Journal of Marine Research, 70, 225–251, 2012

Long-term patterns of an estuarine pathogen along a salinity gradient

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ABSTRACT

Parasitic, disease-causing pathogens can exert strong control over marine populations yet few long-term studies exist that describe these relationships. Understanding the connections to long-term large-scale processes relative to local short-term processes should facilitate better planning for disease impacts in the management of marine resources. We describe a 21-yr dataset of dermo disease (*Perkinsus marinus*) in eastern oysters (*Crassostrea virginica*) in Delaware Bay, USA. Analyses indicated (1) a strong positive association between disease and mortality that was non-linear and defined by thresholds, (2) a clear spatial gradient of increasing disease and mortality with increasing salinity, (3) an apparent 7-year cycle in which peaks were associated with strong positive anomalies of the North Atlantic Oscillation (NAO), (4) an inverse relationship with freshwater inflow, and (5) no obvious response to natural selection from persistent disease pressure. These data quantify the impact of environmental variables on the disease in a wild population and provide new insight into how disease interacts with host populations by linking disease patterns with larger climate controlling processes. Understanding these connections will facilitate prediction of and response to disease outbreaks.

1. Introduction

Parasitic, disease-causing pathogens can exert strong control over host populations (Harvell *et al.*, 2002). Marine and estuarine systems are no less affected and recent reviews point towards an increase in the frequency of emerging marine diseases (Harvell *et al.*, 1999; Lafferty *et al.*, 2004), yet few long-term datasets exist that document disease patterns in marine and estuarine systems. Many molluscan shellfish appear to be free from extensive parasite-induced mortality, but this is not universally true and may be a false impression resulting from our lack of knowledge about molluscan pathology. Those cases for which we have acquired significant information about shellfish pathogens have typically resulted from devastating mortalities of commercially harvested species that have had correspondingly devastating effects on local economies (see Bower *et al.*, 1994, which is updated online at http://www.pac.dfo-mpo.gc.ca/science/species-especes/shellfish-coquillages/diseases-maladies/index-eng.htm). Examples include bonamiasis in

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the European flat oyster *Ostrea edulis* (Grizel *et al.*, 1988), QPX (Quahog Parasite Unknown) in the eastern quahog *Mercenaria mercenaria* (Lyons *et al.*, 2007), MSX (Multinucleated Sphere Unknown) and ROD (Roseovarius Oyster Disease) in the eastern oyster *Crassostrea virginica* (Ford and Tripp, 1996; Boettcher *et al.*, 2005), withering syndrome in abalone *Haliotis* spp.(Friedman *et al.*, 2000) and perkinsosis in a variety of molluscs worldwide (Villalba *et al.*, 2004). Perkinsosis in *C. virginica* is caused by the protozoan *Perkinsus marinus* and is commonly referred to as 'dermo' disease after its initial description assigned the parasite to the genus *Dermocystidium* (Mackin *et al.*, 1950). Here we describe a 21-year data set for *P. marinus* infections in *C. virginica,* which exerts significant control over the population dynamics of that host, a dominant, habitat-forming estuarine species.

Crassostrea virginica inhabits estuaries throughout the East Coast and the Gulf of Mexico of North America across the Yucatan Peninsula and south to Venezuela (Galtsoff 1964). It is a reef-builder that is often a dominant species of both ecological and commercial importance. In Delaware Bay, located along the mid-Atlantic coast of the United States (Fig. 1), oyster mortality is caused by a variety of factors including predation, siltation, freshets, disease and fishing. Following the appearance of MSX disease (caused by the protozoan *Haplosporidium nelsoni*) in 1957, disease mortality has been the primary concern. Through natural selection the native Delaware Bay oyster population has developed a high level of resistance to MSX disease (Ford and Bushek, this issue). As a result, MSX has not been a problem for native oysters in Delaware Bay since 1990 at least (infection prevalence $<<30\%$ with most infections rare and localized). Unfortunately, another major oyster disease-causing protozoan, *Perkinsus marinus*, began causing epizootic mortalities in Delaware Bay in 1990 (Ford, 1996). The back-to-back epizootics have given the native Delaware Bay oyster population little reprieve from pathogen-induced mortality since 1957.

The 1990 epizootic of dermo disease was not the first appearance of *P. marinus* in Delaware Bay, but it has been the most persistent and damaging (Ford, 1996), and continues to this day. The first evidence that *P. marinus* was present in Delaware Bay was in samples of oysters collected in 1953 (Andrews and Hewatt, 1957). Subsequent surveys found that the parasite was associated with large numbers of oysters imported from the lower Chesapeake Bay during the early 1950s for planting on commercially leased bottom (Ford, 1996). Termination of importations into Delaware Bay, followed by a decade of cold winters in the 1960s, resulted in the apparent disappearance of the parasite from the bay except in oysters at an intertidal location in the lower bay, where it persisted until 1980 (Ford, 1996). Through the 1970s and 1980s, *P. marinus* was occasionally detected in histological sections, but without noticeable mortality. The 1990 epizootic was not linked to any known importations, but was associated with a regional warming trend that accompanied a northern range expansion of *P. marinus* (Ford, 1996). Since 1990, dermo disease has been a major source of oyster mortality in Delaware Bay. Many studies have reported the seasonal and spatial variation of dermo disease (see Villalba *et al.*, 2004 and references

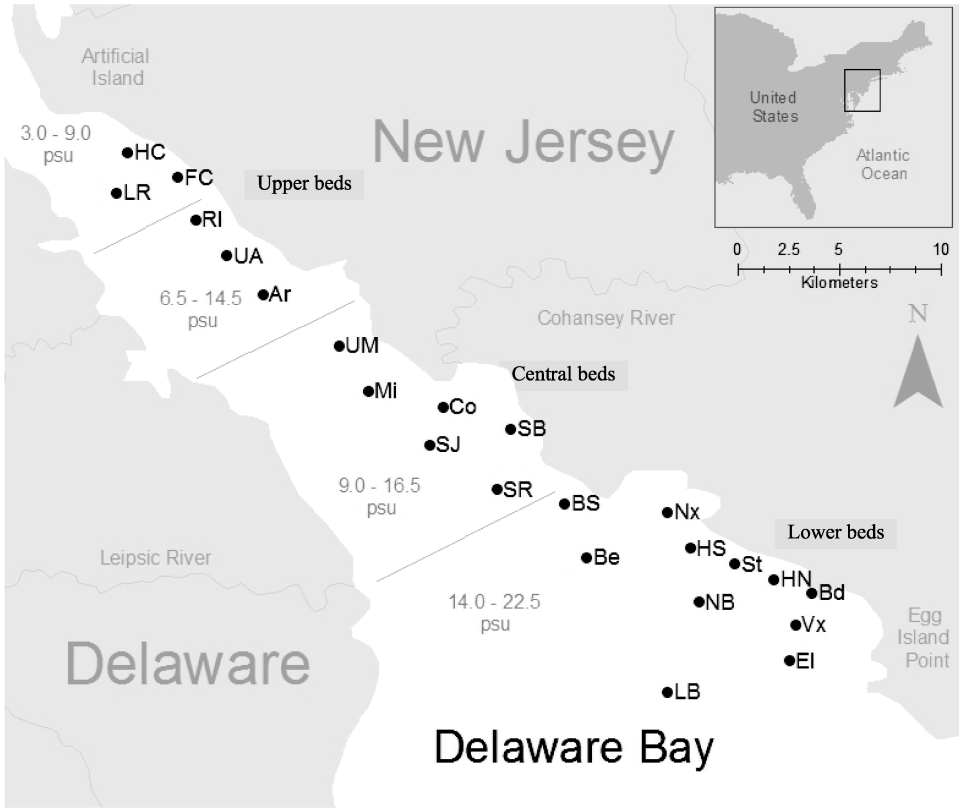

Figure 1. Locations of oyster beds sampled in Delaware Bay. Each point marks the center of a bed. Beds vary in size (see Powell *et al.*, 2008). Cross-bay lines delineate regions differing in salinity regime as described in text. Abbreviations as in Table 1.

therein), but none has reported patterns across more than a few years. This temporal deficiency limits identification of relationships with larger climate drivers such as the North Atlantic Oscillation (NAO), which can control temperature as well as regional sea level and precipitation (Hurrell and Deser, 2009), both of which affect salinity in estuaries. Temperature and salinity have long been identified as major drivers of dermo disease (Burreson and Ragone Calvo, 1996) so it seems reasonable that long-term patterns may well be influenced by cyclical features in the climate such as the NAO. We report the annual patterns of oyster mortality and *P. marinus* infections across a salinity gradient in Delaware Bay since the 1990 epizootic. We examine the data for relationships between dermo disease and oyster mortality as well as relationships with environmental variables, oyster size and oyster age. Finally, we explore the data for evidence of dermo disease resistance in the native oyster population.

2. Materials and methods

a. The study system

The Delaware Bay Estuary is a typical drowned-river estuary (Pritchard, 1967) in which oysters form extensive beds in the upper portion of the bay above Egg Island Point (Fig. 1). Oysters grow very well below Egg Island Point, but this region is comprised of privately leased grounds that are artificially maintained for oyster cultivation (see Ford, 1997 for review). The natural beds in the upper bay are referred to as 'seedbeds' because the commercial oyster industry traditionally gathered oysters from those beds to 'seed' their leases with oysters that could be harvested later. Since 1990, dermo disease has constrained survival of oysters to legal market size on leased grounds, thereby limiting their overall use and productivity. This study focuses on oyster disease and mortality across the seedbeds (Fig. 1).

The seedbeds cover approximately 4,000 hectares in upper Delaware Bay, of which about 90% are on the New Jersey side of the bay. Salinity fluctuates daily with the tides, decreasing overall with distance from the ocean (Fig. 1). Since 1953, the Rutgers University Haskin Shellfish Research Laboratory has worked with the New Jersey Delaware Bay oyster industry and the state of New Jersey to conduct a systematic assessment of the oyster stock on the seedbeds that exist in its jurisdiction (see Powell *et al.*, 2008 for details). Since 1990, *P. marinus* infection prevalence and intensity have been measured as part of the annual stock assessment. The primary seedbeds comprising the oyster fishery are divided into three regions for analysis in this study: the upper region includes Arnolds, Upper Arnolds and Round Island; the central region includes Upper Middle through Shell Rock; the lower region includes Bennies Sand through Ledge and Egg Island (Fig. 1). Prior to 2007, the uppermost seedbeds consisting of Hope Creek, Fishing Creek and Liston Range were considered inconsequential to the overall oyster population and the fishery and were not surveyed.

b. Annual mortality estimates

Powell *et al.* (2008) provides details of the annual fall stock assessment survey methodology. Mortality was assessed from a composite bushel (35.2 liters) created by subsampling three dredge hauls within each sampling location selected by a stratified random sampling design. All live oysters, boxes (articulated oyster valves void of tissue) and gapers (articulated valves that contain tissue) were sorted and counted. Oyster mortality was estimated as the fraction of gapers and boxes compared to live oysters plus boxes and gapers. Based on the half-life of boxes in Delaware Bay, Ford *et al.* (2006) concluded that mortality estimated by fall-survey box counts was a reasonable index to annual mortality.

c. Annual dermo monitoring

During the annual fall stock assessment, samples of oysters that represent the size distribution of the oysters (> one year old) on grids within selected beds (Table 1) were analyzed for *P. marinus* infections. Funding and management priorities determined which beds were sampled during different years with increasing spatial coverage over time. Seven beds that

Table 1. Record of collections for annual fall dermo disease monitoring since 1990. Numbers indicate sample size collected for the corresponding year. Beds are listed more or less by latitude, although some lie at the same latitude with different longitudes (See Figure 1). Beds listed in bold were sampled every year of the study.

SEEDBED	90	91	92	93	94	95	96	97	98	99	2000	01	02	03	04	05	06	07	08	09	10
Hope Creek (HC)																		20	20	20	20
Liston Range (LR)																			20	20	20
Fishing Creek (FC)																			20	20	20
Round Island (RI)	**10**	**10**	**10**	**20**	**10**	**30**	**20**	**20**	**20**	**30**	**20**	**20**	**20**		**20**	**20**	**20**	**20**	**20**	**20**	**20**
Upper Arnolds (UA)				10								10		10		20	20	20	20	20	20
Arnolds (Ar)	**20**	**20**	**21**	**30**	**10**	**20**	**20**	**21**	**20**	**30**	**20**	**30**	**30**	**30**	**20**	**8**	**20**	**20**	**20**	**20**	**20**
Upper Middle (UM)													10				10	20	20	20	20
Middle (Mi)	10	10	20	20	20			20	20	30	30	30	30	30	20	20	20	20	20	20	20
Cohansey (Co)	**30**	**30**	**31**	**20**	**19**	**20**	**20**	**20**	**20**	**30**	**30**	**28**	**30**	**30**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Sea Breeze (SB)										20		10	10		20	20	30	20	20	20	20
Ship John (SJ)	10	10	40	30	30		21			20	20	20	20	20	20	20	20	20	20	20	20
Shell Rock (SR)	**20**	**30**	**42**	**30**	**20**	**20**	**20**	**20**	**20**	**30**	**30**	**30**	**30**	**30**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Bennies Sand (BS)	10	20	20	20	10			16	20	20	20	20	20	40	20	20	20	20	20	20	20
Bennies (Be)	**30**	**30**	**32**	**40**	**20**	**20**	**20**	**20**	**20**	**30**	**20**	**20**	**40**	**20**	**20**	**19**	**20**	**20**	**20**	**20**	**20**
Nantuxent (Na)		30		10		20		20		20	20	20		20		20	20	20	20	20	20
Hog Shoal (HS)		20		10						10		10	10	10	20	30	20	20	20	20	20
New Beds (NB)	**70**	**29**	**31**	**30**	**40**	**20**	**29**	**10**	**20**	**30**	**20**	**20**	**28**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Strawberry (St)	50		10		10								10	10	20	20	16	20	20	20	20
Hawks Nest (HN)	60		20		20		20		20		10		10	10	10	20	20	20	20	20	20
Beadons (Bd)	**20**	**20**	**26**	**10**	**10**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**34**	**20**	**20**	**20**	**20**	**20**
Vexton (Vx)		20		10						10		10	10	10	20	20	20	20	20	20	20
Egg Island (EI)	30	29	30	20	30	24	20	15		14	20	14		10		20		20		20	
Ledge Bed (LB)			20		10				27		10		5		5		6		8		10

span most of the salinity gradient (Bennies to Round Island, Figure 1) have been sampled every year during the time series (Round Island was missed in 2003, Table 1). The shell height of each oyster (distance from umbo to bill along the flat valve) was measured, and *P. marinus* infection prevalence and intensity was diagnosed using mantle and rectal tissues as described by Ray (1952, 1966). Each infection was assigned an intensity rank based on the density of parasites present using the "Mackin scale" from zero (=pathogen not detected) to five (=heavily infected) (Ray 1954). The percent of oysters in the sample with detectable infections is termed the prevalence. Sample intensity (also known as weighted prevalence or WP[3]) was calculated by averaging all infection intensity ranks, including zeros (Mackin,

3. WP can be calculated by multiplying the prevalence and the mean infection intensity, effectively weighting the prevalence by the infection intensity. The term 'weighted prevalence' appears to be unique to the literature on *Perkinsus* spp. and a limited number of other oyster parasites. Therefore, to minimize confusion with the broader audience, we use the term 'mean sample intensity' or simply 'sample intensity' rather than weighted prevalence or WP throughout.

1962). Mean infection intensity was calculated by averaging the infection rank for only those animals with detectable infections, i.e., excluding zeros.

d. Analyses

Perkinsus marinus prevalence, sample intensity and mortality were plotted by bed and against time as well as each other to identify spatial and temporal patterns. Data were arcsine transformed where appropriate to meet assumptions of normality for parametric analyses such as correlation and regression.

In order to assess the relationship between temporal variations and major environmental factors on *P. marinus*, sample intensity was correlated with Delaware River discharge and local temperature. River flow was measured at Trenton, New Jersey (http://waterdata.usgs.gov/nj/nwis/nwisman?site_no=01463500) and is highly correlated with Delaware Bay salinity (Haskin, 1972; Najjar, 2010; Wang *et al.*, this issue). Air temperature for Millville, New Jersey was obtained from the National Climate Data Center (http://www.ncdc.noaa.gov/cdo-web/search#t=secondTabLink) and is correlated with water temperature in the bay (Ford, unpublished). Because the fall measures of *P. marinus* intensity were most likely an integration of conditions over the previous season or year, correlations were computed using (1) mean winter/spring (December–May), mean summer/fall (June–November), or mean annual river flow; and (2) mean winter (December–February) and mean summer (June–August) air temperatures. Additional combinations of months were also examined for each variable, but because they did not change the results, they are not presented. Multiple regressions were completed that combined temperature and river flow for relationships that showed significant p-values in simple regression. To explore potential relationships with the NAO station-based anomalies, data for December through March from 1989 through 2010 were downloaded from the Climate Analysis Section, NCAR, Boulder Colorado (http://www.cgd.ucar.edu/cas/jhurrell/naointro.html), and temporal patterns compared with changes in *P. marinus* sample intensity. Sample intensity was regressed against the winter NAO anomaly and a contingency analysis conducted to calculate odds ratios (OR) and relative risk (RR) of exceeding sample intensity thresholds during positive NAO anomalies. The December through March data were chosen because of the documented importance of winter temperatures to *P. marinus* infection dynamics (Ford, 1996; Cook *et al.*, 1998). The statistics OR and RR are commonly used in epidemiology to express increased or decreased probability of disease in the presence of a factor of interest versus the absence of that factor (Rosner, 2006). Gray *et al.* (2009) used OR to understand the relationship between *P. marinus* infection prevalence and stormwater inputs. Odds are simply prevalence divided by (1-prevalence) and an odds ratio is the odds when a factor of interest is present (positive NAO anomaly) divided by the odds when the factor is absent (negative NAO anomaly). Relative Risk calculated herein describes the likelihood of *P. marinus* sample intensities exceeding 2.0 following positive winter NAO anomalies; calculated as the prevalence of dermo disease when NAO is positive divided by the prevalence when NAO is negative.

The relationships of *P. marinus* infection prevalence and intensity with age and size were examined in two ways. First, during the fall stock assessment surveys for the four years from 1995 through 1998, oysters from major beds were categorized as spat (oysters <1 year old), yearlings or older oysters, the shell height was measured, and each individual was assayed for *P. marinus* infection intensity. These data were examined for differences among age class categories using Analysis of Variance (ANOVA). Secondly, the shell heights of all oysters collected from the major beds for which sampling was done each year from 1990 through 2010, (with the exception of 2002) were placed into one of six size-class bins (<24.9 mm, 25–39.9 mm, 40–59.9 mm, 60–79.9 mm, 80–99.9 mm and >100 mm) and the prevalence, mean sample intensity and mean infection intensity calculated for the oysters in each bin. Data were then inspected for changes in infection levels with increasing size (a correlate of age).

Lastly, we examined the data for evidence of resistance using time-line plots of infection levels and by comparing sample intensity with box-count mortality estimates from the fall survey. First, bay-wide means of sample intensity obtained during the fall surveys from 1990 through 2010 were plotted to identify trends. If resistance to dermo disease was developing in the native oyster population of the bay, we hypothesized that infection prevalence, intensity, or both, should show a decreasing trend with time that could not be explained by environmental fluctuations. Next, data for sample intensity and for box-count mortality obtained at the same time were grouped into four 5-year periods from 1991 to 2010. Mortality (arcsine transformed) was then regressed as a function of sample intensity for seven beds continuously sampled over the four periods. Regressions were compared to investigate the possibility of the development of tolerance (i.e., resistance to mortality caused by a given infection level). Even if infection levels were not decreasing, oysters still might be becoming more tolerant of the parasite, which would be evidenced if the slope of the regression lines relating sample intensity to mortality diminished over the four time periods, or if slopes remained similar but y-intercepts became progressively lower over time, or both.

3. Results

a. Spatial patterns

Mean prevalence and sample intensity of *P. marinus* infections, measured during the fall survey, since 1990 were highly correlated ($r^2 = 0.83$, $p<0.0001$) and increased from the upper to lower beds along the increasing salinity gradient (Fig. 2A). Prevalence frequently reached 100% from Shell Rock downbay, but rarely exceeded 50% at Arnolds and above. Similarly, sample intensity on the upper beds rarely exceeded a value of 1.0 (Mackin Scale), but was often above 2.0 on beds farther downbay and often near or above 3.0 on those beds located farthest downbay. Mortality assessed at the same time also showed a general increase from upbay to downbay (Fig. 2B). As a result, both *P. marinus* prevalence and sample intensity were significantly correlated with mortality ($r^2 = 0.52$, $p<0.0001$; $r^2 = 0.64$, $p<0.0001$; respectively).

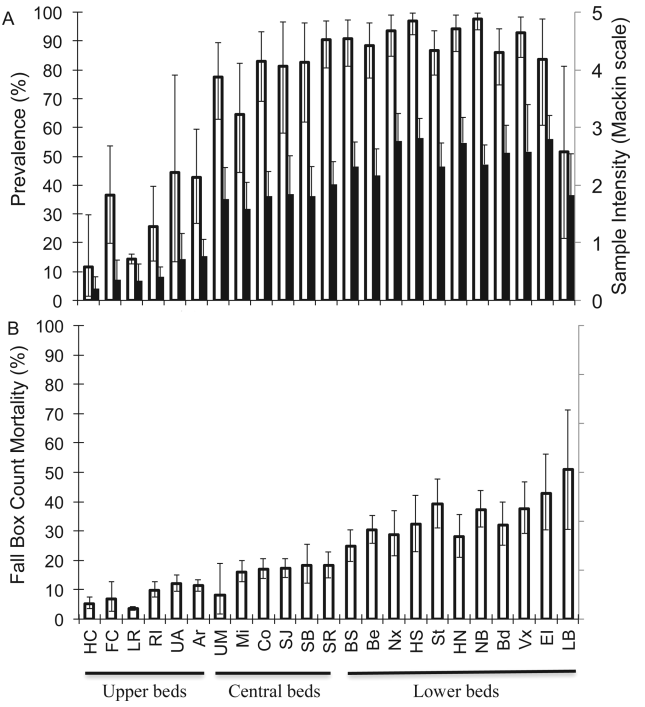

Figure 2. (A) Mean fall prevalence (open bars) and mean sample intensity (solid bars) of *Perkinsus marinus* (dermo) on the New Jersey Delaware Bay oyster seedbeds since 1990 with 95% confidence intervals. (B) Mean annual fall box-count mortality of oysters on New Jersey Delaware Bay seedbeds since 1989 with 95% confidence intervals. Beds are listed from upbay to downbay. Not all beds were sampled every year (see Table 1).

Regressing median mortality against median sample intensity by bed revealed an exponential relationship (Fig. 3). With no *P. marinus* infection, the regression indicates a median background mortality of 5.8%. As infections increase, the data fall into three clusters that indicate thresholds of dermo disease-related mortality at fall sample intensities of about 1.0 and 2.0 (Fig. 3A). Each cluster encompasses a region of the seedbeds that is differentiated from the others by its average salinity regime (Fig. 1). Relatively low mortality (<12%) occurred when *P. marinus* sample intensity was typically below 1.0. This cluster included all beds above Upper Middle bed where salinity typically fluctuated between 3 and 14.5 (Figs. 1 and 2). A region of intermediate mortality (14–20%) was defined by sample intensities roughly between 1.5 and 2.0. This region included beds from Upper Middle through Shell Rock where salinity was typically between 9 and 16.5 (Figs. 1 and 2). Relatively high mortality (≥25%) occurred where median sample intensity routinely exceeded 2.0. This region included all beds below Shell Rock where salinity was typically above 14 (Figs. 1 and 2). When Mackin-scores were converted into parasite densities (i.e., body burden or parasite load) (Choi *et al.*, 1989), it was apparent that the shifts in mortality defining these

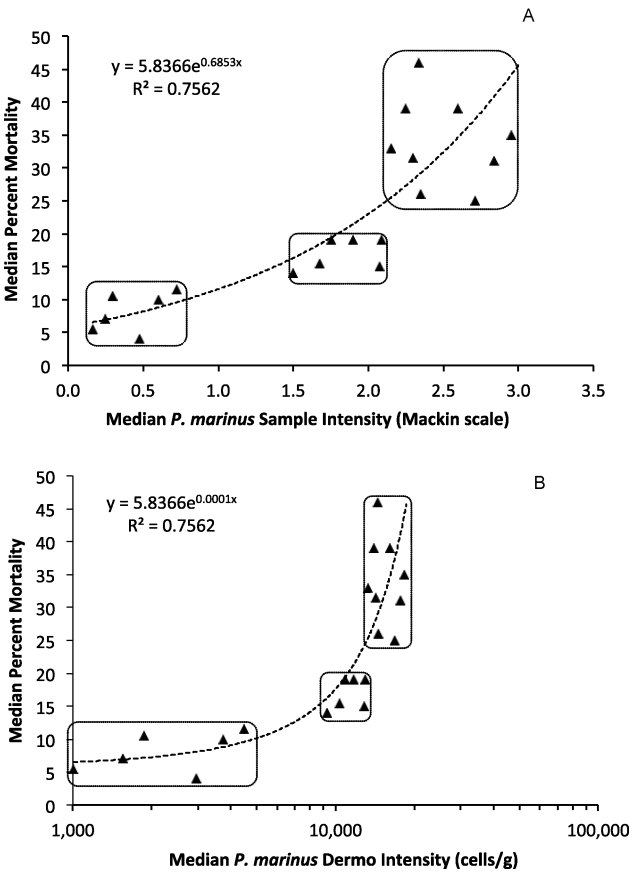

Figure 3. Relationship between long-term median percent fall box count mortality estimate and the long-term median sample intensity of dermo infections since 1990. Data are medians of the entire 1990-2010 study period for individual beds. (A) Mortality as a function of sample intensity. (B) Mortality as a function of parasite body burden converted from sample intensity after Choi *et al.* (1989). Boxes (left to right) enclose beds from upper, central and lower bay regions.

regions occurred at specific parasite densities in host oysters (Fig. 3B). Below a density of 5,000 parasites g^{-1} wet weight, mortality was relatively low (<12%). As parasite body burden approached 10,000 cells g^{-1} mortality rates effectively doubled. As body burdens approached 20,000 cells g^{-1} mortality rates doubled again.

The presentation of data as the median value for each bed across all years (Fig. 3) highlights the thresholds of *P. marinus* infection that separate regions of impact, but use of medians conceals the level of variation present (Fig. 4). Few beds (Mi, Co, SR, BS, Be, St, Bd and Vx – Fig. 1) showed any relationship between *P. marinus* sample intensity and oyster mortality when analyzed separately (data not shown), but the within-region relationships and

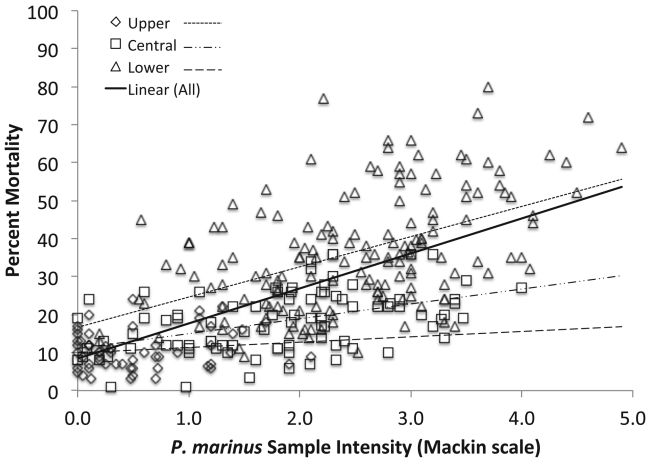

Figure 4. Relationships between estimated fall mortality and *Perkinsus marinus* infection levels (sample intensity). Data are values for individual beds collected during the Random Sampling Program from 1990 through 2010. Diamonds represent upper beds with low mortality in Figure 3, squares represent central beds with intermediate mortality in Figure 3 and triangles represent the lower beds with higher mortality in Figure 3. Solid black regression line is an overall regression for all data combined. Regression equations and statistics are presented in Table 2.

Table 2. Results of linear regression analyses for mortality and *Perkinsus marinus* sample intensity overall and by bay region (see Fig. 4). Mortality was estimated as fall box count frequency and arcsine square root transformed prior to analysis. The upper region includes Hope Creek through Arnolds, the central region is Upper Middle through Shell Rock, and the lower region is Bennies Sand through Ledge (see Fig. 1).

Region	Linear Regression	p-value	Adj R-square
Upper	$y = 1.41x + 9.94$	0.517	0.02
Central	$y = 3.91x + 11.07$	0.000	0.22
Lower	$y = 7.95x + 16.67$	0.000	0.24
Overall	$y = 9.21x + 8.47$	0.000	0.42

the overall relationship plotted for each year by bed were adequately described by simple linear regressions (Fig. 4, Table 2). The change from exponential to linear relationships between Figure 3 and Figure 4 results from the increase in slope from upper to lower bay regions seen in Figure 4 and the condensation of variation into median values in Figure 3, which reveals a more rapid increase in the relationship. The y-axis intercept of the overall linear regression estimated a background mortality of about 8.5% in the absence of dermo disease, which was higher than the value derived from an exponential regression of median values (5.8%, Fig. 3). When examined by region, slopes of regression lines increase about two-fold from upper to central and central to lower regions indicating an increase in the

importance of dermo disease as a source of mortality farther down bay (Fig. 4 and Table 2). Note that the slope of the relationship was not significantly different from zero on the upper beds where disease levels were typically low.

b. Temporal patterns

Because the spatial analyses described above indicated differences among seedbed regions, temporal patterns were examined and compared by region. The spatial component observed when data for all years were pooled by bed (Fig. 2) was readily apparent when data were plotted by region over time (Fig. 5); that is, prevalence, sample intensity and mortality increased from upper to lower regions. Both prevalence and sample intensity followed similar patterns in all regions. Mortality on the central and lower beds roughly tracked the temporal patterns of infection prevalence and intensity, but mortality on the upper beds was often out of phase with prevalence and sample intensity. Pairwise correlations across regions for each metric supported these observations (Table 3) and highlight the different behavior of the upper seedbeds relative to the other regions. Specifically, correlations between prevalence and sample intensity, prevalence and mortality or sample intensity and mortality were less likely to be positive and significant between the upper region and either the central or lower regions than between the central and lower regions. The data also indicated a slight attenuation of *P. marinus* infection intensity and oyster mortality in the central and lower regions over the course of the three successive epizootics. This trend was not observed in the upper region (Fig. 5).

The time series indicated two brief periods of dermo disease remission (low prevalence and sample intensity) that correspond to reduced mortality in 1997 and 2004 (Fig. 2). The spacing of these two periods indicated a seven-year cycle that roughly corresponded to winter (December to March) NAO anomalies (Fig. 6A). No significant correlation was detected between NAO and *P. marinus* intensity either in the same year or when sample intensity was lagged by one or two years. Further inspection of the data revealed an apparent threshold response when winter NAO anomalies became positive (Fig. 6B). Figure 3 indicated that mortality increases dramatically above a *P. marinus* intensity of 2.0. Comparing sample intensities above and below 2.0 with positive and negative winter NAO anomalies shows that the odds of *P. marinus* intensity exceeding 2.0 following a positive winter NAO anomaly was 8:6 whereas the odds following a negative winter NAO anomaly was only 1:6 (Table 4). The resulting OR ($= 8$) was not statistically significant at $\alpha = 0.05$ ($p = 0.078$, Fisher's one-tailed Exact Test of the hypothesis $OR > 1$), but the Relative Risk ($RR = 4$) indicated that the population was four times more likely to exceed a threshold sample intensity of 2.0 when the winter NAO anomaly was positive compared to years when the anomaly was negative.

c. Local environmental correlates

Relationships of sample intensity and mortality on the lower beds with river flow became apparent when a three-point running mean was plotted over time for each parameter (Fig. 7).

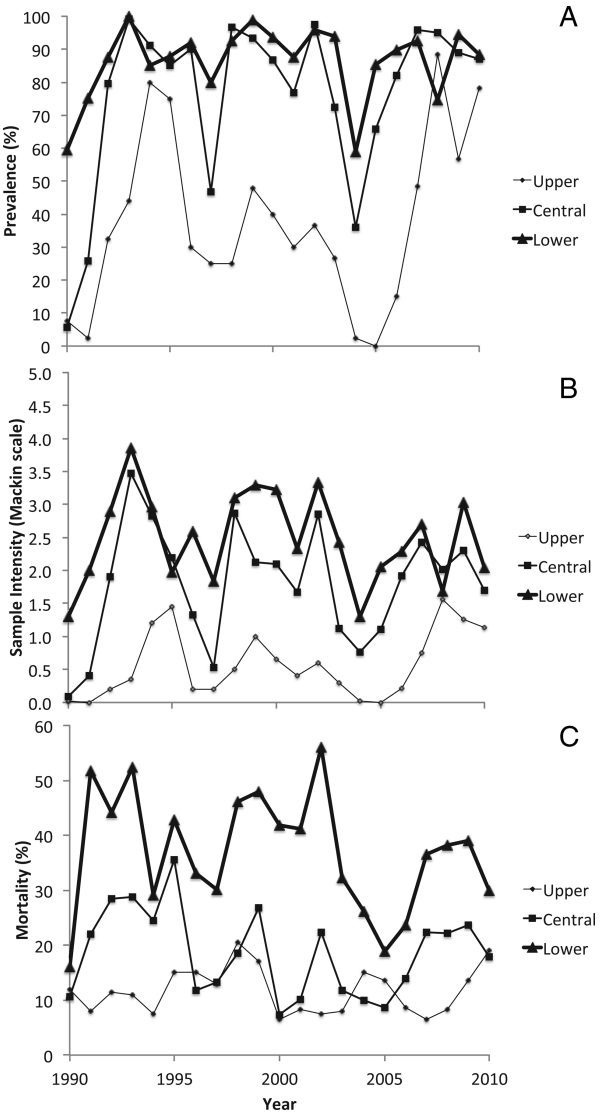

Figure 5. Mean annual fall *Perkinsus marinus* prevalence (A), sample intensity (B), and box count mortality (C) observed on New Jersey Delaware Bay seedbeds during the annual fall surveys (error bars omitted for clarity).

Sample intensity and mortality track each other as has been described above. In comparison, Delaware River flow was nearly out of phase with sample intensity and mortality. Correlations of river flow and temperature with fall sample intensity within individual regions in the same year produced no statistically significant results; however, when sample intensity

Table 3. Correlation matrix among long-term measures of *Perkinsus marinus* prevalence, sample intensity and oyster mortality across upper, central and lower regions of the bay that were identified as being distinct in the spatial analysis (see text and Fig. 5). Non-italicized fonts are correlation coefficients. Italicized fonts are probability levels with significance at $\alpha = 0.05$ indicated by bold face. The pattern highlights the lack of correspondence between the upper seedbeds and the lower seedbeds in general and with mortality.

		Prevalence			Sample Intensity			Mortality		
		Upper	Central	Lower	Upper	Central	Lower	Upper	Central	Lower
Prevalence										
Upper	r	1								
	p									
Central	r	**0.567**	1							
	p	***0.007***								
Lower	r	0.352	**0.681**	1						
		0.117	***0.001***							
Sample Intensity										
Upper	r	**0.920**	**0.477**	0.221	1					
	p	***0.000***	***0.029***	0.337						
Central	r	**0.615**	**0.871**	**0.679**	**0.531**	1				
	p	***0.003***	***0.000***	***0.001***	***0.013***					
Lower		0.295	**0.785**	**0.872**	0.178	**0.794**	1			
	p	0.194	***0.000***	***0.000***	0.440	***0.000***				
Mortality										
Upper	r	−0.022	−0.030	−0.046	0.098	−0.012	−0.099	1		
	p	0.925	0.895	0.843	0.674	0.957	0.670			
Central	r	**0.592**	0.342	0.425	**0.562**	**0.562**	0.371	0.102	1	
	p	***0.005***	0.129	0.055	***0.008***	***0.008***	0.097	0.650		
Lower	r	0.273	**0.454**	**0.613**	0.246	**0.554**	**0.603**	−0.142	**0.673**	1
	p	0.231	***0.039***	***0.003***	0.282	***0.009***	***0.004***	0.529	***0.001***	

was lagged one year behind the physical variables, correlations became significant, but only for certain times and certain regions (Table 5). Neither river flow nor temperature was correlated with sample intensity on the upper beds. Summer/fall and annual Delaware river flow was negatively related to sample intensity in the lower and central regions and winter temperature was positively related with the same measure on the lower beds, but the r^2 values, which ranged from 0.22 to 0.47, indicated that other explanatory factors were equally or more important. Multiple regressions of river flow and temperature with lagged sample intensity on the lower beds increased the r^2 values somewhat (Table 6). The strongest correlation resulted from mean annual Delaware River flow and winter/spring temperature ($r^2 = 0.529, p = 0.0017$), although only mean annual flow was significant as an individual variable (Table 6). These observations indicate that although river flow (= salinity) and temperature play critical roles, other (unknown) factors may have an equally important influence on local variation in *P. marinus* levels in Delaware Bay.

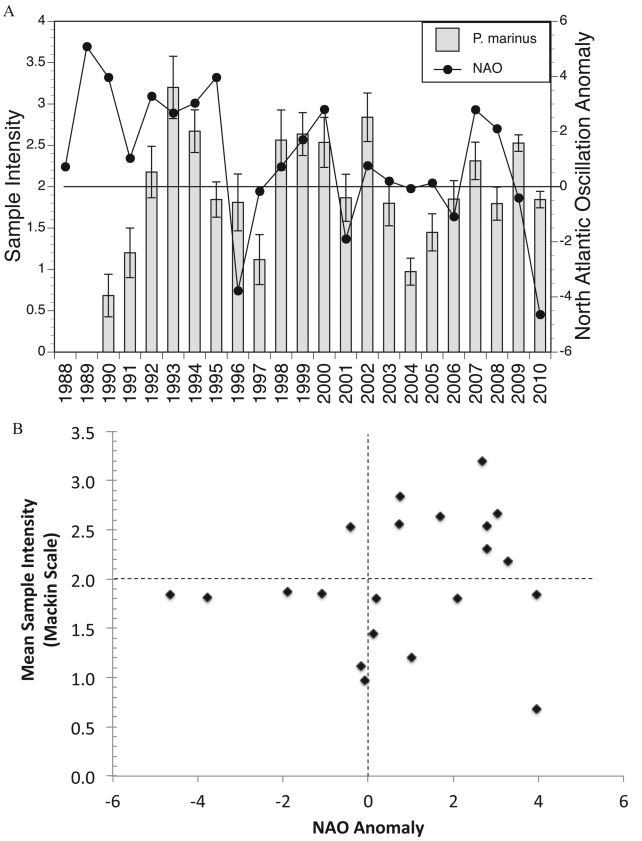

Figure 6. (A) Temporal comparison of *Perkinsus marinus* sample intensity with North Atlantic Oscillation anomalies. Bars represent means of all samples collected during the annual fall survey. Error bars represent one standard error. (B) Scatter plot of mean sample intensity versus winter NAO anomalies. Dashed lines indicated threshold values used in contingency analyses as described in text and Table 4.

Table 4. Contingency table for likelihood of exceeding a threshold *Perkinsus marinus* sample intensity (SI)>2.0 during positive and negative winter NAO anomalies. Counts obtained from Figure 6B. Odds Ratio (OR) of SI>2.0 during positive NAO = (8/6)/(1/6) = 8; Fishers Exact Test p = 0.078. Relative Risk (RR) = (8/14)/(1/7) = 4; 95% CI = 0.75–84.

	SI>2.0	SI<2.0	Row Total
NAO Positive	8	6	14
NAO Negative	1	6	7
Column Total	9	12	21

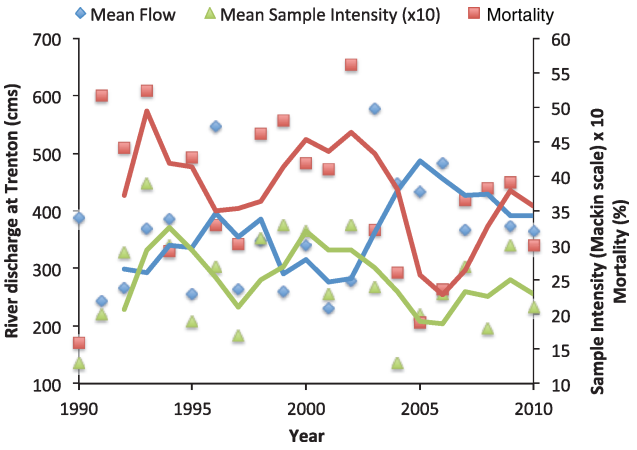

Figure 7. Relationship of Delaware River flow measured at Trenton, NJ with *Perkinsus marinus* sample intensity and oyster mortality on the lower seedbeds. River flow is mean annual discharge. Lines are 3-point running means.

Table 5. Correlations of river flow and temperature for periods shown with *Perkinsus marinus* sample intensity lagged by one year. Non-italicized fonts are correlation coefficients. Italicized fonts are p-values with significance at $\alpha = 0.05$ indicated by bold face.

Flow			Lower	Central	Upper
	Dec-Feb	r^2	0.098	0.002	0.010
		p	*0.192*	*0.872*	*0.678*
	Jun-Nov	r^2	**0.352**	**0.216**	0.112
		p	***0.007***	***0.045***	*0.161*
	Annual	r^2	**0.468**	**0.236**	0.004
		p	***0.001***	***0.035***	*0.785*
Temperature					
	Dec-Feb	r^2	**0.368**	0.055	0.010
		p	***0.006***	*0.331*	*0.678*
	Jun-Aug	r^2	0.005	0.002	0.180
		p	*0.776*	*0.857*	*0.070*
	Annual	r^2	0.200	0.067	0.156
		p	*0.055*	*0.284*	*0.095*

In addition, they suggest a relatively long level of system memory leading to a delayed response.

d. Effects of size/age on dermo

In all four years in which *P. marinus* infections were assayed separately for spat, yearlings and older oysters, sample intensity was lowest in spat ($\leq$0.3) (Fig. 8). In 1995 and 1996,

Table 6. Results of multiple regression models to explain *Perkinsus marinus* sample intensity (SI) on the lower seedbeds. Flows are monthly average levels in cubic meters per second from the USGS station in Trenton, NJ. Winter temperature is monthly mean air temperature recorded at Millville, NJ Airport.

Regression model: SI Lower = mean annual flow + winter temperature						
	Observations	Parameters	Root Mean Square Error	r^2	F	*p*
	20	3	0.4846777	0.5285	9.526929	0.0017
Model	Coefficient	Std. Err.	T	*p*	[95% Conf. Interval]	
Flow	−0.000095	0.0000375	−2.55	0.021	−0.00017	−0.00002
Temperature	0.127026	0.0829852	1.53	0.144	−0.04806	0.30211
Constant	3.536179	0.5944407	5.95	0.000	2.28202	4.79034
Regression model: SI Lower = mean summer-fall flow + winter temperature						
	Observations	Parameters	Root Mean Square Error	r^2	F	*p*
	20	3	0.5198698	0.4575	7.168902	0.0055
Model	Coefficient	Std. Err.	T	*p*	[95% Conf. Interval]	
Flow	−0.0000564	0.0000306	−1.85	0.082	−0.0001209	0.00001
Temperature	0.1633236	0.0860583	1.90	0.075	−0.0182435	0.34489
Constant	2.749054	0.4025817	6.83	0.000	1.899681	3.59843

sample intensity of yearlings was intermediate (∼1.1) between spat (<0.3) and older oysters (∼2). In 1998, yearlings and older oysters were equivalent (2.5), but in 1997 sample intensity of yearlings was higher (1.7) than that of older oysters (1.1). A two-factor ANOVA using year and age class as variables indicated that the interaction just described was significant ($p<0.0001$) because the effect of age class varied across years.

When plotted by shell height size class (Fig. 9), prevalence and sample intensity for oysters pooled across seven beds sampled continuously between 1990 and 2010 increased markedly between individuals with shell height <25 mm and those with shell heights between 25 and 40 mm. Thereafter, both metrics increased steadily, but more slowly, with increasing size. Ninety percent of oysters greater than 100 mm were detectably infected and sample intensity averaged 2.1. A similar marked increase occurred from the <25 mm to the 25–40 mm categories (1.2 to 2.5) when shell height was plotted against infection intensity, but intensity did not increase further, and dropped marginally in oysters >60 mm.

e. Evidence for host resistance

The time-line plots of bay-wide prevalence, sample intensity and infection intensity (Fig. 5) showed no evidence of a decline in prevalence. The slight attenuation of infection level measured by sample intensity corresponded to a small decrease in mortality, indicating that mortality was responding to changes in infection intensity, which itself may have been

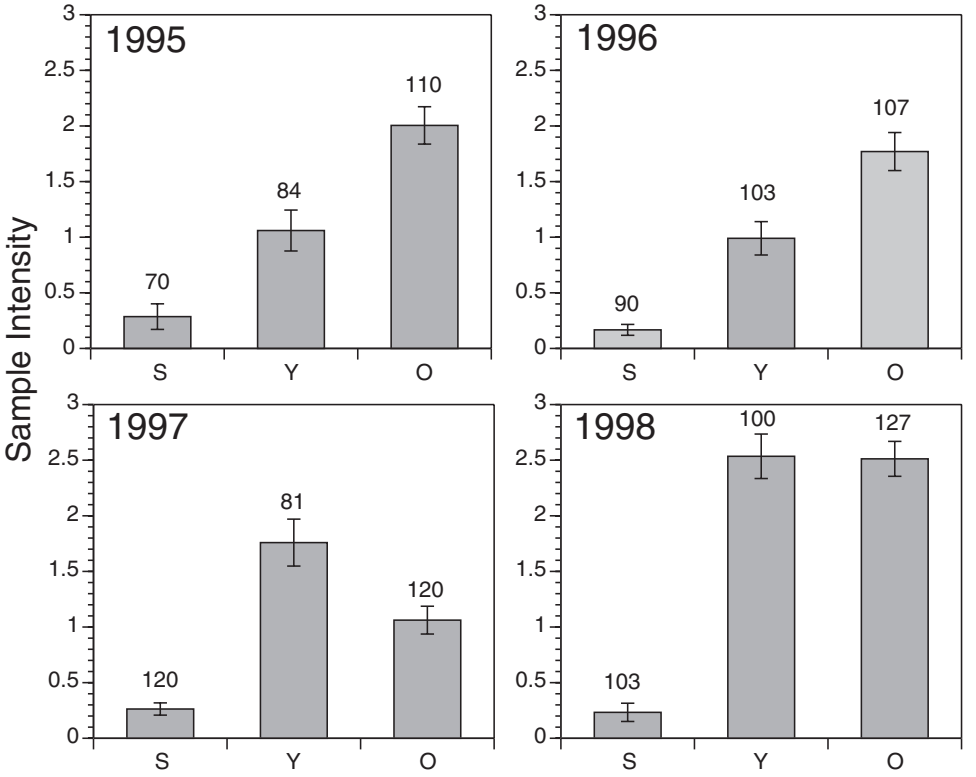

Figure 8. *Perkinsus marinus* sample intensity for different age classes of oysters. S = spat, Y = yearlings, O = older oyster. Error bars represent one standard error. Sample size indicated above bars.

responding to high river flow (Fig. 7). Regressions of mortality against sample intensity for consecutive five-year periods were all significant, but slopes and intercepts were similar indicating no evolution of tolerance (Fig. 10). Collectively, these data indicate no change in resistance to dermo disease by the population over time.

4. Discussion

Infectious diseases of marine organisms often propagate explosively, and there is growing concern that disease-related mortality is increasing in the oceans (Harvell *et al.*, 1999, 2002; Lafferty *et al.*, 2004). Examples include diseases of highly motile marine organisms such as canine distemper in seals (Heidejorgensen *et al.*, 1992) and a variety of fish diseases (e.g., infectious salmon anemia) where contact among hosts is important. These diseases can be described by the typical Susceptible-Infected-Recovered (SIR) model (McCallum *et al.*, 2003; Ogut *et al.*, 2005). In contrast, many diseases of sessile marine organisms are

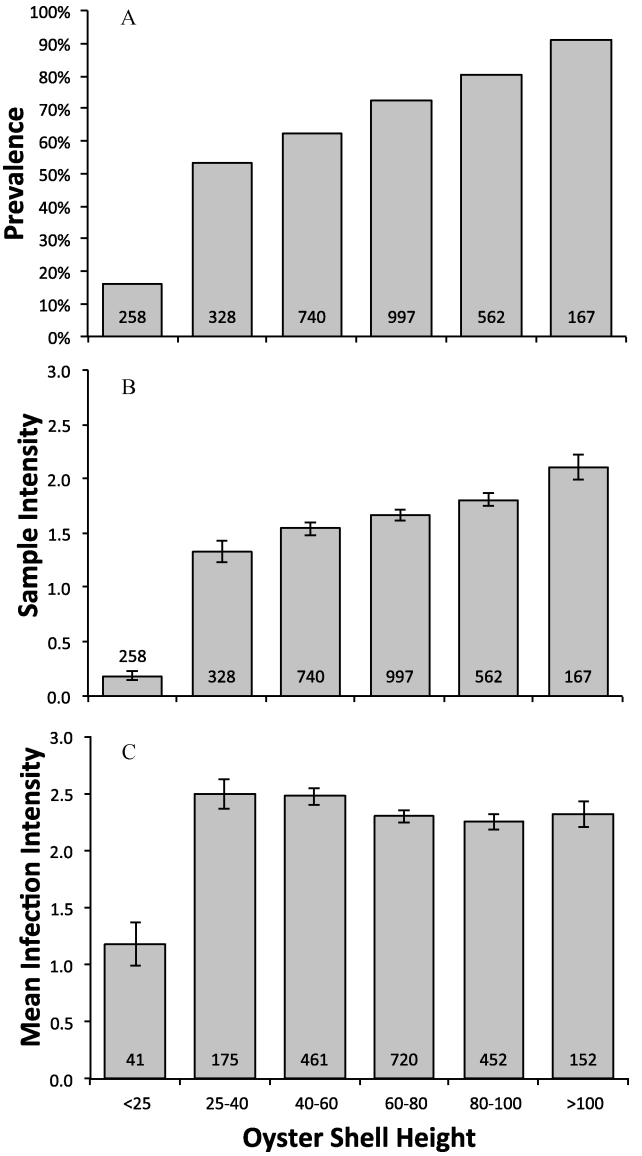

Figure 9. Prevalence (A), sample intensity (B) and infection intensity (C) of dermo disease by size (shell height) class. Error bars represent one standard error.

caused by pathogens transmitted passively in the water column, though other modes of transmission including vectors or alternate hosts may be possible (e.g. Hoese 1963; White *et al.*, 1987; Haskin and Andrews, 1988). Examples include an unidentified disease of sea urchins (Lessios *et al.*, 1984), withering syndrome of abalone (Lafferty and Kuris, 1993;

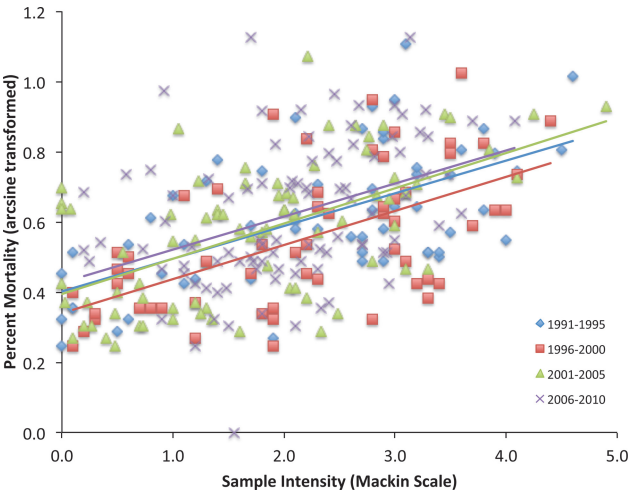

Figure 10. Regressions of mortality as a function of sample intensity for four consecutive time periods over the time series.

Richards and Davis, 1993), and the oyster diseases, MSX and dermo (Ford and Tripp, 1996). Understanding how such diseases interact with host populations over time in response to climate change as well as a myriad of anthropogenic changes is critical to the adaptive management of marine resources and ecosystems (Soniat *et al.* 2009).

The present study summarizes a 21-year dataset on dermo disease and eastern oyster mortality from the onset of a persistent epizootic that began in 1990. Analyses indicated (1) a strong positive association between disease and mortality along the salinity gradient that was exponential and defined by thresholds, (2) a clear spatial gradient of increasing disease and mortality with increasing salinity, (3) an apparent 7-year cycle in which peaks were associated with positive winter anomalies of the NAO, (4) an inverse relationship with freshwater inflow, and (5) a general absence of a response to natural selection despite persistent disease pressure.

Many studies have demonstrated that the population abundance of eastern oysters is strongly influenced by dermo disease (e.g., Ray, 1954; Andrews and Hewatt, 1957; Mackin, 1962; Hofmann *et al.*, 1995; Powell *et al.*, 1996, 2008), and that environmental fluctuations are important influences of epizootics across space and time (e.g., Soniat, 1996; Burreson and Ragone Calvo, 1996; Soniat *et al.*, 2009), but none has spanned 21 years – a time scale relevant to climatic influences. Few studies report results from more than a few years, although some reports have cobbled together data from disparate studies gathered over many years (e.g., Bobo *et al.*, 1997). This situation is not unique to oysters, but is common across many taxa as most studies are undertaken in response to a disease outbreak and support for long-term surveillance is often unavailable. Oyster disease surveillance programs exist in many states and several have accumulated data over many years that are presented in

technical reports (e.g., Quick and Mackin, 1971; Bobo *et al.*, 1997; Carnegie and Burreson, 2009; Tarnowski, 2010; HSRL, 2011), but long-term patterns are not always compiled and the data often start after the disease had become fully epizootic. Furthermore, many studies document experimental work rather than data collected from wild populations. Studies of disease in natural populations often fail to include mortality statistics with disease data due to logistical difficulties of collecting mortality data from wild populations; notable exceptions for dermo disease include Burreson and Calvo (1996), Carnegie and Burreson (2009), Soniat *et al.* (2009) and the present study. Most other studies that have collected *P. marinus* infection and oyster mortality data contemporaneously have used hatchery stocks or wild oysters held in cages. This is not a criticism; rather a distinction that presents different limitations when interpreting results. In the present study, the relatively simple hydrology of Delaware Bay facilitated correlations of disease and mortality to freshwater input, which regulates both flushing and salinity, over a time scale relevant to climatic influences. Our data indicated an epizootic cycling of seven years defined by relatively brief periods of only moderate remission. This periodicity suggests a connection to climate cycles. Other studies have linked the NAO to ecological cycles in populations of marine organisms (e.g., Otterson *et al.*, 2001; Solow 2002; Soniat *et al.* 2009), and we found an indication that high levels of dermo disease were more likely to occur during stronger positive winter NAO anomalies.

Many factors such as temperature, salinity, freshwater inflow and recruitment are known to influence dermo disease, but the confluence of these factors is difficult to predict. Moreover, while there is some understanding of how these factors influence spatial and seasonal variations in dermo disease, it is less clear how they interact to influence inter-annual variation. Powell *et al.* (1992) documented a multi-year cycle of *P. marinus* across the northern Gulf of Mexico that they attributed to larger climate processes that were apparently controlling changes in salinity (i.e., the El Nino Southern Oscillation and particularly La Nina events). The cycling identified in Delaware Bay in the present study roughly corresponded to cycling of the NAO. The relationship of *P. marinus* infection intensity to the NAO and other climate cycles warrants further investigation and analysis.

Epizootics of dermo disease were for many years restricted to oysters in the southeastern and Gulf of Mexico coasts of the United States. Beginning in the mid 1980s, and accelerating in 1990–1992, epizootic outbreaks were recorded in a northward direction from the upper Chesapeake Bay to Cape Cod, Massachusetts (Ford, 1996). This range expansion was associated with a warming trend that was particularly strong in 1990–1992 (Cook *et al.*, 1998), clearly indicating a role for climate change in the outbreaks. Ford (1992), however, pointed to the long history of transplanting oysters from southern regions, where *P. marinus* was prevalent, to northern sites to replenish stocks depleted by overfishing. Ford (1996) suggested that the parasite, which is transmitted directly between oysters, was moved northward at the same time, but remained cryptic because of low temperatures until climate warming triggered the epizootics. Thus, the range extension of dermo disease epizootics may be a combination of anthropogenic introduction and climate warming, although possible between-estuary transport by ocean currents cannot be ruled out.

We found a higher correlation of mortality with sample intensity than with prevalence. This resulted from the fact that very light infections, which increase prevalence, are not likely to cause mortality. Mackin (1962) reported that oyster mortalities could be expected to increase as sample intensity exceeded 1.0 and this was clearly evident in our data (see Fig. 3). It also helps explain why mortality on the upper beds was often out of phase with *P. marinus* prevalence and sample intensity; namely, low infection intensities did not routinely influence mortality on the upper beds. Instead, increased river flow, which reduced disease pressure throughout the bay, put animals on the upper beds at risk of fresh water kill. The y-intercepts of Figure 3 indicate a median background level of mortality of 5.8% and Figure 4 indicates that this ranges from <5% to about 20%, which encompasses the range of median mortalities measured on the upper beds (Fig. 3). Sources of background mortality include freshets (most likely limited to the upper beds) as well as predation, siltation and, occasionally, other parasites and diseases. The association of mortality with salinity observed in the present study is not new to the presence of dermo disease. Predation and MSX disease are well-known to increase with salinity (Haskin and Tweed, 1976; Haskin and Ford, 1982; Ford and Bushek, this issue) and this probably explains some of the higher background mortality on the lower beds shown in Figure 4. The higher annual "background" mortality on the lower beds, however, can also be attributed to dermo disease-induced deaths accruing before the fall sampling. Mortality data are a result of cumulative deaths whereas infection data are instantaneous measurements.

Figure 3 supports the contention that the Mackin scale used to calculate sample intensity approximates a $\log_{10}$ scale of parasite burden g^{-1} wet tissue (Choi *et al.*, 1989; Bushek *et al.*, 1994). This relationship provides insight into how parasite population dynamics influence host mortality. Starting from one cell, it takes more than a dozen doublings to reach 5,000 cells g^{-1}. This means that infections may linger at low levels for relatively long periods with little effect on host mortality. Once infections reach these higher levels, they quickly become lethal with the next two doublings of the parasite population in the host. Note how this dynamic puts the central region beds in a precarious position should parasite intensities increase further with time (Fig. 3). Our data also show that infections develop quickly in new recruits. By the time they are yearlings and/or greater than 25 mm shell height, sample intensity is already approaching the level found in older oysters. It is consequently unlikely that Delaware Bay oysters can grow fast enough to "outgrow" the parasite, as Powell *et al.* (1996) concluded, from numerical simulations, was the case for *P. marinus* proliferation versus oyster growth in the northern Gulf of Mexico.

A few studies indicate that limited resistance of oysters to dermo disease has developed in some populations (Andrews and McHugh, 1957; Bushek and Allen, 1996; Encomio *et al.*, 2005; Brown *et al.*, 2005), but dermo disease remains a significant source of mortality wherever it is not constrained by environmental conditions. This situation contrasts with that for MSX disease: at least one bay-wide population has been shown to have developed a high level of resistance (Ford and Bushek, this issue) and there is evidence that resistance to MSX disease has also developed in other, more localized, populations (Carnegie and

Burreson, 2011). We did not find evidence of resistance to dermo disease developing in Delaware Bay oysters, despite the persistent association of the disease with oyster mortality over a period of 21 years. Using a gene-based mathematical model, Powell *et al.* (2011) simulated the potential for oysters in Delaware Bay to develop resistance to dermo disease. They concluded that resistance develops very slowly: on a decadal time scale with a selective mortality rate of 40% annually and considerably longer at lower rates. Although the studies of resistance to MSX disease have been able to detect differences in mortality among geographically differentiated natural populations or selectively bred stocks of oysters, we are unaware of any comparable studies (c.f., Carnegie and Burreson, 2011; Ford and Bushek, this issue) that have demonstrated similarly strong resistance to dermo disease, documented and persisting over the long term, in any stock that has been under heavy disease pressure. The slight decrease in mortality observed over time in the present study, corresponded to a decrease in disease (Fig. 5), but that can be explained by increased river flow (Fig. 7). We did find that infection intensity decreased marginally in larger, older oysters, suggesting that survivors of selective mortality may be able to restrict infection development to some degree, but infection levels were still high and we found no evidence that oysters have become more capable of tolerating infections and not dying.

It is tempting to compare regressions of mortality against sample intensity between regions as evidence for the development of dermo disease resistance. For example, a sample intensity of 3.0 on the lower region beds corresponds to about double the mortality rate on the central region beds (Fig. 4). This, however, is opposite to the expectations of natural selection since disease pressure, and therefore selection pressure for resistance, is greatest on the lower beds. Monthly monitoring (data not shown) indicates that lower bay beds typically experience higher levels of dermo disease sooner and for longer periods of time resulting in higher mortality rates during the summer and greater cumulative mortality by the annual fall survey.

Temperature, salinity and river flow have long been recognized as prominent environmental controls over dermo disease across spatial and temporal scales (Hoffman *et al.*, 1995; Burreson and Calvo, 1996; Powell *et al.*, 1996; Soniat *et al.*, 2009). Temperature is clearly the major seasonal control over the infection cycle, and is also recognized as the predominant latitudinal control for the species distribution (Ford, 1996). Salinity plays an important role in the distribution of *P. marinus* infections within estuaries, but its seasonal effects are less clear (Soniat, 1985; Soniat and Gauthier, 1989). The interannual effects of temperature and salinity are also unclear, but appear to be linked to larger climate processes (Powell *et al.*, 1992; Soniat *et al.* 2009; this study). Several studies have suggested that warm winters have a large effect by sustaining a higher prevalence and intensity of infections through the winter, particularly in the mid-Atlantic region of North America (Burreson and Calvo, 1996; Cook *et al.*, 1998; Soniat *et al.*, 2009). We found that temperature and river flow (a correlate of salinity) influenced *P. marinus* infections across years, but the relationship was statistically significant only in some regions of the bay. Moreover, the lagged relationship shown in Table 5 indicates a relatively long system memory that warrants further investigation.

Mackin (1962) argued that the relationship with salinity was simply correlative and not causative as salinity can be related to river inflow and flushing (i.e., dilution of infective particles). In Delaware Bay, salinity is largely controlled by freshwater inflow, which also controls the rate of flushing (Wang *et al.*, this issue). As a result, separating the effects of salinity and flushing is virtually impossible. Other studies, however, have clearly indicated that osmotic stress from low salinity ($<\sim 12$) can kill *P. marinus*, reduce transmission, or reduce proliferation (Chu *et al.*, 1993; Ragone and Burreson 1993; Burreson *et al.*, 1994; O'Farrell *et al.*, 2000; LaPeyre *et al.*, 2003; LaPeyre *et al.*, 2006). LaPeyre *et al.* (2009) identified optimal levels of freshwater inflow in both magnitude and duration to depress dermo disease in Louisiana without compromising oyster growth, although they did not consider the effects of flushing from fresh water inflow per se. Subsequently, in laboratory studies, LaPeyre *et al.*, (2010) identified combinations of temperature and salinity that depress *P. marinus* infections in oysters and concluded that low salinity and low temperature combined are likely to produce the greatest impact. Like most previous studies, we found that *P. marinus* infections and mortality were positively associated with temperature and salinity, and negatively associated with river flow. With warm temperature and high salinity favoring the development of dermo disease there may be a continuum of climatic conditions from favorable to unfavorable for the disease: warm dry climate $>$ cool dry climate $\geq$ warm wet climate $>$ cool wet climate. The majority of fresh water entering Delaware Bay comes from the Delaware River and tributaries located above the oyster beds; however, inputs from several tributaries that enter the bay adjacent to the seedbeds combine with the geomorphologic configuration of the shoreline to influence salinity, nutrients, food supply, circulation and flushing in ways that are not completely understood. These factors undoubtedly interact to influence the spatial and temporal variation in prevalence and intensity of disease and mortality on the seedbeds. The three-dimensional circulation model of Delaware Bay (Wang *et al.*, this issue) combined with continued long-term spatial monitoring and directed experimental research should provide additional insights into these dynamics.

Acknowledgments. Many HSRL staff and students provided field, logistical and technical support over the past 21 years. E. Hofmann and two anonymous reviewers provided helpful comments that improved our analyses and discussion. Long-term programmatic support has been provided by the State of New Jersey through the New Jersey Bureau of Shellfisheries, the Delaware Bay Shellfisheries Council and Rutgers University. Additional support was provided by the US Army Corps of Engineers and as part of the National Science Foundation Ecology of Infectious Diseases (OCE06-22672) program.

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Received: *10 October, 2011*; revised: *21 June, 2012*.