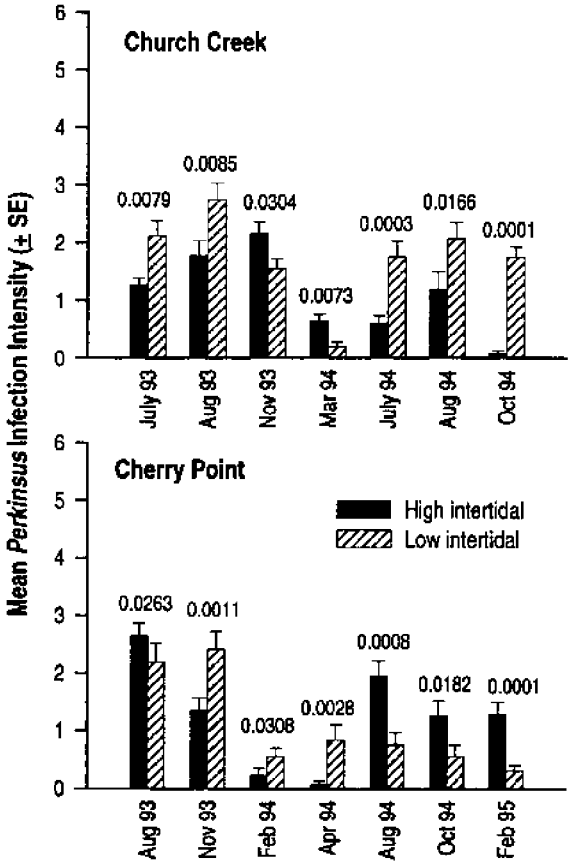

Figure 19. *Perkinsus marinus* mean infection intensity levels at Church Creek and Cherry Point during 1993-1995. Only months with significant statistical differences (paired Mann-Whitney U-test) between high and low intertidal samples are shown.

the monthly samples examined. However, 12 of the 16 (or 75%) sampling months had weighted incidence levels >2.0. The lowest weighted incidence (0.60) and prevalence (47%) levels were observed in February 1988 (see Figure 22).

Folly Creek Winter Transplant

Native oysters transplanted to Folly Creek (Station RE01) in the winter (December 1986) were examined monthly from December 1986 to November 1987. The weighted incidence levels ranged from 0.48 (April 1987) to 3.96 (August 1987). Prevalence levels ranged from 28% (April 1987) to 100%. These values occurred several times during summer and fall 1987 (see Figure 23).

Folly Creek Spring Transplant

Native oysters transplanted to Folly Creek (Station RE02) in April 1987 were examined monthly for *P. marinus* infection intensity and prevalence until March 1988. Weighted incidence levels ranged from 1.04 (March 1988) to 3.84 (August 1987). Prevalence levels were never < 60%, with the lowest (64%) observed in the initial April sample. Nine of the 12 sampling months (or 75%) had prevalence levels >80% (see Figure 23).

Folly Creek Summer Transplant

Native oysters transplanted to Folly Creek (Station RE03) in the summer (July 1987) were examined monthly for *P. marinus* from July 1987 to June 1988. Weighted incidence levels ranged from 1.04 (March 1988) to 3.24 (August 1987). Prevalence levels were never <60% in any of the examined months. The lowest observed prevalence (68%) occurred in March 1988. Prevalence levels > 80% were observed in 11 of the 12 (or 92%) sampling months (see Figure 23).

Folly Creek Fall Transplant

Native oysters transplanted to Folly Creek (Station REO4) in the fall (October 1987) were examined monthly for *P. marinus* from October 1987 to October 1988. During these 13 months of examination, the lowest weighted incidence (0.52) was observed in February 1988, the highest (3.08) occurred in October 1987. The lowest prevalence (40%) was observed in February 1988. Prevalence levels > 80% were observed in 11 of the 13 (or 85%) sampling months (see Figure 23).

1994 *Perkinsus marinus* Monitoring Study

Native oysters from 17 coastal sites across South Carolina were examined for *P. marinus* during the summer of 1994. *Perkinsus marinus* was present in all the sites examined. Weighted incidence levels across all sites ranged from 0.28 (Kiawah Creek) to 3.00 (Koppers Creek, an EMAP degraded site), both occurring in July 1994. Overall, prevalence levels ranged from 24-100% (see Table 6).

Oysters from Ashepoo (Site 46), Cape Romain (Site 8) and Wando River (Site 17) were examined monthly from June to October 1994. Generally, weighted incidence levels never exceeded 2.00, with prevalence levels ranging from 16% in the Wando River to 96% in the Ashepoo River (Figure 24).

Table 6. *Perkinsus marinus* (Dermo) prevalence and intensity in *Crassostrea virginica* from South Carolina creeks sampled during summer of 1994 (n = 25 oysters/site on each sampling date).

Oyster Stations (Site # *)	Date	% Prevalence	Weighted Incidence •
Winyah Bay (3)	14 JUN	44	1.48
Bull Bay (9)	21 JUN	96	2.12
Shem Creek (19)	19 JUL	88	1.96
Noisette Creek (20)	19 JUL	60	1.20
Shipyard Creek (21)	30 JUN	16	1.80
	12 JUL	88	1.96
Diesel Creek (22)	20 JUL	40	1.04
Plum Island Creek (22a)	22 SEP	100	2.28
Koppers Creek (23)	30 JUN	96	1.72
	12 JUL	100	3.00
Metcalf's Creek (25)	13 JUL	88	2.04
Clark Sound (26)	17 AUG	100	1.88
Lighthouse Creek (28)	4 JUN	88	1.80
	12 JUL	92	2.08
Wallace Creek (31)	17 JUN	44	0.64
Kiawah Creek (33)	20 JUN	44	0.56
	29 JUL	24	0.28
	12 AUG	64	1.52
Long Island (34)	20 AUG	80	1.24
Leadenwah Creek (37)	17 JUN	84	1.72
Fripp Inlet (49)	20 JUN	76	1.32
Jenkin's Creek (56)	15 JUN	52	0.84

* See Tables 4 & 5 and Fig. 2 for site locations.
• See Methods for calculation.

Overview of *Haplosporidium nelsoni* (MSX)

Haplosporidium nelsoni (MSX) was first reported in South Carolina in 1992 (Dougherty et al. 1993). The results reported here summarize our subsequent findings and represent the most comprehensive documentation of *H. nelsoni* in South Carolina to date.

A total of 1,924 individuals was examined from 21 sites (Tables 7-9, Figures 25 & 26). Of these, approximately 8% (or 150 individuals) were infected with *Haplosporidium nelsoni* (MSX), with the parasite present in oysters from 52% (or 11 of 21) of the sampling stations included here. Prevalence levels ranged from 0 to 42% (Tables 7-9, Figures 25 & 26), with the highest prevalence occurring in oysters from Toler's Cove sampled in October 1994. Of the 150 individuals with *H. nelsoni* infections, 9% (or 27 of 300) were infected from the Charleston Harbor-Grice station, 3% (or 28 of 824) from the 1994 South Carolina summer oyster study sites, 8% (or 32 of 400) from Inlet Creek and nearly 16% (or 63 of 400) from the Toler's Cove Marina site. Disease intensity among individually infected animals (25 individuals/sample) varied from light to heavy. *H. nelsoni* infections ranged from those localized in the gill epithelium to heavy systemic infections at each of the three sampling sites (see Tables 8 & 9). For the Summer 1994 Survey, 44% (or 8 of 18) of the sampling stations (Table 7) had oysters with *H. nelsoni* infections. Prevalence

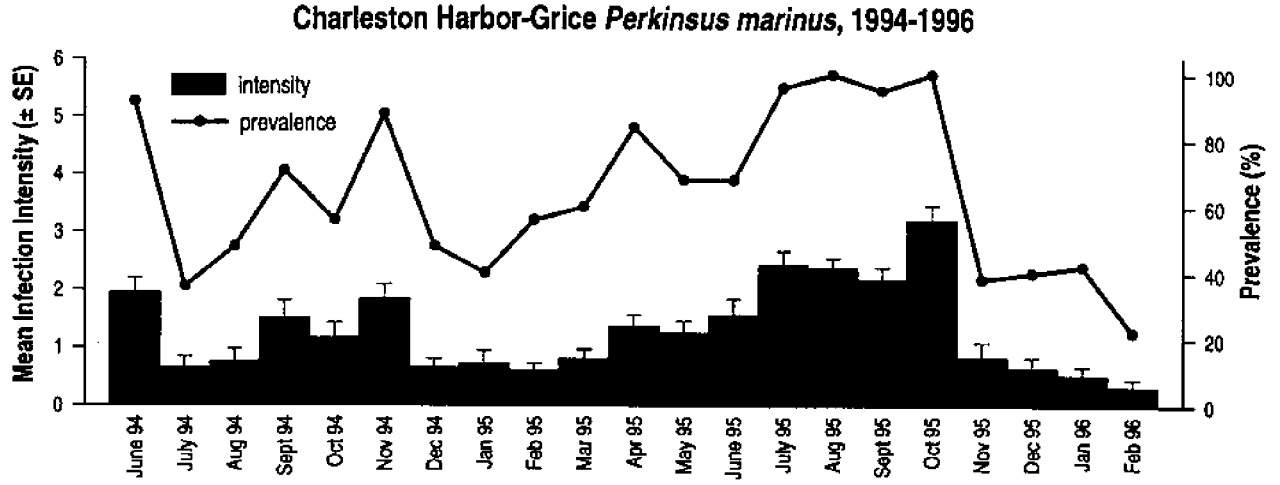

Figure 20. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations from Charleston Harbor-Grice, site #24 (n=25 oysters/month).

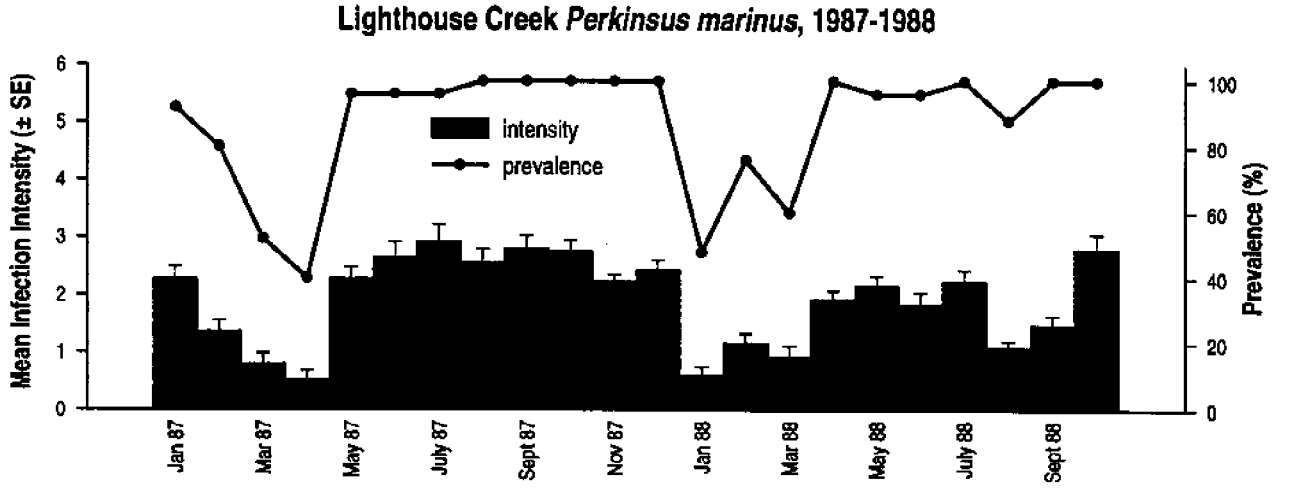

Figure 21. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations from Lighthouse Creek (site #28) sampled from 1987-1988 (n=25 oysters/month).

levels ranged from 0 to 32% (Table 7), with the highest prevalence occurring in oysters from Koppers Creek sampled in June 1994. For the Charleston Harbor-Grice site (Table 8, Figure 25), sampled monthly from June 1994 to June 1995, *H. nelsoni* was found in oysters from 10 of the 12 (or 83%) months examined (Table 8, Figure 25). Peak prevalence (24%) occurred in April 1995. At Inlet Creek (Site 15) and Toler's Cove (Site 16), sampled from September 1994 to December 1995, maximum prevalence levels of 28% and 42% occurred in October 1994. *H. nelsoni* was detected in 13 of the 16 (or 81%) months examined for both sites (Table 9, Figure 26).

DISCUSSION

Diseases caused by *P. marinus* and *H. nelsoni* have had a combined impact to significantly reduce oyster production in the Chesapeake and Delaware Bays, among others (e.g., Lewis et al. 1992, Ewart and Ford 1993, Ford and Tripp 1996). *P. marinus* has been detected in its oyster host, *Crassostrea virginica* (Ray and Mackin 1955, Andrews 1965, Ford and Tripp 1996) in the Gulf of Mexico (Quick and Mackin 1971, Hofsetter 1977, Ray 1987, Soniat 1996), the northeast (Paynter and Burreson 1991) and the southeast (Burrell et al. 1984, Crosby and Roberts 1990, Bobo et al. unpublished data), where the most important oyster producing regions have historically been found

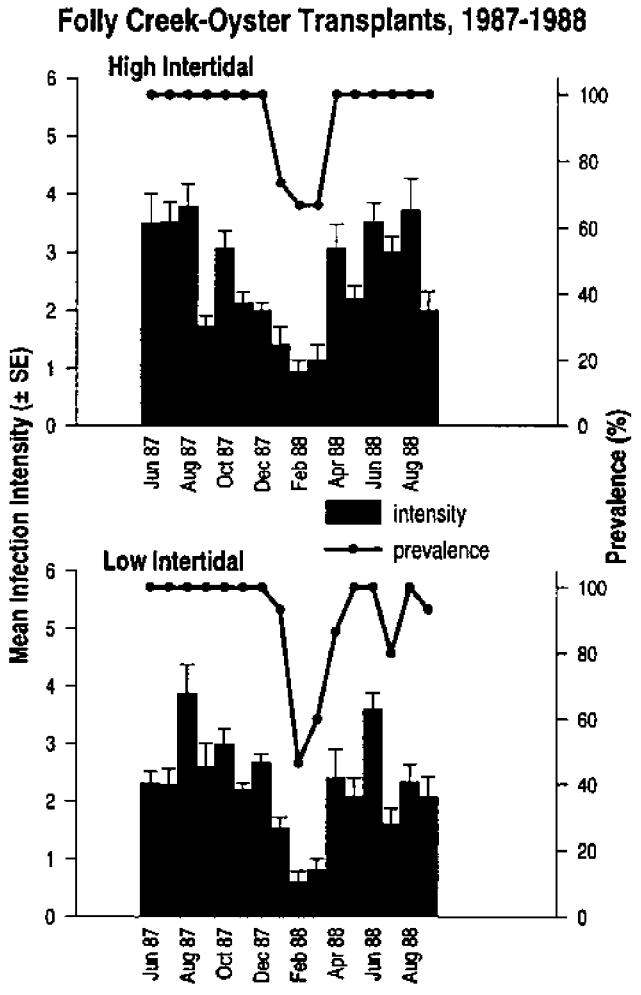

Figure 22. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in oysters transplanted to the high and low intertidal zones at Folly Creek, site #29 (n=25 oysters/month).

in the United States. This pathogen continues to be responsible for significant population declines throughout the northeast (e.g., Maryland, Virginia, New Jersey), southeast (e.g., North Carolina) and Gulf of Mexico.

Similarly since the 1950s, *Haplosporidium nelsoni* (MSX) has contributed to significant oyster population declines in planting areas and seed beds in the Delaware and Chesapeake Bays (Haskin and Andrews 1988). In South Carolina, it has only recently been detected, with little information currently available beyond reporting its presence/absence (Crosby and Roberts 1990, Dougherty et al. 1993, Bobo et al. 1996, Bobo et al. unpublished data).

Perkinsus marinus (Dermo) Patterns

In South Carolina between 1972 and 1996, over 21,000 oysters from over 60 sites were examined for the presence of *Perkinsus marinus*. Although most of these sites were not sampled continuously (i.e. collections were made in conjunction with specific short-term objectives resulting in spatial and temporal inconsistencies overall), several interesting patterns were evident. First, *Perkinsus marinus* was present (as measured by prevalence) at all of the sites examined and throughout all months of the year (see Tables 4 & 5, Figures 2 & 3 for overview). The highest prevalence and intensity levels were observed during the summer and fall months, (see Figures 4 & 8) with the seasonal patterns of infection most similar to observations made for Gulf of Mexico populations (Craig et al. 1989).

Second, average infection intensities (weighted incidence) never exceeded 4.0 on the Quick and Mackin (1971) scale (0-6), with infection intensities rarely exceeding 3.0. Over the 24 year period covered by this report, only 5% (or 42 of 831) of all composite oyster samples equaled or exceeded intensities of 3.0 (Figure 4). Similar low mean prevalence values have also been observed in studies focussed in the North Inlet Estuary, South Carolina (David Bushek unpublished data). These findings are quite different from patterns observed in the northeast, where *P. marinus* intensities often exceed 4.0, with significant *Perkinsus*-related mortalities occurring (Bushek et al. 1994, Ford and Tripp 1996). Weighted incidence values of 4.0 or greater are often common in northeastern oyster populations, especially in heavily infected gapers (Andrews 1988). Meyers et al. (1991) found weighted incidence values approaching or exceeding 4.0 in diploid and triploid *Crassostrea virginica* in Virginia. *P. marinus* severity levels exceeding 4.0 have also been observed at many sites within the Maryland portion of the Chesapeake Bay (Smith and Jordan 1992). Hence, while *P. marinus* prevalence is typically high in South Carolina oysters, infection intensities are relatively low (< 3.0).

A third interesting pattern also emerged in our data. At most of the South Carolina sites surveyed in the 1970s, intensity levels were relatively low (see Figures 5 & 9). Of all the combined oyster samples taken from 1972 to 1979, no single mean value ex-

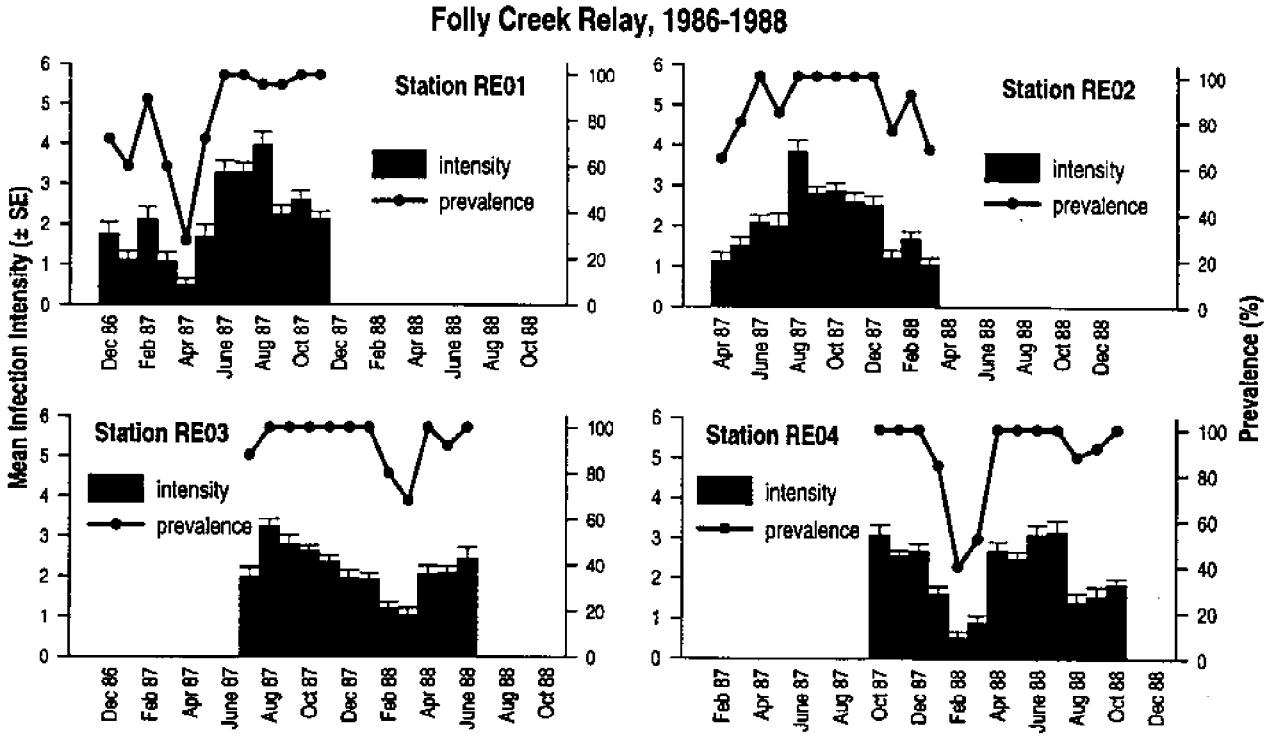

Figure 23. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in oysters transplanted to Folly Creek, site #29 (n=25 oysters/month).

ceeded the *P. marinus* intensity threshold of 3.0; in fact most mean values were less than 0.5 (Figures 5 & 9). In contrast, for samples from the 1980s, 13% (or 30 of 230) of the sample means were greater than or equal to 3.0. In fact, 71% (30 of 42) of all observed "elevated" values from 1972 to 1996 were collected during that period (compare Figures 5 & 9 and 6 & 10). Comparing these two decades, *P. marinus* intensities appear to have risen dramatically during the 1980s, as compared to the previous decade. Finally, during the 1990s, 4.7% (or 12 of 256) of the sample means were greater than 3.0, with 29% (or 12 of 42) of all mean intensities exceeding 3.0 observed. Thus, it appears that *P. marinus* infections increased significantly in the 1980s and have remained elevated during the 1990s. Care must be exercised, however in drawing these temporal interpretations, as sample sizes, spatial scales, associated site attributes (salinities, development, etc.) and included sampling period (7-10 years) have varied considerably. We simply have no sites to compare that were monitored, even sporadically over these three decades.

Specifically, from the late 1980s and 1990s several sites (Toogoodoo, Cherry Point, Church Creek, Toler's Cove, Inlet Creek, and Charleston Harbor) showed a pattern of *P. marinus* infection intensities similar to those documented elsewhere in the southeast (Crosby and Roberts 1990, O'Beirn et al. 1994, 1996a). Intensity increased in the spring, followed by peak levels in late summer/early fall, with infection levels at most sites decreasing during winter months (Figures 4-11). Infection prevalences typically decline to near zero during the late winter in Chesapeake and Delaware Bays (Andrews and Hewatt 1957, Andrews 1988). In contrast, prevalence typically remains high throughout the year in South Carolina estuaries (Burrell et al. 1984, Crosby and Roberts 1990, and this study, Figures 4-11). Similar results have been observed in Georgia and on the east coast of Florida (Quick and Mackin 1971, O'Beirn et al. 1994, 1996a), as well as at sites along the Gulf of Mexico (reviewed by Soniat 1996).

Here, significant differences in *P. marinus* infection levels were observed in fewer than 30% of the high and low intertidal monthly comparisons from Cherry Point (Site 35), Church Creek (Site 32) and Toogoodoo River (Site 36) between 1986 and 1995. Similarly, Burrell et al. (1984) found no significant difference in *P. marinus* levels between subtidal and intertidal oysters sampled from the Wando River and

Table 7. *Haplosporidium nelsoni* (MSX) prevalence and intensity in *Crassostrea virginica* from South Carolina creeks sampled during summer of 1994.

Oyster Stations (Site #*)	Date	*H. nelsoni* Infected/Examined	% Prevalence	Intensity H-M-L•
Winyah Bay (3)	14 JUN	0/25	0	0-0-0
Cape Romain (8)	13 JUN	0/25	0	0-0-0
	13 JUL	0/25	0	0-0-0
	10 AUG	2/25	8	0-1-1
	8 SEP	1/25	4	0-1-0
	6 OCT	1/25	4	0-0-1
Bull Bay (9)	21 JUN	0/25	0	0-0-0
Wando River (17)	3 JUN	0/25	0	0-0-0
	19 JUL	0/25	0	0-0-0
	17 AUG	0/25	0	0-0-0
	15 SEP	0/25	0	0-0-0
	17 OCT	0/25	0	0-0-0
Shem Creek (19)	19 JUL	0/25	0	0-0-0
Noisette Creek (20)	19 JUL	4/25	16	2-0-2
Shipyard Creek (21)	30 JUN	2/25	8	1-0-1
	12 JUL	0/25	0	0-0-0
Diesel Creek (22)	20 JUL	0/25	0	0-0-0
Plum Island Creek (22a)	22 SEP	3/25	12	0-0-3
Koppers Creek (23)	30 JUN	8/25	32	1-1-6
	12 JUL	1/25	4	0-0-1
Metcalf's Creek (25)	13 JUL	0/25	0	0-0-0
Lighthouse Creek (28)	4 JUN	0/25	0	0-0-0
	12 JUL	0/25	0	0-0-0
Wallace Creek (31)	17 JUN	0/25	0	0-0-0
Kiawah Creek (33)	20 JUN	1/25	4	0-0-1
	29 JUL	2/25	8	1-1-0
Leadenwah Creek (37)	17 JUN	1/24	4	0-0-1
Ashepoo River (46)	23 JUN	0/25	0	0-0-0
	25 JUL	1/25	4	0-1-0
	23 SEP	1/25	4	0-0-1
	20 OCT	0/25	0	0-0-0
Fripp Inlet (49)	20 JUN	0/25	0	0-0-0
Jenkin's Creek (56)	15 JUN	0/25	0	0-0-0

* See Tables 4 & 5 and Fig. 2 for site locations.
• See Table 3 for explanations.

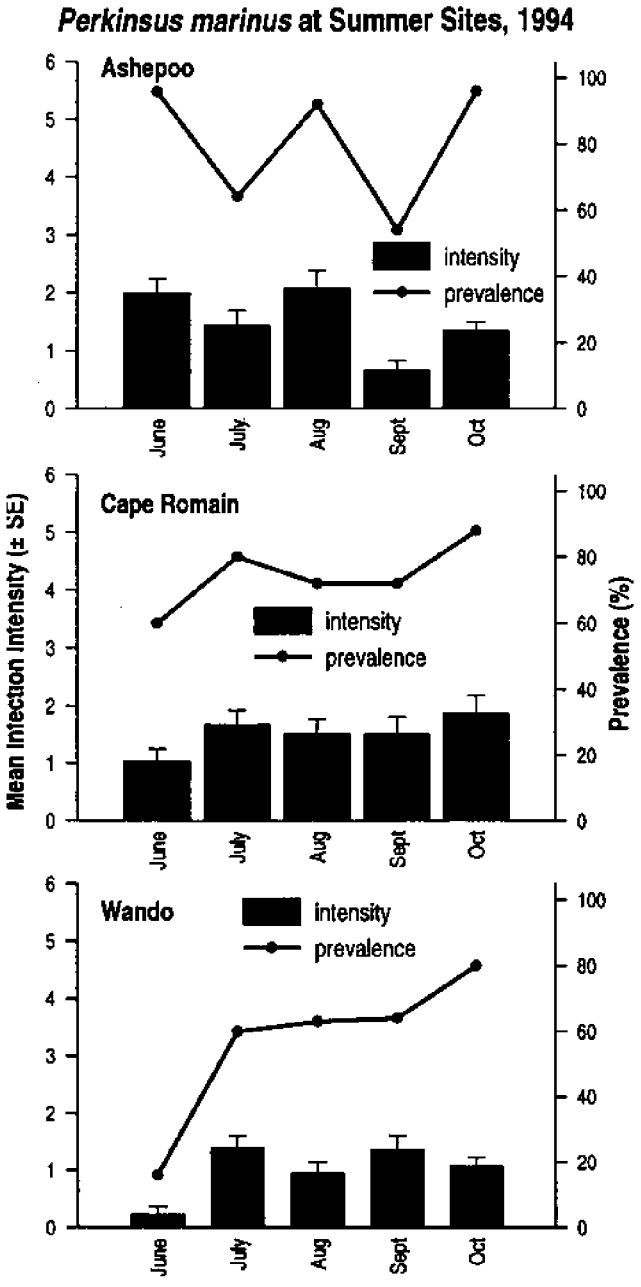

Figure 24. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations sampled during summer of 1994 (n=25 oysters/site on each sampling date).

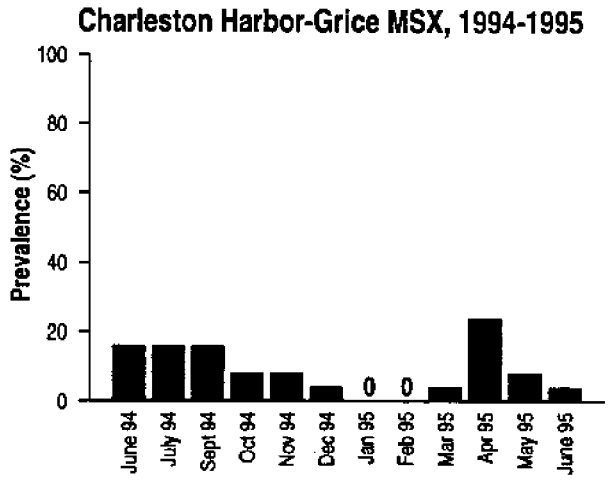

Figure 25. Prevalence of *Haplosporidium nelsoni* (MSX) in native oyster populations from Charleston Harbor-Grice, site #24 (n = 25 oysters/month; see Table 8 for details).

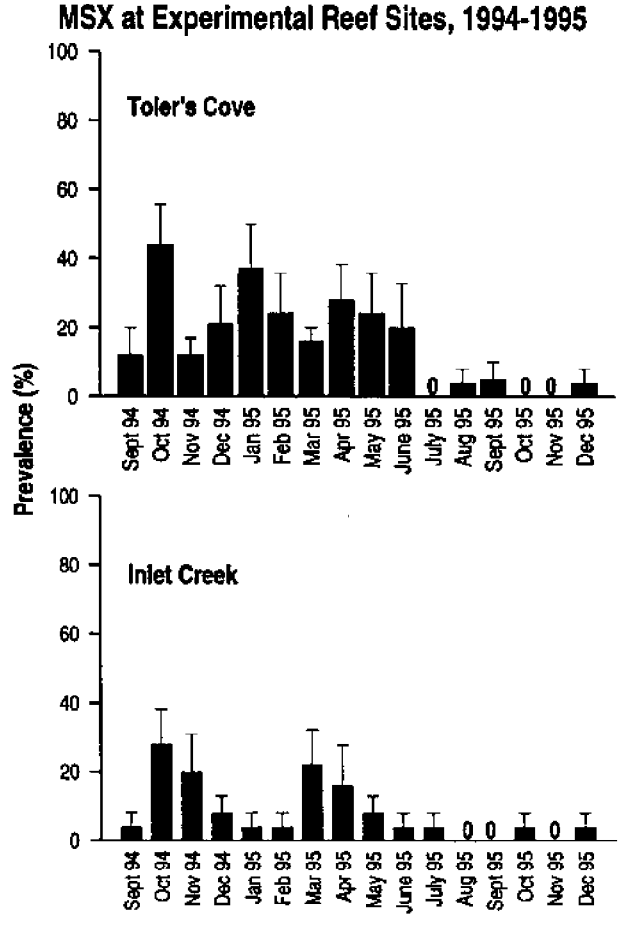

Figure 26. Prevalence of *Haplosporidium nelsoni* (MSX) in native oyster populations from Toler's Cove Marina, site #16 (developed site) and Inlet Creek, site #15 (reference, control site) (n = 25 oysters/site, 5 replicate samples).

Table 8. *Haplosporidium nelsoni* (MSX) infection intensity in *Crassostrea virginica* from Charleston Harbor-Grice in 1994-1995 (n=25 oysters/month, See Fig. 25 for prevalence levels).

Month	Infection intensity H-M-L*	Infection category G-LS-HS*
1994 June	2-0-2	2-0-2
July	3-0-1	4-0-0
Aug	NS	NS
Sep	0-2-2	2-2-0
Oct	1-0-1	1-0-1
Nov	0-1-1	1-1-0
Dec	0-0-1	0-1-0
1995 Jan	0-0-0	0-0-0
Feb	0-0-0	0-0-0
Mar	1-0-0	0-0-1
Apr	3-2-1	1-3-2
May	0-2-0	1-1-0
June	0-0-1	1-0-0

* See Table 3 for explanations.
NS - no sample taken

Cape Romain in South Carolina. Studies in Virginia (Gibbons and Chu 1989) and Georgia (O'Beirn et al. 1994, 1996a) have found similar results.

Results from the Toogoodoo and Cherry Point sites (see Figure 27) were similar to those obtained by Crosby and Roberts (1990) for North Inlet, South Carolina. They described a four-phase cycle for *Perkinsus marinus* epizootiology. **Phase 1** (quiescent period) typically occurs in February, March and April when low mean intensity levels occur. During most of phase 1 there are some oysters with no detectable infections. **Phase 2** (pre-virulent period) generally occurs during May, June and July, when a dramatic increase in monthly mean intensity levels is observed. The transition from a maximum intensity level of 5 to the maximum of 6 is observed in individual (versus mean) oysters. Similar variation in intensity has been observed in our 24 years of sampling, with oysters individually having intensities ranging from 0 to 6.0. However, weighted incidences (i.e. mean intensity levels as discussed above) based on sample sizes of 25 or more oysters rarely yielded average levels greater than 3.0. **Phase 3** (virulent infection stage), which occurs during August, September and October is characterized by peak intensities, with few or no oysters free of *Perkinsus*. This peak in infection intensity has also been observed here (e.g., Toler's Cove, Inlet Creek, Lighthouse Creek) during the same period (see Figures 12 and 21). Although some sampling periods did show individual oysters within the sample population with no infection, 58% (or 30 of 52) of the months sampled showed oysters with infection levels ranging from 1 to 6. Finally, **phase 4** (remission stage) occurs during November, December and January, with mean monthly infection intensities declining and most individual oysters examined having intensity levels <6.0.

Environmental Patterns Affecting *Perkinsus marinus*

Temperature and salinity are important factors affecting the epizootiology of *Perkinsus marinus* (e.g., Mackin 1951, 1962, Hewatt and Andrews 1956, Quick and Mackin 1971, Ogle and Flurry 1980, Soniat 1985, Ray 1987, Burreson and Andrews 1988, Gauthier et al. 1990). In North Inlet Estuary, Crosby and Roberts

Table 9. *Haplosporidium nelsoni* (MSX) intensity in *Crassostrea virginica* from Toler's Cove Marina and Inlet Creek studies in 1994-1995*. (n=25 oysters/month, See Figure 26 for prevalence levels).

Month	Infection intensity		Infection category	
	Inlet (H-M-L)*	Toler's (H-M-L)*	Inlet (G-LS-HS)*	Toler's (G-LS-HS)*
1994 Sep	0-1-0	0-2-1	1-0-0	2-0-1
Oct	2-0-5	7-2-2	5-0-2	4-1-6
Nov	0-3-2	0-2-1	2-3-0	3-0-0
Dec	0-1-1	1-1-3	1-1-0	2-2-1
1995 Jan	0-1-0	6-0-4	1-0-0	5-1-4
Feb	0-0-1	2-2-2	1-0-0	4-2-0
Mar	1-2-2	1-1-2	2-2-1	2-1-1
Apr	1-1-2	0-3-4	2-1-1	3-4-0
May	0-0-2	1-3-2	1-1-0	1-4-1
June	0-0-1	2-1-2	1-0-0	4-1-0
July	0-0-1	0-0-0	1-0-0	0-0-0
Aug	0-0-0	0-0-1	0-0-0	1-0-0
Sep	0-0-0	0-0-1	0-0-0	1-0-0
Oct	1-0-0	0-0-0	1-0-0	0-0-0
Nov	0-0-0	0-0-0	0-0-0	0-0-0
Dec	0-1-0	0-1-0	1-0-0	0-1-0

*See Table 3 for explanations.

(1990) found a positive correlation between elevated water temperature and *P. marinus* infection intensity in South Carolina oysters. In contrast, other studies (e.g., Burrell et al. 1984, Craig et al. 1989) found no relationship between water temperature and infection intensity. In Gulf Coast oysters, neither prevalence nor median infection intensity was correlated with temperature based on samples taken on the day of collection (Wilson et al. 1989, Soniat 1996). However, the overall temperature regime experienced by oysters during the time proceeding sampling is critical for understanding the distribution of *P. marinus.* Crosby and Roberts (1990) measured water temperatures daily over month-long periods and demonstrated that this is a better representation of the history of environmental exposure. Beckert et al. (1972) noted a 6-week time lag between the decline in winter water temperature and a decrease in infection intensity. Ray (1987) has stressed that *P. marinus* infections develop rapidly only above 20°C. Similarly, Lewis et al. (1992) found no statistical correlation between water temperature and disease intensity in their study of oysters in Georgia. For our South Carolina collections, most water temperatures were taken at the time of sampling, with the exception of our long-term oyster reef studies at the Toler's and Inlet sites, using Hydrolabs and intertidal temperature sensors (see Methods Section). Using the extensive data collected at these two sites, we may be better able to examine the relationship between disease intensity levels and physical factors such as air and water temperatures and salinity.

For South Carolina oyster populations, intertidal, rather than subtidal temperature regimes are probably more relevant measurements for understanding oyster physiology and associated disease patterns (D. Bushek pers. comm., L. Burnett pers. comm.). This intertidal habit exposes them to a microenvironment whose winter and summer daily temperature fluctuations often exceed 20°C or more, with extended summer exposed temperatures nearing 54°C or 129°F (Figure A-10, Coen et al. unpubliahed data). Despite this,

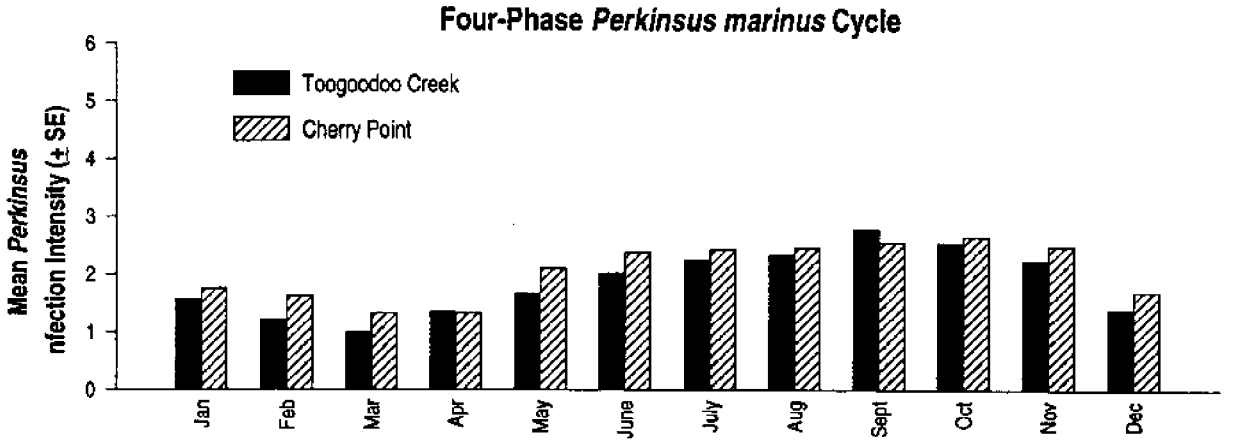

Figure 27. Mean infection intensity of *Perkinsus marinus* in oysters from Toogoodoo Creek (site #36) and Cherry Point (site #35). Monthly data (1986-1991) are combined for each site to illustrate Crosby and Roberts (1990) four phase *P. marinus* cycle.

P. marinus does not appear to produce the high mortalities often observed in the northeast or Gulf of Mexico. As shown in Figure A-9, a great deal of temporal variation in water/air temperatures is encountered by an intertidal oyster (see also O'Beirn et al. 1996b). Future work will explore the relationship between temperature (both intertidal and subtidal) and disease intensity. Ongoing work in L. Burnett's lab (e.g., Dwyer and Burnett 1996) on the interaction between aerial exposure, oyster physiology and *P. marinus* may shed some light in this regard.

Significantly, our extensive 24 year dataset (summarized in Figures 4 & 8) supports the conclusion that the most severe *P. marinus* intensities (> 3.0) always occur during the summer and early fall months, with winter declines. However, no time exists when oyster populations are completely free of the pathogen (Figures 4 & 8). Differences between South Carolina and regions further north, do not appear to explain observed differences in the impact of *P. marinus*. South Carolina's winters are milder and its reproductive season longer, as compared to more northern oyster growing areas. Warmer temperatures generally favor *Perkinsus marinus* (Andrews 1988), suggesting that it should be more of a problem in South Carolina, than in the Chesapeake or Delaware Bays, but oysters here apparently are not subject to the mass mortalities commonly observed in the northeast (Dame 1993 and references therein, Coen et al. 1997, W. D. Anderson SCDNR pers. comm.). Surprisingly, as shown at the long-term oyster research sites, summer intertidal exposure temperatures (often greater than 50°C) should favor very high *P. marinus* levels; however, work in progress suggests that elevated temperatures may kill the parasite (Bushek et al. 1996c).

Many studies have observed a correlation between *Perkinsus marinus* infection intensity and prevalence with variation in salinity (Soniat 1985, Ray 1987, Crosby and Roberts 1990, Powell et al. 1992). For example, Gauthier et al. (1990) found that *P. marinus* infection was highly correlated with large scale, long-term climatic conditions. Many investigations have also indicated that oyster mortality due to *P. marinus* infection has been suppressed at low salinities (Ray 1954, Andrews and Hewatt 1957, Scott et al. 1985, Ragone and Burreson 1993). In fact, Ragone and Burreson (1993) indicated that 9 - 12 ppt was the critical range for *P. marinus* activity; however, *P. marinus* in oysters exposed to low salinities (6 - 12 ppt) for nearly 2 months at temperatures >20°C was not eradicated. Ragone and Burreson (1993) also indicated that infected oysters exposed to low salinities did reduce oyster mortality; however, a decrease in *Perkinsus marinus* prevalence was not observed.

Enigmatically, most South Carolina oyster populations inhabit estuaries with year round salinities typically in the range of 20 - 35 ppt. Crosby and Roberts (1990) noted that although salinity was correlated with *P. marinus* infection in South Carolina, changes in salinity only accounted for 3.6% of the variability in infection intensity. Similarly, Craig et al. (1989) indicated that salinity explained only 20% of the site-to-site variability in infection intensity in Gulf Coast oysters. Only for the Toler's Cove and Inlet Creek sites (Sites 15 & 16) do we have extensive long-term

physical data sets (48 min intervals over two years using Hydrolab sensors). For all other sites, salinity values are from single surface measurements taken on the day of sampling. These values do not reflect the range of salinities and/or temperatures to which an oyster is exposed, which is necessary to fully evaluate the interaction between disease and environmental factors (Craig et al. 1989, Coen et al. 1995, 1997, unpublished data).

Haplosporidium nelsoni Patterns (MSX)

Haplosporidium nelsoni caused high mortalities in oyster planting and seed beds in Delaware and Chesapeake Bays in the late 1950s (Haskin et al. 1965, Haskin and Andrews 1988). Oyster production in Delaware Bay had dropped from about 8 million pounds of meats in 1953 to 167,000 pounds by 1960 partially a direct result of *H. nelsoni* (Sindermann and Rosenfield, 1967). Similarly in Chesapeake Bay, oyster production fell from 39.2 million pounds of oyster meats in 1955 to less than 4.1 million pounds in 1989 (USDOC 1990, as cited in Lewis et al. 1992). *Perkinsus marinus* and *H. nelsoni* had a combined effect in reducing oyster production in Chesapeake Bay to record lows (Lewis et al. 1992).

H. nelsoni has been found previously in South Carolina (Dougherty et al. 1993, Bobo et al. 1996, Bobo unpublished data). However, its geographic distribution in South Carolina, as indicated in this study, is unknown. A total of 1,924 individuals has been examined from 21 sites since 1994. Of these, approximately 8% (or 150 individuals) were infected with *Haplosporidium nelsoni* (MSX), with the parasite present in oysters from 52% (or 11 of 21) of the sampling stations. Disease intensity among individually infected animals varied from light to heavy. Prevalence levels ranged from to 0 - 42%, with the highest prevalence occurring in oysters from Toler's Cove sampled in October 1994. Of the 150 individuals with *H. nelsoni* infections, 9% (or 27 of 300) were infected from the Grice-Charleston Harbor station, 3% (or 28 of 824) were infected from the 1994 South Carolina Summer Oyster Study sites and 8% (or 32 of 400) from Inlet Creek and nearly 16% (or 63 of 400) from the Toler's Cove Marina site.

In comparison, Crosby and Roberts (1990) did not detect *H. nelsoni* in oysters examined in June 1988 from North Inlet Estuary, South Carolina. Elsewhere in the southeast, for example in North Carolina, Morrison et al. (1992) examined oyster hemolymph for *H. nelsoni*. Thirty-one percent of their sites detected *H. nelsoni*. In Georgia, *H. nelsoni* was not observed in oyster samples from 1966 and 1968, but, was first observed in January 1986 (Lewis et al. 1992). Although heavy (7.0 on their scale) *H. nelsoni* intensity levels were observed in a few of their oysters, *H. nelsoni* was probably not the principal agent responsible for mortalities because of its low prevalence and intensity in the sample population; the parasite was diagnosed in only eight animals from four sites during 1986 and 1987 (Lewis et al. 1992). To date, no mortalities have been documented in South Carolina due to *H. nelsoni*, despite our apparently higher prevalence.

Littlewood et al. (1990) studied *Haplosporidium nelsoni* infections in *Crassostrea virginica* grown at five intertidal levels. Although these researchers concluded that there was no statistically significant difference in *H. nelsoni* infection rates of subtidally-derived oysters grown at varying intertidal levels, a different response to *H. nelsoni* may occur in native South Carolina intertidal oysters. South Carolina oysters, which typically encounter long periods of submergence and exposure with extreme temperature ranges, may respond differently to both *H. nelsoni* and *P. marinus* disease challenges as compared to subtidal oyster populations from the northeast or Gulf of Mexico.

Management Implications

Lewis et al. (1992) suggest that the following key strategies be considered to minimize the effects of the disease caused by *P. marinus*: (1) avoid diseased seed stock transplantation; (2) reduce the time oysters are exposed to the disease, either by reducing the legal harvest size of oysters or by planting seed in the fall and winter after the disease progression has been slowed due to decreased water temperatures; and (3) isolate grow-out areas from identified diseased areas (Andrews and Ray, 1988). Bushek and Allen (1996a) recommend that management programs be made aware of the potential danger of spreading *P. marinus* races when relaying oysters, restocking oyster beds, and/or not restricting effluents from shucking houses. Because of elevated water temperatures in the southeast,

Andrews and Ray (1988) suggest that management strategies may be different for the Gulf of Mexico versus the Chesapeake Bay. We do not currently know if *P. marinus* from different locales vary little or consist of many discrete races. Hence, research from one locale may not be transferable to another (Bushek and Allen 1996a), especially if environments vary. These may apply to South Carolina oyster/parasite populations. South Carolina's climate is warmer and oyster beds are primarily intertidal (see Introduction Section). As indicated by Lewis et al. (1992) for Georgia, it may be reasonable to expect differences in the ecology and dynamics of pathogens in the southeast, as compared with the northeastern United States. Recent ongoing studies reported at the National Shellfish Association meetings (Bobo et al. 1996, Coen et al. 1996, Hadley et al. 1996) support this. Ragone and Burreson (1993) suggested that in order to reduce oyster mortality, disease levels and salinities on oyster reefs be closely monitored, and be taken into consideration when deciding harvesting and management strategies.

Haplosporidium nelsoni is typically intolerant of salinities below 10 ppt (Andrews 1964, Haskin and Ford 1982, Ford 1985), leading managers to emphasize the use of lower salinity growout areas to minimize the effects of *H. nelsoni* on oysters (Ford and Haskin 1988). The introduction of infected animals into previously unaffected sites via transplantation of seed and shell stock may be of great concern (Lewis et al. 1992 and references therein). Ewart and Ford (1993) also indicated that transferring wild oysters from infested to non-infested areas should be avoided. Elsewhere, mortalities with long-lasting consequences has been linked to the movement of shellfish stocks (Farley 1992). Perhaps the progressive spread of diseases within Chesapeake Bay may be linked to the movement of infected seed stock (Lewis et al. 1992).

The development of disease-resistant stocks through selective breeding techniques is an alternate management strategy (Haskin and Ford 1979, Ford 1987, Ewart and Ford 1993, Bushek and Allen 1996a,b). Cheng et al. (1994) suggested that *C. virginica* with lathyrose on the hemocyte surface may serve as a marker for innate resistance to *H. nelsoni*. However, Ford and Haskin (1988) noted that extremely heavy *H. nelsoni* infection pressure can overwhelm resistant strains. There has been no success in selecting strains for resistance to *P. marinus* infection (Ewart and Ford 1993).

There has historically been less fishing pressure on South Carolina oysters than those populations in either the Chesapeake or Delaware Bays. This may be the result of: (1) differences in growth form as described above (i.e. dense clusters are more difficult to market); (2) labor shortages; and (3) loss of a cannery-based industry. Because South Carolina's oyster industry has historically been relatively small when compared to that in either Chesapeake and Delaware Bays or the Gulf of Mexico, it has been suggested that harvesting, which selects the larger, more resistant oysters, may slow or even prevent the development of resistant populations (Ray, cited in Ford and Tripp 1996). Less fishing pressure in South Carolina may have enabled more of these 'resistant' oysters to survive and select for resistant population. Bushek and Allen (1996b) in their work with races of *P. marinus* indicate that parasite races may serve an important role in the development of resistant oyster stocks. Hence, South Carolina intertidal oysters may contribute to the production of resistant oysters in future selective breeding efforts.

Recommendations, Current and Future Work

Since 1986, anecdotal reports from oystermen have suggested that oyster die-offs in South Carolina have increased. A variety of factors may have contributed to these dieoffs, including pathogens and environmental conditions. A systematic statewide survey for oyster diseases and associated conditions is required to obtain information needed to assess if these reports are symptomatic of a widespread problem. Such a study would provide baseline information to address this important issue. In addition, knowledge on oyster condition could be compared to our historical baseline data to assess the magnitude and direction of any present and future trends. Recently, we have initiated an intensive long-term study at several sites (Oyster Reef Ecosystem Project) supported by SCDNR, the SC Sea Grant Consortium and SC Marine Recreational Fisheries Stamp Program. This program has begun to gather the information required to assess the value and function of intertidal oyster reef habitats, which are quite distinct from subtidal oyster reef habitats that are predominant in other areas (Wenner et al. 1996, Coen et al. 1997).

This year (1996-1997), MRRI's Shellfish Research Section and OFM's Shellfish Management Section have initiated a joint monitoring program supported by SCDNR and revenue funds to initiate the first state-wide shellfish disease monitoring/research program to assist the SCDNR in its mission to protect and conserve the state's natural resources. We will determine *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) disease levels in native populations from approximately 60 sites throughout South Carolina. The proposed program's objectives are to generate a broad scale *P. marinus* and *H. nelsoni* status and trends evaluation of selected sites in South Carolina estuaries. A smaller scale component will evaluate growth, spat set and the epizootiology of the above diseases by building on our initial Oyster Reef Ecosystem Project results using MRRI's specific-pathogen-free (SPF) oysters. Oyster disease monitoring will provide information on the annual abundance and distribution of *P. marinus* and *H. nelsoni* for resource managers, resource constituents and scientists. Disease abundance and distribution data is critical in making sound management decisions relating to shell transplant efforts, where to plant seed oysters and when to harvest to minimize loses from diseases. The above disease monitoring program will also provide information to better understand the relationship between environmental factors and distribution and abundance of both pathogens. This will increase our future predictive capabilities, as environmental conditions change.

To date, our research efforts on oyster reefs/disease have resulted in: (1) first seasonal data set of *Haplosporidium nelsoni* in SC (also *Perkinsus marinus*) with associated environmental data; (2) initiation of a broader scale *P. marinus* and *H. nelsoni* summer sampling program across South Carolina; (3) large scale production of specific-pathogen-free (SPF) oysters for disease research; (4) assessment of the use of SPF-oysters as an indicator of ecosystem health (growth rates, onset of disease, mortality rates); (5) experimental studies quantifying use of oyster reefs by fishes and crustaceans and; (6) use of experimental oyster reefs to evaluate development, value and functional importance as a critical habitat.

The importance of assessing the intensity and prevalence of oyster diseases in SC includes: (1) disease data gathered will assist in answering questions concerning when die-offs occur. Infection intensity levels are valuable tools in assessing possible causes; (2) it will address why SC oysters have remained abundant in the face of these two diseases, compared to other areas. This is important for management, and requires monitoring the status of our populations; (3) continue monitoring our oyster populations for *H. nelsoni* infections monthly is critical in determining disease patterns. Could there be a potential problem? Is *H. nelsoni* common throughout SC estuaries?; (4) if die-offs start to occur more frequently, we need to assist in a possible relaying management strategy by indicating areas of least intensity and prevalence. However, we should be very cautious regarding moving oysters around the state. Only through a planned monitoring program can we avoid some of the risks that may occur and; (5) experimental studies on the onset of diseases, utilization of the body burden technique for disease certification and to assess very light *P. marinus* infections, and the possible resistance of SC oysters provide important information.

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LITERATURE CITED

Andrews, J.D. 1954. Notes on the fungus parasites of bivalve mollusks in Chesapeake Bay. Proceedings of the National Shellfisheries Association 45:157-163.

Andrews, J.D. 1961. Measurement of shell growth in oysters by weighing in water. Proceedings of the National Shellfisheries Association 52:1-11.

Andrews, J.D. 1964. Oyster mortality studies in Virginia. IV. MSX in James River public seed beds. Proceedings of the National Shellfisheries Association 53:65-84.

Andrews, J.D. 1965. Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. Chesapeake Science 6:60-67.

Andrews, J.D. 1967. Interaction of two diseases of oysters in natural waters. Proceedings of the National Shellfisheries Association 67:38-49.

Andrews, J.D. 1968. Oyster mortality studies in Virginia. VII. Review of epizootiology and origin of *Minchinia nelsoni*. Proceedings of the National Shellfisheries Association 58:23-36.

Andrews, J.D. 1976. Epizootiology of oyster pathogens *Minchinia nelsoni* and *M.* costalis. *Proceedings International Colloquium on Invertebrate Pathology 1:169-171.*

Andrews, J.D. 1979. Oyster diseases in Chesapeake Bay. Marine Fisheries Review 41:45-53.

Andrews, J.D. 1982. Epizootiology of late summer and fall infections of oysters by *Haplosporidium nelsoni*, and comparison to annual life cycle of *Haplosporidium costalis*, a typical Haplosporidium. Journal of Shellfish Research 2:15-23.

Andrews, J.D. 1983. *Minchinia nelsoni* (MSX) infections in the James River seed oyster area and their expulsion in spring. Estuarine, Coastal and Shelf Science 16:255-269.

Andrews, J.D. 1984a. Epizootiology of diseases of oysters (*Crassostrea virginica*), and parasites of associated organisms in eastern North America. Helgoläender Meeresuntersuchungen 37:149-166.

Andrews, J.D. 1984b. Epizootiology of Haplosporidium diseases affecting oysters. Comparative Pathobiology 7:243-269.

Andrews, J.D. 1988. Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. Pages 47-63 *in* W. S. Fisher, editor. Disease processes in marine bivalve molluscs. Volume 18. Special Publication. American Fisheries Society, Bethesda.

Andrews, J.D., and W.G. Hewatt. 1957. Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. Ecological Monographs 27:1-25.

Andrews, J.D., and S.M. Ray. 1988. Management strategies to control the disease caused by *Perkinsus marinus*. Pages 257-264 *in* W. S. Fisher, editor. Disease processes in marine bivalve molluscs. Volume 18. Special Publication . American Fisheries Society, Bethesda.

Anonymous. 1989. Chesapeake Executive Council, Chesapeake Bay oyster management plan, Chesapeake Bay Program, Agreement Commitment Report.

Bahr, L.M., and W.P. Lanier. 1981. The ecology of the intertidal oyster reefs of the South Atlantic coast: a community profile. U.S. Fish and Wildl. Serv. Off. Bio. Serv. Washington, D.C. FWS/OBS-81/15. 105 pp.

Bearden, C. R., R. Low, R. Rhodes, R. Van Dolah, C. Wenner, E. Wenner, and D. Whitaker, editors. 1985. A Review and Analysis of Commercial Shrimp Trawling in the Sounds and Bays of South Carolina. Volume Tech. Rep. 62. South Carolina Marine Resource Center., Charleston, SC.

Beckert, H., D.G. Bland, and E.B. May. 1972. The incidence of *Labyrinthomyxa marina* in Alabama. Alabama Marine Resources Bulletin 8:18-24.

Bobo, M.Y., D.L. Richardson, T.C. Cheng, E. McGovern and L.D. Coen. 1996. Seasonal cycle of *Haplosporidium nelsoni* (MSX) in intertidal oysters *Crassostrea virginica*, in South Carolina. J. Shellfish Res. 15:525.

Blood, E.R., and F.J. Vernberg. 1992. Characterization of the Physical, Chemical, and Biological Conditions and Trends in Winyah Bay and North Inlet Estuaries: 1970-1985, *in* Characterization of the Physical, Chemical and Biological Conditions and Trends in three South Carolina Estuaries: 1970-1985. Volume 2, III-1 through III-117. South Carolina Sea Grant Consortium, Charleston, SC.

Burrell, V.G., M.Y. Bobo, and J.J. Manzi. 1984. A comparison of seasonal incidence and intensity of *Perkinsus marinus* between subtidal and intertidal oyster populations in South Carolina. Journal of the World Mariculture Society 15:301-309.

Burrell, V.G., J.J. Manzi, and C.B. O'Rourke. 1989. Assessment of mechanical transplanting as a means of rehabilitating intertidal oyster beds. Proceedings of the 40th Gulf and Caribbean Fisheries Institute: 228-240.

Burreson, E.M. 1988. Use of immunoassays in Haplosporidium life cycle studies. American Fisheries Special Publication 18:298-303.

Burreson, E.M. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica* (Gmelin, 1791): I. susceptibility of native and MSX-resistant stocks. Journal of Shellfish Research 10:417-424.

Burreson, E.M. 1996. 101 uses for the small subunit ribosomal RNA gene: applications to *Haplosporidium nelsoni*. Journal of Shellfish Research 15:475.

Burreson, E.M., and J.D. Andrews. 1988. Unusual intensification of Chesapeake Bay oyster diseases during recent drought conditions. Oceans '88 Proceedings, pp. 789-802.

Burreson, E.M. and L.R. Calvo. 1994. Status of the major oyster diseases in Virginia- 1993. Summary of the annual monitoring program. VIMS Report 93-5.

Burreson, E.M., R.S. Alvarez, V.V. Martinez and L.A. Macedo. 1994. *Perkinsus marinus* (Apicomplexa) as a potential source of oyster *Crassostrea virginica* mortality in coastal lagoons of Tabasco, Mexico. Diseases of Aquatic Organisms 20:77-82.

Bushek, D., S.E. Ford, and S.K. Allen Jr. 1994. Evaluation of methods using Ray's fluid thioglycollate medium for diagnosis of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*. Annual Review of Fish Diseases 4:201-217.

Bushek, D., and S.K. Allen Jr. 1996a. Races of *Perkinsus marinus*. Journal of Shellfish Research 15:103-107.

Bushek, D., and S.K. Allen Jr. 1996b. Host-parasite interactions among broadly distributed populations of the eastern oyster, *Crassostrea virginica* and the protozoan *Perkinsus marinus*. Marine Ecology Progress Series 139:127-141.

Bushek, D., R. Holley, and M. Kelley. 1996c. Chlorine tolerance of *Perkinsus marinus*. Abstract, International Conference of Shellfish Restoration, Hilton Head, South Carolina, November 1996.

Cheng, T.C., V.G. Burrell, Jr., J.J. Manzi, and F.O. Perkins. 1995. Natural and experimental infection of *Mercenaria mercenaria* by *Perkinsus marinus* from *Crassostrea virginica*. Research and Reviews in Parasitology 55:31-34.

Cheng, T.C., W.J. Dougherty, and V.G. Burrell, Jr. 1994. A possible hemocyte for resistance to *Haplosporidium nelsoni* in the oyster *Crassostrea virginica*. Research and Reviews in Parasitology 54:51-54

Choi, K.S., E.A. Wilson, D.H. Lewis, E.N. Powell, and S.M. Ray. 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. J. Shellfish. Res. 8:125-131.

Chu, F.E., and J.F. LaPeyre. 1991. Effect of salinity in *Perkinsus marinus* susceptibility and defense-related activities in eastern oysters, *Crassostrea virginica*. Journal of Shellfish Research 10:294.

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

Coen, L.D., E.L. Wenner, D.M. Knott, and N.H. Hadley. 1995. Final seed project report, South Carolina Sea Grant Consortium, project entitled "Preliminary studies of oyster reefs as biologically-critical estuarine ecosystems.

Coen, L.D., E.L. Wenner, D.M. Knott, B. Stender, N.H. Hadley, M.Y. Bobo. 1996. Intertidal oyster reefs as critical estuarine environments: evaluating habitat use, development and function. J. Shellfish Res. 15:490.

Coen, L.D., D.M. Knott, E.L. Wenner, N.H. Hadley, and A.H. Ringwood. 1997. Intertidal oyster reef studies in South Carolina: design, sampling and experimental focus for evaluating habitat value and function. *In:* M.W. Luckenbach, R. Mann, J.A. Wesson (eds.), Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches. Virgina Institute of Marine Science Press, Gloucester Point, VA.

Craig, A., E. N. Powell, R. R. Fay, and J. M. Brooks. 1989. Distribution of *Perkinsus marinus* in Gulf Coast oyster populations. Estuaries 12:82-91.

Crosby, M.P., and C.F. Roberts. 1990. Seasonal infection intensity cycle of the parasite *Perkinsus marinus* (and an absence of *Haplosporidium* spp.) in oysters from a South Carolina salt marsh. Diseases of Aquatic Organisms 9:149-155.

Dame, R.F. 1972a. Comparison of various allometric relationships in intertidal and subtidal American oysters. Fishery Bulletin 70:1121-1126.

Dame, R.F. 1972b. The ecological energies of growth, respiration and assimilation in the intertidal American oyster, *Crassostrea virginica*. Marine Biology 17:243-250.

Dame, R.F. 1979. The abundance, diversity, and biomass of macrobenthos on North Inlet, South Carolina, intertidal oyster reefs. Proceedings of the National Shellfisheries Association 69:6-10.

Dame, R.F., ed. 1993. Bivalve filter feeders in estuarine and coastal ecosystem processes. Springer-Verlag, Berlin, 579 pp.

Dame, R., and S. Libbes. 1993. Oyster reefs and nutrient retention in tidal creeks. Journal Experimental Marine Biology and Ecology 171:251-258.

Dame, R.F., R.G. Zingmark, and E. Haskin. 1984. Oyster reefs as processors of estuarine materials. Journal Experimental Marine Biology and Ecology 83:239-247.

DeVoe, M.R. 1992. "Executive Summary". Pages 1-20 *in* Characterization of the Physical, Chemical, and Biological Conditions and Trends in Three South Carolina Estuaries: 1970-1985. Volume 2. South Carolina Sea Grant Consortium., Charleston, SC.

Dougherty, W.J., T.C. Cheng, and V.G. Burrell. 1993. Occurrence of the pathogen *Haplosporidium nelsoni* in oysters, *Crassostrea virginica*, in South Carolina, USA. Trans. Am. Microsc. Soc. 112(1):75-77.

Dwyer, J.J., III, and L.E. Burnett. 1996. Acid-base status of the oyster *Crassostrea virginica* in response to air exposure and to infections by *Perkinsus marinus*. Biological Bulletin 190:139-147.

Engle, J.B., and A. Rosenfield. 1963. Progress in oyster mortality studies. Proceedings of the Gulf and Caribbean Fisheries Institute 15:116-124.

Ewart, J.W., and S.E. Ford. 1993. History and impact of MSX and Dermo diseases on oyster stocks in the Northeast region. NRAC Fact Sheet 200, Northeastern Regional Aquaculture Center, U. Mass. Dartmouth.

Farley, C.A. 1965. Pathologic responses of the oyster, *Crassostrea virginica* (Gmelin), to infection by the protistan parasite, MSX. Am. Malacol. Union Bull. 32:23-24.

Farley, C.A. 1968. *Minchinia nelsoni* (Haplosporida, Haplosporidiidae) disease syndrome in the American oyster, *Crassostrea virginica*. Journal of Protozoology 15:585-599.

Farley, C.A. 1992. Mass mortalities and infectious lethal diseases in bivalve mollusks and associations with geographic transfers of populations. Pages 139-154, *in* A. Rosenfield and R. Mann, editors. Dispersal of living organisms into aquatic ecosystems. Md. Sea Grant Coll. Publ, Univ. MD., College Park, MD.

Fisher, W.S., and R.I.E. Newell. 1986. Seasonal and environmental variation in the protein and carbohydrate levels in the hemolymph from American oysters (*Crassostrea virginica* Gmelin). Comparative Biochemistry and Physiology 85(A):365.

Ford, S.E. 1985. Effects of salinity on survival of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber, and Macklin) in oysters. Journal of Shellfish Research 5:85-90.

Ford, S.E. 1987. Progress on the development of MSX-resistant oyster strains. Pages 12-14 *in* A. White, editor. Shellfish diseases: Current concerns in the northeast. Woods Hole Oceanogr. Inst., Tech Rep. 87-13.

Ford, S.E. 1992. Avoiding the transmission of disease in commercial culture of molluscs, with special reference to *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX). Journal of Shellfish Research 11:539-546.

Ford, S.E. 1996. Range extension by the oyster parasite *Perkinsus marinus* into the northeastern United States: responses to climate change? Journal of Shellfish Res. 15:45-56.

Ford, S.E., and H.H. Haskin. 1982. History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957-1980. Journal of Invertebrate Pathology 40:118-141.

Ford, S.E., and H.H. Haskin. 1988. Management strategies for MSX (*Haplosporidium nelsoni*) disease in eastern oysters. Pages 249-256, *in* W. S. Fisher, editor. Disease processes in marine bivalve molluscs. Volume Special Publication 18. American Fisheries Society, Bethesda.

Ford, S.E., and M.R. Tripp. 1996. Diseases and defense mechanisms. Pages 581-660, *in* A.F. Eble, V.S. Kennedy, R.I.E. Newell, eds. The eastern oyster, *Crassostrea virginica*. Maryland Sea Grant College Park, MD.

Frankenburg, D. 1995. North Carolina Blue Ribbon Advisory Council on Oysters: Final Report on Studies and Recommendations. 41 pp + Appendices.

Fuxa, J. R., and Y. Tanada, editors. 1987. Epizootiology of Insect Diseases. John Wiley & Sons, Inc., New York.

Gauthier, J.D., T.M. Soniat, and J.S. Rogers. 1990. A parasitological survey of oysters along salinity gradients in coastal Louisiana. Journal of the World Aquacultural Society 21:105-115.

Gerritsen, J., A.F. Holland, and D.E. Irvine. 1994. Suspension-feeding bivalves and the fate of primary production: an estuarine model applied to Chesapeake Bay. Estuaries 17:403-416.

Gibbons, M.C., and F.-L.E. Chu. 1989. Does tidal zonation affect the intensity and incidence of *Perkinsus marinus* in juvenile American oysters in Virginia? Journal of Shellfish Research 7:572.

Hadley, N.H., M.Y. Bobo, D.L. Richardson, L.D. Coen and D. Bushek. 1996. Use of specific-pathogen-free (SPF) oysters to measure growth, mortality, and onset of MSX and Dermo disease in South Carolina. J. Shellfish Res. 15:496.

Haskin, H.H. 1961. Delaware Bay oyster mortalities. Proceedings of the Gulf and Caribbean Fisheries Institute 13:109.

Haskin, H.H., and S.E. Ford. 1979. Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. Marine Fisheries Review 41(1-2):54-63.

Haskin, H.H., and S.E. Ford. 1982. *Haplosporidium nelsoni* (MSX) on Delaware Bay seed oyster beds: A host-parasite relationship along a salinity gradient. J. of Invertebrate Pathology 40:388-405.

Haskin, H.H., and J.D. Andrews. 1988. Uncertainties and speculations about the life cycle of the eastern oyster pathogen *Haplosporidium nelsoni* (MSX). Pages 5-22 *in* W. S. Fisher, editor. Disease processes in marine bivalve molluscs. Volume Special Publication 18. American Fisheries Society, Bethesda.

Haskin, H.H., W.J. Canzonier, and J.L. Myhre. 1965. The history of MSX on Delaware Bay oyster grounds, 1957-65. Am. Malacol. Union Bull. 32:20-21.

Haskin, H.H., L.A. Stauber, and J.G. Mackin. 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae) causative agent of the Delaware Bay oyster epizootic. Science 153:1414-1416.

Haven, D.S., W.J. Hargis, and P.C. Kendall. 1978. The oyster industry of Virginia: its status, problems and promise. Virginia Sea Grant Special Paper #4:1024pp.

Heck, K.L. 1987. Lecture notes on coastal and estuarine studies #23. Pages 97-110 *in* K. L. Heck Jr., editor. Benthos, In: Ecological studies in the middle reach of Chesapeake Bay: Calvert Cliffs. Calvert Cliffs, Springer-Verlag, Berlin.

Hewatt, W.G., and J.D. Andrews. 1956. Temperature control experiments on the fungus disease *Dermocystidium marinum* of oysters. Proceedings of the National Shellfisheries Association 46:129-133.

Hofstetter, R.P. 1977. Trends in population levels of the American oyster *Crassostrea virginica* Gmelin on public reefs in Galveston Bay, Texas. Tex. Parks Wildl. Dep. Tech. Ser., 24:1-90.

Hopkins, S.H. 1956. The boring sponges which attack South Carolina oysters, with notes on some associated organisms. Contributions of the Bears Bluff Laboratory 23:30.

Howard, D.W., and C.S. Smith. 1983. Histological techniques for marine bivalve mollusks. NOAA Technical Memorandum NMFS-F/NEC 25:97.

Klemanowicz, K.J. 1985. Effects of a mechanical oyster harvester on macrofaunal community structure. MS Thesis. The College of Charleston. Charleston, SC, 102 pp.

Levine, N.D. 1978. *Perkinsus* gen. n. and other new taxa in the protozoan phylum Apicomplexa. Journal of Parasitology 64:549.

Lewis, E.J., F.G. Kern, A. Rosenfield, S.A. Stevens, R.L. Walker, and P.B. Heffernan. 1992. Lethal parasites in oysters from coastal Georgia with discussions of disease management implications. Marine Fisheries Review 54:1-6.

Littlewood, D.T.J., R.N. Wargo, and J.N. Kraeuter. 1990. Growth, mortality, MSX infection and yield of intertidally grown *Crassostrea virginica*. Journal of Shellfish Research 8:469.

Luckenbach, M., R. Mann, and J. Wesson. 1995. Oyster Reef Restoration Symposium Proceedings, held in Williamsburg, VA, April, 1995.

Lunz, R.G. 1950. Production and yield of the oyster canning industry of South Carolina. Contributions of the Bears Bluff Laboratory 9.

Mackin, J.G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, Collier. Bull. Mar. Sci. Gulf Caribb. 1:72-87.

Mackin, J.G. 1960. Status of researchers on oyster disease in North America. Proceedings of the Gulf and Caribbean Fisheries Institute 13:98-113.

Mackin, J.G. 1962. Oyster diseases caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publications of the Institute of Marine Science, University of Texas 7:132-229.

Mackin, J.G., and J.L. Boswell. 1956. The life cycle and relationships of *Dermocystidium marinum*. Proceedings of the National Shellfisheries Association 46:112-115.

Mackin, J.G., and S.M. Ray. 1966. The taxonomic relationships of *Dermocystidium marinum* Mackin, Owen, Collier. Journal of Invertebrate Pathology 8:544-545.

Mackin, J.G., H.M. Owen, and A.Collier. 1950. Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gemlin). Science (Washington, DC) 111:328-329.

Maggioni, G.J., and V.G. Burrell. 1982. South Carolina oyster industry. Pages 132-137, *in* K. Chew, ed. Proceedings of the North American Oyster Workshop. Special Publication No. 1. Louisiana State University, Baton Rouge, LA.

Mathews, T.D., F.W. Stapor , Jr., C.R. Richter, J.V. Miglarese, M.D. McKenzie, and L.A. Barclay, editors. 1980. Ecological Characterization of the Sea Island coastal Region of South Carolina and Georgia. Volume I: Physical Features of the Characterization Area. U.S. Fish & Wildlife Service, Office of Biological Services, FWS/OBS-79/40, Washington, DC.

Mathews, T.D., M H. Shealy, Jr., and N. Cummings, editors. 1981. Hydrography of South Carolina Estuaries, with Emphasis on the North and South Santee and Charleston Harbor-Cooper River Estuaries. Volume Tech. Report No. 47. South Carolina Marine Resources Center, Charleston, SC.

Menzel, R.W., and S.H. Hopkins. 1955. The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus.* Journal of Parasitology 41:333-342.

Meyers, J.A., E.M. Burreson, B.J. Barber, and R. Mann. 1991. Susceptibility of diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg, 1973) and eastern oysters, *Crassostrea virginica* (Gmelin, 1791) to *Perkinsus marinus.* J. Shellfish Res. 10:433-437.

Monbet, Y. 1992. Control of phytoplankton biomass in estuaries: a comparative analysis of microtidal and macrotidal estuaries. Estuaries 15:563-571.

Morrison, N.M., M.D. Marshall, M.J. Dykstra, and J.F. Levine. 1992. *Haplosporidium nelsoni* (MSX) in eastern oyster populations of North Carolina. Journal of Aquatic Animal Health 4:203-206.

National Oceanic and Atmospheric Administration (NOAA). 1990. Estuaries of the United States: Vital Statistics of a National Resource Base. A Special NOAA 20th Anniversary Report., Silver Spring, MD: Strategic Environmental Assessments Division, ORCA.

Newell, R.I.E. 1988. Ecological changes in Chesapeake Bay: are they the result of overharvesting the American oyster *Crassostrea virginica*? pp. 536-546, Understanding the Estuary: Advances in Chesapeake Bay Research, Chesapeake Research Consortium Publ. 129 CBP/TRS 24/88.

O'Beirn, F.X., C.C. Dean, and R.L. Walker. 1994. Prevalence of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica* in relation to tidal placement in a Georgia tidal creek. Northeast Gulf Science 13:79-87.

O'Beirn, F.X., R.L. Walker, M.L. Jansen, and C.R. Spruck. 1996a. Recruitment, gametogenesis and parasite (*Perkinsus marinus*) prevalence in the eastern oyster, *Crassostrea virginica*, with the Sapelo Island National Estuarine Research Reserve. The University of Georgia Technical Report 96-1.

O'Beirn, F.X., P.B. Heffernan, and R.L. Walker. 1996b. Recruitment of the eastern oyster in coastal Georgia: patterns and recommendations. North American Journal of Fisheries Management 16:413-426.

Officer, C.B., T.J. Smayda, and R. Mann. 1982. Benthic filter feeding: a natural eutrophication control. Marine Ecology Progress Series 9:203-210.

Ogle, J., and K. Flurry. 1980. Occurrence and seasonality of *Perkinsus marinus* (Protozoa: Apicomplexa) in Mississippi oysters. Gulf Research Reports 6:423-425.

Orlando, S.P., Jr., P.H. Wendt, C.J. Klein, M.E. Pattillo, K.C. Dennis, and G.H. Ward, editors. 1994. Salinity Characteristics of South Atlantic Estuaries. National Oceanic and Atmospheric Administration, Office of Ocean Resources Conservation and Assessment., Silver Spring, MD, 117 pp.

Paynter, K. T., and E M. Burreson. 1991. Effects of *Perkinsus marinus* infection in the Eastern oyster *Crassostrea virginica*: II. Disease development and impact on growth rate at different salinities. Journal of Shellfish Research 10:424-431.

Perkins, F.O. 1976. *Dermocystidium marinum* infection in oysters. U. S. National Marine Fisheries Service Marine Fisheries Review 38:19-21.

Perkins, F.O. 1988. Structure of protistan parasites found in bivalve molluscs. American Fisheries Society Special Publication 18:93-111.

Perkins, F.O., and R.W. Menzel. 1966. Morphological and cultural studies of a motile stage in the life cycle of *Dermocystidium marinum*. Proceedings of the National Shellfisheries Association 56:23-30.

Powell, E.N., J.D. Gauthier, E.A. Wilson, A. Nelson, R.R. Fay, and J.M. Brooks. 1992. Oyster disease and climate change. Are yearly changes in *Perkinsus marinus* parasitism in oysters (*Crassostrea virginica*) controlled by climatic cycles in the Gulf of Mexico? Marine Ecology PSZNI 13:243-270.

Preece, A. 1972. A manual for histologic technicians. Little, Brown and Co., Boston, MA., 428 pp.

Quick, JA, Jr., and J.G. Mackin. 1971. Oyster parasitism by *Labyrinthomyxa marinum* in Florida. Florida Dept. Nat. Res. Mar. Res. Lab. Prof. Paper Series 13:55.

Ragone, L.M., and E.M. Burreson. 1993. Effect of salinity on infection progression and pathogenicity of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica* (Gmelin). Journal of Shellfish Research 12:1-7.

Ray, S.M. 1952. A culture technique for the diagnosis of infections with *Dermocystidium marinum*, Mackin, Owen, Collier, in oysters. Science 166:360-361. Washington, DC.

Ray, S.M. 1954a. Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. Rice Institute Pamphlet (special issue). The Rice Institute, Houston, Texas.

Ray, S.A. 1954b. Studies on the occurrence of *Dermocystidium marinum* in young oysters. Proceedings of the National Shellfisheries Association (Convention addresses, 1953.) 44:80-92.

Ray, S.A. 1966. A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. Proceedings of the National Shellfisheries Association 54:55-69.

Ray, S.A. 1987. Salinity requirements of the American oyster, *Crassostrea virginica*. Pages E.1-E.28 *in* A. J. Mueller and G. A. Matthews, editors. Freshwater Inflow Needs of the Matagorda Bay System with Focus on Penaeid Shrimp. U.S. Dept. Commerce, NOAA Tech. Mem. NMFS-SEFC-189.

Ray, S.M., and J.G. Mackin. 1955. Studies of pathogenesis of *Dermocystidium marinum*. Proceedings of the National Shellfisheries Association 45:164-167.

Ray, S.M., J.G. Mackin, and J.L. Boswell. 1953. Quantitative measurement of the effect on oysters of disease caused by *Dermocystidium marinum*. Bull. Mar. Sci. Gulf Caribb. 3:6-33.

Ringwood, A.H., R.F. Van Dolah, A.F. Holland, and M.E. DeLorenzo. 1995. Year One Demonstration Project Studies Conducted in the Carolinian Province by Marine Resources Research Institute: Results and Summaries. Final Report

Rothschild, B.J., J.S. Ault, P. Goulletquer, and M. Héral. 1994. Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. Marine Ecology Progress Series 111:29-39.

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

Sindermann, C.J. 1990. Principal diseases of marine fish and shellfish: diseases of marine shellfish, 2nd Edition. Volume 2. Academic Press, San Diego, 516 pp.

Sindermann, C. J., and A. Rosenfield. 1967. Principal diseases of commercially important marine bivalve mollusca and crustacea. Fishery Bulletin 66:335-385.

Smith, G.F., and S.J. Jordan. 1992. Monitoring Maryland's Chesapeake Bay oysters. A comprehensive characterization of modified Fall survey results, 1990-1991. Maryland DNR Chesapeake Bay Research and Monitoring Division. CBRM-OX-93-3.

Soniat, T.M. 1985. Changes in levels of infection of oysters by *Perkinsus marinus*, with special references to the interaction of temperature and salinity upon parasitism. Northeast Gulf Science 7:171-174.

Soniat, T.M. 1996. Epizootiology of *Perkinsus marinus* disease of eastern oysters in the Gulf of Mexico. Journal of Shellfish Res. 15:35-43.

Sprague, V. 1954. Protozoa. Bull. U.S. Bur. Fish 55:243-256.

Sprague, V. 1970. Recent problems of taxonomy and morphology of Haplosporidia. J. of Parasitology 56:327-328.

Sprague, V. 1978. Comments on trends in research on parasitic diseases of shellfish and fish. Marine Fisheries Review 40:26-30.

Stanley, J.G., and M.A. Sellers. 1986. Species profile: Life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico): American Oyster. Biological Report 82(11.64):25 pp.

Stapor, F.W., Jr. 1984. Sand Transport at Edisto Beach, Colleton County, South Carolina. South Carolina Marine Resources Center Technical Report No. 60.7

Ulanowicz, R.E., and J.H. Tuttle. 1992. The trophic consequences of oyster stock rehabilitation in Chesapeake Bay. Estuaries 15:298-306.

USDOC. 1990. Fisheries of the United States. 1989. U.S. Dept. Commerce NOAA Natl. Mar. Fish. Serv., Corr. Fish. Stat. 8900, 111pp.

Wenner, E.L., H.R. Beatty, and L.D. Coen. 1996. A method for quantitatively sampling nekton on intertidal oyster reefs. Journal of Shellfish Res. 15:769-775.

White, M.E., E N. Powell, S.M. Ray, and E.A. Wilson. 1987. Host to host transmission of *Perkinsus marinus* in oyster (*Crassostrea virginica*) populations by the ectoparasitic snail *Boonea impressa* (Pyramidellidae). Journal of Shellfish Research 6:1-5.

Wilson, E.A., E N. Powell, M.A. Craig, T.L. Wade, and J.M. Brooks. 1990. The distribution of *Perkinsus marinus* in Gulf Coast oysters: relationship with temperature, reproduction, and pollutant burden. International Revue Der Gesamten Hydrobiologie 75:533-550.

Woo, P. T. K., editor. 1995. Fish Diseases and Disorders (Protozoan and Metazoan Infections). Volume 1. Cambridge Press (CAB), Cambridge, 808 pp.

Zimmerman, R., T. Minello, T. Baumer, and M. Castiglione. 1989. Oyster reef as habitat for estuarine macrofauna. NOAA Tech. Mem. NMFS-SEFC-249, 16 pp.

APPENDIX A

Table A-1. *Perkinsus marinus* weighted incidence (mean infection intensity) and prevalence (% infected) in *Crassostrea virginica* from stations in Region B (one time samples) during 1987-1993, (n=25 oysters/site).

Station (Site #)*	Date of Sampling	Weighted Incidence	% Prevalence
Fishing Creek (43)	OCT 87	3.32	100
Fishing Creek (43)	MAR 88	1.00	64
Frampton Creek (40)	APR 88	1.12	71
Alston Creek (18)	JUL 88	3.50	100
Scott Creek (41)	OCT 88	2.72	96
Fishing Creek (43)	OCT 89	2.00	100
South Edisto (45)	OCT 89	1.00	100
Ashepoo River (46)	OCT 89	2.12	100
Long Creek (34)	MAY 90	0.40	32
Inlet Creek (15)	OCT 90	2.76	100
Venning Creek (10)	JUN 91	2.60	100
Wallace Creek (31)	OCT 91	1.84	100
Tom Point Creek (39)	JUL 92	2.76	100
Venning Creek (10)	JUL 92	2.32	98
Secessionville (27)	JUL 92	2.60	100
Swinton Creek (14)	JUL 92	3.29	100
Price's Inlet (11)	APR 93	0.36	28
Long Creek (13)	JUL 93	1.45	67

*See Tables 4 & 5 and Fig. 2 for site locations.

Table A-2. *Perkinsus marinus* weighted incidence (mean infection intensity) and prevalence (% infected) in *Crassostrea virginica* from stations in Region C (one time samples) during 1987-1993, (n=25 oysters/site).

Station (Site #)*	Date of Sampling	Weighted Incidence	% Prevalence
Story Creek (50)	SEP 87	2.83	100
Skull Creek (62)	SEP 88	1.96	92
Hazard Creek (58)	OCT 88	2.28	100
Chowan Creek (54)	OCT 91	1.68	96
Warsaw Flats (52)	OCT 91	2.55	100
Distant Island Creek (53)	NOV 91	2.60	100
Chechesee Creek (60)	JUL 92	2.76	92
Mackay Creek (61)	OCT 92	3.10	100

* See Table A-1

Table A-3. Site characterization chart of all stations sampled. Listed are environmental data ranges, sampling dates and oyster sample habitats. Sites characterized as "degraded" (Ringwood et al. 1995) have clear and distinct elevations in sediment contaminants.

	Station	Temperature Range (°C)	Salinity Range (‰)	Sampling Dates	Habitat +	Remarks
1.	Murrell's Inlet			1988*		OFM
2.	Litchfield			1988 *	high & low intertidal	OFM
3.	Winyah Bay	28.0	36.0	1994 *		
4.	North Santee		5.0 - 22.0	1972 - 1979	subtidal	
5.	South Santee	28.0	22.0	1972 - 1977	subtidal & intertidal	OFM
6.	Alligator Creek			9/72; 11/72; 12/73	subtidal	Native and Wando Transplants
7.	Casino Creek	10.0	17.0	1986*	subtidal	
8.	Cape Romain	32.0	23.0 - 34.0	1989 - 92; 1994	pristine; subtidal	OFM
9.	Bull Bay	7.0 - 30.0	22.0 - 35.0	1977 - 1979; 6/94	subtidal & intertidal	
10.	Venning Creek	28.0	30.0 - 34.0	1991*	subtidal & intertidal	OFM
11.	Price's Inlet	13.0 - 36.0	24.0	1993*		OFM
12.	Capers Inlet	13.0 - 32.0	20.0 - 36.0	1981; 1984; 1989; 1990-91	subtidal & intertidal	
13.	Long Creek	27.0	32.0	1990*	pristine; intertidal	OFM
14.	Swinton Creek			1992*	intertidal	OFM
15.	Inlet Creek ✚	1.2 - 36.0	14.6 - 37.8	10/90; 1994 - 1995	pristine; intertidal	MRRI oyster reef project
16.	Toler's Cove Marina ✚	3.4 - 34.0	19.7 - 35.5	1994 - 1995	developed; intertidal	MRRI oyster reef project
17.	Wando River	7.0 - 32.0	5.0 - 19.0	1973 - 1977; 6/89	subtidal	Temp & salinity ranges are from all stations in Wando
18.	Alston Creek			1988*	subtidal	OFM
19.	Shem Creek	26.5 - 28.0	3.0 - 31.0	1994*	degraded; intertidal	EMAP;CHP
20.	Noisette Creek		10.0	1994*	degraded; intertidal	EMAP
21.	Shipyard Creek	24.5 - 28.0	11.0 - 23.0	11/93; 6 - 7/94	degraded; intertidal	EMAP; CHP
22.	Diesel Creek	27.0 - 34.0	14.0 - 22.0	1994*	degraded; intertidal	EMAP; CHP
22a.	Plum Island Creek		22.4	1994*		
23.	Kopper's Creek	24.0 - 29.0	19.0 - 23.0	10/93; 6 - 7/94	degraded; intertidal	EMAP; CHP
24.	Charleston Harbor/ MRRI/Grice	12.0 - 31.0	15.0 - 25.5	1994 - 1995	pristine; intertidal	MRRI monthly monitoring station
25.	Metcalf Creek/ Stono	28.0 - 35.0	11.0 - 21.0	1994*	degraded; intertidal	CHP
26.	Clark Sound			1994*	intertidal	
27.	Seccessionville Creek			1992*	intertidal	OFM
28.	Lighthouse Creek		29.0 - 32.0	11/93; 6 - 7/94	intertidal	EMAP; CHP
29.	Folly Creek			*	intertidal	
30.	Stono Inlet			*	intertidal	
31.	Wallace Creek	23.0 - 27.5	28.0 - 30.0	1991;1994*	intertidal	OFM
32.	Church Creek	8.0 - 31.0	10.0 - 31.5	2/93 - 2/95	intertidal	
33.	Kiawah Creek			10/77; 6 - 8/94	intertidal	
34.	Long Island	24.0 - 32.0	26.0 - 32.0	1993*	intertidal	OFM
35.	Cherry Point	9.0 - 31.0	21.0 - 36.0	9/86 - 2/95	intertidal	
36.	Toogoodoo Creek	13.5 - 31.0	15.0 - 24.0	9/86 - 12/91	intertidal	
37.	Leadenwah Creek			1994*	intertidal	EMAP
38.	North Edisto Inlet			*	intertidal	
39.	Tom Point Creek			1992*	intertidal	
40.	Frampton Creek	22.0	28.0	1988*	intertidal	
41.	Scott Creek	22.0	35.0	1988*	intertidal	OFM
42.	St. Pierre's Creek		27.5	1980*	pristine; subtidal	Transplanted from Beresford Creek
43.	Fishing Creek	22.0	27.0	1973 - 1974; 3/88	intertidal	OFM
44.	Bailey Creek			1980*	subtidal	Transplanted from Beresford Creek
45.	South Edisto Inlet		20.0 - 25.0	1989*	intertidal	
46.	Ashepoo River	9.0 - 30.0	19.0 - 31.0	1989;1994*	intertidal	
47.	St. Helena Sound		20.0 - 25.0	1994*	pristine; intertidal	EMAP
48.	South Wimbee Creek			1973 - 1974	subtidal	Transplanted from Wando River
49.	Fripp Inlet	28.0	28.0	1994*	intertidal	
50.	Story River		36.0	1988*	intertidal	
51.	Coosaw at Brickyard Point		14.0	1983*	intertidal	
52.	Warsaw Creek	22.0	23.0	1991*	intertidal	OFM
53.	Distant Island Creek			1991*	intertidal	OFM
54.	Chowan Creek	22.0	30.0	1991*	intertidal	
55.	McCally's Creek		13.0	*	intertidal	
56.	Jenkin's Creek	28.0	34.0	1994*	intertidal	
57.	Port Royal Sound		24.0 - 27.0	*	intertidal	
58.	Hazard Creek	22.0	24.0	1988*	intertidal	OFM
59.	Colleton River	22.0 - 30.0	27.0 - 30.0	10/86; 1988; 11/89	high & low intertidal	
60.	Chechesee Creek		24.0 - 27.0	1992*	intertidal	OFM
61.	Mackay Creek	21.0	32.0	1986 - 1987; 10/92	intertidal	OFM
62.	Skull Creek			1986 - 1987; 9/88	intertidal	OFM

* Denotes stations sampled less than 3 times; OFM, stations monitored by the Office of Fisheries Management; EMAP, stations monitored by the Environmental Monitoring Assessment Program; CHP, stations monitored by the Charleston Harbor Project; + Habitat of area from where oyster samples were taken; ✚ see Figure A-10.

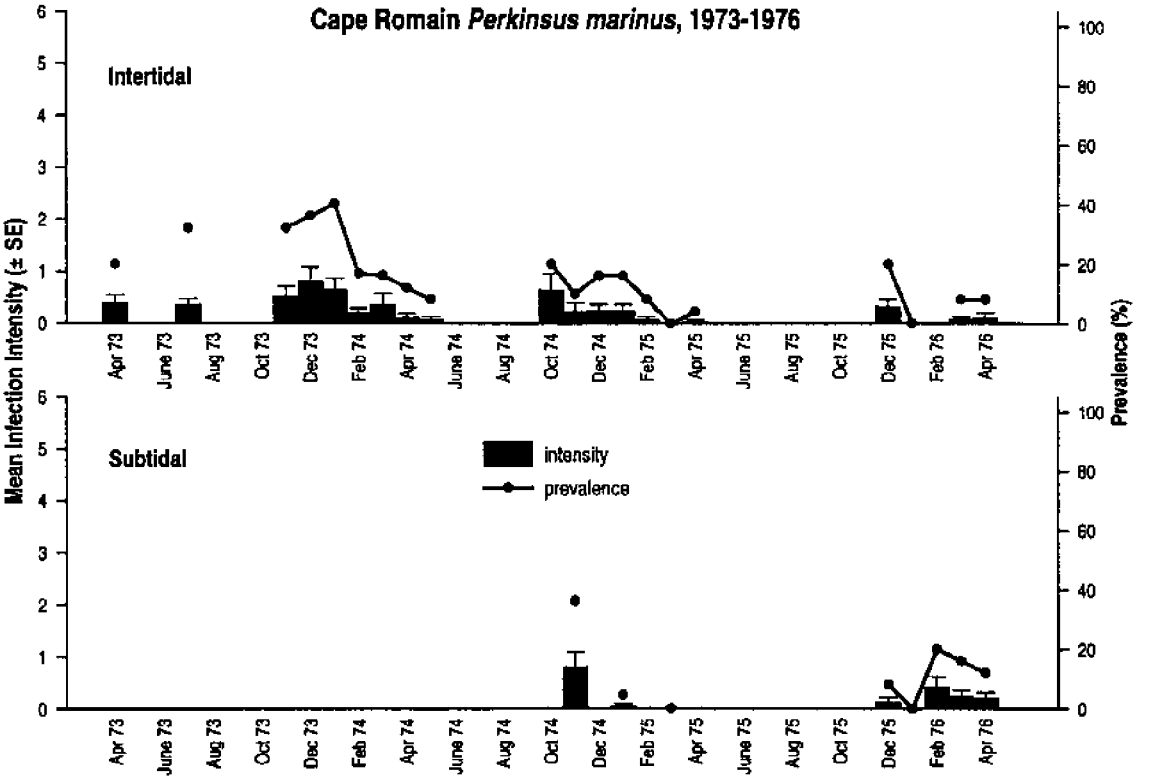


Figure A-1. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations from Cape Romain, SC (site #8), sampled during 1973-1976 (n=25 oysters/sampling date).

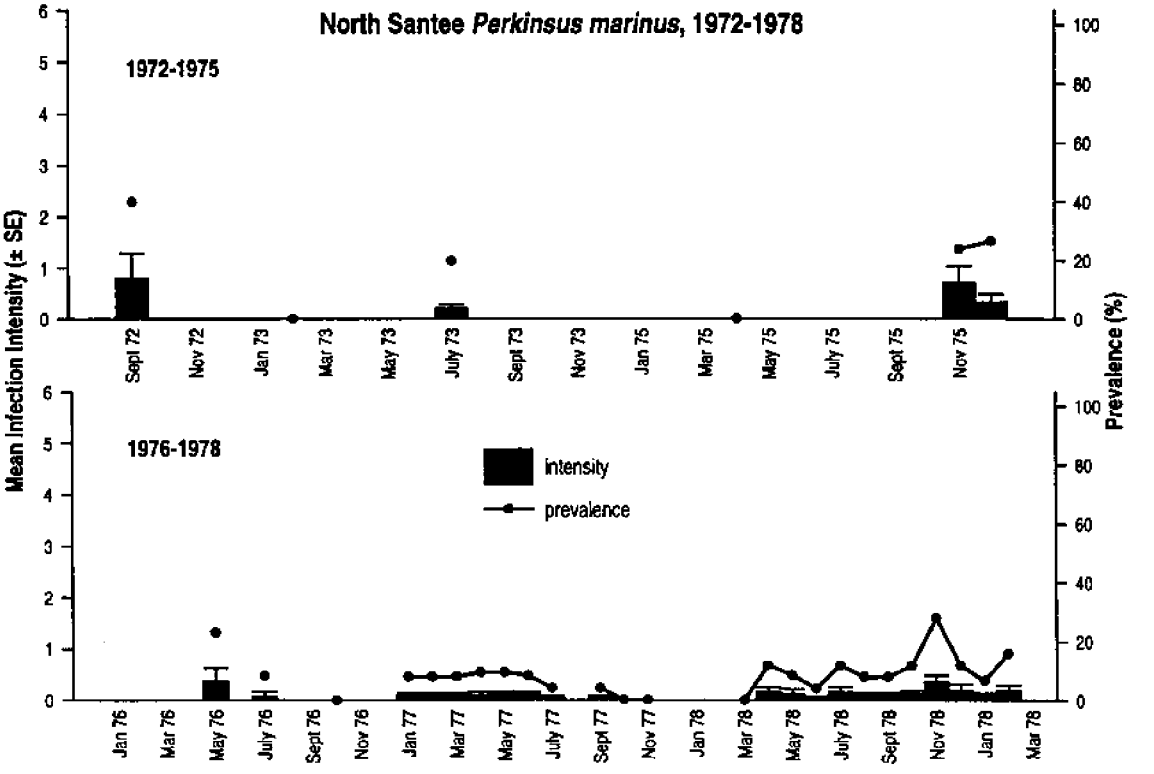


Figure A-2. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations from North Santee, SC (site #4), sampled during 1972-1978 (n=25 oysters/sampling date).

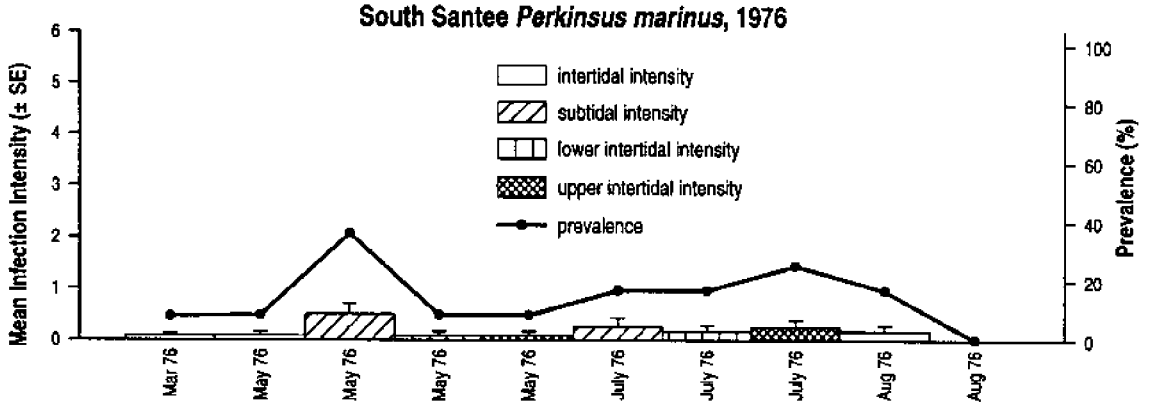


Figure A-3. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations from South Santee, SC (site #5), sampled during 1976 (n=25 oysters/sampling date).

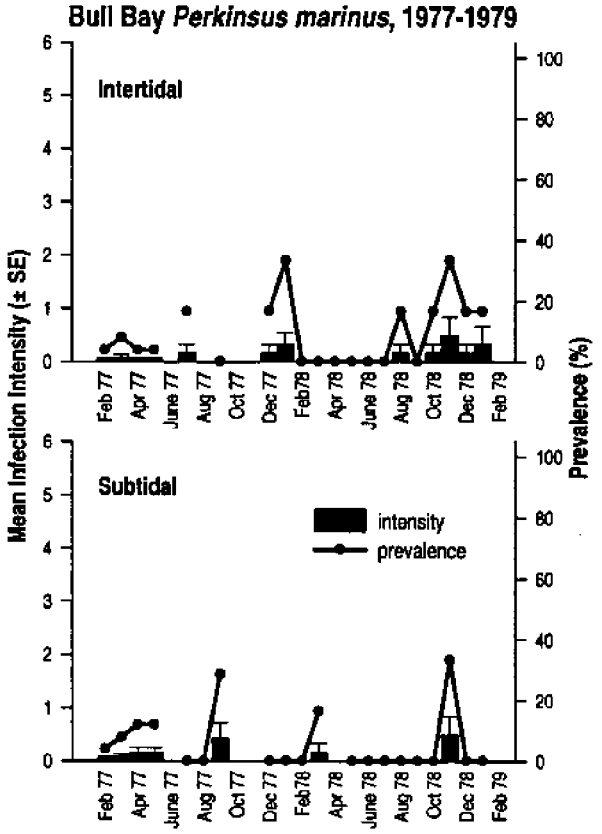


Figure A-4. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations from Bull Bay, SC (site #9), sampled during 1977-1979 (n=25 oysters/sampling date).

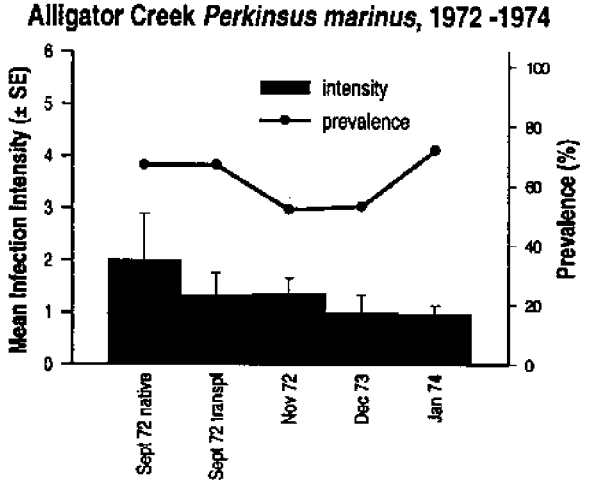


Figure A-5. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations from Alligator Creek (site #6), sampled during 1972-1974 (n=25 oysters/sampling date).

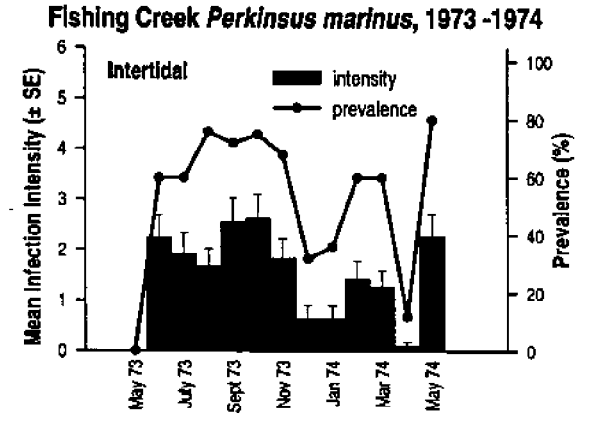


Figure A-6. Prevalence (% infected) and weighted incidence (mean infection intensity) of *Perkinsus marinus* (Dermo) in native oyster populations from Fishing Creek (site #43), sampled during 1973-1974 (n=25 oysters/sampling date).

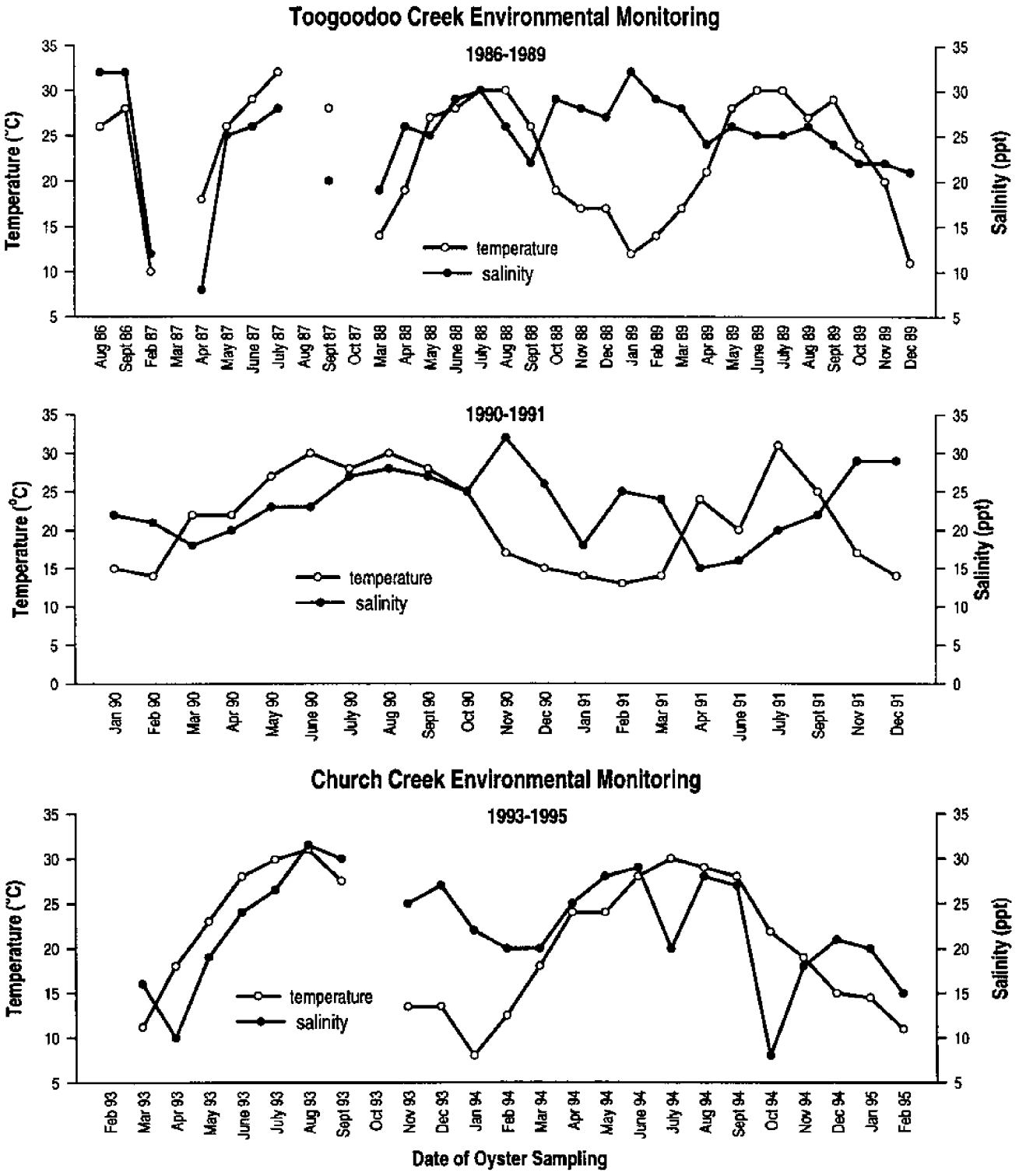


Figure A-7. Temperature and salinity recorded at the time of sampling, generally at low tide.

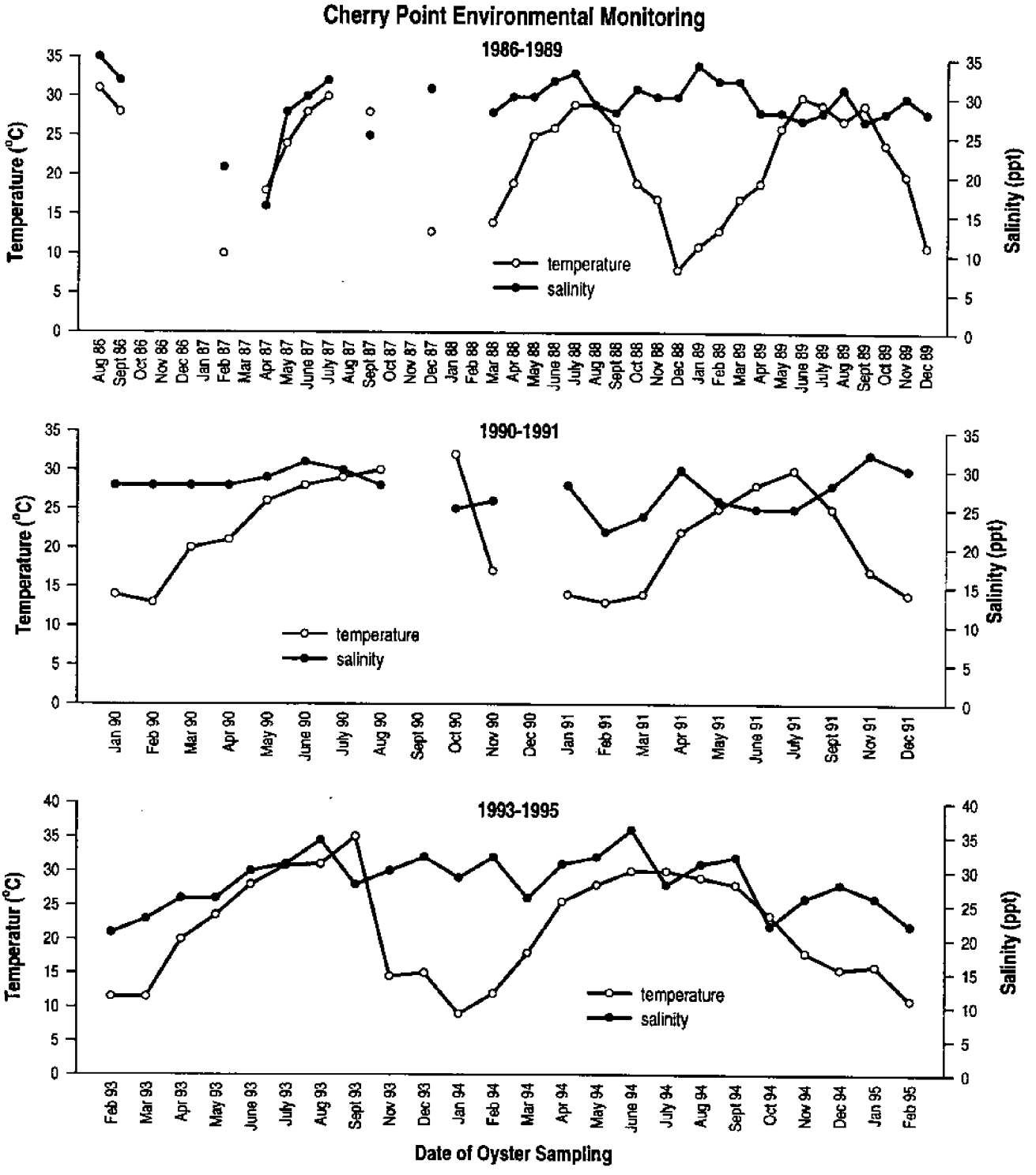


Figure A-8. Temperature and salinity recorded at the time of sampling, generally at low tide.

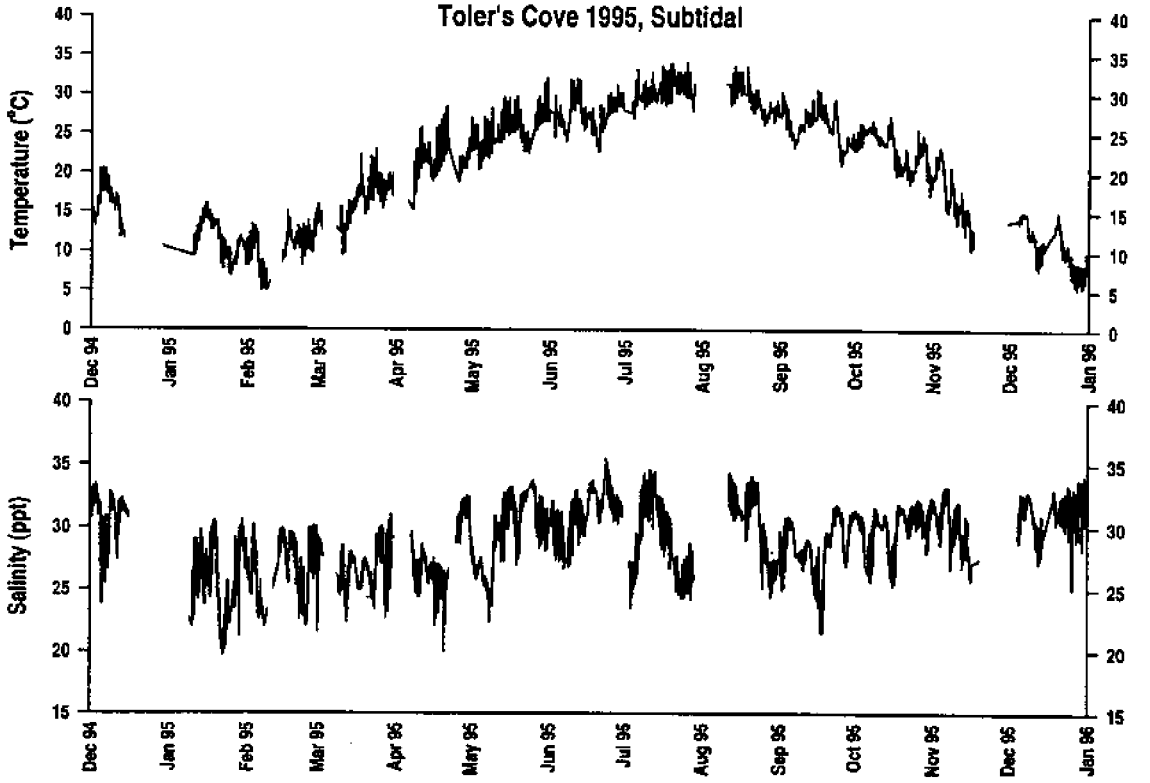


Figure A-9. Subtidal water temperature and salinity at Toler's Cove Marina. Measurements were recorded every 48 minutes using an environmental monitoring sensor (Hydrolab).

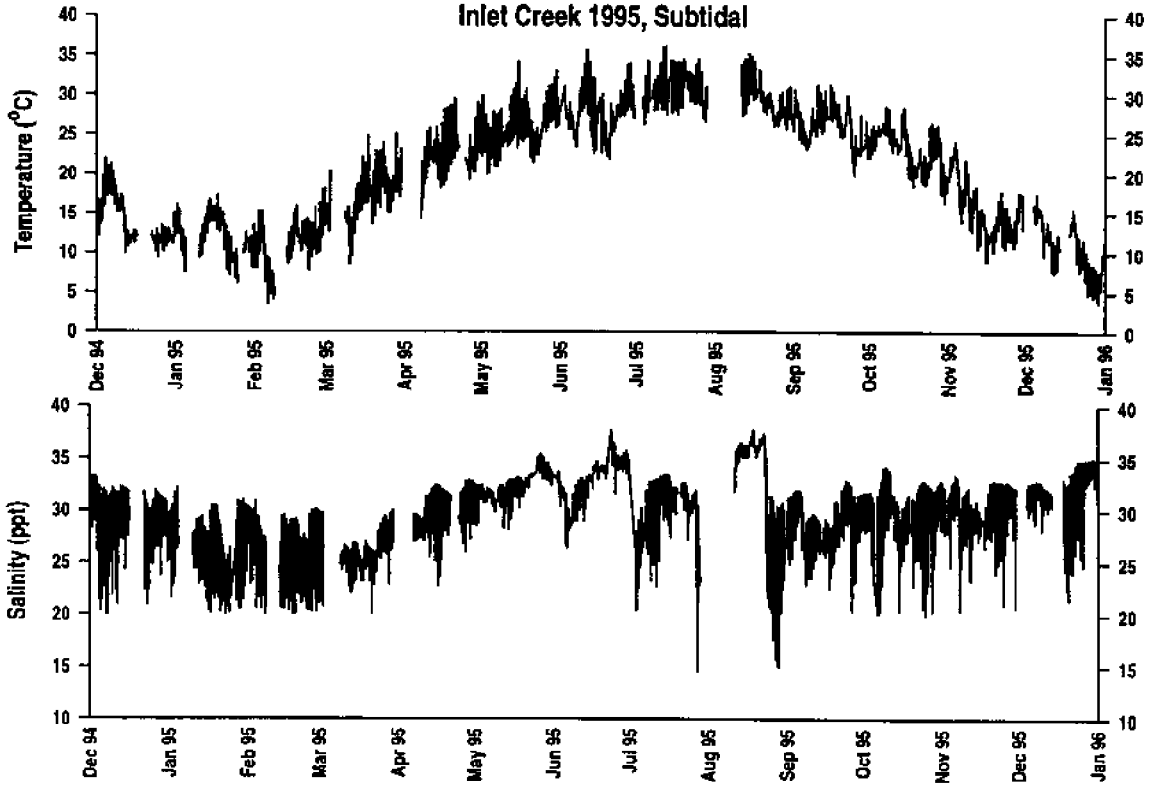


Figure A-10. Subtidal water temperature and salinity at Inlet Creek. Measurements were recorded every 48 minutes using an environmental monitoring sensor (Hydrolab).

APPENDIX B

Perkinsus marinus (DERMO) in *Mercenaria mercenaria*

Perkinsus-like organisms have been reported in 34 species of bivalve molluscs (Perkins 1988), including the hard clam, *Mercenaria mercenaria* (Andrews 1954, Ford 1992). In 1994 we surveyed natural populations of hard clams in South Carolina to determine the extent of infection with *P. marinus*. We sampled pond-raised clams, known to have been exposed to *Perkinsus marinus*, and also examined uninfected clams which were deployed into areas of potential disease exposure to determine disease prevalence and intensity. Diagnosis was determined using Ray's (1966) fluid thioglycollate medium (RFTM). Infection intensity was rated by the Quick and Mackin (1971) scale from 0 (uninfected) to 6 (heavy) (See Methods Section, this report).

Native clams were sampled from Cape Romain, Grice Cove and Ashepoo River during summer 1994 and tested for *Perkinsus marinus* infection. Clam prevalence levels ranged from 0 to 13%. Infection intensities ranged from 0 to 0.13 (See Table B-1). In many individuals only 1 or 2 *Perkinsus*-like organisms were observed. This is consistent with prior reports in the literature, which indicated that *Perkinsus*-like organisms may be observed in many species (including *M. mercenaria)* but always at very low intensity levels (Ray 1954a, Andrews 1954).

Two year old clams, cultured in three ponds at the Waddell Mariculture Center (WMC) for at least a year, were tested for *P. marinus* in November 1993 and again in February 1994. Subsequently, the remaining clams were consolidated into one WMC pond. These were again sampled in May, June, July, and August 1994. In November 1993, prevalence levels ranged from 25% to 52% in the three ponds. Mean infection intensities were very low (0.25 - 0.56). In February, there was no detectable *P. marinus* in the samples from any of the ponds. In May, 21% of the clams were infected, with a mean infection of 0.21. In June, there was no

Table B-1. *Perkinsus marinus* (Dermo) prevalence and weighted incidence (mean infection intensity) in native and cultured *Mercenaria mercenaria* populations.

Site	Date	Sample Size	% Prevalence	Weighted Incidence
Cape Romain (N)	5/94	14	7	0.07
Grice Cove (N)	5/94	15	13	0.13
Ashepoo River (N)	5/94	9	0	0
MRRI nursery (C)	2/94	30	0	0
MRRI nusery (C)	3/94	30	6	0.07
Kiawah River (C)	8/94	40	8	0.08
Clark Sound (C)	8/94	30	6	0.06
Long Island (C)	8/94	30	3	0.03
Waddell- 2yr (C)	11/93	70	42	0.44
Waddell -2yr. (C)	5/94	29	21	0.21
Waddell-2yr. (C)	6/94	30	0	0
Waddell- 2yr. (C)	7/94	20	10	0.10
Waddell -2yr. (C)	8/94	30	0	0

(N) = native clams
(C) = cultured clams

detectable infection, but in July 10% of the sampled clams were infected, with a mean infection of 0.10 (very light). In August, no *P. marinus* infections were detected (See Table B-1). It is interesting that this clam population, which exhibited infections in 1993, did not experience much infection in 1994.

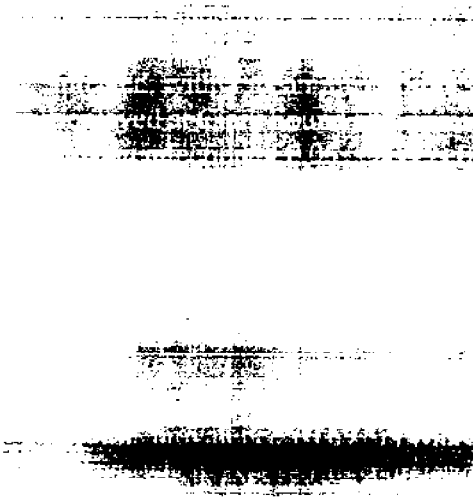
One year old seed clams were deployed in March/ April 1994 at three field sites. These clams had been previously maintained in a flowing seawater nursery at MRRI and were, therefore, potentially already infected. A subsample tested at the time of deployment had a low prevalence (6.6%) and intensity (0.07). As a control, a subsample was quarantined in the MRRI hatchery, to reduce any potential for exposure. Because *P. marinus* infections appear, in oysters, to be related to water temperature, a subsample of the hatchery-maintained clams was kept in a heated (30°C) aquarium for 90 days. The field planted seed clams and the control group were tested in August. Native oysters from adjacent banks at each of the three sites were tested simultaneously. At Kiawah, the seed clams had a prevalence of 8% and mean infection intensity of 0.08. Sixty four percent of the native oysters were infected, with a mean infection of 1.[illegible]ed clams deployed [illegible] Island had a prevalence of 6% and a mean infection of 0.06. Oysters from the same area had a 100% prevalence with a mean infection of 1.92. At Long Island, the deployed seed clams had a prevalence of 3% and mean infection intensity of 0.03. Eighty percent of the adjacent oysters were infected with an intensity of 1.55.

Clams from the MRRI nursery exhibited very low disease prevalence (6.6% in March). These same clams, when exposed to increased [illegible] in aquaria at MRRI, did not experience an increase in infection intensity. When out-planted to the field, prevalence increased, however it was still very low, despite proximity to infected oysters. Even when prevalence was high (50%), no heavy intensities were noted. This contrasts with the situation observed in ponds in 1993 (T. Cheng pers. comm.), when most of the infected individuals had heavy infections. Perhaps environmental conditions were more favorable for disease development in 1993, which was characterized by a hot, dry summer in comparison to the fairly cool and much wetter than normal summer of 1994; however, oyster infection levels observed in 1994 were fairly typical.

Perkinsus-like organisms have been reported in clams at very light infections (Andrews 1954, Andrews 1988, Perkins 1988). Observations made in SC in 1993 (T. Cheng pers. comm.) suggested that heavy infection levels and related mortality were occurring. Cheng et al. (1995) indicated that *P. marinus* can be transmitted from infected *Crassostrea virginica* to *Mercenaria mercenaria*. The observations of *Perkinsus*-like organisms in clams examined in 1994 are probably more typical levels of those that regularly occur in this clam species. At this time there is no evidence that *Perkinsus marinus* is a threat to native or cultured populations of hard clams in South Carolina.

Adapted from:

Coen, L.D., N.H. Hadley, M.Y. Bobo. 1995. Preliminary investigations of Dermo infections in hard clams (*Mercenaria mercenaria*). Sea Grant "Seed" Project Report.



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