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The influence of multiple environmental stressors on susceptibility to parasites: An experimental determination with oysters

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Abstract

A large-scale field experiment was used to test whether exposure to a suite of potential environmental factors (flow speed, temperature, salinity, and low dissolved oxygen) influences the level of parasitic infection of the oyster *Crassostrea virginica.* The parasite was the protozoan *Perkinsus marinus,* which has decimated populations of oysters in estuaries of North America. The environmental factors were considered stressors because they influence the physiological condition of either the host or parasite. Between December 1994 and July 1995, flow speed, temperature, salinity, dissolved oxygen concentration (DO), *Perkinsus* infection, and mortality of oysters were monitored across 24 experimental oyster reefs in the Neuse River estuary, North Carolina. Eight reef treatments were created consisting of an orthogonal combination of three factors: water depth (3 m vs. 6 m deep), reef height (2 m vs. 1 m tall), and position on reef (base vs. crest). Principal component analysis revealed that there was clear separation of environmental factors among reefs and that a majority of the variation (96.2%) among treatments could be explained by two principal component axes (PCs): one (24.3% of variation explained) was formed by flow and the other (71.9% of variation explained) by temperature, salinity, and DO. Oysters with the highest proportion of individuals infected (prevalence), highest intensity of infection, and highest mortality were located at the base of reefs, where flow speeds and food quality were lowest and sedimentation rates highest. However, there was no significant effect of hydrographic conditions on *Perkinsus* infection or mortality of oysters, despite large differences in mean salinity, DO, and exposure to hypoxia-anoxia. Temperature did not vary among treatments. Correlation of disease responses (infection prevalence and intensity, and mortality) with the first two PCs showed that these response variables were significantly (and negatively) correlated with flow only. Oysters in low flow were hypothesized to have the greatest susceptibility to *Perkinsus* infection because of their poor physiological condition. The restoration of oyster reefs increases reef height and thus flow speeds (by elevating oysters higher above the boundary and by actively influencing flow), thereby reducing the negative effects of disease by restoring reef morphology to its natural size.

Interactions between parasites and their hosts can dictate host population dynamics (Burdon 1987), alter food webs (Marcogliese and Cone 1997), and influence the functioning of ecosystems (Real 1996). Environmental conditions, including natural and anthropogenic stressors, often have strong influence on parasite–host interactions because they regulate the physiological condition, reproduction, and survival of both parasites and their hosts. When a host suffers physiological stress induced by extreme environmental conditions, its susceptibility to parasitism and associated disease may increase (e.g., Chapin 1991; Gustaffson et al. 1994; Urawa 1995), while its ability to survive infection decreases (e.g., Sousa and Gleason 1989). This is an example of a more general phenomenon, described by Peterson and Black (1988), that poor physiological condition from past environmental stress predisposes organisms to greater risks from exposure to additional stressors. Alternatively, environmental stress may have a deleterious effect on parasites causing infection rate and intensity within host populations to decrease (Lafferty 1997). It has long been hypothesized that synergism of multiple environmental stressors has a greater impact on host-parasite dynamics than do single stressors (*see* Myers 1995; Lafferty and Kuris 1999), but there is little empirical evidence supporting this hypothesis (*but see* Valtonen et al. 1997).

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Eastern oysters (*Crassostrea virginica* Gmelin: Bivalvia) are historically important components of estuarine ecosystems along the Atlantic and Gulf coasts of North America (e.g., Newell 1988; Baird and Ulanowicz 1989) and support a multi-million dollar fishery (MacKenzie 1996). Unfortunately, populations and harvests of eastern oysters have declined more than 90% during the last century (e.g., Hargis and Haven 1988; Rothschild et al. 1994), in part because of heavy mortalities caused by the introduced protozoan parasite *Perkinsus marinus* (e.g., Ford and Tripp 1996). Controlling the disease caused by *Perkinsus* infection ("Dermo") is considered a high priority by oyster fishery managers in Atlantic and Gulf coast states (Kennedy and Breisch 1981; Hargis and Haven 1988; Frankenberg unpubl. rep.). At present, we know very little about how environmental factors influence *Crassostrea–Perkinsus* interactions beyond the fact that virulence of the parasite and rates of infection in oysters increase with water temperature and salinity (Paynter and Burreson 1991; Chu et al. abstr.). Many workers have proposed that exposure to multiple stressors such as sediment loading, anthropogenic pollutants, and hypoxia-anoxia, increase the susceptibility of oysters to *Perkinsus* infection (e.g., Barber 1987; Fischer et al. abstr.; Kennedy 1996). In laboratory tests, oysters have exhibited increased susceptibility to *Perkinsus* infection when exposed to toxic contaminants (Chu and Hale 1994; Anderson et al. 1996), but experiments testing the effects of multiple stressors have not been conducted under field conditions.

Oysters create biogenic reefs in the intertidal and subtidal portions of estuaries that are habitat to species-rich communities of fish (Arve 1960; Breitburg 1992) and invertebrates (Wells 1961; Bahr and Lanier 1981). The physical structure and location of oyster reefs regulate environmental conditions for oysters, including flow speed, sediment deposition and accumulation, and exposure to hydrographic conditions, including temperature, salinity, and dissolved oxygen concentrations. In turn, these environmental factors influence the recruitment, growth, physiological condition, and survival of oysters (Lenihan and Peterson 1998; Lenihan 1999). Experiments with restored oyster reef habitat show that destructive harvesting practices reduce the height of reefs by removing not only oysters, but the shell matrix on which oysters are attached (Lenihan and Peterson 1998). The reduction in reef height causes local flow speed to decrease and rates of sedimentation and reef burial to increase (Lenihan 1999). In addition, when harvest-degraded reefs are located in deep water subject to density stratification and bottom water hypoxia-anoxia, exposure to low oxygen stress increases in frequency and duration. Oysters exposed to the combined effect of low flow speed (and associated high sediment deposition) and prolonged hypoxia on harvest-degraded reefs suffer poorer growth, physiological condition, and survival (Lenihan 1999). Thus, the structure and location of oysters reefs may control exposure to environmental stressors with subsequent effects on the prevalence and intensity of *Perkinsus* infection in oysters.

In this paper, we test the general hypothesis that exposure to multiple environmental stressors influences the prevalence (i.e., the proportion of infected individuals) and intensity (i.e., the concentration of parasite cells per individual) of parasitic infection of a host species. The environmental stressors to which we exposed oysters were of natural and anthropogenic origin. Temperature and salinity are factors that vary naturally with water depth and season within estuaries and can significantly influence the virulence of *P. marinus*. Dissolved oxygen also varies naturally within estuaries, but the temporal and spatial extent of hypoxia-anoxia has increased in many estuaries subjected to anthropogenic nutrient loading (Dyer and Orth 1994). Oxygen stress may also influence *Perkinsus* infections in oysters by reducing the physiological condition and thereby increasing the susceptibility of the host. Flow speed varies over oyster reefs as a function of reef height, a physical characteristic of natural oyster reefs that has been reduced over the last century due to fishery-related disturbance. Reduced flow speed on harvest-disturbed reefs results in greater sediment deposition, reduced quality of suspended food material, and subsequent decrease in the physiological condition of oysters. We hypothesized that acting in concert, these natural and anthropogenic stressors would have a greater positive effect on *P. marinus* infection than they would acting separately. To test this hypothesis, we performed a field experiment in the Neuse River estuary, North Carolina, using experimental oyster reefs established in a factorial design. We varied reef height as a means of controlling local flow speed and water depth, as a means of manipulating exposure to variation in temperature (T), salinity (S), and dissolved oxygen concentration (DO). By manipulating water depth and reef height independently, we were able to test experimentally whether flow (and associated variables), hydrography (T, S, and DO) or the combined effect of flow and hydrography influence parasitic infection in oysters.

Methods

Study organisms—The eastern oyster *C. virginica* (Ostreacea: Mollusca) inhabits estuarine and coastal waters from the Gulf of St. Lawrence, Canada, to Argentina (Carriker and Gaffney 1996). Oysters live in water temperatures of 0–36°C and salinities of 0–40 psu but have the highest growth and reproductive rates and lowest overall mortality in temperatures ranging from 20 to 30°C and salinities ranging from 15 to 30 psu (Shumway 1996). Healthy adult oysters can survive several weeks without oxygen if water temperature is <5°C but cannot withstand hypoxic conditions (<2 mg O_2 liter^{-1}) for more than about 10 d in 18°C water (Jordan et al. 1992). Temperature, salinity, and dissolved oxygen concentration are abiotic factors that most profoundly influence the development, feeding, growth, reproduction, and survival of oysters (Shumway 1996) because they control their physiological condition. When parasitic infection is absent, growth rate, physiological condition, and survival of oysters increase with flow speed (Lenihan et al. 1996) and salinity (Shumway 1996) and decrease with sedimentation and burial (Ali 1981).

P. marinus is a protozoan having a complex life cycle composed of free-living larvae (zoospores) and adult stages that live within oysters (Andrews 1988). Propagules of *Perkinsus* are ingested by oysters as they filter the water for

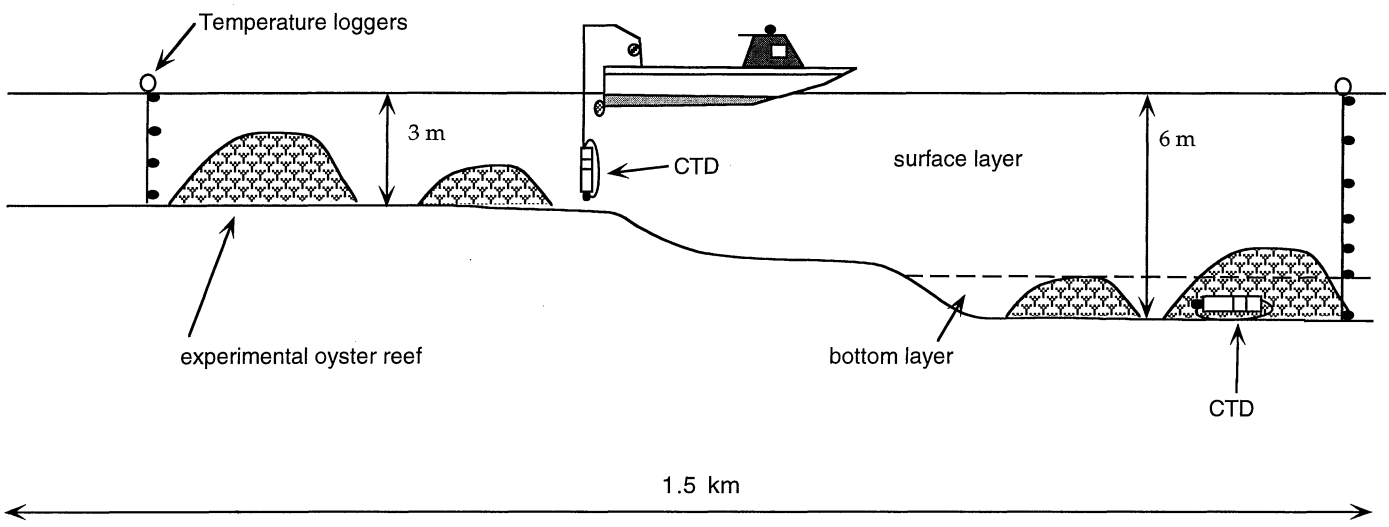

Fig. 1. Experimental design of study showing placement of experimental oyster reefs in the Neuse River estuary. Tall (2 m) and short (1 m) reefs (n = 6 of each reef type) were located at 3- and 6-m water depths. Horizontal scale of river width is grossly compressed. Tall reefs were 6.5–7.5 m in diameter; short reefs were 4.5–5.5 m in diameter. The actual distance between any two reefs was 10–32 m.

food and the parasite proliferates within the tissues and sinuses of the host when the water temperature and salinity increase in spring and summer (Ford and Tripp 1996). Infection reduces the growth rate and physiological condition of oysters (Paynter and Burreson 1991), and mortality rates within infected populations are usually high (Andrews 1988). Although the specific mechanism causing death is unknown, *Perkinsus* probably kills oysters by lysing and replacing oyster tissue until the oyster's organs cease to function (Paynter 1996). Upon death, oysters gape open and parasite propagules are released into the water column where they can be ingested by other oysters. Infection within an oyster population spreads rapidly but usually disappears within infected oysters during winter months when *Perkinsus* becomes dormant (Menzel and Hopkins 1955).

Environmental stressors—We exposed oysters to different flow speeds on experimental oyster reefs because oysters in low flow (<4 cm s^{-1}) have lower growth rates, poorer physiological condition, and higher mortality than oysters in high flow (7–20 cm s^{-1}) in both the laboratory (Lenihan et al. 1996) and field (Lenihan 1999). Greater flow speed is thought to enhance the efficiency of active suspension feeding, thereby reducing the energy required for feeding and respiration (Newell and Langdon 1996; Lenihan et al. 1996). Results of earlier experiments (Lenihan 1999) indicate that the structure of oyster reefs enhances ambient flow speed such that flow is 50–60% greater over the crests of reefs than at similar heights above the seafloor but away from reefs, that flow is much greater (400–600%) over the crests than bases of reefs (*see Fig. 1*), that the quality of suspended food material (the ratio of particulate organic matter to particulate inorganic matter) is greatest higher up on oyster reefs, and that areas of lower flow are depositional environments, where sedimentation rates are higher (Lenihan 1999) and oysters presumably expend a greater amount of energy processing and removing non-food materials from their filtering apparatus. Oysters at low flow speed may attain higher prevalence and intensity of *Perkinsus* infection than oysters in high flow primarily because their general physiological condition is reduced in environments with low flow speed and high sedimentation rates.

Oysters were exposed to different temperature and salinity regimes because these fundamental hydrographic variables influence the prevalence and intensity of *Perkinsus* infection. *Perkinsus* infection in oysters increases, and growth and physiological condition of infected oysters decrease, with increasing temperature and salinity (Chu et al. abstr.). Oysters were exposed to different temperature and salinity by placing them on experimental oyster reefs of different heights (1 and 2 m tall) located at two water depths (3 and 6 m) in a stratified estuary. This dispersion of oysters across experimental reef treatments effectively placed oysters at different depths (1, 2, 3, 4, 5, and 6 m) within the water column of the estuary (*see below*), which is subject to temperature and density stratification (Lenihan and Peterson 1998). Therefore, placing oysters at different positions (base and crest) on experimental reefs of two different heights located at two water depths provided not only a means of manipulating exposure to different flow speeds but also different hydrographic conditions.

Oysters were exposed to the stress of low oxygen (hypoxia-anoxia) to reduce their overall physiological condition (e.g., Paynter 1996) and test their ability to suppress and survive *Perkinsus* infection. Hypoxia-anoxia is likely to increase the intensity of *Perkinsus* infection in oysters and mortality caused by infection because oysters are immunologically suppressed due to reduced energy supply and the disruption of cellular function (*see* Paynter 1996), infected oysters rapidly become acidotic (i.e., decreased tissue pH) when hypoxic, thereby benefiting *Perkinsus,* which prefers a low pH environment (Paynter 1996), and infected oysters expire at faster rates than uninfected oysters when exposed to hypoxia (Dwyer and Burnett 1996). Thus, rather than in-

creasing the likelihood of contracting the parasite, prolonged exposure to hypoxia-anoxia probably decreases an oyster's ability to suppress proliferation of *Perkinsus.* This intensifies the infection and increases mortality rates of infected oysters and may cause more oysters to remain infected once they ingest the parasite.

Study site—Our experiment was conducted in the Neuse River estuary (*see* fig. 1 of Lenihan and Peterson 1998), which is well described in the literature (e.g., Mallin and Paerl 1994). This estuary is a typical mesohaline habitat for *C. virginica,* with salinity that ranges from 5 to 30 psu and water temperature that ranges from 0 to 32°C (Paerl et al. 1995). The Neuse River estuary is subject to frequent, prolonged periods of hypoxia (<2 mg O_2 liter^{-1}) and anoxia in bottom waters (i.e., water depths >5 m) during periods of vertical stratification in the water column during late spring and summer (Paerl et al. 1995; Lenihan and Peterson 1998). Periods of stratification and hypoxia-anoxia can last up to 10 weeks (H. Lenihan unpubl. data) but most often last for periods ranging from several days to 2 weeks. Mass mortality of both oysters and sedentary fish occurs on harvest-degraded oyster reefs in deep water during prolonged hypoxic-anoxic events (Lenihan and Peterson 1998). The North Carolina Division of Marine Fisheries (NCDMF) has monitored oysters for *P. marinus* infection annually throughout the estuary since 1989 and has found that oysters have suffered moderate to high prevalence and intensity of *Perkinsus* infection since 1990 (NCDMF unpubl. data).

Experimental reefs—We conducted our field experiment using experimentally restored oyster reefs constructed for use in this study and two companion studies (*see* Lenihan and Peterson 1998; Lenihan 1999). For this experiment, six replicate experimental oyster reefs (i.e., mounds of oyster shells) of each of two morphologies, tall (2 m high, roughly hemispherical in shape) and short (1 m high, hemispherical), were constructed at two water depths (3 and 6 m) in the estuary (24 total reefs, see Fig. 1). Oysters were sampled at the base of half of the reefs (n = 3) in each water-depth × reef-height combination, while oysters at the crests of the reefs were sampled from the other half. Reefs were constructed in July 1993 with help from NCDMF. Our experimental design provided eight locations throughout the water column where naturally occurring and experimental oysters were exposed to different levels of flow, sedimentation, food quality, S, T, and DO: the base of short reefs at 6-m water depth (=6 m deep); the base of tall reefs at 6 m (=6 m deep); the crest of short reefs at 6 m (=5 m deep); the crest of tall reefs at 6 m (=4 m deep); the base of short reefs at 3-m water depth (=3 m deep); the base of tall reefs at 3 m (=3 m deep); the crest of short reefs at 3 m (=2 m deep); and the crest of tall reefs at 3 m (=1 m deep). Based on the results of previous work (Lenihan 1999), in which flow speed, sedimentation, and food quality were measured many times under varying environmental conditions, we anticipated that oysters placed on crests of experimental reefs would be exposed to higher flow speeds and food quality and lower sedimentation. Oysters placed at progressively deeper locations were also expected to be exposed to progressively higher salinity and greater oxygen stress but lower temperature (Lenihan and Peterson 1998). Tall reefs were 6.5–7.5 m in diameter and 33–38 m^2 in area and were used to mimic tall natural oyster reefs that once existed in the estuary. Short reefs were 4.5–5.5 m in diameter and 16–20 m^2 in area and were used to mimic large reefs that had been reduced in height by several decades of oyster dredge-harvesting. Short reefs also provided a mimic of the most common morphology of restored oyster reef constructed in North Carolina's oyster reef restoration program. Replicate reefs at both water depths were 10–32 m apart from one another. All reefs were constructed in July 1993. Larval oysters colonized experimental reefs primarily in three distinct settlements between August and October of 1993–1995. Reefs supported dense populations of adult and juvenile oysters (300–500 oysters m^{-2}) by the beginning of our experiment in December 1995.

Environmental measurements—Methods used to measure flow speed on experimental oyster reefs as a function of water depth, reef height, and position on reefs are given by Lenihan (1999). Briefly, flow measurements were made with InterOcean S-4 current meters placed on each position on reefs for one 15-min interval. Flow speeds reported are mean flow speeds of 60 measurements taken during each 15-min interval. Flow measurements were taken in April 1995 at the base (on the side facing into the direction of flow) and crest of three replicate reefs of each height at each water depth. Flow was measured during a period of moderate freshwater discharge when wind velocity was 5 m s^{-1} from the SW (Lenihan 1999), conditions that cause estuarine circulation and ambient water velocities of ~10–15 cm s^{-1} (normal flow conditions) at both the 3-m and 6-m stations (H. Lenihan unpubl. data). Freshwater discharge and wind velocity are the primary factors controlling tidal level and current velocity within the estuary, and our measurements of flow speed were taken during relatively normal discharge and wind conditions for the Neuse River (Wells and Kim 1989). In addition, flow speeds over reefs measured in this study correspond closely to other flow measurements taken over the same experimental reefs for periods of 15 min to 10 d during winter, spring, and summer months in 1993 and 1994 (Lenihan 1999).

Hydrographic conditions were determined by measuring water T, S, and DO around experimental oyster reefs between December 1994 and July 1995. Hydrographic data presented in this study are from Lenihan (1999), and methods used to collect data are presented therein. Briefly, vertical T profiles were collected at each water depth using a string of temperature loggers which recorded temperature at the surface of the water and at multiple positions in the water column corresponding to where oysters were deployed on experimental reefs (Fig. 1). T was taken at the surface and at water depths of 1, 2, and 3 m at one location among experimental reefs located at 3-m water depth. At 6-m water depth, T was taken at the surface and at water depths of 4, 5, and 6 m, also at one location. Hydrographics were sampled at only one location at each site because horizontal variability in T, S, and DO among experimental reefs (an area of ~3,000 m^2 at each site) was very low in three previous years (1992–1994; H. Lenihan unpubl. data). S and

DO were also measured at a water depth of 5.5 m (at the base of a tall reef at 6 m deep) using a moored Sea-Bird SeaCat 16-DO CTD. A profiling CTD was deployed from a boat once a week, or daily during periods of stratification, throughout the duration of the experiment to provide additional T, S, and DO measurements (Fig. 1).

Sampling of oysters—To determine the influence of environmental stressors on the dynamics of *Perkinsus* infection, we sampled both naturally colonized oysters and genetically similar, hatchery-raised oysters on experimental oyster reefs as a function of water depth (3 m vs. 6 m), reef height (2 m vs. 1 m tall) and position on reefs (base vs. crest). Naturally colonized adult oysters (>35 mm in shell ht) were sampled four times in 1994–1995 (2 December 1994, and 11 February, 1 May, and 22 June 1995) to follow the seasonal progression of *Perkinsus* infection in natural populations of oysters on experimental reefs. On each of the four sampling dates, 30 adult naturally colonized (background) oysters were haphazardly selected from the crest or base of three short and three tall reefs located at water depths of 3 and 6 m. To avoid statistical non-independence, we sampled no oysters from the base and crest of the same reefs.

Genetically similar, hatchery-raised oysters were spawned from North Carolina broodstock by ARC, Inc., in October 1994 and kept in upwelling tanks at the Institute of Marine Sciences in Morehead City until they were transplanted to experimental reefs. Only oysters with a shell height of 23.8–24.4 mm were used in the experiment (n = 4,620 oysters total). On 12 December 1995, a subsample of 300 hatchery-raised oysters was collected from upwellers and analyzed for prevalence and intensity of *Perkinsus* infection. On 13–15 December, oysters were transported in coolers from upwellers to the estuary where they were deployed onto experimental reefs. We placed 180 oysters on each of the three replicate water depth × reef height × position-on-reef treatment combinations. The 180 oysters were divided into six groups of 30 oysters each and each group was placed on a cement sampling substrate that was dug into the surface of the reef. Sampling substrates were 30 × 30-cm square slabs of cement (6 cm high) upon which 100 single valves of dead and bleached adult oysters shells (55–70 mm high) had been attached by sinking the ventral side of the shells several centimeters into the cement before it hardened. Experimental oysters were wedged in between the oyster shells of the slabs. We anticipated that a relatively high proportion of oysters would be lost from the cement slabs due to predation by crabs and fish. We chose to use slabs and not predator-exclusion cages to mimic the topographic complexity of the experimental oyster reefs and minimize potential hydrodynamic artifacts. Sampling substrates at the base of reefs were placed on the side most often facing into flow (the upstream side at 3 m deep and downstream side at 6 m deep). On 10 May 1995, all oysters from two haphazardly selected sampling substrates were collected by divers and returned to the laboratory for measurements of *Perkinsus* infection. Recovered oysters were scored as live vs. dead in the field. All four sampling substrates that remained after the May sampling were collected on 27 June 1995.

Infection of *P. marinus* was determined by removing a 3–5-mm-long section of the rectum of each oyster and analyzing for the presence of *Perkinsus* using the thioglycolate staining method (Ray 1963; Paynter and Burreson 1991). Each rectum sample was stained with thioglycolate solution, mounted on a microscope slide after a 5-d incubation period, and the number of *Perkinsus* cells (hypnospores) counted in each of three haphazardly selected visual fields using a compound microscope at 10×. The mean number of hypnospores was then determined for each oyster based on the counts from the three visual fields. Prevalence of infection was calculated as the proportion of oysters infected in each group of 30 oysters collected. The weighted intensity of infection was calculated using Mackin's scale (e.g., Quick and Mackin 1971), in which the concentration of parasite cells from each oyster was assigned a score of 1, 3, or 5. Oysters with 0–100 *Perkinsus* cells per field of a microscope slide were considered lightly infected (score = 1), oysters with 100–1,000 cells were considered mildly infected (score = 3), and oysters with >1,000 cells were considered heavily infected (score = 5). Weighted intensity of infection for each replicate group of 30 oysters was determined by multiplying each Mackin's scale score (1, 3, or 5) by the total number of oysters with each score and then summing the total of the scores.

The mean proportion of transplanted oysters infected (i.e., prevalence) for each treatment replicate was determined by calculating the mean proportion of infected oysters from each of the sampling substrates and then calculating a grand mean (weighted by numbers from each replicate) based on the two (May) or four (June) means. Mean weighted intensity for each treatment was calculated in a similar manner. Mean prevalence and weighted intensity of infection in background oysters were based simply on the 30 oysters collected from each treatment replicate on each of the four sampling dates. Only living oysters were sampled for *Perkinsus* infection because scavenging organisms removed all the soft tissue from dead oysters before we could sample them. Mortality of transplanted oysters was measured across experimental reefs by counting the number of live and dead oysters recovered on sampling substrates collected in June. A relatively large number of oysters (8–18) were missing from each sampling substrate and most likely were removed by crabs and fish who carried the oysters away to open and consume them (H. Lenihan pers. obs.). The missing oysters were not used in the calculation of proportional mortality.

Statistical analyses—A three-factor ANOVA was used to test whether mean flow speed differed among treatments as a function of water depth (3 m vs. 6 m), reef height (2 m vs. 1 m tall), position on reef (base vs. crest), and their interactions. Differences in T, S, DO, and number of days in hypoxia were compared among water depths where oysters were located using quadratic regressions. Regression was used because hydrographic conditions were measured with water depth over time at the 3- and 6-m stations, not over each replicate oyster reef as was flow speed. Environmental variables (mean flow speed, mean salinity, mean temperature, and the total number of days spent in hypoxia) were condensed into principal components using correlation-based principal component analysis (PCA; sensu Chatfield and

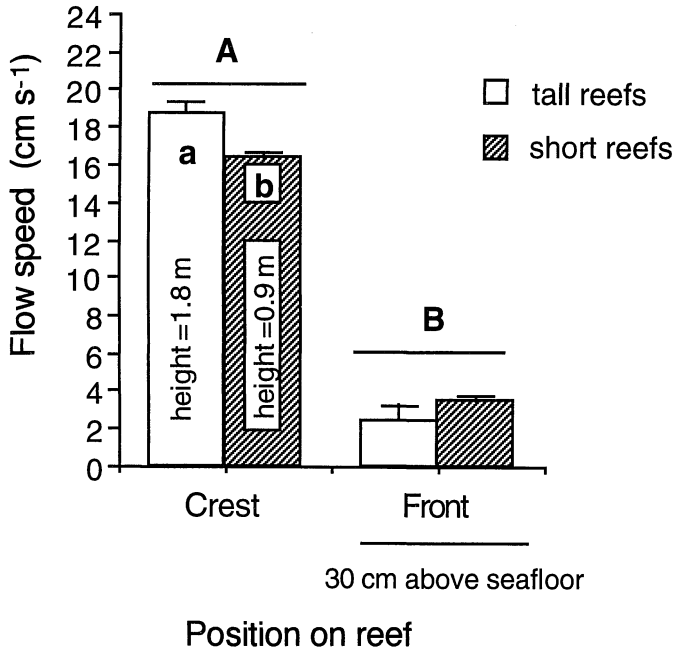

Fig. 2. Flow speeds measured on bases and crests of tall and short oyster reefs at water depths of 3 and 6 m. Measurements at the base were taken on the side of reefs facing into the flow. Data are means (±1 SE) of flow measured over three replicate reefs of each reef height × position-on-reef combination. There was a significant interaction in a three-way ANOVA between reef height and position on reefs but no significant effect of water depth. Accordingly, data shown were pooled from across reefs at 3 and 6 m. Letters represent results of SNK a posteriori comparisons: A > B and a > b at $\alpha = 0.05$.

Collins 1980). Differences among treatments in mean prevalence and weighted intensity of *Perkinsus* infection of naturally colonized and transplanted oysters, and proportional mortality of transplanted oysters, were determined using three-factor ANOVAs identical to that used for flow speed. A correlation matrix using Pearson correlation coefficients was erected to determine whether the prevalence and weighted intensity of infection and mortality of transplanted oysters were correlated with the first two principal components of environmental factors generated from the PCA. Before ANOVA, homogeneity of variances was tested using Cochran's test (at $\alpha = 0.05$). Variances were never heterogeneous. Proportional data were arcsin, square-root-transformed before analyses. Post-hoc multiple comparisons tests were performed on mean response variables using the Student-Newman-Keuls (SNK) method (Day and Quinn 1989).

Results

Results of a three-way ANOVA indicated that flow speed varied significantly with reef height and position on reefs (ANOVA; two-way interaction, reef height × position; $P = 0.002$) but did not vary with water depth ($P = 0.11$). Flow speed was greater over the crests of tall reefs than over crests of short reefs (SNK, $P < 0.05$; Fig. 2) but did not differ between the bases of tall and short reefs ($P > 0.05$). Flow speed was much greater over the crests than over bases of both tall and short reefs at both water depths.

There was a normal seasonal change in water temperature at the 3- and 6-m stations. At the 3-m station, there was very little difference in T among reef treatments (Fig. 3; Table 1), except during a 2-week period in June when temperature at the surface and crest of tall reefs (1 m deep) was 3–4°C higher than at the crest of short reefs (2 m) and base of all reefs (3 m). The same seasonal pattern in T was observed at the 6-m station (Fig. 4), but differences among the 4-, 5-, and 6-m treatments were not discernible throughout the course of the experiment. There was no significant difference in mean T among water depths (1–6 m) during the course of the experiment (1–6 m deep; $r^2 = 0.86$, $P = 0.055$).

Mean salinity at the 6-m station (16.9–17.6 psu) was higher than at the 3-m station over the course of the experiment (11.9–14.3 psu; Table 1; $r^2 = 0.96$, $P = 0.01$). However, the range of S was greater at the 3-m (4–19.0 psu) than at 6-m (13–19 psu) station during periods of stratification in late June and early July (Figs. 3 and 4). There was a large difference in S among treatments at the 3-m station during February and March (Fig. 3). Due to heavy storm runoff during that period, S at the surface and at 1 m deep was 3–7 psu lower than at 2 and 3 m deep. During a 2-week period in late May, density stratification developed at the 3-m station during which time S values at 2 and 3 m deep were 1–5 psu greater than at 1 m deep. This period was followed by another 2-week period in which S varied greatly among all treatments but was frequently 4–8 psu higher at 2 and 3 m than at 1 m deep. At the 6-m station, there were three periods of strong density stratification: during December–February, February–March, and beginning in mid-June and lasting until the end of the experiment on 1 July 1995 (Fig. 4). These stratification events were characterized by low S at the surface compared with that at 4, 5, and 6 m deep. The only discernible difference in S among treatments at the 6-m station occurred during the last 2 weeks of June, when S was 1–5 psu higher at 5 and 6 m deep than at 4 m.

Mean dissolved oxygen concentration varied with water depth over the course of the experiment ($r^2 = 0.96$, $P = 0.01$). Between December 1994 and 16 June 1995, DO did not drop below 5 mg O_2 liter^{-1} at the 3-m station (Fig. 3). The number of days of hypoxia-anoxia also varied with water depth during the course of the experiment (Table 1; $r^2 = 0.94$, $P = 0.01$). Beginning on 16 June, hypoxic conditions (0.5–2.0 mg O_2 liter^{-1}) developed at 2 and 3 m deep for short (1–2 d), interrupted periods. In contrast, hypoxia and anoxia occurred several times at the 6-m station (Fig. 4) beginning in the latter part of April. May and June had recurrent, short periods of hypoxia and anoxia that lasted 1–2 d at 5 and 6 m. Beginning on 16 June, hypoxia-anoxia developed at 4, 5, and 6 m deep and persisted for the remainder of the experiment. The bases of reefs at 6 m were exposed more frequently to hypoxia-anoxia than all other reef treatments (Table 1). Oysters located at the base of reefs at the 6-m station experienced a much greater number of days in hypoxia-anoxia (i.e., <2 mg O_2 liter^{-1}) than other reef treatments. In addition, there was a large difference in mean DO among treatments, characterized by low mean DO at bases of reefs located at 6 m deep (Table 1).

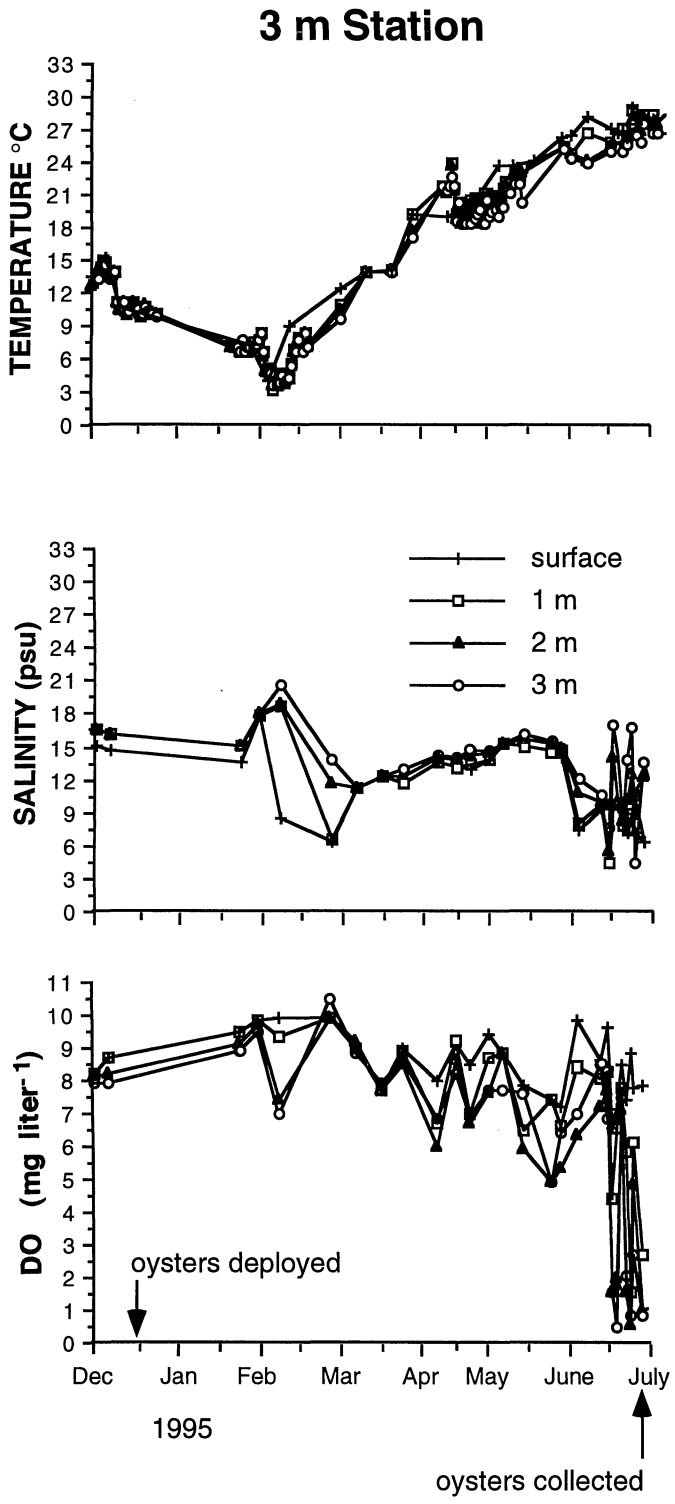

Fig. 3. Hydrographic profiles of water temperature, salinity, and dissolved oxygen taken at the 3-m station around experimental oyster reefs in the Neuse River estuary. Measurements were taken at water depths that corresponded to those where oysters were sampled on reefs.

Principal component analysis (PCA) revealed that 96.2% of the variation in the environmental factors flow speed, mean daily salinity and temperature, and number of days in hypoxia could be explained by two principal components (PC 1 and 2). PC 1 explained 71.9% of the total variation in environmental factors among treatments (Table 2) and was determined largely by T (eigenvector = −0.56), S (0.57), and hypoxia (0.56). Results of a correlation matrix (using Pearson correlation coefficients) showed that mean temperature was negatively correlated with PC 1 (P = 0.0001; see Table 2), while mean salinity and days in hypoxia were both positively correlated with PC 1 (P = 0.0001 for both variables). Consequently, PC 1 characterized mainly differences in T, S, and hypoxia-anoxia among treatments. Therefore, despite the lack of statistical differences in T among treatments, T still varied enough to help segregate treatments in the PCA. PC 2 explained an additional 24.3% of the total variation in environmental factors among treatments (Table 2) and was determined largely by flow (0.94). Results of a second correlation matrix showed that flow was the only variable that significantly correlated with PC 2 (P = 0.0001). PCA revealed that the original eight experimental reef treatments separated into six groups that were characterized by distinctly different environmental conditions (Fig. 5). Group 1 consisted of the crests of tall reefs at 3 m deep, which experienced high flow speed, the highest mean temperature, and the lowest mean salinity and number of days in hypoxia-anoxia. Group 2 consisted of the crests of short reefs at 3 m, which experienced high flow speed, high mean temperature, and low mean salinity and number of days in hypoxia-anoxia. Group 3 consisted of the bases of tall and short reefs at 3 m, which experienced low flow speed, relatively high temperature, and low salinity and number of days in hypoxia-anoxia. Group 4 consisted of the crests of tall reefs at 6 m deep which were characterized by high flow speed, low temperature, and moderately high salinity and number of days in hypoxia. Group 5, consisting of crests of short reefs at 6 m, experienced conditions very similar to those experienced by group 4 but with higher salinity and number of days in hypoxia. Finally, group 6 consisted of the bases of short and tall reefs at 6 m, which experienced low flow speed, the lowest temperature, highest salinity, and greatest number of days in hypoxia.

In December 1994, naturally colonized (background) oysters sampled on reefs and hatchery-raised oysters sampled from upwellers were not infected by *Perkinsus*. Background oysters on experimental reefs were also uninfected in February 1995. In May, the proportion of background oysters and hatchery-raised oysters infected on experimental oyster reefs was extremely low (0–1.5%) across all treatments, and there were no significant differences among treatments for either group of oysters (three-way ANOVAs; P = 0.68–0.98). The weighted intensity of infection averaged for all oysters (with and without disease) in May was also low, ranging from 0 to 1.0 for both groups. Again, there were no differences among treatments (three-way ANOVAs; P = 0.67–0.83).

Results of a three-way ANOVA comparing mean proportion of infected background oysters sampled on 22 June 1995 among reef treatments showed no significant interac-

Table 1. Mean (±1 SD) temperature (°C), salinity (practical salinity units; psu), and dissolved oxygen concentration (DO; mg O_2 $liter^{-1}$), and number of days experiencing hypoxia (<2 mg O_2 $liter^{-1}$) for each experimental reef treatment over the 7-month experiment (December 1994–July 1995). N = 54–144 days sampled for each hydrographic measurement at each reef treatment.

	3-m station				6-m station			
	Tall reefs		Short reefs		Tall reefs		Short reefs	
Hydrographics	crest (1 m)	base (3 m)	crest (1 m)	base (3 m)	crest (4 m)	base (6 m)	crest (5 m)	base (6 m)
T	16.4 ± 8.6	15.9 ± 8.2	16.2 ± 8.5	15.9 ± 8.2	14.4 ± 7.6	14.4 ± 7.1	14.4 ± 7.0	14.4 ± 7.1
S	11.9 ± 3.7	14.3 ± 2.7	13.0 ± 3.1	14.3 ± 2.7	16.9 ± 2.2	17.6 ± 2.1	16.9 ± 2.0	17.6 ± 2.1
DO	7.4 ± 2.1	6.1 ± 3.0	6.2 ± 3.0	6.1 ± 3.0	4.8 ± 3.0	4.0 ± 3.1	4.7 ± 2.8	4.0 ± 3.1
No. days in hypoxia	1	4	4	4	11	26	12	26

tions among the main factors water depth, reef height, and position on reef (Table 3). However, the proportion of background oysters infected differed significantly between reef heights and between positions on reefs (Table 3). The mean (±1 SD) proportion of infected oysters was significantly greater on short reefs than on tall reefs, regardless of water depth (Fig. 6). Prevalence of infection was also significantly greater on the bases than on the crests of reefs (Fig. 6). The proportion of infected background oysters ranged from 10 to 53% among treatments (Table 4). In a three-way ANOVA comparing mean weighted intensities of *Perkinsus* infection in background oysters (i.e., those with and without infection) sampled on 22 June, there were no significant interactions among the main factors (i.e., water depth, reef height, and position on reef; Table 5). There appeared to be a trend for greater infection intensity at 3 m than at 6 m deep (Table 4) and for higher intensity of infection on bases than on crests of reefs, especially on short reefs at a water depth of 3 m. However, differences between these treatments were not statistically significant at $\alpha = 0.05$ (Table 5), even within an ANOVA in which insignificant interaction terms were pooled (water depth: $P = 0.06$; position: $P = 0.06$).

There were large differences between reef crests and bases in both the prevalence and intensity of *Perkinsus* infection in transplanted oysters on 27 June (Table 4). Results of a three-way ANOVA showed that infection prevalence was greater on the bases than crests of reefs ($P = 0.0001$; Table 6, Fig. 7), but there were no significant effects of water depth or reef height and no significant interactions among the main factors ($P = 0.26$–0.92; Table 6). The weighted intensity of *Perkinsus* infection of transplanted oysters (i.e., considering all oysters with and without infection) sampled in June showed a similar pattern (Table 4). Results of ANOVA revealed that infection intensity varied with position on reefs ($P = 0.005$; Table 7, Fig. 8) but there was no significant effect of reef height or water depth and no significant interactions among the main factors ($P = 0.37$–0.91; Table 7). Infection intensity was higher on the bases than crests of reefs (SNK, $P < 0.05$).

Mean proportional mortality of transplanted oysters varied with position on reefs ($P = 0.05$; Table 8, Fig. 9) but did not vary significantly as a function of water depth or reef height nor were there any significant interactions among main factors. Predation mortality of oysters remaining on the sampling substrates (i.e., excluding those that were missing) caused by crabs and large fish, such as sheepshead (*Archosargus probatocephalus*), seemed to be very low. We deduced this from the fact that only a few oysters per treatment had cracked or crushed shells—damage indicative of crab and fish predation (Micheli 1997). We do not have data on how many oysters died from factors that do not cause noticeable shell damage, including flatworm predation and parasitic infection. In contrast, the percentage of oysters missing from each treatment was fairly high (26–60%) and may have been caused by the colonization of sampling substrates by benthic fish that do not seem to eat oysters (H. Lenihan pers. obs.), mainly two species of blennies (*Chasmodes bosquinas* Lacépéde, *Hyposoblennius hentzi* Lesueur). These two species were observed dislodging and displacing experimental oysters from the substrates throughout the experiment in order to construct burrows (H. Lenihan pers. obs.). We do not know how many of the missing oysters were removed and consumed by crabs and large fish.

Results of a correlation matrix using Pearson correlation coefficients showed that the prevalence and weighted intensity of *Perkinsus* infection and the mortality of transplanted oysters were significantly negatively correlated with PC 2 ($P = 0.0001$), the principal component describing almost entirely differences in flow speed among reefs treatments (Table 9). Infection prevalence and intensity, and mortality were not significantly correlated with PC 1 ($P = 0.10$–0.63), the principal component describing mainly differences in temperature, salinity, and exposure to hypoxia-anoxia among reef treatments.

Discussion

Our experiment was designed to determine whether the exposure of oysters to multiple anthropogenic and natural stressors in an estuary influences the susceptibility, prevalence, and intensity of *P. marinus* infection in oysters. We considered reduced flow speed over oyster reefs an anthropogenic stressor because it results from fishery-related disturbances that decrease the size (i.e., height) of oyster reefs. Oysters in low flow speeds can have reduced physiological condition, thus leaving them more susceptible to *Perkinsus* infection. Salinity was considered a natural stressor because it varies with water depth in estuaries and controls levels of *Perkinsus* infection in oysters. There was no significant dif-

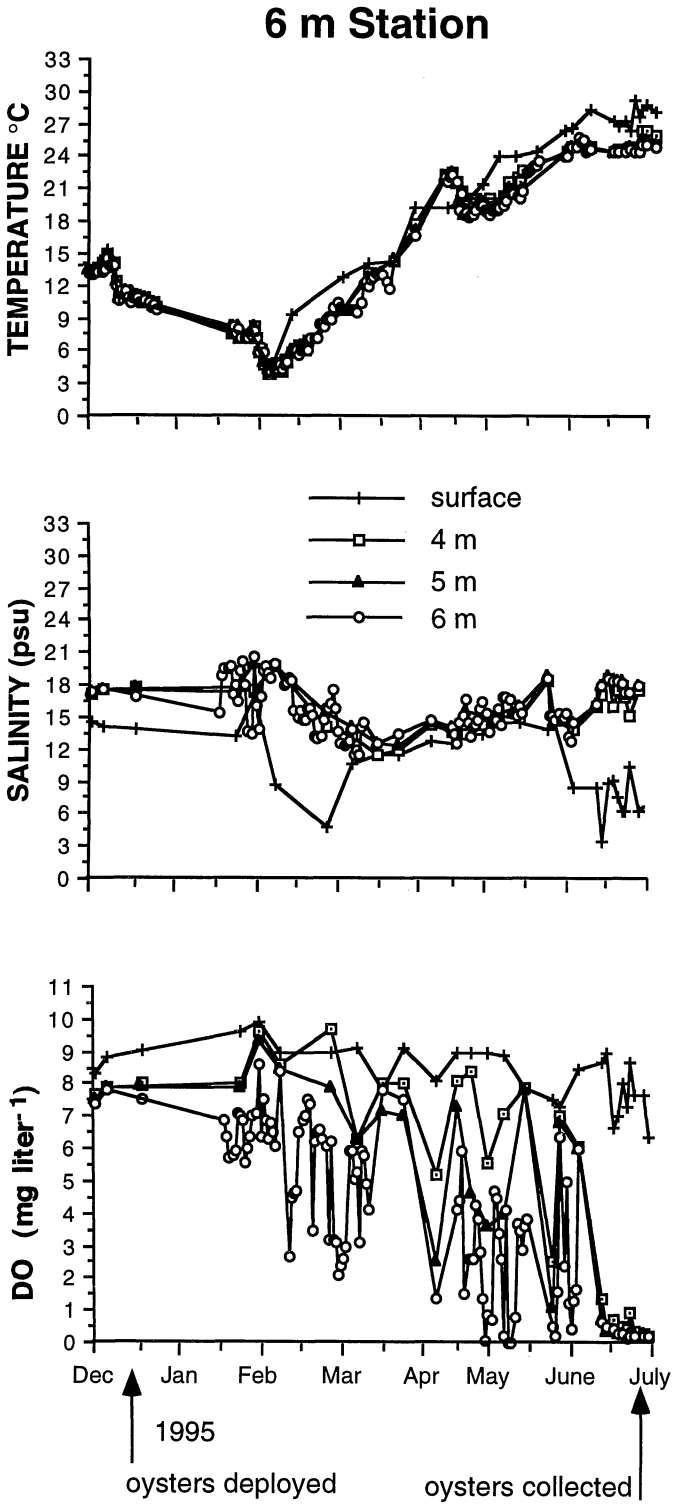

Fig. 4. As Fig. 3, but at the 6-m station.

ference in temperature among treatments, so it was not considered a stressor in this experiment. Low oxygen stress, which also reduces the physiological condition of oysters, was considered an anthropogenic stressor because it has increased in frequency and duration in many estuaries due to nutrient loading and resulting eutrophication. We were able to expose oysters to various combinations and levels of flow speed, salinity, and dissolved oxygen by placing them at the crest or base of tall and short experimental oyster reefs located at water depths of 3 and 6 m in the estuary. Our factorial design of two water depths × two reef heights × two positions on reef provided eight experimental reef treatments within which eight different combinations of the environmental stressors were predicted to occur. Results of PCA using the average values of flow speed, temperature, salinity, and number of days in hypoxia-anoxia, and results of ANOVA testing for differences in flow speed among treatments, revealed that only six experimental treatments were created (see Fig. 5). The eight factorial combinations were in effect condensed into six treatments because environmental conditions at the bases of tall and short reefs at 3-m water depth (group 3) were very similar, as were those at the bases of reefs at 6 m (group 6). Oysters located at increasing overall water depth segregated into groups experiencing increasing mean salinity and days in hypoxia. In addition, oysters located on the crests of reefs at both 3 and 6 m deep experienced greater flow speeds than oysters on the bases of reefs at both water depths.

Table 2. Results of a correlation matrix erected using Pearson correlation coefficients to determine the relationship between key environmental factors and the first two principal components axes (PC 1 and 2). PC 1 and 2 were calculated in a principal component analysis (PCA) used to determine how sites (i.e. reef treatments) grouped together and segregated on the bases of the environmental variables measured at each site and how those environmental variables contributed to explaining patterns of segregation. Underlined values are those that were significantly correlated with the PCs at $P = 0.0001$.

Environmental variables	PC 1	PC 2
Flow speed	−0.37	0.93
Mean T	−0.94	−0.29
Mean S	0.97	0.16
Days in hypoxia	0.95	−0.09
Component variance (*Eigenvalue*)	2.87	0.97
% of total variance explained	71.89	24.28

The results of our large-scale field experiment indicate that exposure to low flow significantly influenced the susceptibility of genetically similar, hatchery-raised oysters—the host species—to infection by *P. marinus*—their parasite. Oysters on the bases of tall and short reefs at 3- and 6-m water depths (groups 3 and 6) had significantly greater prevalence and intensity of *Perkinsus* infection (Figs. 7 and 8) and greater levels of mortality (Fig. 9) than oysters on the crests of reefs at both water depths (groups 1, 2, 4, and 5). There were no significant differences in these response variables between groups 3 and 6 nor between groups 1, 2, 4, and 5. Consequently, it appears that environmental conditions at the base of reefs had a greater influence on the susceptibility of oysters to *Perkinsus* infection than did environmental conditions at the crests of reefs. Oysters in group 3 were exposed to low flow speed and oysters in group 6

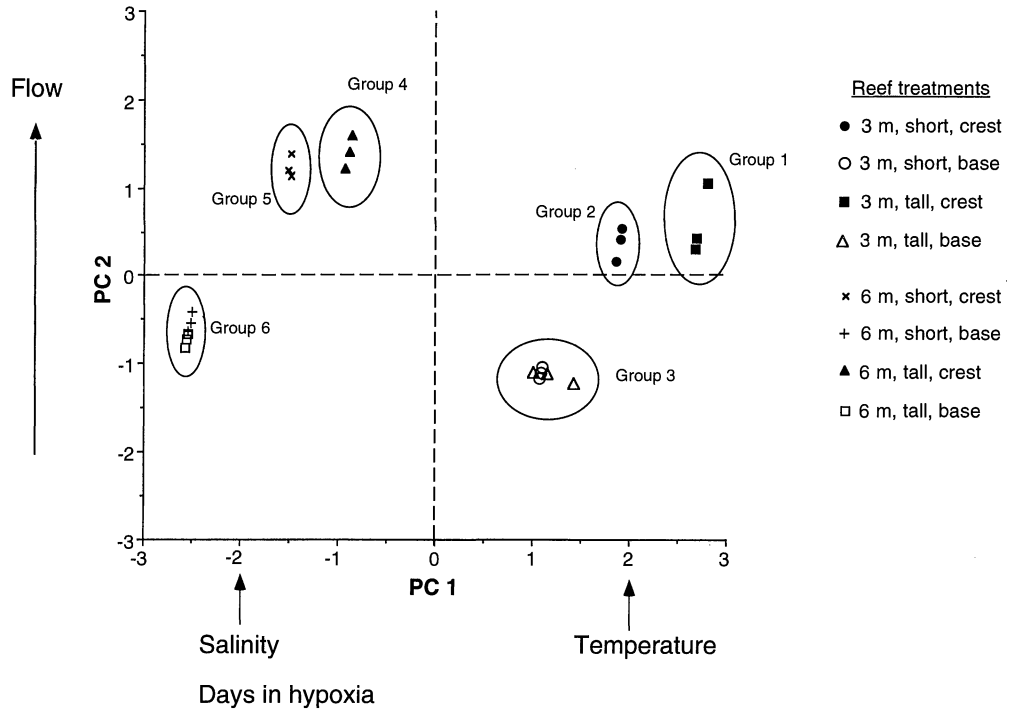

Fig. 5. Principal component analysis (PCA) ordination of environmental factors across the eight water depth × reef height × position-on-reef treatment combinations. PC 1 accounted for 71.9% of the total variance in environmental factors among treatments and was explained almost entirely by variation in temperature (PC loading = −0.56), salinity (0.57), and days exposed to hypoxia (0.56; *see Table 2*). PC 2 accounted for 24.3% of the total variance in environmental factors among treatments and was explained almost entirely by variation in flow speed (PC loading = 0.94). The eight reef treatments shown in the legend were visually condensed into six treatment groups (indicated by the six circles) because the distribution of environmental factors was very similar at the bases of all reefs.

were exposed to low flow speed and relatively high salinity and low dissolved oxygen. Results of a correlation matrix (Table 9) showed that prevalence and intensity of infection and mortality were correlated with PC 2, which primarily described differences in flow speed among treatments. In contrast, none of the response variables was significantly correlated with PC 1, which described differences in temperature, salinity, and oxygen stress. Therefore, we conclude that under the conditions realized in this experiment, position on the reef had a greater influence on the dynamics of *Perkinsus* infection than did water depth and reef height. We also conclude that of the environmental conditions measured, flow speed (and potentially other factors associated with flow speed) had a greater influence on parasitic infection than did salinity, low oxygen stress, or the interaction of these two factors.

Table 3. Results of a three-way ANOVA testing whether the proportion of background (i.e. naturally colonized) oysters infected with *Perkinsus marinus* varied as a function of water depth, reef height, position on reef, and their interactions. ANOVA was performed on arcsin (square-root)-transformed data.

Source	df	ms	*F*	*P*
Depth (D)	1	0.01	0.17	0.68
Reef height (H)	1	0.17	4.86	0.04
Position (P)	1	0.38	10.99	0.004
D × H	1	0.01	0.35	0.56
D × P	1	0.04	1.28	0.72
H × P	1	0.10	2.91	0.11
D × H × P	1	0.02	0.62	0.44
Residual	16	0.03		

We know of no study that has linked flow speed with parasitism in the aquatic environment but can identify several possible explanations for why flow speed may affect *Perkinsus* infection. First, recent studies (e.g., Breitburg et al. 1995; Lenihan 1999) reveal that oyster reefs influence the behavior of water flow and that flow speed increases from the base to the crest of reefs in a manner similar to that shown in Fig. 2. The importance of flow speed on the population dynamics of oysters and other suspension-feeding bivalves is well recognized (e.g., Peterson and Black 1987; Fréchette et al. 1993). On experimental reefs similar to those used in this study, Lenihan (1999) found that oyster growth, physiological condition, and survival were greater on the crests of reefs, where flow speed was highest, than on the bases of reefs, where flow speed was lowest. One potential mechanism explaining why flow speed and elevation on reefs enhances physiological condition is that oysters at the base of reefs in low flow are situated in a depositional en-

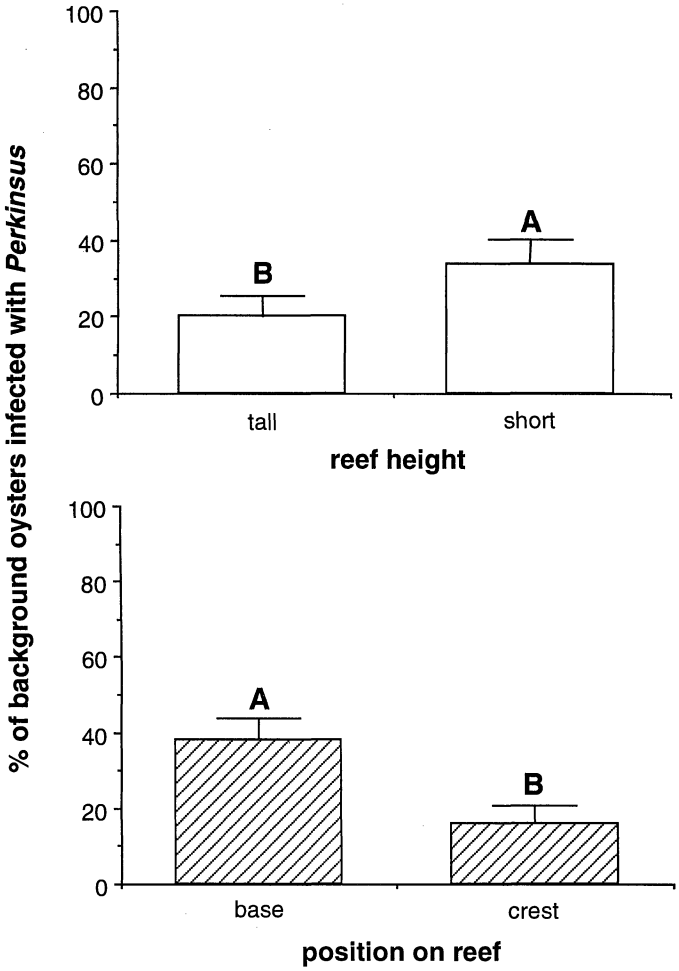

Fig. 6. Percent of naturally colonized (i.e., background) oysters, *Crassostrea virginica,* on experimental reefs infected with *Perkinsus marinus.* Oysters were sampled on 22 June 1995. Graphs illustrate significant differences among reef height and position on reef treatments (*see Table 2*). Top graph shows significant difference in infection prevalence between tall and short reefs pooled across water depths of 3 and 6 m. Bottom graph shows significant difference in infection prevalence between bases and crests of reefs pooled across both water depths. A > B at $\alpha = 0.05$ (ANOVA).

Table 5. Results of a three-way ANOVA testing whether the weighted intensity of *Perkinsus marinus* infection in background oysters varied as a function of water depth, reef height, position on reef, and their interactions.

Source	df	ms	*F*	*P*
Depth (D)	1	0.28	3.85	0.07
Reef height (H)	1	0.00	0.00	0.98
Position (P)	1	0.29	3.97	0.06
D × H	1	0.09	1.31	0.27
D × P	1	0.00	0.00	0.96
H × P	1	0.02	0.34	0.57
D × H × P	1	0.11	1.49	0.24
Residual	16	0.07		

vironment where suspended particles concentrate (e.g., Breitburg et al. 1995), rates of sedimentation and burial are highest (Lenihan 1999), and the quality of suspended food, measured as the ratio of particulate organic to particulate inorganic matter, is reduced (Lenihan 1999). Consequently, oysters in low flow and reduced food quality are in poorer physiological condition and less likely to fight off parasitic infection than oysters in high flow. Second, if we assume that *Perkinsus* propagules are evenly distributed in the water column and that they act as passive particles (e.g., Andrews 1988), it is likely that the highest concentration of propagules near an oyster reef would be at the base where other suspended particles accumulate. Consequently, oysters may be exposed to the highest concentrations of *Perkinsus* propagules at the base of reefs.

An alternative scenario is that oysters in higher flow at the crest of reefs are subjected to a greater flux of *Perkinsus* propagules and, therefore, would have a greater probability of being exposed to the parasite than oysters in lower flow at the base of reefs. We believe that this alternative scenario did not hold for our experiment for the following reasons. Oysters are active suspension-feeders that closely regulate the volume of water they filter based on the size and content of suspended particles (Newell and Langdon 1996). The feeding rate of active suspension-feeders, like oysters and clams, is generally independent of flow speed (Fréchette et al. 1993), unlike passive suspension-feeders in which feed-

Table 4. Mean (±1 SD) percent of background (i.e. naturally colonized) and transplanted, hatchery-raised oysters infected with *Perkinsus marinus.* Given also are the mean weighted intensity of *Perkinsus* infection for both sets of oysters and percent mortality of transplanted oysters (excluding those missing from sampling substrates). Background oysters were sampled on 22 June 1995 and transplanted oysters on 27 June 1995. Means are based on $n = 3$ samples per treatment, with each sample consisting of 68–80 individual oysters.

	3-m station				6-m station			
	Tall reefs		Short reefs		Tall reefs		Short reefs	
	crest	base	crest	base	crest	base	crest	base
Background oysters								
% infected	10.0 ± 5.0	30.0 ± 7.0	13.0 ± 5.0	48.0 ± 11.0	20.0 ± 20.0	21.0 ± 29.0	22.0 ± 0.1	53.0 ± 2.0
Weighted intensity	0.50 ± 0.10	0.78 ± 0.53	0.45 ± 0.17	0.59 ± 0.10	0.29 ± 0.02	0.31 ± 0.46	0.22 ± 0.14	0.64 ± 0.12
Transplanted oysters								
% infected	30.0 ± 7.0	44.00 ± 8.0	25.0 ± 1.0	50.4 ± 7.2	24.0 ± 15.4	51.3 ± 2.0	24.3 ± 11.0	53.2 ± 0.1
Weighted intensity	0.57 ± 0.22	0.89 ± 0.21	0.44 ± 0.21	0.59 ± 0.15	0.37 ± 0.28	0.85 ± 0.35	0.30 ± 0.20	0.65 ± 0.24
% mortality	30.0 ± 0.5	45.0 ± 2.0	30.0 ± 2.0	36.0 ± 23.0	30.0 ± 6.0	46.0 ± 13.0	33.0 ± 11.0	42.0 ± 10.0

Table 6. Results of a three-way ANOVA testing whether the proportion of transplanted oysters infected with *Perkinsus marinus* varied as a function of water depth, reef height, position on reef, and their interactions.

Source	df	ms	*F*	*P*
Depth (D)	1	0.00	0.00	0.97
Reef height (H)	1	0.00	0.10	0.80
Position (P)	1	0.39	34.22	0.0001
D × H	1	0.00	0.01	0.92
D × P	1	0.01	1.35	0.26
H × P	1	0.00	0.41	0.53
D × H × P	1	0.00	0.45	0.51
Residual	16	0.01		

Table 7. Results of a three-way ANOVA testing whether the weighted intensity of *Perkinsus marinus* infection in transplanted oysters varied as a function of water depth, reef height, position on reef, and their interactions.

Source	df	ms	*F*	*P*
Depth (D)	1	0.04	0.67	0.42
Reef height (H)	1	0.18	3.13	0.10
Position (P)	1	0.63	10.84	0.005
D × H	1	0.01	0.19	0.67
D × P	1	0.05	0.85	0.37
H × P	1	0.03	0.59	0.45
D × H × P	1	0.00	0.01	0.91
Residual	16	0.06		

ing rates scale with flow speed (e.g., Okamura 1984). Therefore, greater flux of parasite propagules does not necessarily mean greater rates of exposure for oysters, especially if oysters feed at lower relative rates where food quality is high (i.e., at the crest of reefs). In addition, oysters in higher flow are likely to be in better physiological condition than oysters in low flow, so increased exposure to *Perkinsus* in higher flow does not necessarily correspond to increased prevalence and intensity of infection. Greater physiological condition may counterbalance greater exposure to *Perkinsus*. Finally, that oysters in low flow speed had the highest prevalence and intensity of *Perkinsus* also agrees with predictions generated by the mathematical models of Hofmann et al. (1995) and Powell et al. (1996). These models predict that oysters experiencing the lowest food availability and quality, and greatest exposure to suspended sediments, have the lowest physiological condition and the greatest chance of contracting *Perkinsus* infection because they must filter the greatest volume of water to survive, grow, and reproduce.

Hydrographic conditions can have a large effect on *Perkinsus* infection in oysters. Results of several studies indicate that salinity and(or) temperature are the most important environmental factors influencing *Perkinsus* infection in oysters (e.g., Paynter and Burreson 1991; Burreson and Calvo 1996; Chu 1996). Differences in salinity among treatments in our study (11.9–17.6 psu; Table 1) were high enough to have potentially influenced the susceptibility of oysters to *Perkinsus* infection. For example, *Perkinsus* infects oysters at much higher rates in salinities ranging from 16 to 20 psu than in salinities ranging from 8 to 12 psu in Chesapeake Bay (Paynter and Burreson 1991) and North Carolina (Sherman and Shelton unpubl. rep.). Oysters infected with *Perkinsus* die at higher rates than uninfected oysters when exposed to hypoxia (Dwyer and Burnett 1996). However, we know of no study that has tested whether infection prevalence and intensity increase with oxygen stress. If oxygen stress does increase the susceptibility of oysters to *Perkinsus* infection as we hypothesize (*see also* Dwyer and Burnett

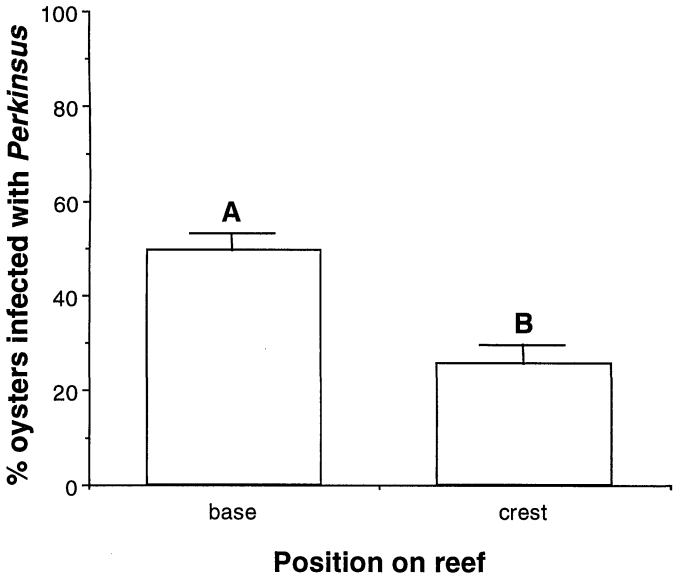

Fig. 7. Percent of transplanted, hatchery-raised oysters infected with *Perkinsus marinus* on experimental reefs sampled on 27 June 1995. Graph illustrates significant differences between positions on reefs, pooling across other factors which were insignificant in ANOVA (*see Table 6*). A > B at $\alpha = 0.05$ (ANOVA).

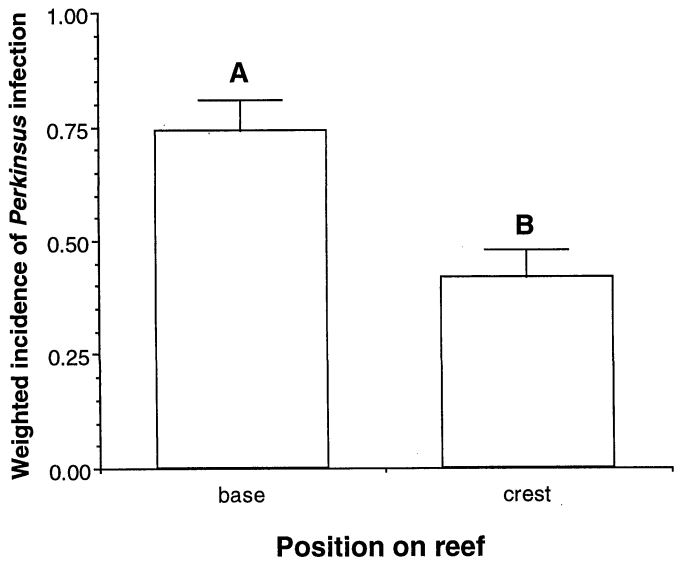

Fig. 8. Weighted incidence of *Perkinsus marinus* infection of transplanted oysters on experimental reefs sampled on 27 June 1995. Graph illustrates significant differences between positions on reefs, pooling across other factors which were insignificant in ANOVA (*see Table 7*). A > B at $\alpha = 0.05$ (ANOVA).

Table 8. Results of a three-way ANOVA testing whether the proportion of transplanted oysters found dead varied as a function of water depth, reef height, position on reef, and their interactions.

Source	df	ms	*F*	*P*
Depth (D)	1	0.00	0.29	0.59
Reef height (H)	1	0.00	0.24	0.63
Position (P)	1	0.09	4.42	0.05
D × H	1	0.00	0.19	0.66
D × P	1	0.00	0.04	0.84
H × P	1	0.01	0.54	0.47
D × H × P	1	0.00	0.01	0.93
Residual	16	0.02		

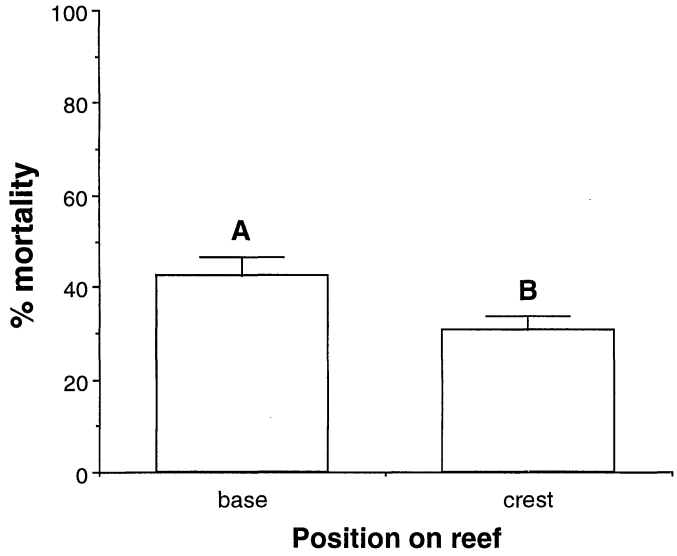

Fig. 9. Percent of transplanted oysters recovered on sampling substrates found dead on experimental reefs sampled on 27 June 1995 (*see Table 8*). Mortality presented here did not include oysters that were missing from substrates. A > B at $\alpha = 0.05$ (ANOVA).

1996; Paynter 1996), the large differences in dissolved oxygen concentration (Figs. 3 and 4) and duration of hypoxia (Table 1) among reef treatments should have influenced *Perkinsus* infection in our experimental oysters. In spite of these differences in hydrographic conditions, oysters exposed to the highest salinity and the greatest oxygen stress in our study did not have the highest overall prevalence or intensity of *Perkinsus* infection. For example, oysters at 6 m (groups 4–6) did not have greater prevalence or intensity of infection than oysters located at 3 m (groups 1–3), even though groups 4–6 were exposed to higher mean salinity (17.9–19.0 psu) and number of days in hypoxia (11–26) than groups 1–3 (13.9–15.8 psu; 1–4 d in hypoxia). Therefore, the effects of salinity and hypoxia alone—at least over the ranges realized in this experiment—did not have a large influence on *Perkinsus* infection.

The lack of significant effects of salinity and hypoxia in our experiment may have resulted for several reasons. First, the final sampling period occurred in early summer before the highest levels of disease prevalence and intensity are usually found in North Carolina (Sherman and Shelton unpubl. rep. 1991) and other regions of the U.S. (e.g., Ford and Tripp 1996). Oysters on experimental reefs in the Neuse River estuary sampled in late June may not have been infected at high enough weighted intensities (0.30–0.89; see Table 4) for the deleterious effects of the salinity and hypoxia to have caused significant increases in the response variables. Consequently, if the experiment were to have run throughout summer 1995, thereby exposing more intensely infected oysters to higher salinity and longer periods of hypoxia, a significant effect of these stressors on disease prevalence and intensity and oyster mortality might have been observed. We were unable to test the effects of higher levels of and longer exposure to environmental stressors because the experiment ended when prolonged stratification and hypoxia-anoxia began in early July.

We terminated the experiment on 1 July because weather forecasts called for an extended period of high temperature and calm winds—conditions that usually cause severe stratification and hypoxia-anoxia (Lenihan and Peterson 1998). In fact, stratification and hypoxia-anoxia lasted for >9 weeks in the estuary beginning in mid-June (H. Lenihan unpubl. data). Had we left experimental oysters on reefs, all would have perished as did natural populations of oysters on experimental reefs (H. Lenihan unpubl. data). By sampling oysters on 27 June, we were able to salvage the experiment from the fatal effects of an intense and prolonged natural disturbance. Second, salinity and hypoxia alone may not have had a significant effect on *Perkinsus* infection because of the counteracting effect of one or more of the other stressors. For example, relatively low water temperature at 6 m deep may have reduced the influence of elevated salinity and hypoxia on *Perkinsus* infection. Finally, salinity, hypoxia, and temperature may have influenced *Perkinsus* infection but only in concert with low flow speed. High salinity and hypoxia in concert with low flow speed and slightly elevated temperature in concert with low flow may have caused higher levels of infection in groups 3 and 6 than in any of the other groups.

Although the results of our experiment conducted on subtidal oyster reefs do not necessarily extend to another common type of oyster habitat—intertidal reefs—similar stressors and levels of *Perkinsus* infection are found in both habitats. Intertidal reefs are exposed daily to air (i.e., oxygen stress), solar heating (i.e., high temperature), and reduced flow speed on harvest-degraded reefs. In addition, intertidal oysters located in highly urbanized tidal creeks are often exposed to high loads of anthropogenic chemical contaminants. Intertidal oyster reefs are also located closer to the ocean inlets of estuaries, so oysters are exposed to higher

Table 9. Results of a correlation matrix using Pearson correlation coefficients erected to determine the relationship between disease response variables in oysters and PC 1 and 2. Underlined values are those that were significantly correlated at $P = 0.0001$.

Response variables	PC 1	PC 2
% infected	0.34	−0.77
Weighted intensity	0.10	−0.66
Mortality	0.29	−0.44

salinity (a better environment for *Perkinsus*) than subtidal reefs, which are usually found in the back positions of estuaries away from inlets. Consequently, oysters on intertidal reefs are probably exposed to even higher levels of multiple environmental stressors than oysters on subtidal reefs. Exposure to these multiple environmental stressors may be to blame for the high rates of *Perkinsus* infection and the resulting mortality in oysters living on some intertidal reefs in North and South Carolina (Sherman and Shelton unpubl. rep.; L. Coen pers. comm.).

The physical structure of habitat seems to have a strong effect on the dynamics of host–parasite interactions (e.g., Sousa 1991). For example, the frequency of parasitism in host populations changes with varying levels of habitat fragmentation (Kruess and Tscharntke 1994; Marino and Landis 1996; Roland and Taylor 1997) and within-habitat spatial heterogeneity (Hassell and May 1988; Murdoch et al. 1989; Grosholz 1993). The mechanism used to explain the results of these tests of whether habitat structure influences parasitism was that habitat structure and location control the frequency of physical contacts between hosts and parasites. The findings of our experiment indicate that habitat structure may have another, indirect effect on host–parasite interactions through the alteration of environmental factors. Specifically, the structure of oyster reef habitat, which changes with the level of fishery-related physical disturbance, influences flow with subsequent effects on the dynamics of *Perkinsus* infection. Oyster reefs influence flow because they project above the seafloor where flow speed naturally increases and reefs block ambient flow causing it to accelerate over and around the reef (Lenihan 1999). Flow has a subsequent effect on oysters by regulating the concentration of suspended food material and the rate of particulate deposition (Lenihan 1999), which together appear to influence the prevalence and intensity of *Perkinsus* infection. We suggest that protecting some natural reef habitat from fishery disturbance and that restoring oyster reefs so that they are tall enough to significantly influence local flow and associated factors will likely reduce levels and deleterious effects of parasitic infection in oysters. The relationship between reef height and *Perkinsus* infection is an example of how the effects of an introduced species interact with habitat management.

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