ABSTRACT

Title of thesis: THE EFFECTS OF INTERTIDAL EXPOSURE ON DISEASE, MORTALITY, AND GROWTH OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*

Jennafer Christine Malek, Master of Science, 2010

Thesis directed by: Adjunct Professor Denise Breitburg
Department of Biology

Disease, mortality, and growth of benthic organisms can be influenced by and determine spatial distributions. The eastern oyster, *Crassostrea virginica*, an economically and ecologically important species in Chesapeake Bay, is found in both the intertidal and subtidal in Virginia, but only in the subtidal in Maryland. I used field experiments and sampling to determine whether disease (Dermo) mortality, and growth of oysters vary among tidal heights during summer in the Maryland and Virginia regions of Chesapeake Bay. Results indicated that Dermo prevalence and mortality decreased and growth increased with decreasing durations of intertidal air-exposure. Dermo prevalence was higher in habitats with long durations of air-exposure than in subtidal habitats but progression of the disease did not differ consistently among tidal heights. Patterns in summer mortality, growth, and disease in combination with recruitment, winter mortality, and predation likely contribute to the variation in tidal distributions of oysters within Chesapeake Bay.

THE EFFECTS OF INTERTIDAL EXPOSURE ON DISEASE, MORTALITY, AND GROWTH OF THE EASTERN OYSTER, *CRASSOSTREA VRGINICA*

by

Jennafer Christine Malek

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Master of Science
2010

Advisory Committee:

Professor	Roger I. E. Newell, Chair
Adjunct Professor	Denise L. Breitburg, Co-Chair
Assistant Professor	Eric J. Schott

Acknowledgements

I would like to acknowledge the following organizations for helping fund my research:

Smithsonian Institution
Smithsonian Institution Women's Committee
National Oceanic and Atmospheric Administration
University of Maryland

I would like to acknowledge the following organizations for aiding in my research:

Smithsonian Environmental Research Center
    Maine Ecology Laboratory
Cooperative Oxford Laboratory
Morgan State University Estuarine Research Center
Virginia Institute of Marine Science
Virginia Institute of Marine Science – Eastern Shore Laboratory
Marinetics Inc.

Table of Contents

List of Tables

List of Figures

Chapter 1: Effects of Intertidal Exposure on Mortality and Growth of the eastern oyster, *Crassostrea virginica*

Introduction

Physical factors and biotic interactions determine geographic and tidal distributions of benthic marine and estuarine invertebrates. Geographic ranges of benthic species can be limited by heat and freezing tolerance, salinity, competition for space and food, and the presence of predatory species and pathogens (Dayton 1971; Mouritsen & Poulin 2002). Within their ranges, species may inhabit the intertidal or subtidal zones, or both. Organisms in the intertidal zone experience an array of physiological stressors due to exposure to extreme and rapidly fluctuating environmental factors. During periods of emersion and as the tide changes, heat stress, wave stress and damage, desiccation, freezing, limited ability to acquire O_2, and reduced feeding can occur (Dayton 1971). At locations with colder winter temperatures, ice scour plays a large role in determining the upper limits of species' spatial distribution (Wethey 1985; Heaven & Scrosati 2008). Ice scour can damage or kill intertidal organisms or entire communities, resulting in frequent recolonization of intertidal habitats in some areas. Mobile intertidal species may avoid exposure to stressful environmental conditions by retreating to less exposed areas (tidepools, rock crevices, algal cover) while sessile species must tolerate conditions or die. Organisms in the subtidal tend to be affected by factors such as water flow patterns, increased siltation, and increased exposure to aquatic predators (Crosby et al. 1991). However, there are fewer factors that cause physiological stress in the subtidal than in the adjacent intertidal.

The eastern oyster, *Crassostrea virginica*, is capable of living intertidally, subtidally, or in both habitats, depending on geographic location. There are intertidal oysters in the coastal bays of Virginia and in the southern portion of Chesapeake Bay, but none within the Maryland portion of the Chesapeake or to the north in Delaware Bay. Conversely, areas south of Virginia tend to have intertidal but not subtidal oyster populations. Proposed explanations for these spatial distributions include ice scour during winter (Taylor & Bushek 2008) and low recruitment (Tarnowski 2008) in northern Chesapeake Bay, and predation, disease, and shifting sediments in Delaware Bay (Taylor & Bushek 2008) and areas south of the Chesapeake (Burrell 1986; Crosby et al. 1991). Lutz et al. (1970) found that oyster larvae in the laboratory were stimulated to settle after exposure to a rapid increase in temperature of 5°C, such as might be experienced in intertidal waters during a flood tide. The small tidal range in most of the Maryland region of Chesapeake Bay constrains the actual area of the intertidal zone, thereby reducing the chances of spat settlement in intertidal habitats. Additionally, other settlement cues that larvae may require, such as stimuli from existing adult intertidal populations (Lutz et al. 1970), are currently non-existent in Maryland, further reducing the likelihood of intertidal spat settlement.

C. virginica is intertidal primarily south of Chesapeake Bay, although oysters have recently been observed in the intertidal zone in estuaries of New Hampshire and Maine as well (Capone et al. 2008). High salinity and local water temperatures influence the array of both competitors and predators that inhabit the subtidal in areas North and South of the Chesapeake and on the Atlantic coast of Maryland and Virginia (e.g. oyster drills and

starfish inhabit higher salinity environments). Increased competitors and predators in these areas cause high subtidal mortality and restrict oysters primarily to the intertidal zone to the south (Burrell 1986; Crosby et al. 1991). However, colder waters to the north of the Chesapeake likely reduce or alter the diversity of subtidal predators, allowing oyster to persist in the subtidal and intertidal zones.

The eastern oyster has been an ecologically important species in Chesapeake Bay since the last ice age and was an important food resource for both Native Americans and European colonists (Kennedy & Breisch 1981; Bartol & Mann 1997). *C. virginica* plays a key role in ecosystems in which it occurs by creating large three-dimensional reefs that serve as hunting, nursery, settlement, and refuge grounds for large numbers of fish and invertebrate species, including larval oysters (Kennedy 1996). After being the largest oyster producer in the world in the late 1800s (Kennedy & Breisch 1981; NRC 2004), the Chesapeake suffered a serious decline in oyster abundance, with current stocks at extremely low levels (Newell 1988; Jordan & Coakley 2004). This decline can be attributed to numerous factors, including overfishing (Jordan & Coakley 2004; Smith et al. 2005), habitat destruction (Rothschild et al. 1994; Coen et al. 2007), increased sedimentation and nutrient inflow (Rothschild et. al 1994), and disease (Andrews 1965; Andrews 1988). Reduced oyster abundances have led to a decrease in reefs resulting in diminished habitat for numerous other species (Coen et al. 2007), as well as reduced shore protection from wave effects (Meyer et al. 1997; Grizzle et al. 2002) and decreased phytoplankton consumption in the Chesapeake (Newell 1988; Cerco & Noel 2007; Fulford et al. 2007).

Until the last half century, overfishing and the resultant habitat (reef) destruction were the most important cause of oyster declines in Chesapeake Bay. However, in the 1950s in Virginia (Andrews 1988) and in the mid-1980s in Maryland, the oyster diseases MSX (caused by parasite *Haplosporidium nelsoni*) and Dermo began to take a heavy toll on the already heavily overfished populations, leading to greater decreases in harvest and abundance. The protistan parasite, *Perkinsus marinus*, which causes Dermo disease in oysters, was discovered in Chesapeake Bay in 1950, but did not cause substantial mortalities in the Maryland portion of the Chesapeake until the 1980s (Andrews 1988), although it caused annual mortalities in areas of the Chesapeake to the south of the Rappahannock River prior to that (Andrews 1996). Dermo has been a major obstacle in oyster restoration efforts throughout the Chesapeake due to the high mortality it causes (Mann & Powell 2007).

Adult *C. virginica* can withstand extreme air and water temperatures from 0°C to higher than 40°C. Intertidal oysters can survive temperatures of 46-49°C when exposed to air (Galtsoff 1964; Ingle et al. 1971) and can resume full metabolic functions after being frozen in ice over winter (Loosanoff 1965). Temperature also influences *P. marinus* infections, which spread and intensify rapidly at 25-30°C (Andrews 1965). Changes in water temperature, such as increases between winter and summer (from 1°C to 30°C in Chesapeake Bay) result in increased transmission and proliferation of the parasite in the oyster tissues, usually leading to mortality of the host. However, high temperatures and large temperature fluctuations (≥15°C), as well as increased CO_2 levels like those

experienced by intertidal oysters, can reduce growth of *P. marinus* in the laboratory (Milardo 2001), suggesting that oysters in the intertidal may have reduced *P. marinus* infections compared to oysters in the subtidal. Sudden changes in salinity or prolonged exposure to very high or low salinities may make oysters more susceptible to other stresses. Salinity also influences *P. marinus* prevalence and intensity and thus oyster mortality, with higher growth and proliferation of the parasite at salinities of 12 or above (Mackin 1956).

Chesapeake Bay oysters are preyed upon by both invertebrates and finfish (for a review see White & Wilson 1996), including the blue crab, *Callinectes sapidus* (Menzel & Hopkins 1956), the flatworm, *Stylochus ellipticus* (Landers & Rhodes 1970; Newell et al. 2000), and the cow-nosed ray, *Rhinoptera bomasis* (Krantz & Chamberlain 1978). Other major oyster predators such as the oyster drill, *Urosalpinx cinerea,* and the starfish, *Asterias forbesi*, are restricted to salinities of 15 or higher (Zachary & Haven 1973) and 18 or higher (Galtsoff 1964), respectively. Osmotic restrictions prevent these predators from inhabiting a large portion of the Maryland region of Chesapeake Bay and limit their influence on the spatial distribution of *C. virginica*.

Growth of *C. virginica* is influenced by many of the same factors that influence mortality. The amount of energy accrued through filtering suspended particles from the water plays the largest role in determining oyster growth. Water temperature, in turn, influences the amount and type of food that is available, the feeding behavior of oysters, and metabolic rates. In the warmer waters of the Gulf of Mexico, oysters can grow to market size (76

mm) in approximately 2 yr. In the colder waters of Long Island Sound, it can take 4-5 yr to reach the same size (Loosanoff & Nomejko 1946, Shumway 1996). The relationship between growth and salinity is not as well established within the range of salinities at which survival is high. Shaw (1966) found no difference in growth between oysters from the same area that were deployed at high and low salinity sites, but a recent study in Delaware Bay observed a salinity-based growth gradient, with higher growth occurring in areas of higher salinity (Kraeuter et al. 2007). Menzel and Hopkins (1955) observed that oysters of the same age with light *P. marinus* infections had slower growth rates than uninfected oysters, and those with heavy infections ceased growing altogether. Paynter and Burreson (1991) found that *P. marinus* infection can negatively affect *C. virginica* growth, particularly in moderate (12-15) to somewhat higher (16-20) salinity areas. Because higher *P. marinus* infections are most prevalent at moderate to high salinities, a negative relationship between infection intensity and oyster growth could counteract any positive effects of salinity on *C. virginica* growth where *P. marinus* is present in an oyster population.

Oysters that inhabit the intertidal are subject to physiological stress that can influence both mortality and growth. During intertidal air exposure *C. virginica* keep their shells tightly closed preventing gas exchange with the surroundings. Oxygen reserves are consumed, creating hypoxic internal conditions and elevated CO_2 levels (hypercapnia). Combined with other factors such as infection by *P. marinus* or extreme temperature changes, hypercapnia and hypoxia may cause elevated mortality. Hemolymph pH decreases during intertidal exposure as well, causing oysters to dissolve their calcium

carbonate shells and relieve some of the effects of acidosis (Dwyer & Burnett 1996; Burnett 1997). Duration of emersion, shell thickness, and overall physiological condition may influence how *C. virginica* responds to these intertidal stresses. The duration of emersion is a major determinant of how long oysters can feed. Oysters will feed constantly if food is available but growth tends to decrease with increasing emersion time (Burrell et al. 1984; Roegner & Mann 1995; O'Beirn et al. 1994; Bartol et al. 1999), as oysters become more physiologically stressed and are unable to feed during air exposure. Energy that would normally be used for somatic growth may be allocated towards metabolic functions as a response to intertidal stress (Dame 1972; Newell 1979). Also, once oysters become reproductive, gamete production further reduces resources available for somatic and shell growth. With decreased feeding time caused by air exposure, older, reproductive oysters are likely to experience substantially slower growth in the intertidal zone.

The objective of this study was to examine differences in mortality and growth of intertidal and subtidal oysters, with the goal of understanding how temperature, salinity, and *P. marinus* infections influence the spatial distribution of oysters. The following questions were posed: 1) Does tidal height affect *C. virginica* summer mortality and growth, and is the effect of tidal height similar in areas that have naturally occurring intertidal oyster populations (Virginia) than in those that do not (Maryland)? and 2) Does *P. marinus* infection intensity affect oyster growth at all tidal heights and sites during summer? I hypothesized that mortality of caged oysters would be lower in the subtidal than the intertidal, at sites with and without naturally occurring intertidal oyster

populations due to decreased physiological stress in the subtidal. I also hypothesized that at sites with and without naturally occurring intertidal oyster populations, oyster growth would be faster in the subtidal than in the intertidal due to increased feeding and decreased physiological stress in the subtidal. Lastly, I hypothesized that oyster growth would decrease with increasing *P. marinus* infection intensity at all tidal heights and sites.

Methods

Study Sites

Crassostrea virginica were deployed in field experiments conducted from June through September or October of 2008 and 2009 to evaluate spatial variations in mortality and growth between intertidal and subtidal oysters in Chesapeake Bay. Sites (Fig. 1) were chosen to span a range of environmental parameters, including temperature, salinity (Fig. 2a & 2b), and proximity to local oyster populations where heavy *P. marinus* infections have been documented (referred to herein as disease pressure), that potentially influence mortality, growth, and *P. marinus* infection of *C. virginica*. In 2008, study sites were in the Patuxent River at the Morgan State University Estuarine Research Center (MSUERC) on the western shore of Maryland and on the Atlantic coast of Virginia at the Virginia Institute of Marine Science Eastern Shore Laboratory (VIMS-ESL). Tidal ranges varied +0.09 to +0.79 m, and -0.18 to +1.77 m from mean low water (MLW) at MSUERC and VIMS-ESL respectively, depending on the lunar cycle (NOAA). In 2009, I conducted experiments at MSUERC, in the Rhode River at the Smithsonian Environmental Research Center (SERC), and in the York River at the VIMS laboratory located in

Gloucester Point, VA (Fig. 1). Tidal ranges varied +0.06 to +0.58 m, and -0.09 to +0.97 m from MLW, at SERC and VIMS, respectively, depending on the lunar cycle. Both VIMS-ESL and VIMS had natural populations of intertidal oysters, but at VIMS-ESL there were distinct, abundant reefs while at VIMS intertidal oysters settled on nearby pilings and experimental racks rather than forming distinct reefs. There was a gradient of increasing salinity and local disease pressure from North to South in both years (Tarnowski 2008).

Oyster Sources

Field experiments were conducted with 1-year-old oysters that were initially uninfected with *P. marinus* and older oysters with mid to high prevalence of pre-existing infections. Simultaneous use of these two oyster types allowed me to assess whether acquisition and progression of the parasite within *C. virginica* varied with tidal height at each location in Chesapeake Bay (see Malek Chapter 2). In both 2008 and 2009 initially uninfected oysters grown in the Choptank River were purchased from Marinetics Inc., Cambridge MD. Oysters averaged 53.6 mm ± 0.6 shell height (n=450) in 2008 and 54.5 mm ± 0.2 (n=1080) in 2009 (mean ± 1SE). Initially infected oysters were of mixed-ages, collected in the Patuxent River for the MSUERC deployments (98.9 mm ± 1.2 shell height, n=300) and near Wachapreague, VA for the VIMS-ESL deployments (79.55 mm ± 1.8 shell height, n=150) in 2008. The starting *P. marinus* prevalence and intensity for the 2 sites were 90% and 1.4 (±0.2, n=35 and ± 0.2, n=41; see Disease Analyses section for explanation of prevalence and intensity scales), and 90% and 1.5 (± 0.2, n=41) respectively. Two-year-old oysters were purchased from Marinetics Inc. for 2009

experiments (74.95 mm ± 0.4 shell height) and had starting *P. marinus* prevalence and intensity of 39% and 1.4 (±0.3, n=40), respectively (see Malek Chapter 2).

Study Design

At each of the study sites, oysters were deployed in cages set in the intertidal and subtidal zones. Intertidal cages were placed where they would receive varying amounts of air exposure during low tide and subtidal cages were placed where they would be continuously submerged. There are currently no intertidal oysters in the Maryland portion of the Chesapeake Bay, so three intertidal treatments, high, mid, and low, were used to cover the range of air exposures that might provide suitable habitat for oysters. At VIMS-ESL, where abundant wild intertidal oysters occur, only one intertidal treatment was used and its tidal height was based on the location of existing natural reefs. Although VIMS also had wild intertidal oysters, three intertidal treatments were used because most intertidal oysters at this site were scattered and a single appropriate intertidal height could not be easily determined. Each cage contained 45-50 oysters of one initial disease treatment in 2008 and 45-50 oysters of both initial disease treatments in 2009 (~100 oysters cage^{-1}).

To create three distinct intertidal height treatments three polypropylene oyster cages (www.fukuina.com; 61 cm x 61 cm x 20 cm, 32.5 mm diagonal mesh) were secured in a single line to strips of rebar and deployed perpendicular to the water line (see Malek Chapter 2 for more specific details). Cages were covered in 0.28 mm gillnetting (monofilament nylon, Memphis Net and Twine Co., Memphis, TN) stretched tight across

the top and sides of the cages. The netting was used instead of polypropylene covers that would shade oysters and prevent maximal exposure to intertidal conditions. Sets of cages were weighted with approximately 35-45 kg of metal weights and natural rock and chained to 45.7 cm spiral stakes (www.petco.com) screwed into the substrate. Cages were deployed at low tide with the bottom edge of the low intertidal cage placed at the mean low water mark (MLW). At VIMS-ESL, the single intertidal treatment cages were covered with gillnetting and weighted with cinder blocks. The subtidal treatment at all sites consisted of single oyster cages closed with a polypropylene lid (23 mm mesh) and secured by cable ties. The polypropylene lids were used instead of gillnetting because it was not necessary to maximize air and sun exposure for this treatment. Cages were deployed in ~1-2 m of water at low tide, weighted with cinder blocks, and marked with buoys.

Each cage of an intertidal set of 3 represented one replicate of a particular treatment (high, mid, or low). A single submerged cage was used for each replicate of the subtidal treatment. In 2008, there were 5 replicates of each tidal height treatment for each initial disease treatment. Initially uninfected oysters were deployed ~5 m away from initially infected oysters to reduce *P. marinus* transmission between experimental treatments. Because 2008 final *P. marinus* prevalence in the initially uninfected oysters was quite low and these experiments were also intended to test effects of intertidal exposure on *P. marinus* acquisition and progression (see Malek Chapter 2), the two initial disease treatments were combined in the same cage in 2009 to promote acquisition of *P. marinus* in the initially uninfected oysters. All tidal height treatments were deployed June 5th-25th,

2008 and June 3rd -12th, 2009 and were scheduled to be retrieved in late September-early October. Due to the threat of Tropical Storm Hannah in 2008, MSUERC oysters were retrieved September 4th after 11 wk of deployment while VIMS-ESL oysters were retrieved September 22nd. Experimental oysters in 2009 remained in the field for 16-17 wk as planned and were retrieved September 29th- October 12th.

Oysters (~15) were removed from cages for disease analyses at the mid-point of the study after 7 to 8 wk of deployment, and again at the conclusion (see Malek Chapter 2). Mortality was also assessed at these intervals, but shell height was assessed only at deployment and final retrieval. Cages were cleaned every 3 wk during deployment when oyster mimics were replaced (see below). Minor maintenance was done as needed to tighten the netting or repair rips on intertidal cages. Predators such as blue crabs, *Callinectes sapidus*, and fouling species such as tunicate sea squirts, *Mogula manhattensis*, were removed from subtidal cages (these species were not found in intertidal cages). Temperature and salinity data were collected from local water quality monitoring stations in 2008 (http://mddnr.chesapeakebay.net/eyesonthebay) and using a YSI 85 in 2009 during trips to change mimics (Fig. 2a & 2b).

Mimics

Oyster mimics were used in intertidal and subtidal cages during both years to predict internal temperatures and duration of air exposure experienced by experimental oysters. A full description of results are provided in Malek Chapter 2 and are summarized here to provide background data on conditions experienced by oysters at the different study sites.

Mimics consisted of oyster shells filled with silicon in which a temperature logger was embedded (iButton data loggers, Dallas Semiconductor; accuracy of ±0.5°C). These were deployed in the mid intertidal treatment, which was expected to have the highest survival based on duration of air exposure, and also in the subtidal treatment. Each site had 2-3 mimics per treatment and temperature measurements were taken every 15 min. Temperature data were used to estimate the duration during which oysters were influenced by air, including partial exposure at the beginning and end of the tidal cycle for the different intertidal treatments; from herein, the word 'exposure' will refer to the total amount of air exposure experienced by oysters during the tidal cycle. Mimic data allowed me to estimate internal temperatures reached in oysters, since high temperatures may influence *P. marinus* acquisition and proliferation (Milardo 2001; Malek Chapter 2).

The approximate durations of exposure for the mid intertidal treatments at each site were determined by comparing the mean temperatures for the mid intertidal and subtidal mimics for individual days at each site (see Appendix 1a for details and examples of how exposure was calculated). Mimic data allowed me to compare exposure of intertidal treatments at each site. I found that the high intertidal treatments at MSUERC in 2008 and VIMS in 2009 were more similar in the duration of exposure to the mid intertidal treatment at SERC than to the high intertidal treatment (Table 1). Comparisons across sites therefore considered cages with similar estimated exposure durations rather than initial tidal height designations.

Mortality

To assess mortality, shells from dead individuals were removed from each cage during the mid and final disease assessments and shell heights were measured. Dead oysters were examined for attached tags (see next section) and the presence of oyster tissue (noted as fresh dead). Percent mortality for each sampling period was calculated as [# dead individuals/ starting # of oysters deployed in each cage]*100.

Mortality of experimental oysters at VIMS-ESL was assessed three times: twice during the course of the experiment (July 31st, August 28th) and a third time at the end (September 23rd). Minimal mortality was observed in the initially uninfected oysters in the intertidal treatment during the standard 8 week assessment. However, when cages were cleaned at the end of August, I noted that substantial mortality had occurred. I therefore removed and measured dead oysters while cleaning the cages in order to prevent shells from being damaged further, which could have affected shell measurements during the remaining month of deployment.

Growth

In 2008, average growth was estimated for each initial disease treatment and tidal height treatment by measuring the initial shell height of a random subsample of 15 individuals in each cage and subtracting that from the mean end shell height of the subsample of individuals used for disease analysis at the end of the experiment. All shell heights were measured to the nearest millimeter using a flexible ruler from umbo to bill over the curve of the right valve.

In 2009, growth was assessed using shell heights of tagged individuals measured at deployment and experiment completion. Electrical wire tags with unique alpha-numeric combinations (Ideal Wire Marker Booklet) were attached with marine grade epoxy (West Marine) to the right valve of 15 randomly selected individuals from each cage. Prior to tagging, oysters were cleaned to remove any dirt or fouling agents and air-dried. Tagged oysters were not used for the mid-study disease analysis to allow growth to continue for the remainder of the experiment. Those that survived to the end of the experiment however, were used for the final disease analysis and measured prior to dissection. There were 720 individually tagged and measured oysters per site. Growth was calculated for surviving tagged individuals by subtracting the initial height from the end height. Weekly growth rates were also calculated as [average growth per cage/# of weeks deployed] for both years.

Some initially uninfected oysters at MSERUC were lost over the course of the experiment in 2008. Small initial heights allowed oysters to be forced out of cages by wave action, but these oysters could sometimes be found tangled in the gillnet covers and returned to the cage. In 2009, uninfected oysters were larger in initial height and fewer were lost.

Disease Assessment

P. marinus infections were assessed using the Ray's Fluid Thiogylcollate Medium (RFTM) method (Ray 1966a). Rectal tissue samples were removed from oysters,

incubated in media for 5-7 d and stained with Lugol's iodine to make parasite spores visible. Prevalence and mean intensity of infection were calculated for each cage for both initial disease treatments. Prevalence was calculated as the percentage of oysters from each cage that was infected with *P. marinus.* Mean intensity, based on a 6 point scale from 0.5-5 (0 no infection, 0.5 very light, 1 light, 2 and 3 moderate, 4 and 5 heavy/lethal; Mackin 1962) was calculated by averaging the intensity scores of oysters infected with *P. marinus.* Infection intensities of tagged individuals were used to determine if there was a relationship between infection intensity and oyster growth.

Statistical Analyses

The mean mortality, growth, and *P. marinus* intensity were found for each replicate of both tidal initial disease and all tidal height treatments and these cage means were used for all analyses except the comparison of *P. marinus* infection intensity and individual oyster growth. Percent mortality was arcsine, square root transformed to eliminate heterogeneity in variances (Sokal & Rohlf 1995). For some sites this transformation did not result in homogeneity so rank transformations were used (Potvin & Roff 1993). To test for an effect of tidal height on mortality, a 2-way analysis of variance (ANOVA) was used to determine if percent mortality differed by initial disease treatment or tidal height, or if there was an interaction between these main effects. If the interaction term was not significant it was dropped from the final statistical model. In addition, I ran a 1-way ANOVA with planned specific comparisons of each intertidal treatment against the subtidal treatment using T-tests for both initial disease treatments separately.

Growth data were tested for homogeneity of variances and growth was $\log_{10}$ transformed where necessary. In some cases, rank transformations were needed. The two initial disease treatments were analyzed separately. To test for an effect of tidal height on growth in the 2009 experiments, an analysis of covariance (ANCOVA) was used with the initial shell height of tagged individuals as the covariate. Planned specific comparisons for each initial disease treatment using T-tests tested whether each intertidal treatment differed from the subtidal treatment at that site.

I used a 3-way ANOVA to test for a relationship between *P. marinus* intensity and oyster growth, within each initial disease treatment. The model used growth as the response variable and infection intensity, site, and tidal height as independent variables. Linear regressions were used to determine which tidal heights at each site had significant relationships between intensity and growth, and if the overall slope indicated a positive or negative relationship (Sigmaplot 11). Except where noted, analyses were performed using the SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Mortality analyses including both initial disease groups

Mortality patterns differed between sites with and without wild intertidal oyster populations. At the Maryland sites with no intertidal populations, high intertidal and sometimes mid intertidal mortality was substantially and significantly higher than subtidal mortality (Fig. 3). In contrast, at the Virginia sites with wild intertidal oyster populations, subtidal mortality tended to be similar to intertidal mortality, with no

significant differences in mortality between tidal heights (Fig. 4). Additionally, at Maryland sites in 2009, the younger, initially uninfected oysters (Fig. 3a) had significantly lower mortality across tidal height treatments than the initially infected oysters (Fig. 3b; Table 2). In contrast, at VIMS-ESL both initial disease treatments had similar mortality, and older initially infected oysters had higher mortality than younger, initially uninfected oysters at VIMS (Table 2, Fig. 4). Due to heterogeneous variances, statistical analyses were performed on rank transformed data for all experiments except VIMS-ESL

Mortality at Maryland Sites

Mortality of oysters at MSUERC in 2008 varied significantly with tidal height; oysters in the high intertidal treatment had significantly higher mortality than subtidal oysters, and low intertidal oysters had significantly lower mortality in analyses combining both initial disease treatments (Table 2a). Mortality of initially uninfected oysters ranged from ~4-40% (Fig. 3a), with the highest mortality in the high intertidal and the lowest mortality in the low intertidal. Mean mortality in the high intertidal was more than twice that in the subtidal, although planned comparisons indicated that only mortality in the low intertidal was significantly lower than mortality in the subtidal. The initially infected oysters followed a similar trend but mortality ranged from ~4-11% and the effect of tidal height was not statistically significant (Fig. 3b; Table 2b). The majority of mortality in both disease treatments and all tidal heights occurred during the first 8 wk of deployment, but the low absolute mortality measured at this site was likely the result of the early retrieval in anticipation of storm damage.

The 2009 results at MSUERC differed somewhat from 2008 results at the same site. Mortality of the initially uninfected oysters ranged from 6-43%, with the highest mortality in the high intertidal and the lowest in the mid intertidal (Fig. 3a). Planned comparisons indicated that mortality in the high intertidal was significantly higher than mortality in the subtidal (Table 2b). Mortality of the initially infected oysters ranged from 13-59%, with the highest in the high intertidal and lowest in the subtidal. Planned comparisons indicated that mortality in the high intertidal was significantly higher than mortality in the subtidal (Fig. 3b). Mortality occurred evenly throughout the duration of the experiment in both disease treatments and in all tidal heights.

At SERC in 2009, mortality of the initially uninfected oysters ranged from 3-65%, with the highest mortality in the high intertidal and the lowest in the subtidal. Planned comparisons indicated that mortality in the high and mid intertidal was significantly higher than mortality in the subtidal (Table 2b). The initially infected oysters followed a similar trend (Fig. 3b), but mortality ranged from 10-70%. Planned comparisons indicated that mortality in the high and mid intertidal was significantly higher than morality in the subtidal (Table 2b). The bulk of the mortality for both initial disease treatments and all tidal heights occurred during the first 8 weeks of deployment.

Mortality at Virginia Sites

Mortality of the initially uninfected oysters ranged from 35-50% at VIMS-ESL. Planned comparisons indicated that mortality in the intertidal was significantly lower than

mortality in the subtidal (Table 2b). Mortality of the initially infected oysters was ~39% for both tidal height treatments. Most mortality of the initially uninfected oysters in the intertidal occurred after the mid-study assessment while most mortality in the subtidal occurred during the first 8 weeks of deployment. Mortality of initially infected oysters occurred evenly throughout the experiment in both tidal height treatments.

At VIMS, percent mortality did not vary significantly among tidal heights for either initial disease treatment (Table 2a; Fig. 4a & 4b). Mortality of the initially uninfected oysters ranged from 25-38% and was similar across tidal heights whereas mortality in the initially infected oysters ranged from ~50-63%, and tended to be highest in the subtidal (Fig. 4a & 4b). Mortality in the initially uninfected oysters occurred evenly over the 16 weeks while the initially infected oysters had more mortality occur during the last 8 weeks of deployment.

General patterns in oyster growth

At all sites in both years there was a trend of increased shell growth with lower tidal height in initially uninfected oysters (Fig. 5a & 6a). Growth was faster in high and mid intertidal treatments at Virginia sites than at Maryland sites, but decreased with increasing durations of air-exposure among all sites (Fig. 11). The effect of tidal height on growth of initially infected oysters varied between sites in 2008, and intertidal growth was slower than subtidal growth at all sites and for both initial disease treatments in 2009 (Fig. 5b & 6b).

Several of the intertidal treatments had negative average growth. Each of these negative averages was tested to determine if they were significantly different from 0, or no growth, by using a Student's T-test. In all but one case, initially infected oysters in the mid intertidal treatment at MSUERC in 2008 (T-test result: $t=-3.47$, $p=0.02$), growth was not found to be significantly different from 0 ($p \leq 0.05$). Negative or no growth was likely the result of erosion or breakage of shell margins. Additionally, very low growth that occurred in initially uninfected oysters in the high intertidal treatments in Maryland experiments was tested to determine if it was significantly higher than 0 and in all Maryland experiments, growth in the high intertidal was not significantly higher than 0, suggesting that no growth occurred in that tidal habitat.

Growth at Maryland Sites

At MSUERC in 2008, growth of initially uninfected oysters differed significantly among tidal heights (Table 3b). No growth occurred in the high intertidal (Fig. 5a). Planned comparisons indicated that growth in the high intertidal was significantly slower than growth in the subtidal and growth in the mid intertidal also tended to be slower than growth in the subtidal (Table 3b). Growth rates averaged 0.41, 0.74, and 0.83 mm^{-1} wk for the mid, low, and subtidal treatments, respectively. Growth in the initially infected oysters did not vary significantly among tidal heights. Positive growth was measured only in the low intertidal (Fig. 5b), where oysters grew at a rate of 0.41 mm^{-1} wk.

In 2009, the effect of initial shell height on growth of initially uninfected oysters at MSUERC varied among tidal heights (Table 3a). High intertidal oysters of smaller initial

shell heights grew more than oysters with larger initial shell heights, but growth of the other 3 tidal height treatments was similar for all initial shell heights. Planned comparisons indicated that growth in all intertidal treatments was significantly slower than growth in the subtidal (Fig. 5a). Positive growth occurred in all treatments with average rates of 0.09, 0.36, 0.42, and 1.06 mm^{-1} wk in high, mid, low, and subtidal treatments, respectively. Growth of initially infected oysters was not significantly affected by initial shell height but did vary significantly among tidal heights (Table 3a). No growth occurred in the high or mid intertidal. Planned comparisons indicated that growth in all 3 intertidal treatments was significantly slower than growth in the subtidal (Fig. 5b). The low intertidal and subtidal average growth rates were 0.03 and 0.54 mm^{-1} wk, respectively.

At SERC in 2009, growth of initially uninfected oysters was not significantly affected by initial shell height but varied significantly among tidal heights. No growth occurred in the high intertidal (Fig. 5a). Planned comparisons indicated that growth in all intertidal treatments was significantly slower than growth in the subtidal (Fig. 5a). Growth rates averaged 0.2, 0.47, 0.97 mm^{-1} wk for the mid, low intertidal, and subtidal treatments, respectively. Growth of initially infected oysters was significantly affected by initial shell height and varied significantly among tidal heights (Table 3a). No growth occurred in the high intertidal (Fig. 5b) and growth in the mid and low intertidal was negligible (Fig. 5b). Planned comparisons indicated that growth in all intertidal treatments was significantly slower than growth in the subtidal, which had a rate of 0.37 mm^{-1} wk.

Growth at Virginia Sites

Growth of initially uninfected oysters at VIMS-ESL was significantly slower in the intertidal than in the subtidal (Fig. 6a; Table 3b). Average growth rates for the intertidal and subtidal were 0.88 and 1.47 mm^{-1} wk, respectively. In contrast, growth of initially infected oysters was significantly faster in the intertidal than in the subtidal (Fig. 6b; Table 3b). Average growth rates were 0.87 and 0.41 mm^{-1} wk, respectively.

At VIMS, growth of initially uninfected oysters was not significantly affected by initial shell height (Table 3a) and there was a non-significant trend towards an effect of tidal height. Planned comparisons indicated that growth in the high intertidal was significantly slower than growth in the subtidal (Fig. 6a; Table 3b). Average growth rates for the high, mid, low, and subtidal were 0.36, 0.47, 0.46, and 0.72 mm^{-1} wk, respectively. Growth of the initially infected oysters was not affected by initial shell height and there was a non-significant trend towards an effect of tidal height (Table 3a & 3b). No growth occurred in any of the intertidal treatments (Fig. 6b). Planned comparisons indicate that growth in the mid intertidal was significantly slower than growth in the subtidal, which had the only positive growth, at a rate of 0.12 mm^{-1} wk.

Effects of *P. marinus* Intensity on Oyster Growth

The relationship between *P. marinus* intensity and growth varied among sites and tidal heights in initially uninfected oysters (Table 4). There were significant negative relationships between *P. marinus* infection intensity and oyster growth in 4 of the 12 initially uninfected oyster tests conducted and an overall negative relationship between

factors (Table 5). At MSUERC and VIMS respectively, the low intertidal treatment and the mid, low intertidal and subtidal treatments had significant negative relationships between these factors (Fig. 7a & 9a). All 12 regressions of initially uninfected oyster disease intensity and growth had negative slopes ($p=0.00024$, assuming equal probability of positive or negative slopes using a Binomial Distribution). In the initially infected oysters there were significant interactions between *P. marinus* intensity and tidal height and between site and tidal height in the final growth model (Table 4). There were significant negative relationships between *P. marinus* infection intensity and oyster growth in 2 of the 12 initially infected oyster tests conducted - the subtidal treatments at SERC and VIMS (Fig. 8b & 9b; Table 5). Of the 12 initially infected oyster disease intensity and growth tests, 7 had negative slopes, while 5 had positive slopes, indicating no clear relationship between these factors ($p=0.19$, assuming equal probability of positive or negative slopes using a Binomial Distribution).

Discussion

Field experiments indicated that the summertime effects of intertidal exposure on mortality and growth of the eastern oyster, *Crassostrea virginica*, in Chesapeake Bay varied between sites with (Virginia) and without (Maryland) wild populations of intertidal oysters. The positive relationship between the duration of exposure and mortality in high and mid intertidal treatments was similar at all study sites in both initial disease treatments (Fig. 10a & 10b). In contrast, subtidal mortality was substantially higher at Virginia than at Maryland sites in both initial disease treatments even with the exclusion of large predators. Growth decreased with increasing durations of exposure in

Maryland and Virginia, though intertidal growth tended to be slower in Maryland than Virginia (Fig. 11). Subtidal growth was generally faster than intertidal growth except for initially infected oysters deployed in 2008. Environmental (reduced feeding opportunities, high temperature) and internal (decreased O_2, increased CO_2) stresses experienced by oysters during prolonged durations of exposure likely caused the increased mortality and decreased growth. There was also a negative relationship between *P. marinus* infection intensity and oyster growth in oysters with newly acquired *P. marinus* infections. Results of this study suggest that in combination with lower recruitment and more severe winter conditions in Maryland compared to Virginia, summer patterns of mortality and growth may help explain the differences in tidal distributions in northern, low salinity areas and southern, high salinity areas of Chesapeake Bay.

Oyster Mortality

Mortality at sites in Maryland decreased with decreasing tidal height. The duration of exposure, the time during which oysters were influenced by air, including partial exposure at the beginning and end of the tidal cycle, differed between tidal heights and sites. Tidal height treatments with long durations of exposure (>3.8 h, except SERC; Table 2b) had significantly higher mortality than subtidal treatments when both disease treatments were combined, except at MSUERC in 2008, when the two disease treatments were considered separately. High mortality observed in treatments with long durations of exposure, such as the high and mid intertidal treatments at SERC (Table 1), may have been the result of numerous days with large internal temperature fluctuations and high

maximum internal temperatures. For example, mimics indicated that oysters in the mid intertidal treatment at SERC (exposure of 3.6 h) experienced daily internal temperature fluctuations as large as 30°C and maximum internal temperatures of 47.5°C.

In contrast to Maryland, mortality at high salinity Virginia sites was similar across tidal heights; the primary difference between Maryland and Virginia sites was the magnitude of subtidal mortality. At VIMS-ESL, mortality in the subtidal was significantly higher than in the mid intertidal. The relationship between mortality and exposure was similar, however, in high and mid intertidal treatments for initially infected and uninfected oysters in both Maryland and Virginia (Fig. 10a & 10b).

Increased temperature (Potter & Hill 1982), hypoxia, and hypercapnia that can occur within oysters during prolonged durations of exposure can cause mortality (Burnett 1997). Shorter durations of exposure usually cause less internal stress and survival tends to increase. Results from my study support this observation as I found a strong trend ($0.05 \leq p \leq 0.10$) towards increasing mortality as the durations of exposure increased in Maryland and Virginia (Fig. 10). Mortality patterns from Maryland sites were in agreement with the findings of Bartol et al. (1999), who compared the mortality of small (~5wk post-set) and large (~10 month post-set) juvenile oysters in the Piankatank River (which has salinities intermediate to MSUERC and VIMS in the current study) where they found that treatments with long durations of air exposure had higher mortality than treatments with short durations in a small tidal range environment (0.36 m). Results from Virginia sites, where intertidal and subtidal mortality were similar, were in agreement

with O'Beirn et al. (1994), who found no difference between on-bottom intertidal and subtidal oyster mortality at a high salinity site in Georgia. This pattern is different from the results of Roegner and Mann (1995), who found that during summer months at VIMS there was considerably higher mortality in oyster spat that settled on substrate ≥25 cm above MLW compared to spat that settled in areas with shorter durations of air exposure. My results from VIMS may differ from theirs because older oysters, not spat, were used and there may be variations in mortality factors that affect oysters at different life stages.

Absolute rates of oyster mortality in the low intertidal and subtidal were much higher in Virginia than in Maryland, indicating the presence of additional mortality factors in these habitats at the two highest salinity sites (Fig. 3 & 4). Ambient *P. marinus* levels and salinity were higher at Virginia than Maryland sites. Ingestion of higher number of *P. marinus* zoospores in the low intertidal and subtidal treatments due to more prolonged feeding times (relative to high and mid intertidal treatments) could have increased prevalence in test oysters, and high salinity could have caused acquisition and progression to occur more quickly than in Maryland. However, Malek (Chapter 2) found that prevalence and intensity of *P. marinus* infections were similar among tidal height treatments at both Virginia sites, in contrast to the differences in prevalence among tidal height treatments observed at Maryland sites (higher prevalence in the high intertidal than in the subtidal). Another oyster parasite, *Haplosporidium nelsoni,* which causes MSX, also persists in both the coastal bays of Virginia and in the York River, as well as throughout the Chesapeake at salinities > 12. MSX is capable of causing mortality in the first year of infection (Ford & Haskins 1982) and has been responsible for large oyster

mortalities in the Chesapeake in the past (Andrews 1988). Subtidal oysters of both initial disease treatments from VIMS were tested for MSX using PCR by the Shellfish Pathology Laboratory at VIMS, but no evidence of the parasite was found (R. Carnegie Virginia Institute of Marine Science, pers. comm.). Oysters from VIMS-ESL experiments were not tested for MSX, but wild oysters in areas ~24 km south of VIMS-ESL had a 4-8% prevalence of MSX. Disease patterns at VIMS-ESL may have been similar (R. Carnegie, pers. comm.) and such a low prevalence would not have resulted in high mortality. There was an outbreak of MSX in the Maryland region of Chesapeake Bay in 2009 (C. Dungan Cooperative Oxford Laboratory, pers. comm.), but prevalence near study sites was very low (<5%).

In addition to differences in disease between the regions of Chesapeake Bay, it has been suggested that an increased abundance and diversity of predators found at salinities above 15-18 reduces oyster survival in the subtidal in Virginia and areas further south (Burrell 1986; Roegner & Mann 1990). At VIMS-ESL, the oyster drill *Urosalpinx cinerea* was found on and sometimes in subtidal cages (predator access was restricted on intertidal cages due to the mesh covering) but no drill damage was found on shells removed from subtidal cages. Roegner and Mann (1990) found oyster predators such as blue crabs, oyster drills, and flatworms during a previous study conducted at VIMS. In the current study, blue crabs were occasionally found in subtidal cages at both Virginia and Maryland sites, where they likely entered cages to molt and then were too large to escape, but even after molting, crabs were usually too small to cause substantial damage to oysters within the cage. Other predators found at these sites, such as flatworms, are less

conspicuous and are capable of causing high oyster mortality (Hofsetter 1977; White & Wilson 1996; Newell et al. 2000). Therefore, it is plausible that small invertebrate predators were responsible for the high mortality that occurred in the low intertidal and subtidal treatments in Virginia.

It is likely that the progression of second year *P. marinus* infections contributed to the significantly higher mortality observed in initially infected oysters compared to initially uninfected oysters at MSUERC 2009 and SERC. At least 40% of initially infected oysters in 2009 had acquired the parasite the previous year. Oysters that survive the first year of *P. marinus* infection rarely survive a second year as infections usually become lethal during mid-summer of that year (Andrews 1988), and disease-related mortality tends to occur in late summer and early fall (Andrews and Hewatt 1957; Ford & Tripp 1996). It is not clear if the difference in mortality of the two disease treatments could be attributed to *P. marinus* infections at VIMS; prevalence and intensity were similar by the mid-study assessment and much of the difference in mortality between the initial disease treatments occurred after the mid-study assessment. In contrast to other sites, initially infected oysters at MSUERC 2008 had lower mortality than initially uninfected oysters. A comparison of 2008 and 2009 experiments at MSUERC suggests that differences in oyster source (wild vs. cultured initially infected oysters) may have contributed to the varied patterns of mortality observed at this site. The low overall mortality at MSUERC in 2008, however, may reflect the early retrieval of oysters at the site. Results from Maryland and Virginia sites in 2009 are in agreement with Burrell et al. (1981) who found that younger seed oysters (~1 yr) from the Wando River in South Carolina

experienced substantially lower mortality than older oysters (>1 yr, of unknown age) from the same area when deployed subtidally in racks 20 cm above the bottom.

Oyster Growth

Contrary to patterns in mortality, and as predicted, growth of oysters in Maryland increased with decreasing tidal height. Tidal heights with the longest durations of exposure and highest mortality tended to have the slowest growth and there was slower growth in the high and mid intertidal at Maryland sites than at Virginia sites (Fig. 5a, 5b, & 11). Exposure to air reduces the time oysters can spend feeding, thus reducing the energy they have available for growth. Increased temperature can cause energy otherwise available for somatic growth to be used for metabolic maintenance (Dame 1972; Newell 1979). Subtidal oysters had the fastest growth of all tidal height treatments (except MSUERC 2008 initially infected oysters), and in 2009 subtidal growth was significantly faster than all intertidal treatments. Oysters that are continuously submerged will feed constantly if food is available, resulting in faster growth than in oysters submerged for less than 100% of the time each day. The pattern of faster growth in the subtidal compared to the high and mid intertidal at Maryland sites is in agreement with the results of numerous other studies (Loosanoff 1932; Ingle & Dawson 1952; Roegner & Mann 1995), indicating that air exposure affects *C. virginica* growth similarly at low salinity, small tidal range sites in Chesapeake Bay.

Oyster growth tends to be slower in intertidal than subtidal habitats (most sites/disease treatments in this study; Loosanoff 1932; Ingle & Dawson 1952; Burrell 1982; Roegner

& Mann 1995; Bartol et al. 1999), though some results suggest the opposite pattern (Crosby et al. 1991). Across sites the fastest growth rates occurred at the highest salinity sites (VIMS-ESL), and growth varied between Maryland sites and VIMS, with faster growth at VIMS than at MSUERC and SERC (Fig. 11). This suggests that oyster growth may increase with increasing salinity in Chesapeake Bay, as seen in Delaware Bay (Kraeuter et al. 2007).

The growth observed at VIMS was highest in the subtidal, as at Maryland sites. But in contrast to Maryland sites, growth was similar among intertidal heights (Fig. 6a). The difference in the duration of exposure among intertidal treatments was shortest at VIMS (0.9 h difference) and longer at Maryland sites (1.5-2 h difference). These differences in exposure may have contributed to the growth patterns observed in the two regions of the Chesapeake. Intertidal growth also tended to be higher in Virginia than in Maryland at comparable durations of exposure (Fig. 11), suggesting that intertidal air exposure may not reduce growth as much at high salinities compared to low and moderate salinities.

Growth varied between initial disease treatments. Initially uninfected oysters grew substantially more than initially infected oysters at all tidal heights. Growth in continuously submerged treatments tended to be at least twice as fast in initially uninfected oysters as in initially infected oysters, indicating that uninfected oysters grow faster than infected oysters. Age and initial disease status were confounded in this experiment as they often are in the field. Shell growth (measured as the change in shell height) naturally decreases with age; as they increase in size, oysters become

reproductively mature, producing energy-consuming gametes and increasing meat mass relative to shell height. Butler (1953) found that during the summer months, third year oysters experience almost no increase in shell height, although tissue weight and shell volume usually increase. Also, Burrell et al. (1981) found that younger seed oysters grew faster than older seed oysters in South Carolina. The initially infected oysters used in this study were between their second and third year and could have followed growth trends similar to those observed by Butler (1953) and Burrell et al. (1981); i.e. they had slower increases in shell height.

I measured oyster growth as change in shell height, but it can be also measured by changes in shell area or shell volume. Based on growth patterns observed by Loosanoff and Nomjeko (1949) in Milford Harbor CT, oysters had the largest increases in shell length (height) in May and June, but shell growth slowed in August through September, when the largest increases in oyster volume were observed. Similar seasonal patterns in shell and soft tissue growth have been observed in other intertidal bivalves such as the mussel, *Mytilus edilus* (Hilbish 1986). Results of these previous studies suggest that shell height may be a good indicator of growth in the early summer, but volume may best represent growth later in the summer. By measuring shell height only, the current study did not account for the multiple types and seasonality of growth oysters experience during the time period when they were deployed. Negative or negligible growth in older, initially infected oysters may reflect increases in volume, as opposed to shell height, and growth likely did occur in this initial disease treatment, but was not detected due to the methods used.

Shell growth at all tidal height treatments and at all sites was likely affected by deployment of oysters in hard plastic cages. Oysters in all tidal height treatments were subject to water movement within cages, which likely led to some shell chipping (a possible cause of 'negative' growth in initially infected oysters). Field experiment sites differed in coastal exposure and water movement characteristics, ranging from exposed rip-rap in an area with a large fetch and high wave energy (MSUERC) to open mud flats or a beach where waves caused by wind and a large fetch could dissipate with little disturbance to oysters in cages (VIMS-ESL, VIMS). The differences between sites likely influenced the shell growth observed, particularly in intertidal oysters, as local water activity affected the frequency and intensity of oyster movement within hard plastic cages that may have resulted in abrasion of the growing edge of the shell.

Effects of *P. marinus* Intensity on Oyster Growth

I found a negative relationship between *P. marinus* infection intensity and oyster growth in initially uninfected oysters. My results are in agreement with Paynter and Burreson (1991), who observed that oysters newly infected by *P. marinus* experienced a decrease in growth. My results suggest that *P. marinus* infection intensity does not affect oyster growth similarly among all sites. Though the relationship between disease and growth was negative at all tidal heights at all sites and 4 of the individual regressions were significant, *P. marinus* infection intensity explained very little of the variation in observed growth. However, it is possible that not all *P. marinus* infections in initially

uninfected oysters were detected as the disease assay used (RFTM) can be insensitive to low level infections (Bushek et al. 1994).

My results suggest that there may not be a strong relationship between *P. marinus* infection intensity and oyster growth in oysters with existing infections. However, slow growth may have made it difficult for a relationship between factors to be identified. Menzel and Hopkins (1955) observed a strong negative relationship between increasing *P. marinus* infection intensity and decreasing oyster growth in individuals similar in age to the initially infected oysters used in this study.

Conclusions

The different patterns in summer mortality and growth in Maryland versus Virginia suggest that these factors, in addition to recruitment, salinity, predators, winter conditions, and tidal range, play important roles leading to difference in the tidal height distributions of *C. virginica* in the two regions of Chesapeake Bay. High mortality in intertidal treatments with long durations of exposure across all sites indicates that the physiological stress that occurs in these habitats reduces oyster survival. Other studies suggest that long intertidal exposure also causes high mortality of spat or discourages settlement all together (Kenny et al. 1990). Field studies from Delaware Bay to Florida have found that oyster larvae settlement is higher in the subtidal than in the intertidal (McDougall 1942; Hidu & Haskin 1971; Roegner & Mann 1990; Bartol & Mann 1997), but post-settlement mortality appears to alter the initial distribution, resulting in the primarily intertidal population patterns observed in many areas (Roegner & Mann 1990).

The primary source of mortality that eliminates or severely limits oyster populations in subtidal habitat is thought to be predation (Burrell 1986; Roegner & Mann 1990). But, other sessile species such as encrusting ascidians and bryozoans can compete with oyster spat for space and food, contributing to high subtidal spat mortality and further altering distributions (Osman et al. 1989). Higher mortality in the low intertidal and subtidal at Virginia sites than at Maryland sites may suggest that predators, which increase in abundance and diversity in salinities >15-18, strongly influence tidal height distributions in Virginia. In this study, most large predators were excluded from cages, but smaller, micropredators may have contributed to the mortality observed in the subtidal. Newell et al. (2000) found high mortality associated with subtidal predation by the flatworm, *Stylochus ellipticus*, on newly settled subtidal oyster spat in the in the Choptank River in Maryland, indicating that these predators contribute to subtidal mortality in Maryland, though this was not observed in the current study, where larger oysters were used. But other studies indicate that the same species of flatworm also causes subtidal oyster mortality in the York River (Roegner & Mann 1990) and may have contributed to the mortality observed in the current study.

Low oyster recruitment rates in Maryland likely also contribute to the limited tidal height spatial distribution of oysters in northern Chesapeake Bay. Both field and laboratory studies (Lutz et al. 1970; Hidu & Haskin 1971) have shown that exposure to increased temperatures, such as would be experienced during a flood tide in large tidal flat areas stimulates larval settlement in the intertidal. Microtidal habitats in most of Maryland reduce the occurrence of sudden increases in temperature needed to stimulate intertidal

settlement. However, subtidal oysters in Maryland experience lower mortality and faster growth than intertidal oysters and these positive attributes appear to allow subtidal oyster populations to persist in Maryland despite low recruitment.

The duration of these field experiments (11-17 wk) may have influenced the patterns observed in oyster mortality and growth. My experimental design involved deploying oysters from June to early October to investigate summer patterns in disease. However, by only leaving oysters in the field for one season, mortality that may have resulted from winter conditions or the possible progression of *P. marinus* infections to lethal levels during a second summer, could not be accounted for. Patterns in growth between locations and tidal heights may also be different in oysters held for longer time periods. The time of year for fastest shell growth may not occur at the same time both regions of Chesapeake Bay, so there may be larger differences in growth between the two regions that was not detected in this study. Multiple-year studies in the Patuxent River, MD, looking at mortality and growth of oysters that were initially Dermo-disease-free found that mortalities increased during the second year of deployment and growth was slower in the second year as acquired *P. marinus* infections increased in intensity (Albright et al. 2007). Although that study took place during two drought years where salinities were higher than average, different patterns seen in the two years suggest that longer periods of deployment may more accurately represent natural patterns and help identify long-term differences between different areas, such as the Maryland and Virginia regions of Chesapeake Bay.

In conclusion, my study indicates that summer oyster mortality and growth differ across regions of Chesapeake Bay with (Virginia) and without (Maryland) intertidal oyster populations. Physiological stresses from long durations of air exposure cause high mortality in intertidal oysters in both regions, but higher salinity and increased abundance and diversity of predators (Burrell 1986; Roegner & Mann 1990) are the most likely cause of the high subtidal mortality that occurs in Virginia. Physiological stresses also cause slower growth in the intertidal than subtidal throughout Chesapeake Bay, though intertidal growth is faster in Virginia than in Maryland. My results suggest that oysters in Maryland experience the highest survival and fastest growth in the subtidal, and oysters in high salinity areas of Virginia are most successful overall in the intertidal. Understanding the factors that shape tidal distributions of benthic marine organisms in different habitats within a species range is critical to developing and implementing conservation efforts. This study suggests that oyster conservation efforts could be customized in the two regions of the Chesapeake to account for the different factors that shape tidal distributions and make the best use of the habitats that are currently most successful within each region.

Chapter 2: Effects of Intertidal Exposure on Dermo disease in the eastern oyster, *Crassostrea virginica*

Introduction

Diseases caused by pathogens and parasites can reduce the range, local spatial distribution, and abundance of host species (Lafferty 2003; Smith et al. 2006). Many disease-causing organisms flourish in ecosystems that have been disrupted such as by habitat fragmentation and eutrophication that can cause increased transmission through changes in disease vectors and intermediate host availability (Lafferty 2003). In addition, changing environmental conditions such as temperature, salinity, and pollutant concentrations can increase the geographic range of disease-causing organisms and also cause stress in host species, increasing susceptibility to infection (Harvell et al. 2002). Continuously increasing anthropogenic impacts (e.g. habitat alterations, eutrophication, species introductions) and changing climate will likely result in larger pathogen ranges and faster disease transmission as well as reduced host distributions and abundances which can affect management and conservation efforts.

Diseases caused by protistan parasites of the genus *Perkinsus* in molluscan hosts can result in severe population reductions. A number of *Perkinsus* species have been identified and are known to infect gastropods such as abalone (Lester & Davis 1981) and bivalves including scallops (Blackbourn et al. 1998), clams (Azevedo 1989; McLaughlin et al. 2000), and oysters (Mackin et al. 1950), and range from Australia (Lester & Davis 1981) and Korea (Park et al. 2006) in the Pacific Ocean to Portugal (Azevedo 1989) and Spain (Casas et al. 2004) in the Eastern Atlantic. Both the east and west coasts of the

United States, Canada, and Mexico fall within the range of *Perkinsus* sp. (Blackbourn et al. 1998; Mclaughlin et al. 2000; Caceres-Martinez et al. 2008). Extensive mortalities due to *Perkinsus* sp. infections have been observed in important commercial species such as the greenlip abalone, *Haliotis laevigata*, off Australia (Goggin & Lester 1995), Manila clams, *Ruditapes philippinarum*, in South Korea (Park & Choi 2001), and eastern oysters along the Atlantic coast of the US (Andrews 1988). The most studied *Perkinsus* species in terms of ecological and economic impacts on its host is *Perkinsus marinus,* which causes Dermo disease in the oyster *Crassostrea virginica* (La Peyre et al. 2008).

The eastern oyster, *C. virginica*, supported a historically important commercial fishery and is an ecologically important species within the Chesapeake Bay and in other coastal ecosystems. However, overfishing (Jordan & Coakley 2004; Smith et al. 2005), increased sedimentation and nutrient inflow (Rothschild et. al 1994), disease (Andrews 1965; Andrews 1988), and habitat destruction (Rothschild et al. 1994; Coen et al. 2007) have lead to extreme declines in oyster abundance in the Chesapeake since the late 1800s. As a result there has been a reduction in habitat for fish and other invertebrate species (Coen et al. 2007), phytoplankton consumption in the Chesapeake (Newell 1988; Cerco & Noel 2007), and shoreline protection (Meyer et al. 1997; Grizzle et al. 2002). The combination of fisheries removals and disease (MSX, Dermo) has led to decreases in harvests and abundance since the 1950s. Disease is a major concern for recovery efforts; associated with increased salinities due to severe drought in the mid-1980s, Dermo began causing mass mortalities of *C. virginica* in the upper portion of Chesapeake Bay (Andrews 1988).

Dermo has been causing heavy annual mortalities south of the Rappahannock River in Virginia since the 1950s (Andrews 1996).

P. marinus infections on the East coast of the United States have been reported from the Gulf of Mexico (Mackin et al. 1950) to as far north as Maine (Ford 1996). Infections are most prevalent at temperatures and salinities above 25°C and 12, respectively (Andrews 1965; Mackin 1956), though the parasite can survive temperatures as low as 4°C for up to 6 wk (Burreson & Ragone Calvo 1993) and salinities of 4 for up to 28 d (Chu & Green 1989). Under the preferred environmental conditions, epizootics can develop over 1-2 yr in a population and are characterized by 80-100% prevalence (percentage of individuals in a population with infection; Andrews & Hewatt 1957; Burreson & Andrews 1988), and intensities (severity of individual infections) of 1.5 or greater (Mackin scale 0-5, see *Disease Analysis* section below; Soniat & Kortright 1998). Infection of *C. virginica* by *P. marinus* can lead to decreased shell and soft tissue growth (Menzel & Hopkins 1955; Paynter & Burreson 1991; Ford & Tripp 1996; Malek Chapter 1), reduced gametogenic development (Dittman 1993), altered biochemical composition (Soniat & Koenig 1982; Wilson et al. 1988), and ultimately host mortality. The parasite spreads to the water and surrounding oysters through feces produced by living oysters (Bushek et al. 1994a) and through decaying tissue of dead, infected oysters (Andrews & Hewatt 1957).

Transmission and progression of *P. marinus* infections are closely linked to variability in rainfall that alters salinity and seasonal temperatures (Ford & Tripp 1996; Powell et al. 1996; Kim & Powell 1998; Soniat et al. 2006). Studies in the Gulf of Mexico have

indicated that infection prevalence and intensity are associated with El Niño southern-oscillation (ENSO) patterns (Soniat et al. 2006). Similar disease trends have been seen in other benthic marine invertebrates such as the black abalone (*Haliotis cracherodii*) in California. Raimondi et al. (2002) found that abalone experienced higher disease-related mortalities associated with withering syndrome during El Niño years than in non- El Niño years. For *C. virginica*, in the Gulf of Mexico warmer waters and reduced rainfall resulting from La Niña events can lead to *P. marinus* epizootics, while El Niño years tend to have more subdued *P. marinus* activity (Powell et al. 1996; Kim & Powell 1998). In Chesapeake Bay, *P. marinus* infections and disease-related mortality peak in the late summer and fall during seasonal high temperatures and salinities. Cold winter temperatures may slow parasite growth in infected individuals that survive the summer (Ford & Tripp 1996).

Laboratory experiments testing the effects of temperature on *P. marinus* suggest that oyster survival in the second year of infection may be influenced by the effects of water temperatures on host-parasite interactions in the spring (La Peyre et al. 2008). Some studies have found a decrease in *P. marinus* prevalence and intensity in Chesapeake Bay as waters warm in the spring, perhaps due to elimination of the parasite by the host (Burreson & Ragone Calvo 1996; Ragone Calvo et al. 2001). However, this pattern is not consistent among years. If spring waters are warm, the oyster's ability to eliminate the parasite may be reduced, leading to high infection intensities while spring waters that are cooler could promote parasite elimination, resulting in decreased intensities later in the season (La Peyre et al. 2008). If this is the case, changes in rainfall variability and

temperature could affect seasonal infection dynamics with spring temperatures influencing disease trends that occur through the rest of the year.

Oysters are currently restricted to subtidal waters in Maryland but can be found intertidally from portions of Chesapeake Bay in Virginia and southward. Intertidal populations have also recently been found to extend sporadically as far north as New Hampshire and Maine (Capone et al. 2008). Factors such as ice scour (Taylor & Bushek 2008), larval recruitment patterns, and high intertidal mortality and slow intertidal growth during summer (Malek Chapter 1) potentially limit the tidal spatial distribution of intertidal oysters in northern Chesapeake Bay. In contrast, further south, predation and disease may restrict spatial distributions to the intertidal zone (Burrell 1982; Crosby et al. 1991).

Oysters that inhabit the intertidal are subject to substantial physiological stress during air exposure, including high or low temperatures, internal hypoxia and hypercapnia, and acidification of hemolymph (Burnett 1997; Milardo 2001). Unlike some other intertidal bivalve species, such as the blood cockle, *Anadara granosa* (Davenport & Wong 1986) and the ribbed mussel *Geukensia demissa* (Huang & Newell 2002), *C. virginica* does not use aerial respiration, which reduces some of the stresses experienced during intertidal exposure. Because *P. marinus* lives within the host oyster, the parasite itself is exposed to high temperature, low oxygen, high CO_2 and low pH, and this may cause *P.* marinus infections to differ between intertidal and subtidal oysters.

Laboratory experiments by Milardo (2001) indicated that increased internal temperature and CO_2 levels in oysters reduces the ability of *P. marinus* to proliferate quickly. These changes in internal conditions are similar to those that occur in oysters during intertidal exposure, and suggest that the intertidal zone may be refuge from development of lethal infections. In these experiments, *P. marinus* cells had significantly reduced growth *in vitro* when subjected to temperatures differing by 15°C (25-40°C) compared to a control of constant temperature (29°C). Decreases in *P. marinus* growth were also seen under conditions of high internal CO_2 levels, such as those experienced during hypercapnia, while low CO_2 levels at the same temperature (35°C) had no effect on parasite growth (Milardo 2001). There was no significant influence of pH on *P. marinus* oxygen uptake at high CO_2 levels, suggesting that the low pH that occurs during hypercapnia may not inhibit growth of the parasite (Milardo 2001). In addition, *P. marinus* hypnospores died at 37°C when incubated in Ray's Fluid Thioglycollate Medium (Ray 1954). These laboratory results indicate that infection intensities may decrease under conditions of increased temperature and CO_2 that can occur during emersion. In South Carolina, intertidal oyster populations have almost 100% *P. marinus* prevalence during summer, but suffer lower mortality than subtidal populations (Milardo 2001), perhaps due to slower proliferation of *P. marinus* that experience the physiological stress associated with aerial exposure.

Despite laboratory results suggesting that there should be a difference in intensity of *P. marinus* infections in oysters physiologically stressed by air exposure, previous field studies investigating differences in *P. marinus* infection between intertidal and subtidal

oysters have not found an effect of air exposure on prevalence or intensity (Burrell et al. 1984, O'Beirn et al. 1994; Milardo 2001; Ybanez 2007). Several field studies found no differences between *P. marinus* infections or survival of oysters in the intertidal and the subtidal (Burrell et al. 1984, O'Beirn et al. 1994) but another field study showed that though infected intertidal and subtidal oysters had similar infection intensity, intertidal oysters had higher survival than infected subtidal oysters, suggesting an advantage of living intertidally (Ybanez 2007). These previous studies have been conducted in high salinity areas with tidal ranges from 0.3 to 2 m (Texas, Georgia, South Carolina, respectively) where oyster populations are primarily intertidal.

My study used a combination of field experiments, a controlled air-exposure experiment, and sampling of wild oyster populations to examine the effect of intertidal exposure on the acquisition (prevalence) and progression (intensity) of *P. marinus* infections in *C. virginica*. The following questions were addressed: 1) Does *P. marinus* acquisition and progression vary among tidal heights at sites in Maryland, where wild intertidal oyster populations are absent, and Virginia, where wild intertidal oyster populations are present? 2) Does *P. marinus* acquisition and progression differ between oysters that experience experimentally controlled durations of air exposure and oysters that are continuously submerged? and 3) Does *P. marinus* prevalence and intensity vary among tidal heights in wild populations at sites across the Atlantic Coast range of *C. virginica*? I hypothesized that 1) acquisition and progression of *P. marinus* would be lower in intertidal or air-exposed oysters than in subtidal or continuously submerged oysters due to reduced proliferation of *P. marinus* experiencing physiological stress related to air exposure of

host oysters and 2) prevalence and intensity of *P. marinus* would be lower in intertidal oysters than in subtidal oysters in wild populations due to reduced acquisition and progression of *P. marinus*. Results from these experiments and field surveys indicated that, in contrast to previous field studies (Burrell et al. 1984, O'Beirn et al. 1994), *P. marinus* infection prevalence tended to be higher in high intertidal than subtidal habitats. There was no effect of intertidal exposure on *P. marinus* progression, contrary to findings from laboratory experiments (Milardo 2001). My findings contribute to a more complete understanding of disease dynamics in oysters in Chesapeake Bay and elsewhere and may contribute to the development of effective management strategies and restoration efforts in the future.

Methods

Field Experiments

Field experiments were designed to address my first question: Does *P. marinus* acquisition and progression in *C. virginica* vary among tidal heights? Experiments were conducted at sites in the Maryland region of Chesapeake Bay, where there are currently only subtidal oyster populations, as well as in the Virginia region of the Chesapeake and on the Atlantic coast of Virginia, where oyster populations are predominately intertidal, and subtidal oysters are scarcer.

Study Sites

Sites were chosen to encompass a range of environmental conditions that can influence *P. marinus* infection of *C. virginica*, including temperature, salinity, and proximity to oyster

beds where heavy *P. marinus* infections have been documented (referred to hereafter as disease pressure). In 2008, study sites were in the Patuxent River at the Morgan State University Estuarine Research Center (MSUERC) on the Western Shore of Maryland and on the Atlantic coast of Virginia at the Virginia Institute of Marine Sciences Eastern Shore Laboratory (VIMS-ESL; Fig. 1). In 2009, experiments were conducted at MSUERC, in the Rhode River at the Smithsonian Environmental Research Center (SERC) in Maryland, and in the York River at the VIMS main laboratory in Gloucester Point, Virginia. Salinity and background Dermo disease prevalence and intensity increase from North to South in the Chesapeake Bay (Tarnowski 2008), and maximum mean monthly temperatures varied by 1-2°C between different sites used in the same year (Fig. 2a & 2b). Both VIMS-ESL and VIMS had wild intertidal oyster populations, although the reefs at VIMS-ESL were more abundant and more clearly defined intertidal oyster habitat.

Oyster Sources and Initial Disease Status

Oysters initially uninfected by *P. marinus* were used to test for acquisition of the parasite. Experimental 'initially uninfected', 1-year-old oysters were purchased in 2008 and 2009 (53.6 mm ± 0.6, n=450; 54.5 mm ± 0.2, n=1080; mean ± 1 SE shell height) from Marinetics Inc., a hatchery in the Choptank River in MD. I tested these oysters multiple times for *P. marinus* and yielded zero prevalence (see Disease Assessment methods below). Oysters with a moderate to high prevalence of existing *P. marinus* infections were used to test for progression of infections (change in intensity of a pre-existing infection). Experimental 'initially infected' oysters for MSUERC in 2008 were collected

from reefs in the Patuxent River using an oyster dredge (98.9 mm ± 1.2 shell height, n=300; unknown ages but >1yr based on size). Tests for *P. marinus* indicated a starting prevalence of 90% and mean intensity of 1.4 (±0.2 SE, n=35; scale 0-5, see *Disease Analysis*). Initially infected oysters for VIMS-ESL were collected from local reefs in Wachapreague, Virginia (79.5 mm ± 1.7 shell height, n=150; unknown ages but>1yr based on size). Starting prevalence and mean intensity were 90% and 1.51 (± 0.2, n=41), respectively. In 2009, initially infected, 2-year-old oysters (74.9 mm ± 0.4 shell height, n=1075) were purchased from Marinetics Inc. so that all individuals for the initially infected disease treatment placed at all experimental sites came from the same stock and conditions. Tests indicated a moderate prevalence and light-moderate intensity (39% and 1.3± 0.3, n=40, respectively).In order to differentiate between the disease treatments, the initially infected oysters were marked with nail polish on both valves, though initially infected oysters tended to be larger than initially uninfected oysters.

Using oysters from two different sources in 2008 increased genetic variability among initial disease treatments. Wild Patuxent River and Wachapreague oysters were used in the initially infected treatment and local adaptations to disease, such as *P. marinus* tolerance or resistance may have influenced the response of these oysters to ambient salinity and *P. marinus* levels compared to the naïve, initially uninfected Choptank River hatchery oysters. In 2009, there was less geographically-based genetic variability between initial disease treatments (both of hatchery origin), which may have reduced variation in responses to *P. marinus* exposure and different salinities.

2008 and 2009 Field Deployments

There are currently no intertidal oysters in the Maryland portion of the Chesapeake Bay on which to estimate correct intertidal placement of oysters. Therefore, a range of placements was estimated to test for the effects of tidal height on *P. marinus* acquisition and progression. I used a design with three intertidal treatments that covered a range of air exposure durations referred to as: high, mid, and low intertidal for MSUERC, SERC, and VIMS. At VIMS-ESL, oysters naturally inhabit the intertidal zone and there was a distinct tidal height at which oysters occurred. Because of this, I used only 1 intertidal treatment, placing experimental oysters at the same height as wild oysters on the surrounding mud flats. A single, defined tidal height was not obvious for intertidal oysters at VIMS, so 3 intertidal height treatments were used at this site, even though intertidal oysters occur in the area.

In order to create 3 distinct air exposure treatments, three polypropylene oyster cages (www.fukuina.com; 61cm x 61cm x 20 cm, 32.5 mm diagonal mesh) were attached in series along two parallel 3m pieces of rebar (see Fig. 12) or to rebar racks (VIMS only). Each set represented 1 replicate of each intertidal treatment. Intertidal cages were covered tightly with 0.95 cm^2 gillnetting (monofilament nylon, Memphis Net and Twine Co., Memphis, TN) to allow maximum exposure to air and environmental conditions. Sets of cages were weighted with approximately 35-45 kg of metal weights and natural rock and chained to 45.7cm spiral stakes (www.petco.com) screwed into the substrate. Cages were deployed at low tide with the bottom edge of the low intertidal cage placed at the water

line at mean low water (MLW). Cages were placed in locations that sloped up from the water on natural gradients created by either rip-rap shorelines (MSUERC) or sandy beaches (SERC, VIMS) which differed between sites and created variations in the duration of air exposure in each tidal height treatment at each site (Malek Chapter 1). At VIMS-ESL, the single intertidal treatment cages were weighted with cinder blocks and deployed during low tide. The subtidal treatment consisted of individual cages containing oysters. Cages were covered with a polypropylene lid with the same mesh as the cage sides and bottom, secured by cable ties, weighted with cinder blocks, and either hung off a dock (SERC) or deployed in ~1-2 m of water at low tide and marked with buoys (MSUERC, VIMS-ESL, VIMS). The polypropylene lids were used on subtidal cages because maximum air exposure was not necessary for this treatment.

In 2008, each cage had 45-50 oysters of an initial disease treatment and each site had 5 replicates of each of the 4 tidal height treatments (high, mid, low, subtidal) for each initial disease treatment. Oysters of the two initial disease treatments were deployed in separate cages with 3-4.5 m between them to minimize transmission of *P. marinus* between experimental oysters. Low *P. marinus* acquisition in 2008 at MSUERC in the initially uninfected oysters made it difficult to test for differences in disease between tidal height treatments. In 2009, the two initial disease treatments were combined and each cage had 45-50 oysters of both initial disease treatments (100 oysters cage^{-1}) for each tidal height treatment. This change provided a local source of *P. marinus* even if background levels of infective cells were low, and increased the chance of detecting differences among tidal heights. There were 6 replicates for each tidal height treatment in 2009.

Oysters were deployed from 5-25 June in 2008 and 3-12 June in 2009. I assessed disease at the mid-point (7 to 8 wk) and at the end of the experiment. Up to 15 individuals were removed from each cage to be tested for *P. marinus* infection, depending on the number of surviving oysters (Malek Chapter 1). The final sampling occurred early at MSUERC in 2008 (early September) due to landfall by Tropical Storm Hannah potentially disrupting the cages with experimental oysters. Final sampling occurred on schedule after 16-17 wk (September 29th- October 12th) at VIMS-ESL and all sites in 2009.

Water temperature and salinity data were collected monthly from water quality monitoring stations near each site (http://mddnr.chesapeakebay.net/eyesonthebay) in 2008 and were taken every 3 wk using a YSI 85 at MSUERC and VIMS in 2009. SERC data were collected from a YSI datasonde deployed 1 m below surface at the laboratory dock (C. Gallegos, Smithsonian Environmental Research Center). Every 3 wk, cages at each site were cleaned, fouling organisms were removed, and temperature-logging mimics were replaced (see below). Minor maintenance was done to repair rips or tighten the netting on intertidal cages. Predators such as blue crabs, *Callinectes sapidus,* as well as the tunicate, *Mogula manhattensis*, which fouled cages, were removed from subtidal cages.

Controlled Air Exposure Experiment

In summer 2008, a preliminary field study was conducted to test under controlled conditions the effects of controlled durations of air exposure on progression of *P.*

marinus in infected oysters (S. Khadke, unpubl.). This experiment indicated a trend of slower progression due to air exposure similar to that predicted from laboratory experiments (Milardo 2001). In summer 2009, I expanded this experiment and used initially uninfected, as well as initially infected oysters to test for effects of air exposure on *P. marinus* acquisition and progression.

I initially used 3 treatments which varied in the length of air-exposure: 0 (control), 2 h, and 4 h. Cages were hung off the dock at SERC, ~3 m apart and ~0.5 m above the river bottom. At midday 5 d wk^{-1}, the 2 and 4 h treatment cages were removed from the water and oysters exposed to ambient conditions (cages were pulled regardless of weather). Each cage contained 50 oysters of each initial disease group and each treatment had 5 replicates. After 3 wk there were too few surviving oysters in the 4 h treatment to adequately assess disease acquisition and progression. The 4 h treatment was therefore removed and replaced with a new 1 h treatment with its own 0 h control.

At the mid-point of the experiment, 15 oysters per cage were removed from the 1 h, 1 h control, and 2 h d^{-1} control treatments to be tested for *P. marinus* infection. Removing animals for a mid-experiment disease analysis in the 2 h d^{-1}treatment was not feasible due to high mortality. The experiment continued until the middle of September, when oysters from all treatments were tested for *P. marinus* infections.

Mimics

Oyster mimics were used to predict internal temperatures experienced by experimental oysters, to describe the durations of exposure (the amount of time an oyster was exposed to air, including partial exposure at the beginning and end of the tidal cycle; Malek Chapter 1) and to interpret disease results. Mimics consisted of silicon filled oyster shells with an embedded temperature logger (iButton data loggers, Dallas Semiconductor; accuracy of ±0.5°C). Silicon has heat properties similar to water and thus to bivalve tissue, resulting in temperature readings similar to internal temperatures experienced by the organism (Helmuth 2002; Schneider & Helmuth 2007).

Mimics were deployed in mid intertidal and subtidal cages in field experiments and in air-exposed and control cages in the controlled air-exposure experiment. Each site had 2 to 3 mimics at each of these tidal heights or air-exposure treatments. Temperature measurements were taken every 15 min and mimics were replaced every 3 wk to download data and reset the loggers. In 2008, mimics were similar in size to initially infected oysters, but in 2009, I constructed mimics of various sizes out of shells from both initial disease treatments. The initially uninfected oysters were small with thinner shells, so it was important to know whether the internal temperature readings would differ from those of larger, initially infected oysters with thicker shells.

I conducted a calibration study to determine differences between mimic and live oyster internal temperatures by comparing iButton readings taken from both under simulated intertidal temperature cycles using an incubator (Percival I36VLC8). Mimics made from

both initially uninfected and initially infected oysters were used to find possible differences in temperatures due to shell size and thickness. Complete results of this experiment are in Malek Chapter 1, Appendix 1b. During the first simulated cycle, temperatures differed between mimics and oysters by ~5°C, but during the second cycle, temperatures were almost identical. After the experiment it was found that the silicon inside the mimics had not solidified completely, despite having been made 48 h prior to the experiment. Because the silicon likely continued to solidify during the experiment, the results from the second cycle suggested similar temperatures in both mimics and oysters, and silicon was completely solidified in mimics retrieved from field experiment cages, the temperatures recorded by mimics used in the field were likely accurate estimates of temperatures experienced in intertidal oysters.

Field Surveys

To examine whether *P. marinus* prevalence and intensity vary consistently among tidal heights in wild populations along the Atlantic Coast, I conducted field surveys of wild oysters from different tidal heights at sites ranging from Maine to North Carolina. Between 2008 and 2009, nine sites were sampled (Fig. 13; Table 6). Sites were chosen based on the presence and accessibility of naturally occurring intertidal and subtidal oysters. Tissue samples for disease analyses and size measurements were taken on 30-40 individuals from each tidal height at each site. Table 6 shows temperature and salinity recorded at the time of oyster collection site in both years using a YSI 85.

At each location, 40-50 oysters were randomly collected along a 15-25 m transect (depending on size of the reef or sampling area) at as many of the 3 tidal heights – high intertidal, mid intertidal, and subtidal – at which oysters occurred. The first oysters exposed as the tide ebbed during a standard tidal cycle were collected as high intertidal oysters, while mid intertidal oysters were designated as those exposed at the mid-point of the tidal cycle. Subtidal oysters were collected below the water line at low tide. In 2008, sampling was conducted in Wachapreague, VA where VIMS-ESL is located, and in the Lynnhaven River, VA (Fig. 13). Both locations have large populations of intertidal oysters that are infected with *P. marinus*. At Wachapreague, the collection sites were West Wye (70.7 ± 1.0 mm, average shell height, n=90), the Hummocks (74.7 ± 1.4 mm, average shell height, n=90), and Bradford's Bay (85.7 ± 1.7 mm, average shell height, n=90). In the Lynnhaven, the collection sites were Hume's Marsh (74.4 ± 1.2 mm, average shell height, n=120), Great Neck Point (86.2 ± 1.9 mm, average shell height, n=120), and Western Branch (78.7 ± 1.6 mm, average shell height, n=120). Collected wild oysters tended to be similar in size to initially infected oysters used in field experiments.

In 2009, I sampled in the Damariscotta River, ME because of recent reports of intertidal oysters in estuaries in New Hampshire and Maine (Capone et al. 2008). Oysters from both high and mid intertidal heights were collected from a site called Sugarloaf (78.4 ± 2.9 mm, average shell height, n=60). Subtidal oysters could not be collected without use of a boat and oyster dredge, so 2-5 year old oysters (88.5 ± 3.4 mm, average shell height, n=30) were purchased from a nearby hatchery, Mook Sea Farms, which raises oysters

subtidally in the river. Sampling was also conducted in North Carolina (Fig. 13) in Calico Bay, at Moorehead City (86.0 ± 1.8 mm, average shell height), and on Bear Island, off of Swansboro (66.7 ± 1.8 mm, average shell height). Due to above average wind and tides, only high and mid intertidal oysters were collected from Bear Island.

Disease Assessment

For all studies, I tested for *P. marinus* infection prevalence and intensity using the Ray's Fluid Thiogylcollate Media (RFTM) method (Ray 1954). This involved dissecting out rectal tissue samples from each oyster and incubating these for 5-7 d in individual tubes filled with media. Samples were then stained with Lugol's iodine so that *P. marinus* hypnospores could be identified. Samples were scored using the Mackin scale (Mackin 1962), a 6-point scale measuring the intensity of infection observed (0 no infection, 0.5 very light, 1 light, 2 and 3 moderate, 4 and 5 heavy/lethal). From these scores, prevalence and mean intensity were calculated for oyster from each cage for both initial disease treatments and for each field sampling site. Prevalence was calculated as a percentage of individuals with infections out of the total number sampled. Intensity was calculated by averaging intensity of *P. marinus* infections from individuals with Mackin scores of 0.5 to 5 (Soniat et al. 2006). Calculating intensity this way provides a measure of disease that is independent of the prevalence in the population.

Statistical Analyses

Means for each initial disease treatment and each tidal height treatment were used for analyses. Prevalence data were arcsine, square root transformed to eliminate

heterogeneity of variances (Sokal & Rohlf 1995). In some cases, this was not sufficient and rank transformations were used (Potvin & Roff 1993). Intensity data for all experiments was tested for homogeneity and $\log_{10}$ or rank transformations were performed where necessary.

Analysis of variance (ANOVA) was used to determine if tidal height had an effect on *P. marinus* acquisition and progression. T-tests were used for planned comparisons of each intertidal treatment against the subtidal treatment comparing prevalence and intensity data separately for each initial disease treatment. Separate analyses were run for the mid-point and final sampling data for all sites in both years.

Field surveys were not based on replicated sampling so a G-test (R x C test of independence) was used to determine whether there was an effect of tidal height on prevalence (Sokal & Rohlf 1995). When a significant effect of tidal height was observed (i.e., $p \leq 0.05$), the prevalence of each intertidal height was compared to the subtidal samples using a G-test. Intensity data from the field surveys was analyzed using a 1-way ANOVA to test for variation in infection intensity among tidal heights. Planned comparison T-tests were used to determine which intertidal heights differed significantly from the subtidal treatment. ANOVA was used to determined differences in *P. marinus* acquisition and progression under controlled durations of air exposure. Planned comparison T-tests were used to compare prevalence and intensity of each air-exposed treatment with the appropriate control treatments. Except where noted, analyses were performed using the SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Field Experiments

Environmental Conditions:

Water temperatures at field experiment sites peaked in July in 2008 and in August in 2009 (Fig. 2a). The lowest and highest temperatures were at the two Virginia sites, VIMS-ESL and VIMS, respectively. Salinity increased from sites in Northern to Southern ends of Chesapeake Bay, with the highest salinities at VIMS-ESL (Fig. 2b). At all sites, salinity remained fairly constant during the duration of field deployments.

Overview:

There was no effect of tidal height on *P. marinus* acquisition or progression in either initial disease treatment at sites in Maryland (without wild intertidal oysters) or Virginia (with wild intertidal oysters) regions of Chesapeake Bay in 2008. In 2009, however, *P. marinus* prevalence in initially infected oysters at sites without wild intertidal oysters was significantly higher in the high intertidal than in the subtidal. At the lowest salinity site (SERC), infection progression in the high intertidal was significantly slower than progression in the subtidal; at MSUERC (2008), there was a trend toward slower progression in the high intertidal than in the subtidal (Fig. 14a). Statistical analyses were performed on rank transformed data for all experiments.

Maryland *P. marinus* Infections*:*

In 2008, acquisition and progression of *P. marinus* did not vary significantly among tidal heights in either initial disease treatment at MSUERC (Table 7a & 7b). No acquisition of *P. marinus* occurred in the initially uninfected oysters during the first 8 wk of deployment, but by the end of the experiment, oysters in all treatments had acquired the parasite, with very light to light intensity infections (Fig. 15a & 15b). Prevalence of *P. marinus* in the initially infected oysters remained fairly constant during the experiment. There was a subtle trend toward slower progression in high intertidal than subtidal oysters and infections among tidal heights progressed to light-moderate intensity infections (Fig. 15a & 15b).

In 2009, acquisition and intensity of *P. marinus* at MSUERC did not vary significantly among tidal heights in initially uninfected oysters. Acquisition occurred in all treatments during the first 8 wk of deployment, increasing to greater than 90% by the end of the experiment (Fig. 16b). Intensity substantially increased in the mid, low intertidal and subtidal treatments after the mid-study sampling (Fig. 16a), resulting in light to moderate infections. Prevalence but not progression of *P. marinus* varied significantly among tidal heights in initially infected oysters (Fig. 16a & 16b). Planned comparisons indicated that prevalence was significantly higher in the high intertidal than in the subtidal (Table 7a). Prevalence increased to 90-100% in all treatments by the end of the experiment and intensity increased during the final 9 wk, resulting in moderate intensity infections (Fig. 16a & 16b).

Acquisition and intensity of *P. marinus* at SERC did not vary significantly among tidal heights in initially uninfected oysters (Table 7a & 7b). Acquisition occurred in 3 of 4 tidal height treatments during the first 8 wk of deployment, with greater than 50% acquisition in all treatments by the end of the experiment (Fig. 17b). Intensity increased substantially in all treatments to light infections after the mid-study sampling (Fig. 17a). Prevalence varied significantly among tidal heights in the initially infected oysters and planned comparisons indicated that prevalence at the end of the experiment was significantly higher in the high intertidal than in the subtidal (Table 7a). By the end of the experiment, prevalence had increased in all tidal height treatments to greater than 75% with a pattern of decreasing prevalence with decreasing tidal height (Fig. 17b).Though progression did not vary significantly overall, planned comparisons indicated that intensity was significantly lower in the high intertidal than in the subtidal (Fig. 17b), with light to moderate intensity infections across tidal height treatments.

Virginia *P. marinus* Infections:

At VIMS-ESL, acquisition and progression of *P. marinus* did not vary significantly among tidal heights in either initial disease treatment (Table 7a & 7b). Acquisition occurred in both tidal height treatments in the initially uninfected oysters during the first 8 wk of deployment and increased to an average of 50% across treatments during the remainder of the experiment (Fig. 18b). Intensity more than doubled in the subtidal treatment after the mid-study sampling period (Fig. 18a) with light infections in both treatments. Prevalence increased from 90 to 100% in the initially infected oysters during the first 8 wk, but decreased slightly by the end of the experiment (Fig. 18b), perhaps as a

result of mortality of heavily infected individuals. Intensity was higher in the first 8 wk but either decreased or stayed the same until the end of the experiment (Fig. 18a), resulting in light to moderate intensity infections.

Acquisition of *P. marinus* varied significantly among tidal heights in initially uninfected oysters after 8 wk of deployment at VIMS (Table 7a). Planned comparisons indicated that acquisition was significantly faster in all intertidal treatments than in the subtidal at the mid experiment sampling date. By the conclusion of the experiment, however, acquisition reached ~100% in all tidal height treatments (Fig. 19b). There was no overall significant effect of tidal height on intensity, but planned comparisons indicated that intensity was significantly higher in the low intertidal than in the subtidal, though visual representation of the data suggest that this is unlikely to be biologically significant due to the small difference between treatments and large standard errors (Fig. 19a; Table 7b). All treatments increased to moderate intensity infections after the mid-study sampling (Fig. 19a). Prevalence and progression did not vary significantly among tidal heights in the initially infected oysters (Table 7a & 7b). Prevalence increased to 100% in all treatments in the last 9 wk and infections intensified more quickly in all treatments during the second half of the experiment, resulting in moderate intensity infections (Fig. 19a & 19b).

Mimics:

The average daily maximum temperatures recorded by mimics was higher in the mid intertidal than the subtidal treatment at all sites (Fig. 20-22, Table 8). Data indicate that mid intertidal oysters at all sites except VIMS-ESL experienced body temperatures

greater than 35°C during some exposures, and that oysters in Maryland sometimes experienced body temperatures ≥40°C. In the mid intertidal mimics, the highest average daily maximum, daily fluctuation, and maximum difference between high and low temperatures in a day (Table 8) were at MSUERC (2008) and SERC, the two sites with small tidal ranges. These variables at the two sites were considerably higher than at sites with larger tidal ranges (VIMS-ESL, VIMS) that had wild intertidal oyster populations. Temperatures at MSUERC in 2009 were lower than the previous year. There was little difference in monthly air temperatures between the years (www.wunderground.com; Historic Data, Leonardtown, MD weather station), but more cloud cover during durations of air exposure in 2009 could have contributed to the lower mimic temperatures.

Mimic data indicate that VIMS-ESL had the largest daily fluctuation in subtidal treatment temperature of all sites tested. Due to the large tidal range at this location, subtidal cages may have been in fairly shallow water during low spring tides, compared to other sites. Monitoring data (S. Fate VIMS-ESL, pers. comm.) indicate that water temperatures at VIMS-ESL were the lowest of all sites (Fig. 2a); however the temperatures were recorded at the VIMS-ESL dock, which was some distance from the actual study site. Mimic data also indicate that the maximum daily fluctuation for subtidal temperatures at MSUERC (2009), SERC, and VIMS-ESL were approximately 5°C higher than MSUERC (2008) and VIMS (Table 8).

Controlled Air Exposure Experiment

Results from mid-study analyses indicated that *P. marinus* prevalence in the 1 h d^{-1} air-exposed treatment was significantly higher than the control for initially infected oysters (Table 9a). Final acquisition and intensity did not vary significantly between 1 h d^{-1} air-exposed and control treatments for either initial disease treatment, though there was a trend ($0.10>p>0.05$) toward higher prevalence in the air-exposed treatment than in the control for both disease treatments (Table 9a). Initially uninfected oysters had light intensity infections and initially infected oysters had light to moderate infections (Fig. 23a).

In the 2 h d^{-1}air-exposed treatment, there was a trend toward higher acquisition in the air-exposed treatment than in the subtidal control for initially uninfected oysters. Intensity in the 2h d^{-1} air-exposed treatment was significantly higher than in the subtidal control for this disease treatment. There was no affect of air exposure on prevalence in initially infected oysters in the 2 h d^{-1} air-exposed treatments, but intensity tended to be higher in the air-exposed treatment than in the subtidal control (Fig. 23b; Table 9b).

Mimic data indicate that the 2 h d^{-1} air-exposed treatment had higher daily maximum temperatures and larger daily differences between maximum and minimum temperatures than the 1 h d^{-1} air-exposed treatment, but both air-exposed treatments had temperatures comparable to mid intertidal mimic data from field experiments (Table 8).

P. marinus Progression in Field and Controlled Air-Exposure Experiments

There was a significant relationship between the number of days that internal temperatures (measured by mimics in the mid intertidal treatment) fluctuated by ≥15˚C and the difference in subtidal and high intertidal *P. marinus* infection intensity (i.e. subtidal intensity – intertidal intensity) among sites in initially infected oysters (Fig. 14a). Sites that had more days with large internal temperature fluctuations had the largest difference in intensity between the two tidal height treatments. There was no relationship between these factors in initially uninfected oysters.

However, increased *P. marinus* prevalence in initially infected oysters at all tidal heights suggests that acquisition occurred in this disease treatment during the experiment. Newly acquired, light intensity infections are not true representations of disease progression and likely caused larger differences in intensity to be calculated between the tidal heights than actually occurred by progression of infections alone. To address this, the frequency of each intensity score for *P. marinus* infections (0.5-5, Mackin scale) for high intertidal and subtidal oysters at each field experiment site and the controlled air-exposure experiment were plotted (Fig. 14b-f). Because the initial intensity of the infected oysters was 1.3, I examined the frequencies of infections with scores of ≥2 (moderate infections). With the exceptions of MSUERC in 2008 (Fig. 14b) and SERC (Fig. 14d), when considering only moderate to lethal infections, there was no difference in *P. marinus* intensity between the tidal heights and there was no relationship between the number of days with large

temperature fluctuations and the difference between high intertidal and subtidal intensity (not graphed).

Field Surveys

Prevalence or intensity of *P. marinus* varied significantly among tidal heights at 6 of the 9 sites sampled in 2008 and 2009 (Table 10). When results were significant, prevalence was higher in intertidal samples than in subtidal samples from the same site. In contrast, the tidal height with the highest disease intensity varied – at two sites with significant differences intensity was highest in the subtidal and at another two sites, intensity was highest in the intertidal.

Wachapreague:

Prevalence but not intensity of *P. marinus* varied significantly among tidal heights at West Wye (Fig. 24; Table 5). Pairwise comparisons indicated that prevalence was significantly higher in the mid intertidal than in the subtidal (Fig. 24b; Table 10). All tidal heights had light intensity infections. Neither prevalence nor intensity varied significantly among tidal heights at the Hummocks (Fig. 24b; Table 10), which had the lowest prevalence in all 3 tidal heights of the 6 sites sampled in 2008. Intensity was comparable to the other sites and decreased from light to very light infections with decreasing tidal height (Fig. 24a). Prevalence did not vary significantly among tidal heights at Bradford's Bay (Table 10), but planned comparisons indicated that intensity was significantly lower in the high intertidal than in the subtidal. Intensity increased with decreasing tidal height

from light infections in the high intertidal to moderate infections in the subtidal. Statistical analyses were performed on $\log_{10}$ transformed data for all Wachapreague sites.

Lynnhaven:

At Hume's Marsh, prevalence and intensity of *P. marinus* did not vary significantly among tidal heights overall (Fig. 25; Table 10). However, planned comparisons indicated that the mid intertidal intensity was significantly higher than the subtidal intensity. At Great Neck Point, both prevalence and intensity varied significantly among tidal heights (Fig. 25; Table 10). Prevalence was significantly higher in the high intertidal than in the subtidal and intensity was significantly lower in the mid intertidal than in the subtidal. Prevalence but not intensity varied significantly among tidal heights at Western Branch (Fig. 25; Table 10). Prevalence was significantly higher in both the high and mid intertidal than in the subtidal. Infection intensities were light to moderate at all tidal heights at all sites.

Maine:

No *P. marinus* infections were detected in the samples from the Damariscotta River, despite salinities being conducive to *P. marinus* infections and some samples having 4-5 yr of subtidal exposure.

North Carolina:

Prevalence differed significantly among tidal heights at Calico Bay and pairwise comparisons indicated that prevalence was significantly higher in the high intertidal than

in the subtidal (Fig. 26a; Table 10). Although there was no overall significant effect of tidal height on intensity, planned comparisons indicated that the high intertidal intensity was significantly higher than the subtidal and all tidal heights had light to moderate infections (Fig. 26a). Both prevalence and intensity tended to decrease with decreasing tidal height. With no subtidal disease data, comparisons could not be made for Bear Island. However, prevalence in the high and mid intertidal followed a pattern similar to Calico Bay (Fig. 26b) and intensity had an opposite pattern of increased intensity with decreasing tidal height (Fig. 26a).

Discussion

A combination of field experiments, a controlled air-exposure experiment, and field surveys of wild oyster populations indicated that there was an effect of tidal height on *P. marinus* infection prevalence from Maryland to North Carolina. Prevalence was higher in the high intertidal than in the subtidal in 11 of the 13 total experiments and field survey sites at which *P. marinus* occurred and at which I had both high intertidal and subtidal data ($p=0.0017$, assuming equal probability of higher or lower prevalence in high intertidal than subtidal treatment using a Binomial Distribution; Fig. 27). Prevalence was significantly higher ($p\leq0.05$) in the high intertidal than in the subtidal at 6 of 13 experiments and field sites surveyed. In contrast, progression and intensity of *P. marinus* infections varied inconsistently among tidal heights. There was a significant relationship between the number of days mid intertidal oysters experienced predicted internal temperature fluctuations of $\geq15°C$ and the difference between subtidal and high intertidal disease progression in initially infected oysters in the field and controlled experiments; as

the number of days with large fluctuations increased, subtidal infection intensity increased relative to high intertidal intensity (Fig. 14a). However, when newly acquired, light infections were removed from progression analyses, there was no relationship between days with large temperature fluctuations and the difference in subtidal and high intertidal progression, and there was no overall effect of tidal height on *P. marinus* progression. Infection progression was only substantially higher subtidally than in the high intertidal at two sites. As summarized in the Introduction, previous research indicates that intertidal exposure causes physiological stress in both oysters and *P. marinus*, and hence this stress likely contributed to the observed pattern of prevalence. The results from my study suggest that long durations of air exposure result in higher prevalence of *P. marinus* infections compared to subtidal habitats, but do not consistently affect disease progression. High *P. marinus* prevalence and as I reported previously (Malek Chapter 1), high oyster mortality and slow growth, occur in the high intertidal which suggests that this habitat does not provide a refuge from disease and may not provide a suitable, successful habitat for oysters, particularly in Maryland.

Prevalence

A trend or statistically significant pattern of higher *P. marinus* prevalence in initially infected oysters in the high intertidal than in the subtidal was observed in 2 of 3 field experiments in Maryland, as well as in oysters exposed to air 1 and 2 h d^{-1} in the controlled air-exposure experiment. Based on mimic data the high intertidal treatment in field experiments experienced 3.8 – 5.9 h of exposure (the duration of time that oysters are exposed to air, including partial exposure during ebb and flood tides; see Malek

Chapter 1). The experiments with the longest durations of exposure (MSUERC 2009 and SERC) had significantly higher prevalence in the high intertidal than in the subtidal (Fig. 15b & 16b). Despite shorter durations of exposure, conditions in 1 and 2 h d^{-1} treatments of the controlled air-exposure experiment were likely more severe than in field experiments as oysters were exposed directly in the middle of the day with no shade or relief from water spray or damp sand or rocks. Also, there was no period of partial exposure and therefore no gradual change from submersion to exposure.

In contrast to Maryland, *P. marinus* prevalence in Virginia field experiments did not differ significantly among tidal heights (Fig. 17b & 18b); prevalence in initially infected oysters at VIMS-ESL and VIMS approached 100% by the end of the experiment. However, mid-study analyses from VIMS in 2009 suggested a trend similar to Maryland experiments, with higher prevalence in the intertidal than in the subtidal (Fig. 18b). Prevalence was also higher in the high intertidal than in the subtidal at all field survey sites in Virginia, as well as at Calico Bay in North Carolina. Of the 7 sites with higher prevalence in the high intertidal than in the subtidal, 3 had significantly higher prevalence in the high intertidal. These results suggest that the pattern observed in Maryland field and controlled air-exposure experiments occurs in wild intertidal populations. Genetic differences between hatchery-raised oysters and wild oysters that may have local adaptations to disease (*P. marinus* tolerance) could have caused the differences in prevalence among field experiments and field surveys in Virginia.

Unlike the pattern in initially infected oysters, there was no consistent effect of tidal height on acquisition of *P. marinus* by initially uninfected oysters in Maryland or Virginia field experiments. The change in experimental design, that involved placing both initial disease treatments in the same cage, may have increased acquisition in 2009. The presence of a disease source within cages may have made it difficult to detect differences between tidal heights as maximum prevalence and acquisition occurred, preventing the detection of true differences that may exist among tidal heights. Acquisition reached 98-100% at all tidal heights at VIMS in 2009, but did not reach 100% in initially uninfected oysters at VIMS-ESL in 2008 despite considerably higher salinity (Fig. 2b) and more abundant intertidal populations; in wild populations sampled, prevalence rarely reached 100% (Fig. 24b & 25b). Additionally, mid-study results from VIMS indicated a pattern similar to initially infected oysters at other sites (higher prevalence in the intertidal than the subtidal) that may have been more reflective of disease dynamics in that area. Maximum acquisition (100%) did not occur at Maryland sites in 2009 despite the change in experimental design, likely due to slower disease transmission in low and moderate salinities.

Though there was no consistent effect of tidal height, *P. marinus* acquisition in initially uninfected oysters increased from northern to southern Chesapeake Bay in both years. In 2008, average acquisition among all tidal heights was lower at MSUERC (~7%), the intermediate salinity/disease site than at VIMS-ESL (~53%), the high salinity/disease site. Average acquisition among all tidal height treatments in 2009 increased from ~70% at the low salinity/disease site (SERC) to ~93% at the intermediate salinity/disease site

(MSUERC) and finally to ~100% at the high salinity/disease site (VIMS). There tends to be high acquisition in young oysters when they are exposed to high concentrations of infective *P. marinus* spores and conditions favorable for transmission, such as high salinity (Andrews & Hewatt 1957). Additionally, *C. virginica* filters water faster at high salinities (Hopkins 1936; Galtsoff 1964), potentially increasing exposure to infectious stages of *P. marinus* and thus acquisition.

Results from my study suggest that different salinities and ambient disease levels found within Chesapeake Bay can influence acquisition of *P. marinus* by young, uninfected oysters. This conclusion is in contrast to results from McCollough et al. (2007) in the Patuxent River, MD, who found that specific-disease-free oysters acquire *P. marinus* infections rapidly even when isolated by up to 5 km from other infected oysters. My results suggest that oysters may not always acquire disease, even when they are in close proximity to other infected oysters. Data from MSUERC in 2008, when oysters of each initial disease treatment were placed 5 m apart, indicate that even though *P. marinus* was present in the surrounding waters, uninfected oysters did not rapidly acquire the parasite (Fig. 15b). It was only after combining the initial disease treatments in the same cage in 2009 that rapid acquisition occurred (Fig. 15b). Also, results from SERC, the low salinity site, in 2009 show that acquisition in initially uninfected oysters was lower compared to other sites. This suggests that even when uninfected oysters are in close proximity to an infection source (in the same cage), acquisition of infection is greatly influenced by local conditions, such as the low salinity recorded at SERC.

Knowing how background disease levels in an area may affect disease acquisition would be a useful tool in determining the placement of oysters for restoration. Placing oysters in a potential restoration system the year prior to deployment may allow for differences in local conditions to be evaluated that will create more successful site selection. In this case it would also be useful to know how environmental conditions in the year of testing compare to local averages, as years of drought or freshet can influence disease patterns (McCollough et al 2007).

Intensity

In contrast to prevalence, there was no consistent effect of tidal height on *P. marinus* progression in initially infected oysters from field experiments or *P. marinus* intensity in oysters from field surveys of wild populations. In only 2 of 13 experiments and field survey sites was progression significantly slower in high intertidal than subtidal oysters (1 of 5 field experiments (SERC; Table 7b) and 1 of 7 field surveys (Table 10)). Mimic temperature data from SERC indicated that mid intertidal oysters were subjected to numerous days of internal temperature fluctuations of ≥15°C (Table 8) and high intertidal oysters were likely subject to at least that many days of fluctuations of this amplitude, possibly contributing to the slower progression of disease in the higher intertidal than the subtidal. There was also no difference in disease intensity at the end of the 8 wk deployment for initially uninfected oysters from any field experiment, suggesting no effect of intertidal air-exposure on intensity of newly acquired *P. marinus* infections.

Despite no overall effect of tidal height on *P. marinus* progression and intensity among experiment and survey sites, there was a significant relationship between the number of days predicted to have large internal body temperature fluctuations (≥15°C) in the mid intertidal and the difference in subtidal and high intertidal intensity in the field and controlled experiments. I found that as the number of days with internal temperature changes of this magnitude increased, the difference in progression between subtidal and high intertidal oysters increased, with faster progression in the subtidal than in the high intertidal (Fig. 14a). The sites with the largest differences in progression and the most days with internal temperature fluctuations of ≥15°C were in the Maryland region of Chesapeake Bay (MSUERC 2008, SERC) and had the largest average daily fluctuations between minimum to maximum temperatures (Table 8). However, based on increased prevalence over the course of the study, acquisition occurred in initially infected oysters, potentially leading to overestimated differences among tidal heights in progression. When only moderate to lethal infections (2-5, Mackin scale) were considered, there was no relationship between the number of days with large temperature fluctuations and the difference in progression between subtidal and high intertidal oysters. The only experiments to have a difference between tidal heights based on these advanced infections were MSUERC (2008) and SERC, suggesting progression was in fact slower in the high intertidal than the subtidal at these sites.

During air exposure, oysters experience increased (or decreased) internal temperatures, low O_2, high CO_2, and low hemolymph pH (Milardo 2001). Physiological stress during long durations of air exposure can suppress the cell lysis capability/capacity of oyster

hemocytes, thereby reducing their ability to kill parasites, such as *P. marinus* (Allen & Burnett 2008) and potentially increasing their susceptibility to disease. Boyd and Burnett (1999) found that production of reactive oxygen intermediates (ROIs), which are an important defense mechanism, was reduced by 66% in *C. virginica* under simulated hypoxic conditions. Under field conditions, this change in ROI production may reduce the ability of intertidal oysters that experience internal hypoxia during air exposure to resist infection by *P. marinus*. The pattern of higher prevalence in the high intertidal than the subtidal in 11 of 13 total experiments and field sites strongly indicates that oysters exposed to long durations of air exposure are more susceptible to *P. marinus* infections than oysters that are always submerged, even though there are longer feeding periods and thus exposure to the parasite in the subtidal. The differences in high intertidal and subtidal prevalence observed in this study indicate that *P. marinus* infections do vary among tidal heights, but results showed a pattern opposite of my original hypothesis that prevalence would be lower in the intertidal than in the subtidal.

My results from Maryland field experiments and Virginia and North Carolina field surveys are in contrast to the findings of Burrell et al. (1984) and O'Beirn et al. (1994) who found no difference in *P. marinus* prevalence in intertidal and subtidal oysters from low to moderate and high salinity sites in South Carolina and a high salinity tidal creek in Georgia. These previous studies may not have found a tidal height effect because they compared *P. marinus* prevalence from oysters at only 1 intertidal height, that may not have represented differences in exposure (and potentially disease) experienced within the population, to oysters in the subtidal. The current study compared disease prevalence

from oysters at intertidal heights with different durations of air exposure, and potentially different physiological conditions that can affect *P. marinus* infections.

Slow progression of *P. marinus* infections in high intertidal oysters at the site with the longest duration of air exposure (SERC), the highest number of days with large internal temperature fluctuations, and the highest maximum intertidal temperature suggests that the parasite is also heavily stressed by intertidal exposure, especially during days of large internal temperature fluctuations (≥15°C) and this is in agreement with Milardo's results (2001). However, significantly slower progression in intertidal compared to subtidal oysters was observed at only 2 of 13 total experiments and sites and indicated no overall effect of tidal height on *P. marinus* progression or intensity. My results are in agreement with findings from previous field studies that ranged from low to moderate and high salinity sites in South Carolina to high salinity sites in Georgia and Nueces Bay, Texas (Burrell et al. 1984; O'Beirn et al. 1994; Ybanez 2007) and found no difference in *P. marinus* intensity between intertidal and subtidal oysters. Results of my study and others indicate that despite reduced growth of *P. marinus* under simulated intertidal conditions in the laboratory, air exposure does not negatively affect the parasite under field conditions, or does not inhibit parasite growth enough to significantly reduce progression of infections. Growth of *P. marinus* could be reduced during intertidal exposure, but it is possible that during periods of low stress, such as during immersion, parasite growth is fast enough to compensate for periods of high stress, resulting in an overall increase in infection intensity. Additionally, my mimic data indicated that internal temperatures in intertidal organisms in northern Chesapeake Bay, where the tidal range is small, can

reach as high as 47.5°C, which is higher than those used in the laboratory experiments (Milardo 2001), suggesting the temperatures in my field experiments might have been high enough for an effect to be detected.

The nature of the tidal regime in the Maryland region of Chesapeake Bay may contribute to high *P. marinus* prevalence and oyster mortality, and slow oyster growth in the high intertidal compared to the subtidal. The duration of physiological stress that intertidal oysters in Maryland may experience is variable, as the microtidal habitat available is strongly influenced by the atmospheric nature of tides in Chesapeake Bay. During storms, winds may cause the intertidal zone to be either exposed or submerged for days, potentially leading to mortality or severe stress in intertidal organisms. Under these conditions, intertidal oysters could experience higher mortality and differences in growth and disease depending on frequency and duration of exposure and submersion; more days submerged may increase growth as oysters can feed longer or increased exposure may lead to higher prevalence of *P. marinus* infections.

In Virginia, mortality tends to be similar among tidal heights, and growth is fastest in the subtidal but similar among intertidal treatments (Malek, Chapter 1). Although my field experiments showed no difference in *P. marinus* prevalence between tidal heights, results from my Virginia field surveys indicated that prevalence was higher in the high intertidal than in the subtidal. Combined with mortality and growth patterns from field experiments (Malek, Chapter 1), disease results suggest that high intertidal oysters have a disadvantage in terms of disease, mortality, and growth throughout Chesapeake Bay.

Additionally, although *P. marinus* prevalence tends to be lower and growth tends to be faster in the subtidal than the high intertidal in Virginia, high mortality in this habitat caused by the increased abundance and diversity of predators found in high salinity areas (Burrell 1986; Roegner & Mann 1990; Crosby et al. 1991) creates a disadvantage for oysters in this habitat as well.

Research by Andrews (1988) showed that *P. marinus* infections tend to progress to lethal intensities in the second year of infection, though mortality can occur during the first year. Because my study documented effects of tidal height on *P. marinus* acquisition and progression only during single summer seasons, it is possible that multiple years of deployment may yield different patterns in acquisition and progression. In addition, though patterns in prevalence from field surveys were consistent with each other and with results from Maryland field experiments, disease data from these surveys represent the local prevalence and intensity from a single date during the year and should not be considered averages for the areas sampled. To strengthen results from field surveys, multiple samplings from the different wild populations could be conducted to better follow seasonal, and maybe even yearly disease patterns. Multi-year field experiments and surveys of wild populations could further define patterns observed in this study (higher prevalence in the high intertidal than in the subtidal) and potentially identify patterns not detected from single-year experiments and sampling.

Conclusions

Overall, this study indicated that prevalence of *P. marinus* infections is affected by tidal height, though in the opposite way than originally predicted. Progression of *P. marinus* infections, however, is not affected consistently by tidal height. In contrast to findings in laboratory studies suggesting an advantage of intertidal over subtidal habitats for oysters with *P. marinus* infections (Milardo 2001), my results indicate that oysters in the high intertidal have a higher prevalence of *P. marinus* infections, as well as higher oyster mortality and slower oyster growth, as compared to oysters in the subtidal. Under current conditions, intertidal habitats, especially those with long durations of air exposure, do not provide a refuge for oysters from lethal *P. marinus* infections, reducing the potential of using intertidal oyster restoration as a means of lowering disease-related mortality in Maryland. It is possible that consistent differences in *P. marinus* infections among tidal heights that may be favorable for oysters, such as slower progression in the intertidal than the subtidal, could occur in the future, allowing oyster populations to potentially survive *P. marinus* infections. Such differences could be exploited to the benefit of restoration programs by directing efforts towards habitats that may decrease *P. marinus*-related mortality. However, if *P. marinus* prevalence remains higher in the high intertidal than in the subtidal and there continues to be no refuge for oysters from lethal infections , oyster populations will continue to be heavily impacted by *P. marinus* infections, further hindering restoration efforts and possibly leading to the loss an ecologically and economically valuable species in Chesapeake Bay.

Table 1. Tidal range and mimic data for all sites: **a.** High and Low intertidal approximate exposures determined from mean mid intertidal exposure based on mimic recordings (see Appendix 1) and **b.** Average daily maximums and fluctuations in estimated oyster internal temperatures calculated using average mid intertidal and subtidal mimic temperatures from each site (MSUERC 2008, n=5738; MSUERC 2009, n=9110; SERC, n=6426; VIMS-ESL, n=6162; VIMS, n=7270). Historic tidal data was obtained from http://tidesandcurrents.noaa.gov/ and averaged to get the mean tidal range at each site during the periods when oysters were deployed.

a. Tidal range, approximate and mean exposure durations						
Year	**Site**	**Mean Tidal Range (m) ± (1SE)**	**Rate of Tidal Change (mhr^{-1})**	**Approx. High Intertidal Exposure Duration (hr)**	**Mean Mid Intertidal Exposure Duration (hr) ± (1SE)**	**Approx. Low Intertidal Exposure Duration (hr)**
2008	MSUERC	0.37 (0.012)	0.06	3.82	2.3 (0.32)	0.78
2009	MSUERC	0.36 (0.009)	0.06	4.64	2.8 (0.26)	0.96
2009	SERC	0.28 (0.007)	0.04	5.95	3.6 (0.36)	1.25
2008	VIMS-ESL	1.18 (0.024)	0.2	NA	2.5 (0.31)	NA
2009	VIMS	0.70 (0.012)	0.11	3.8	2.9 (0.40)	2
b. Daily average temperatures for mid and subtidal treatments						
		Mid Intertidal Mean Daily Max (°C)± (1SE)	**Mid Intertidal Mean Daily Fluctuation (°C)± (1SE)**	**Subtidal Mean Daily Max (°C)± (1SE)**	**Subtidal Mean Daily Fluctuation (°C)± (1SE)**	
2008	MSUERC	31.2 (0.5)	8.6 (0.6)	28.2 (0.1)	2.01 (0.1)	
2009	MSUERC	28.4 (0.43)	5.9 (0.3)	26.77 (0.3)	2.44 (0.2)	
2009	SERC	31.9 (0.62)	8.7 (0.7)	29 (0.2)	2.57 (0.2)	
2008	VIMS-ESL	28.7 (0.3)	7.0 (0.3)	28.35 (0.2)	4.95 (0.2)	
2009	VIMS	28.5 (0.3)	5.7 (0.4)	26.89 (0.2)	2.05 (0.1)	

Table 2. Statistical analyses of oyster mortality **a.** 2-way ANOVA of initial disease group and tidal height and **b.** planned comparisons of each intertidal treatment against the subtidal treatment for both initial disease groups. Interaction terms that were not significant (in parentheses) were not included in the final statistical model. * indicate analyses conducted on rank transformed data. For VIMS-ESL, I=intertidal treatment.

a. 2-way ANOVA of Initial Disease Group and Tidal Height											
		Initial Disease Group			**Tidal Height**			**Interaction**			**Heights Significantly Different from Subtidal ($p \leq 0.05$)**
Year	**Site**	**df**	**F**	**p**	**df**	**t**	**p**	**df**	**F**	**p**	
2008	MSUERC*	1, 32	10.88	**0.002**	3, 32	7.25	**0.0009**	(3, 32	1.53	0.22)	**H>S, L<S**
2009	MSUERC*	1, 38	20.23	**<0.0001**	3, 38	19.53	**<0.0001**	(3, 38	1.88	0.15)	**H>S**
2009	SERC*	1, 40	6.49	**0.01**	3, 40	55.17	**<0.0001**	(3, 40	1.43	0.24)	**H>S, M>S**
2008	VIMS-ESL	1, 17	0.04	0.83	1, 17	1.29	0.27	(1, 16	3.46	0.08)	
2009	VIMS*	1, 36	21.61	**<0.0001**	3, 36	0.87	0.46	(3, 36	0.30	0.82)	
b. Planned comparisons											
2008	MSUERC	Uninfected			3, 16	7.44	**0.002**				**L<S**
2009	MSUERC	Uninfected			3, 19	11.80	**0.0001**				**H>S**
2009	SERC	Uninfected			3, 20	42.38	**<0.0001**				**H>S, M>S**
2008	VIMS-ESL	Uninfected			1,8	6.77	**0.03**				**I<S**
2009	VIMS	Uninfected			3, 18	0.31	0.81				
2008	MSUERC	Infected			3, 16	1.58	0.23				
2009	MSUERC	Infected			3, 19	9.22	**0.0006**				**H>S**
2009	SERC	Infected			3, 20	17.76	**<0.0001**				**H>S, M>S**
2008	VIMS-ESL	Infected			1,8	0.40	0.69				
2009	VIMS	Infected			3, 18	0.76	0.52				

Table 3. Statistical analyses of oyster growth **a**. ANCOVA of initial shell height and **b.** ANOVA and planned comparisons using T-tests of each intertidal treatment against the subtidal treatment. Interaction terms that were not significant (in parentheses) were not included in the final statistical model. * indicate analyses conducted on rank transformed data. For VIMS-ESL, I=intertidal treatment.

a. ANCOVA of Initial Shell Height											
			Initial Shell Height			**Tidal Height**			**Interaction**		
Year	**Site**	**Disease Group**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
2009	MSUERC	Uninfected	1, 16	2.97	0.10	3, 16	3.30	**0.04**	3, 16	3.52	**0.03**
		Infected	1, 19	0.01	0.90	3,19	23.97	**<0.0001**	(3, 16	0.11	0.95)
2009	SERC	Uninfected	1, 19	0.06	0.12	3, 19	37.70	**<0.0001**	3, 16	2.59	0.08
		Infected	1, 19	7.26	**0.01**	3,19	15.53	**<0.0001**	(3,16	0.62	0.61)
2009	VIMS	Uninfected	1, 19	0.29	0.59	3,19	1.72	0.19	(3,16	0.25	0.86)
		Infected	1, 19	3.09	0.09	3,19	2.06	0.13	3, 16	2.69	0.08

b. Planned comparisons						
		Initial Disease Group	**Tidal Height**			**Heights Significantly Different from Subtidal (p≤0.05)**
Year	**Site**		**df**	**F**	**p**	
2008	MSUERC	Uninfected	3,15	7.44	**0.002**	**H<S**
2009	MSUERC*	Uninfected	3,20	10.49	**0.0002**	**H<S, M<S, L<S**
2009	SERC*	Uninfected	3,20	42.24	**<0.0001**	**H<S, M<S, L<S**
2008	VIMS-ESL	Uninfected	1,8	14.12	**0.005**	**I<S**
2009	VIMS	Uninfected	3,20	2.32	0.10	**H<S**
2008	MSUERC	Infected	3,16	2.08	0.14	
2009	MSUERC*	Infected	3,20	27.39	**<0.0001**	**H<S, M<S, L<S**
2009	SERC*	Infected	3,20	10.60	**0.0002**	**H<S, M<S, L<S**
2008	VIMS-ESL	Infected	1,8	13.97	**0.005**	**I>S**
2009	VIMS	Infected	3,20	2.5	0.08	**M<S**

Table 4. Statistical analyses of relationship between oyster growth and *P. marinus* infection intensity **a.** Results from 3-way ANOVA for each initial disease treatment with total growth as the response variable and site, tidal height, and *P. marinus* intensity as the independent factors. Final models by initial disease treatment, interaction terms that were not significant (in parentheses) were not included in final model. **b.** Growth results of 2-way ANOVAs for each initial disease treatment with site, tidal height, and *P. marinus* intensity as independent factors.

a. 3 way ANOVA

Initial Disease Treatment	**Site**			**Tidal Height**			***P. marinus* infection intensity**			**Interaction**		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Uninfected	2, 632	12.54	**<0.0001**	3, 632	32.17	**<0.0001**	1, 632	24.83	**<0.0001**	(6, 632	0.92	0.48)
Infected	6, 507	6.86	**0.0012**	3, 507	10.55	**<0.0001**	1, 507	8.49	**0.0037**	(6, 507	1.94	0.07)

b. 2-way ANOVA

		Site			**Tidal Height**			***P. marinus* infection intensity**		
		df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Uninfected	Site				6, 632	1.78	0.10	2, 632	2.99	**0.05**
	Tidal Height	6, 632	1.78	0.10				3, 632	0.20	0.89
Infected	Site				6, 507	2.10	**0.05**	2, 507	2.59	0.07
	Tidal Height	6, 507	2.10	**0.05**				3, 507	3.35	**0.02**

Table 5. Regression analyses of *P. marinus* infection intensity and oyster growth for each initial disease treatment by site in 2009.

		Initial Disease Group							
Site	**Tidal Height**	**Uninfected**				**Infected**			
		n	**R^2**	**p**	**Slope (+/-)**	**n**	**R^2**	**p**	**Slope (+/-)**
SERC	High	14	0.21	0.09	-	28	0.08	0.07	+
	Mid	47	0.0003	0.91	-	46	0.01	0.36	-
	Low	87	0.02	0.07	-	71	0.0008	0.81	+
	Sub	78	0.02	0.21	-	75	0.12	**0.0021**	-
MSUERC	High	32	0.02	0.42	-	48	0.006	0.59	+
	Mid	81	0.016	0.25	-	66	0.01	0.25	-
	Low	62	0.08	**0.02**	-	63	0.0024	0.70	+
	Sub	79	0.0001	0.92	-	67	0.005	0.57	-
VIMS	High	47	0.04	0.16	-	22	0.13	0.09	-
	Mid	41	0.13	**0.01**	-	21	0.0007	0.91	+
	Low	43	0.09	**0.04**	-	15	0.0049	0.80	-
	Sub	44	0.15	**0.009**	-	7	0.56	**0.05**	-

Table 6. Temperature and Salinity data from field survey sites in both years.

Site	Year	Temperature (°C)	Salinity	Latitude/Longitude
West Wye, VA	2008	22.6	29.1	037°36’28.3/075°37’46.2
The Hummocks, VA	2008	23.5	29.2	037°37’11.6/075°38’48.1
Bradford’s Bay, VA	2008	22.5	28.8	037°34’40.4/075°40’46.9
Hume’s Marsh, VA	2008	19.6	21.3	036°53’88.3/076°05’28.5
Great Neck Point, VA	2008	20	20.7	036°53’74.0/076°05’02.5
Western Branch, VA	2008	20.3	21.4	036°53’73.8/076°05’45.0
Sugar Loaf, ME	2009	21.1	29	044°03’14.0/069°32’52.0
Calico Bay, NC	2009	25.3	30.7	034°43’20.7/076°42’21.4
Bear Island, NC	2009	25.7	30.8	034°38’22.8/077°42’14.4

Table 7. Results of planned comparisons of *P. marinus* **a.** prevalence **b.** intensity for each initial disease treatment for mid and final disease samplings. U=initially uninfected treatment, I=initially infected treatment. * indicate all analyses were performed on rank transformed data. + indicate that only mid study disease sampling analyses were preformed on rank transformed data. Ωindicate that only final disease sampling analyses were performed on rank transformed data.

a. Planned comparisons of *P. marinus* Prevalence										
		Initial Disease Treat.	**Prevalence**						**Heights Significantly Different from Subtidal (p≤0.05)**	
Year	**Site**	**U/I**	**Mid**			**Final**			**Mid**	**Final**
			df	**F**	**p**	**df**	**F**	**p**		
2008	MSUERC*	U				3, 15	0.59	0.63		
		I	3, 15	0.44	0.73	3, 16	0.92	0.45		
2009	MSUERC*	U	3, 16	0.06	0.97	3, 20	0.88	0.47		
		I	3, 16	1.54	0.23	3, 20	3.81	**0.02**		**H>S**
2009	SERC +	U	3, 14	1.09	0.38	3, 19	0.68	0.57		
		I	3, 16	0.90	0.46	3, 20	7.35	**0.0016**		**H>S**
2008	VIMS-ESL+	U	1, 8	3.7	0.09	1, 8	2.43	0.16		
		I	1, 8	2.62	0.14	1, 8	0.22	0.65		
2009	VIMS Ω	U	3, 20	7.09	**0.002**	3, 17	0.49	0.69	**H>S, M>S, L>S**	
		I	3, 20	0.84	0.49	3, 17	0	1		
b. Planned comparisons of *P. marinus* Intensity										
			Intensity						**Heights Significantly Different from Subtidal (p≤0.05)**	
			Mid			**Final**			**Mid**	**Final**
			df	**F**	**p**	**df**	**F**	**p**		
2008	MSUERC*	U				3, 15	0.51	0.68		
		I	3, 15	0.68	0.58	3, 15	2.19	0.13		
2009	MSUERC*	U	3, 16	1.45	0.26	3, 20	0.96	0.43		
		I	3, 19	0.58	0.64	3, 20	1.54	0.24		
2009	SERC +	U	3, 14	2.11	0.14	3, 19	0.62	0.61		
		I	3, 16	0.24	0.87	3, 20	2.09	0.13		**H<S**
2008	VIMS-ESL+	U	1, 8	3.12	0.11	1, 8	0.25	0.63		
		I	1, 8	2.63	0.14	1, 8	2.75	0.13		
2009	VIMS Ω	U	3, 20	0.14	0.93	3, 17	1.48	0.25		**L>S**
		I	3, 20	1.25	0.32	3, 17	0.62	0.61		

Table 8. Summarized temperature data from **a.** mid-intertidal **b.** subtidal mimics in field experiments from all sites and 2 h air exposed treatment from the controlled air exposure experiment. All calculations based on average temperatures from all mid-intertidal mimics at each site. (MSUERC 2008, n=5738; MSUERC 2009, n=9110; SERC, n=6426; VIMS-ESL, n=6162; VIMS, n=7270)

a. Mid Intertidal							
Year	**Site**	**Mean Exposure Duration (hr) ± (1SE)**	**Avg. Daily Max. (°C)**	**Avg. Daily Min. (°C)**	**Mean Daily Fluctuation (°C)± (1SE)**	**Max. Daily Min.-Max. Difference (°C)**	**Days Daily Min.-Max. Difference >15°C**
2008	MSUERC	2.3 (0.32)	31.2 (0.5)	22.1 (0.2)	8.6 (0.6)	23.5 (0.6)	7
2009	MSUERC	2.8 (0.26)	28.4 (0.4)	22.4 (0.2)	5.9 (0.3)	14.5 (0.3)	0
2009	SERC	3.6 (0.36)	31.9 (0.62)	23.2 (0.3)	8.7 (0.7)	30 (0.7)	13
2008	VIMS-ESL	2.5 (0.31)	28.7 (0.3)	21.7 (0.2)	7.0 (0.3)	16.0 (0.3)	2
2009	VIMS	2.9 (0.40)	29.8 (0.3)	22.9 (0.2)	6.8 (0.4)	22.0 (0.4)	6
2009	Cont. Air Exposure Exp. 1hr	N/A	30.9 (1.2)	25.1 (0.5)	5.8 (0.9)	20 (0.9)	4
2009	Cont. Air Exposure Exp. 2hr	N/A	32.9 (0.8)	25.8 (0.3)	7.1 (0.7)	20 (0.7)	10
b. Subtidal							
2008	MSUERC	N/A	28.2 (0.1)	26.2 (0.1)	2.0 (0.1)	4.2 (0.1)	
2009	MSUERC	N/A	26.8 (0.3)	24.3 (0.3)	2.4 (0.2)	10.0 (0.2)	
2009	SERC	N/A	29.0 (0.2)	26.4 (0.2)	2.6 (0.2)	9.7 (0.2)	
2008	VIMS-ESL	N/A	28.3 (0.2)	21.5 (0.2)	4.9 (0.2)	16.0 (0.3)	
2009	VIMS	N/A	26.9 (0.2)	24.8 (0.2)	2.0 (0.1)	4.5 (0.1)	
2009	Cont. Air Exposure Exp.		27.5 (0.2)	26.5 (0.2)	0.9 (0.1)	2.0 (0.1)	

Table 9. Results of statistical analyses of Controlled Air Exposure Experiment **a.** Planned comparisons by initial disease treatment of 1hr air exposed treatment to its control treatment. **b.** Planned comparisons by initial disease treatment of 2hr air exposed treatment to its control treatment. Analyses preformed on rank transformed data.

a. Planned Comparisons of 1hr air-exposure and control treatments												
Initial Disease Treatment	**Prevalence**						**Intensity**					
	Mid			**Final**			**Mid**			**Final**		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Uninfected+	1, 8	2.76	0.13	1, 8	3.47	0.09	1, 8	0.18	0.67	1, 8	0.47	0.51
Infected+	1, 8	5.28	**0.05**	1, 8	3.83	0.08	1, 8	0.97	0.35	1, 8	2.08	0.19
b. Planned Comparisons of 2hr air-exposure and control treatments												
	Prevalence						**Intensity**					
	Mid			**Final**			**Mid**			**Final**		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Uninfected+	NA			1, 8	3.87	0.09				1, 8	7.68	**0.02**
Infected+	NA			1, 8	0.33	0.58				1, 8	1.22	0.30

Table 10. Results of planned comparisons of *P. marinus* prevalence and intensity at each field survey site for each year. + indicates no *P. marinus* was detected at site. * indicate intensity analyses conducted on $\log_{10}$ transformed data. Ωindicates no subtidal comparison could be made.

Planned comparisons for each field survey site							
		Prevalence	**Prevalence: Tidal Heights Significantly Different from Subtidal (p≤0.05)**	**Intensity**			**Intensity: Tidal Heights Significantly Different from Subtidal (p≤0.05)**
Site	**Year**			**df**	**F**	**p**	
Sugarloaf, ME+	2009	NA		NA	NA	NA	
West Wye, VA*	2008	**p≤0.05**	**M>S**	2, 52	1.32	0.27	
The Hummocks, VA*	2008	p>0.10		2, 17	1.20	0.32	
Bradford's Bay, VA*	2008	p>0.10		2, 67	3.07	**0.05**	**H<S**
Hume's Marsh, VA	2008	p>0.10		2, 109	2.08	0.13	**M>S**
Great Neck Point, VA	2008	**p≤0.05**	**H>S**	2, 105	3.35	**0.04**	**M<S**
Western Branch, VA	2008	**p≤0.05**	**H>S, M>S**	2, 102	1.06	0.35	
Calico Bay, NC	2009	**p≤0.05**	**H>S**	2, 58	2.78	0.07	**H>S**
Bear Island, NCΩ	2009	NA		NA	NA	NA	

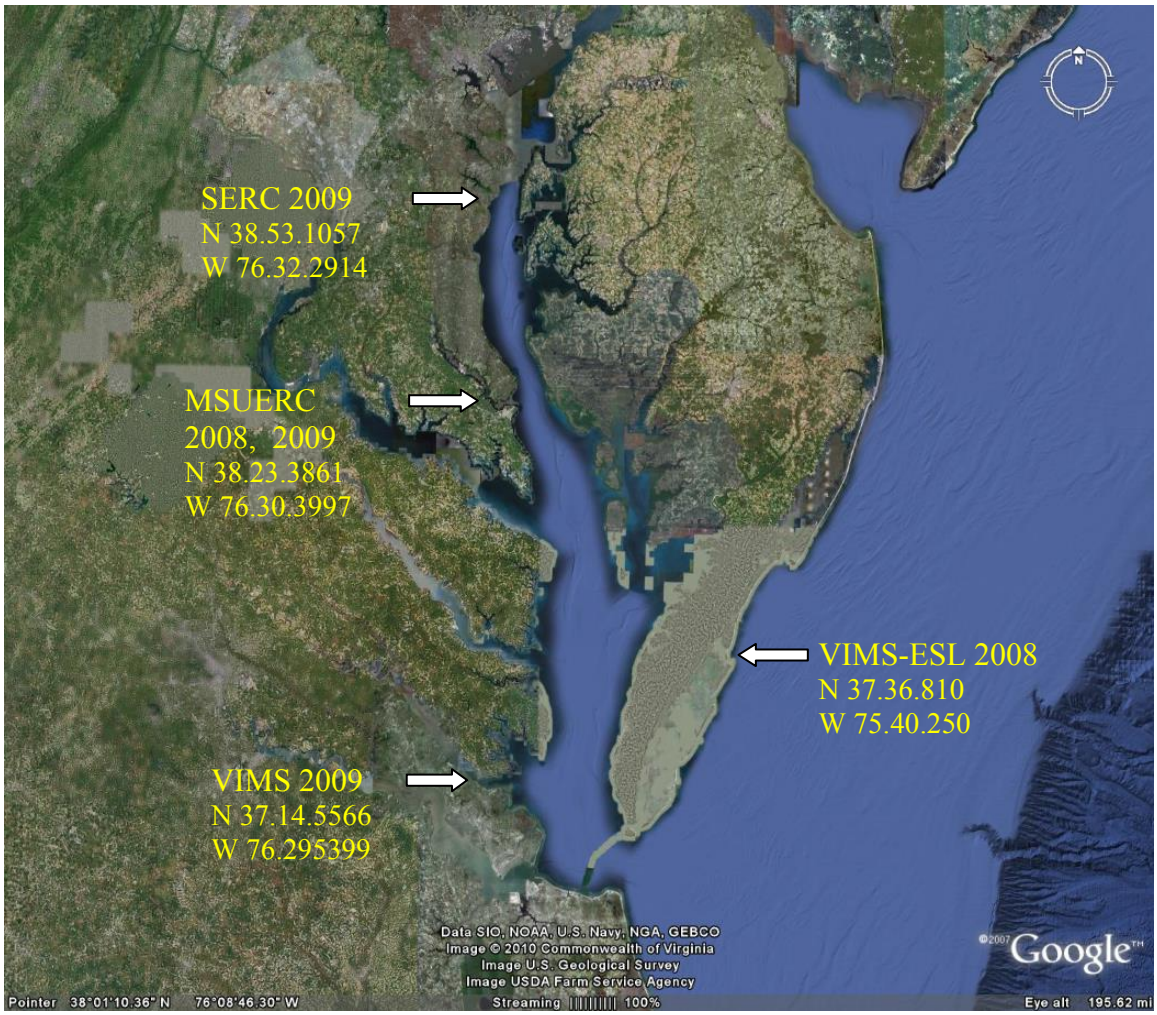
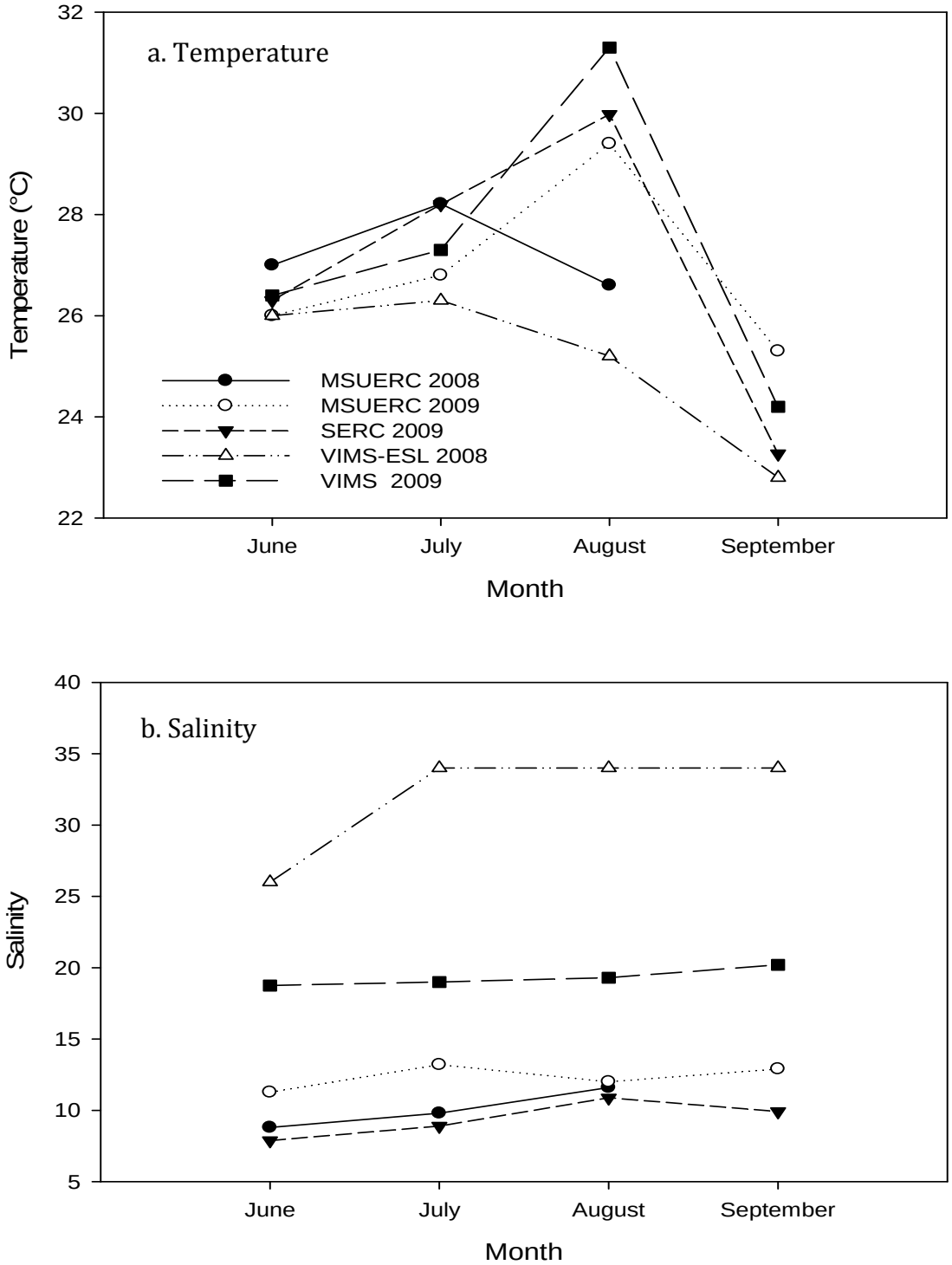
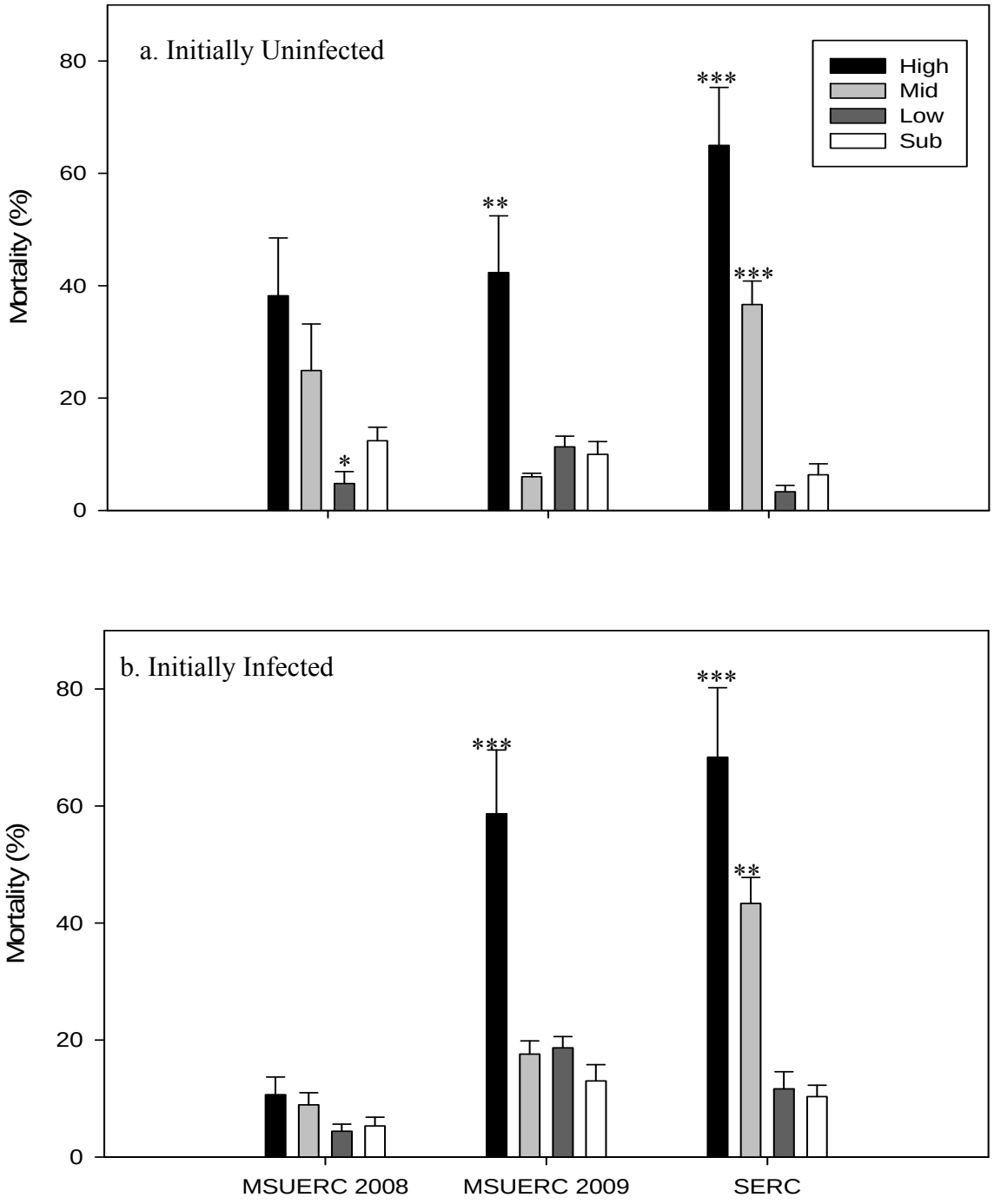


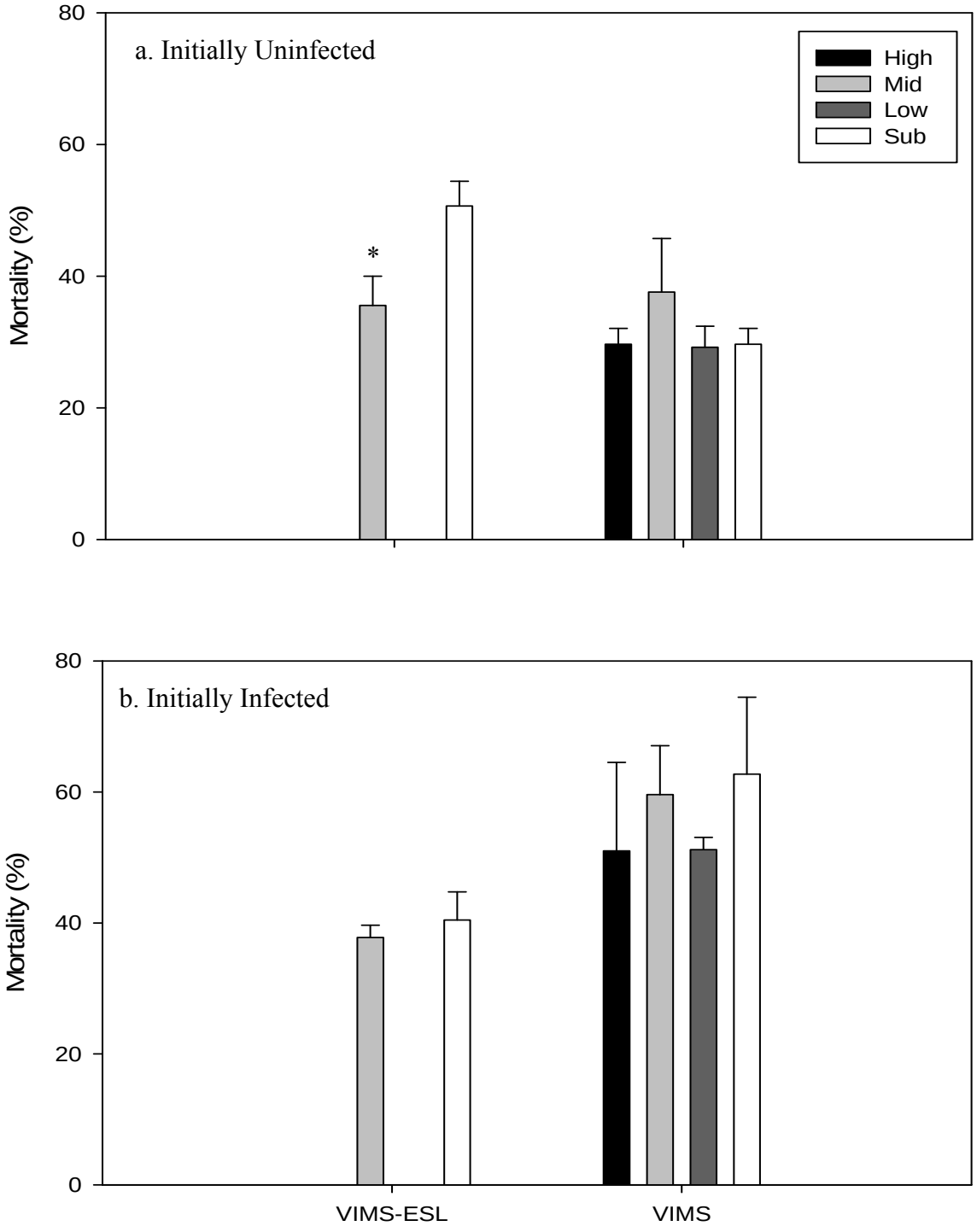
Figure 1. Map showing location of study sites where oysters were deployed in cages in 2008 & 2009.



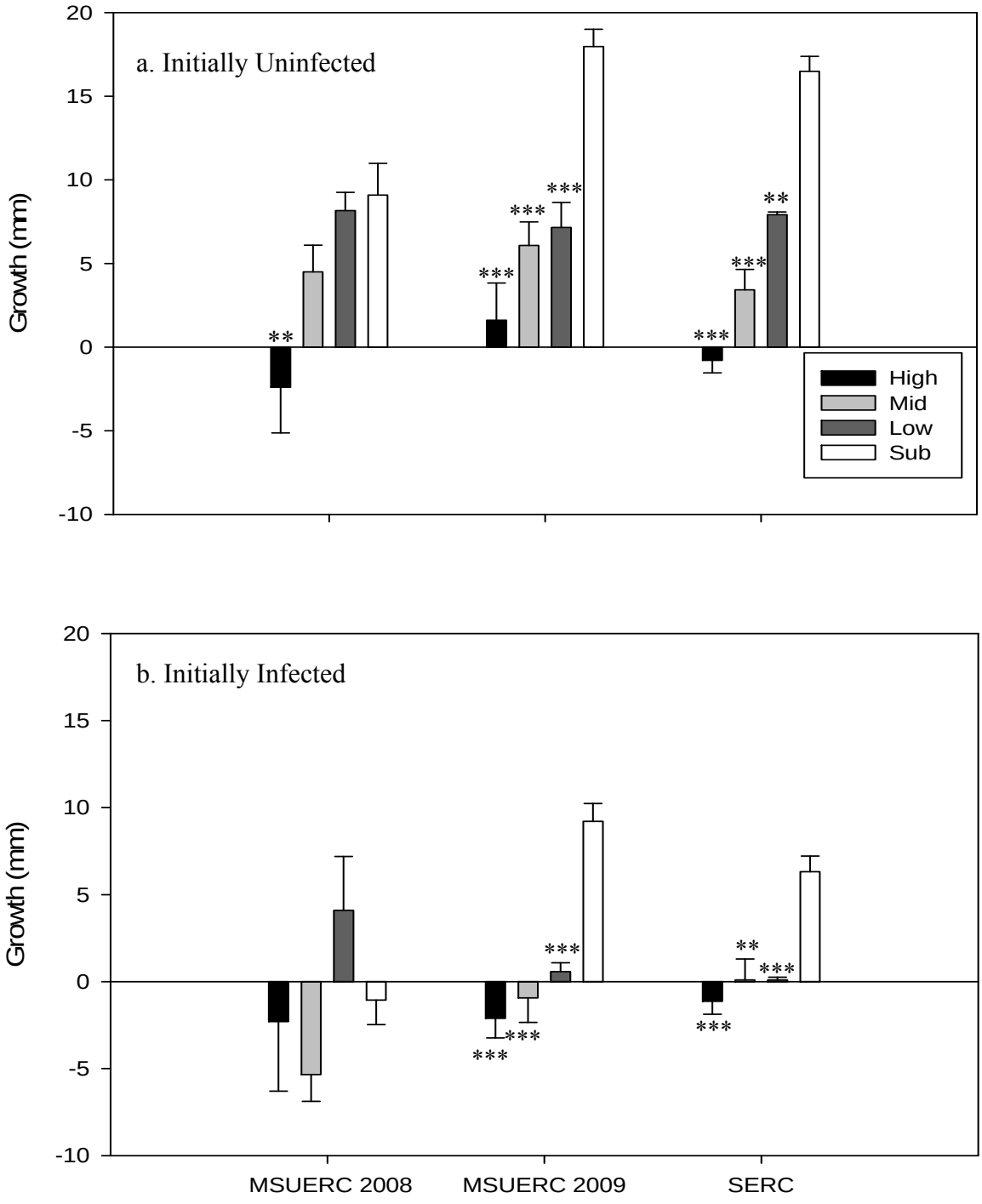
Figures 2a & 2b. Monthly **a.** Temperature **b.** Salinity from study sites in 2008 and 2009 (see methods for data collection details).



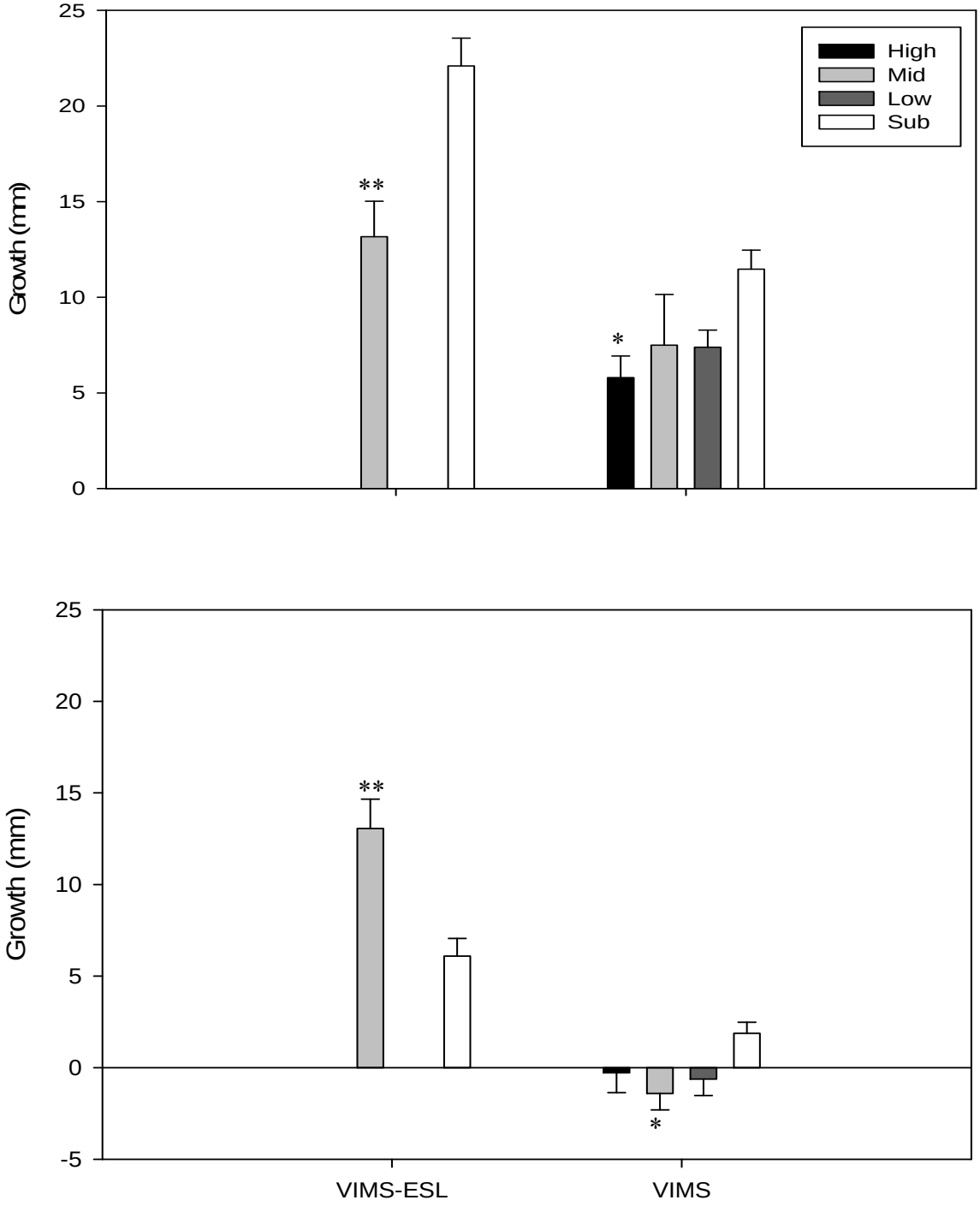
Figures 3a & 3b. Percent mortality of **a.** Initially Uninfected **b.** Initially Infected oysters for each tidal height treatment at MSUERC in 2008 and 2009, and SERC in 2009. * ($0.01 \leq p \leq 0.05$), ** ($0.0001 \leq p \leq 0.01$), *** ($p < 0.0001$) indicates which intertidal treatment were significantly different from the subtidal treatment.



Figures 4a & 4b. Percent mortality of **a.** Initially Uninfected **b.** Initially Infected oysters for each tidal height treatment at VIMS-ESL in 2008 and VIMS in 2009. * ($0.01 \leq p \leq 0.05$) indicates which intertidal treatments were significantly different from the subtidal treatment.



Figures 5a & 5b. Growth in shell height of **a.** Initially Uninfected **b.** Initially Infected oysters for each tidal height treatment at MSUERC in 2008 and 2009, and SERC in 2009. * ($0.01 \leq p \leq 0.05$), ** ($0.0001 \leq p \leq 0.01$), ***($p < 0.0001$) indicates which intertidal treatments were significantly different from the subtidal treatment.



Figures 6a & 6b. Growth in shell height of **a.** Initially Uninfected **b.** Initially Infected oysters for each tidal height treatment at VIMS-ESL in 2008 and VIMS in 2009. * ($0.01 \leq p \leq 0.05$), ** ($0.0001 \leq p \leq 0.01$) indicates which intertidal treatments were significantly different from the subtidal treatment.

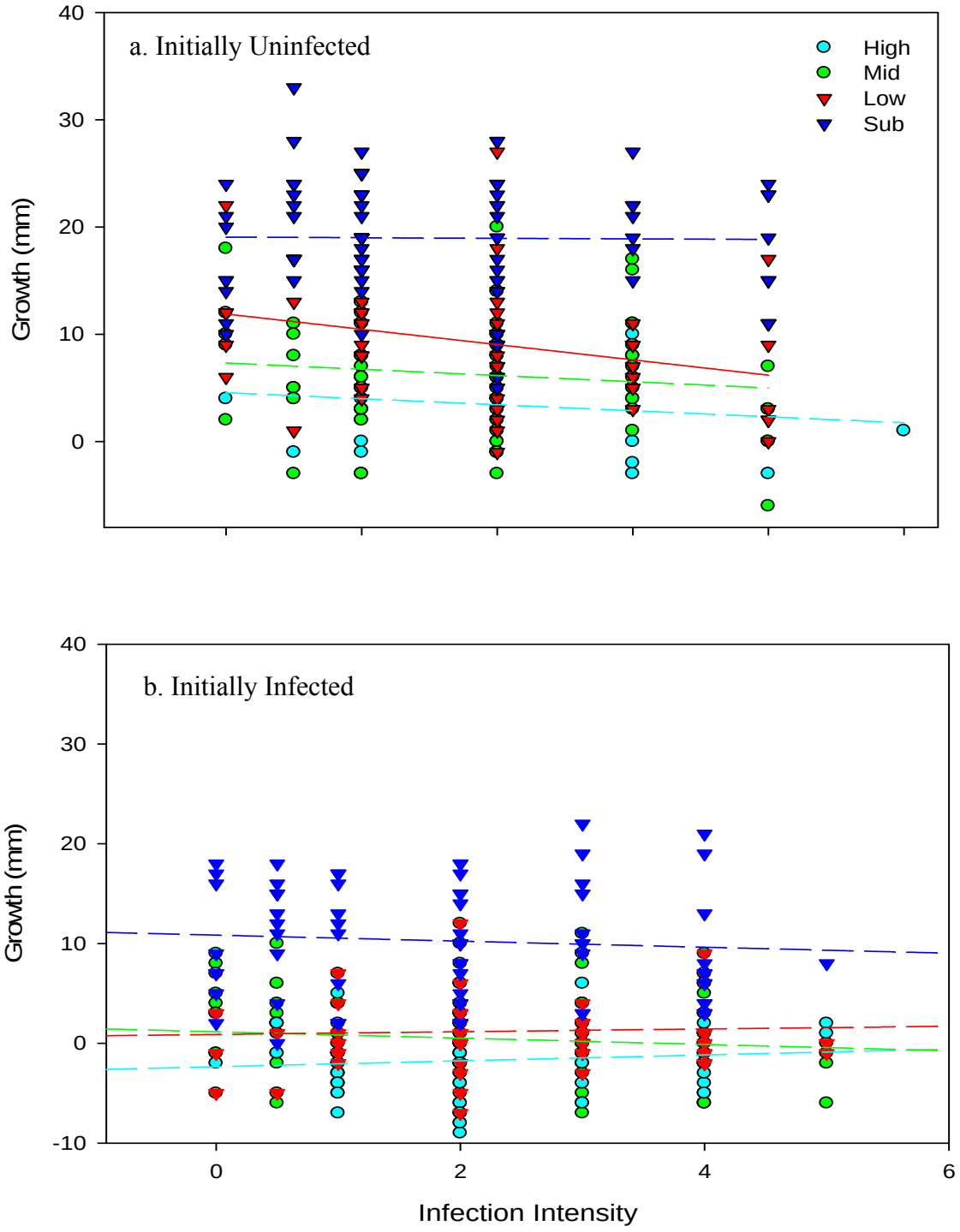


Figure 7a & 7b. Relationship between *P. marinus* infection intensity and growth for **a.** Initially Uninfected **b.** Initially Infected oysters at MSUERC in 2009. Dashed regression lines represent no significant relationship ($p>0.10$), solid lines represent a significant relationship ($0.05\leq p\leq 0.10$). Regression information is presented in Table 5.

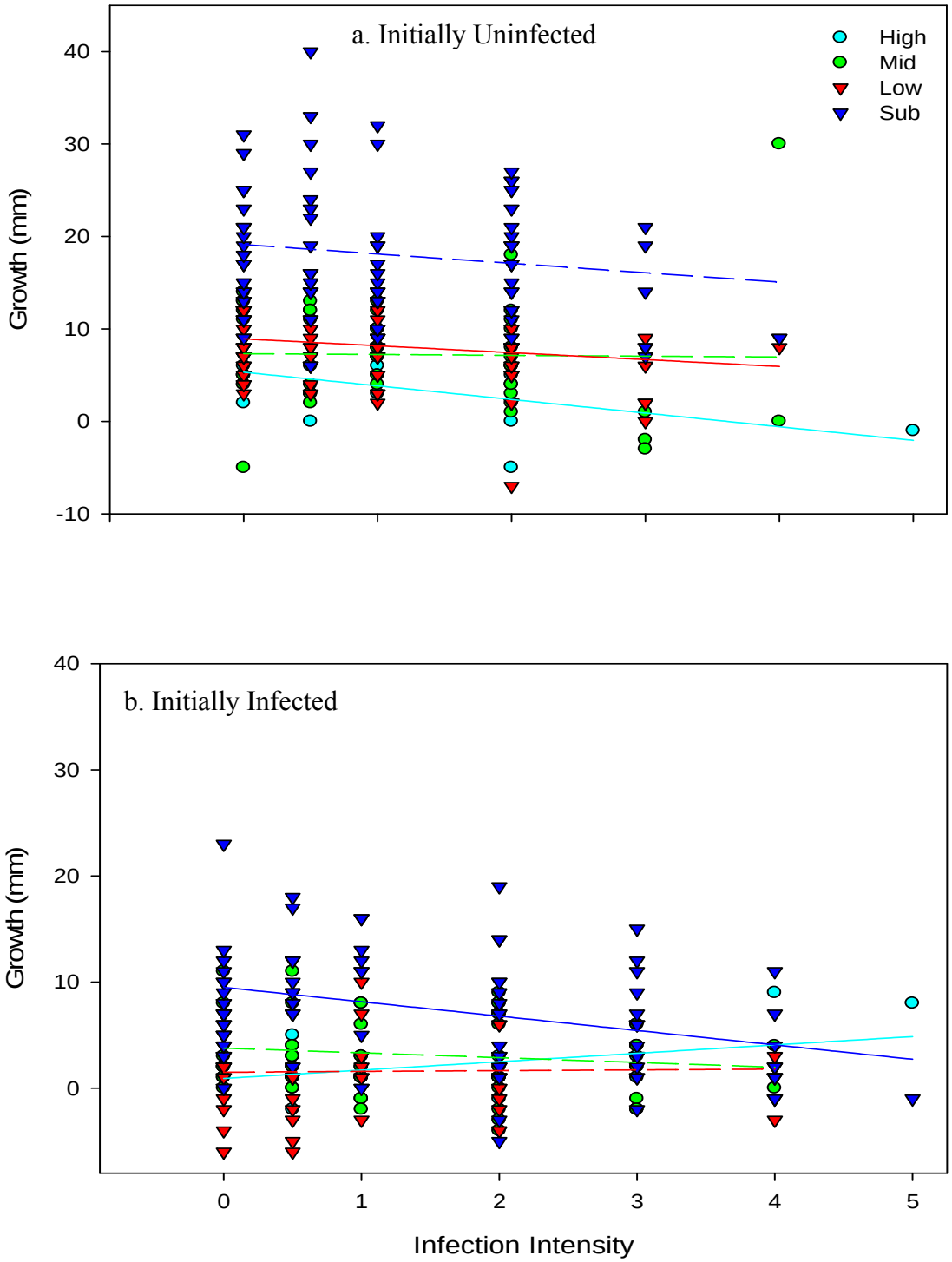


Figure 8a & 8b. Relationship between *P. marinus* infection intensity and growth for **a.** Initially Uninfected **b.** Initially Infected oysters at SERC in 2009. Dashed regression lines represent no significant relationship ($p>0.10$), solid lines represent a significant relationship ($0.05\leq p\leq 0.10$). Regression information is presented in Table 5.

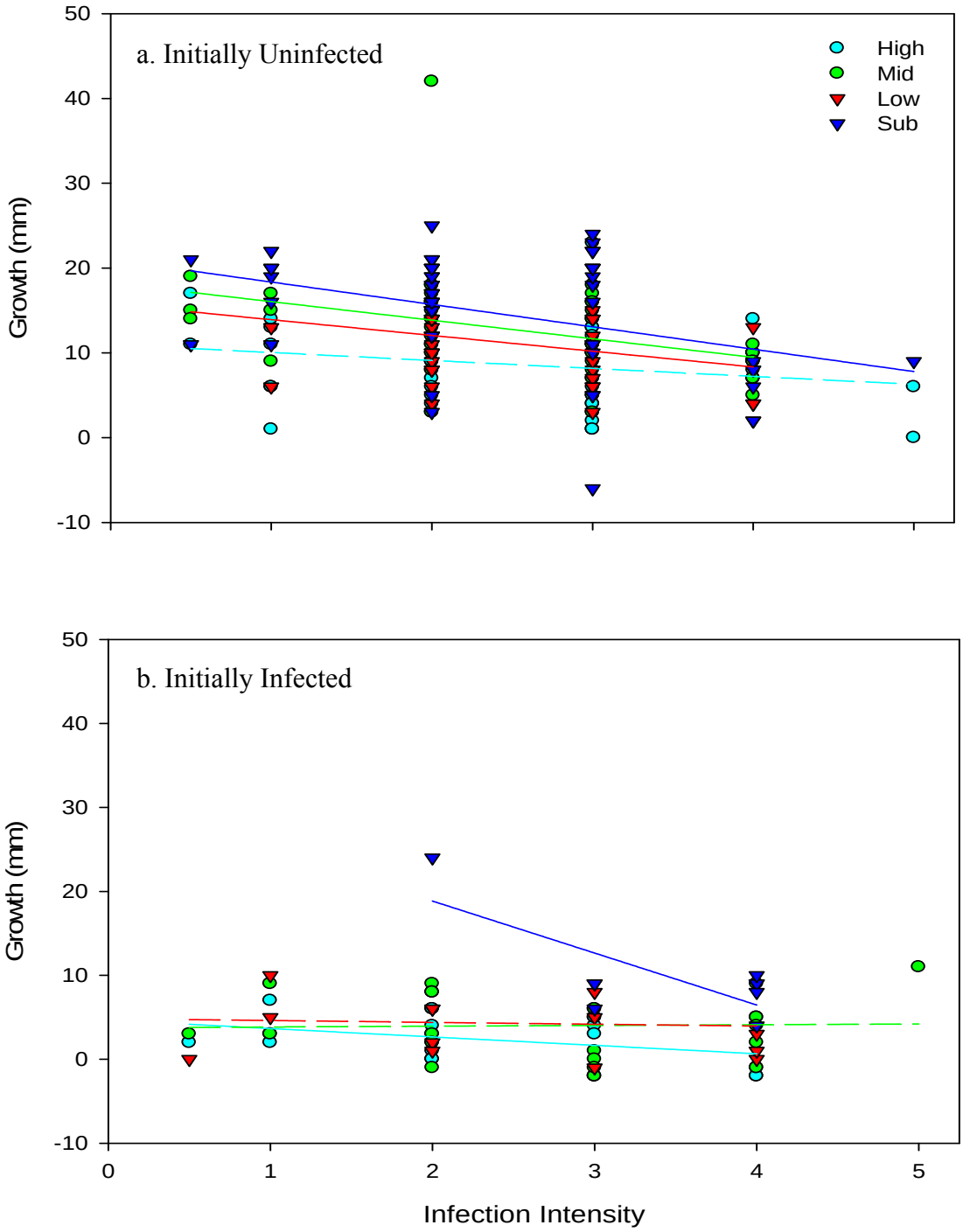


Figure 9a & 9b. Relationship between *P. marinus* infection intensity and growth for **a.** Initially Uninfected **b.** Initially Infected oysters at VIMS in 2009. Dashed regression lines represent no significant relationship ($p>0.10$), solid lines represent a significant relationship ($0.05\leq p\leq 0.10$). Regression information is presented in Table 5.

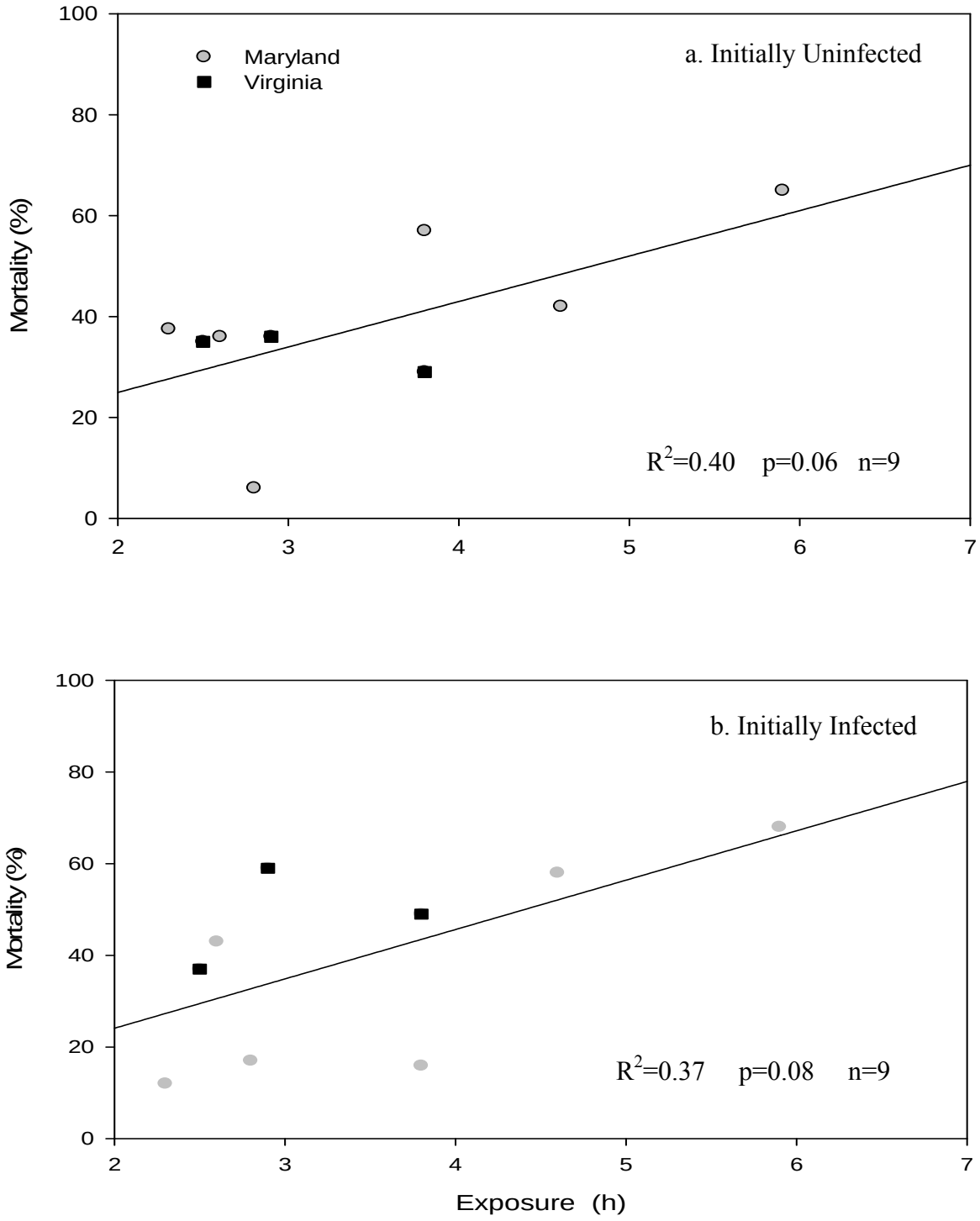


Figure 10a & 10b. Mortality of high and mid intertidal **a.** Initially Uninfected **b.** Initially Infected oysters at Maryland and Virginia sites by duration of intertidal exposure (MSUERC 2008 data were adjusted due to early retrieval). Results of regression analyses presented.

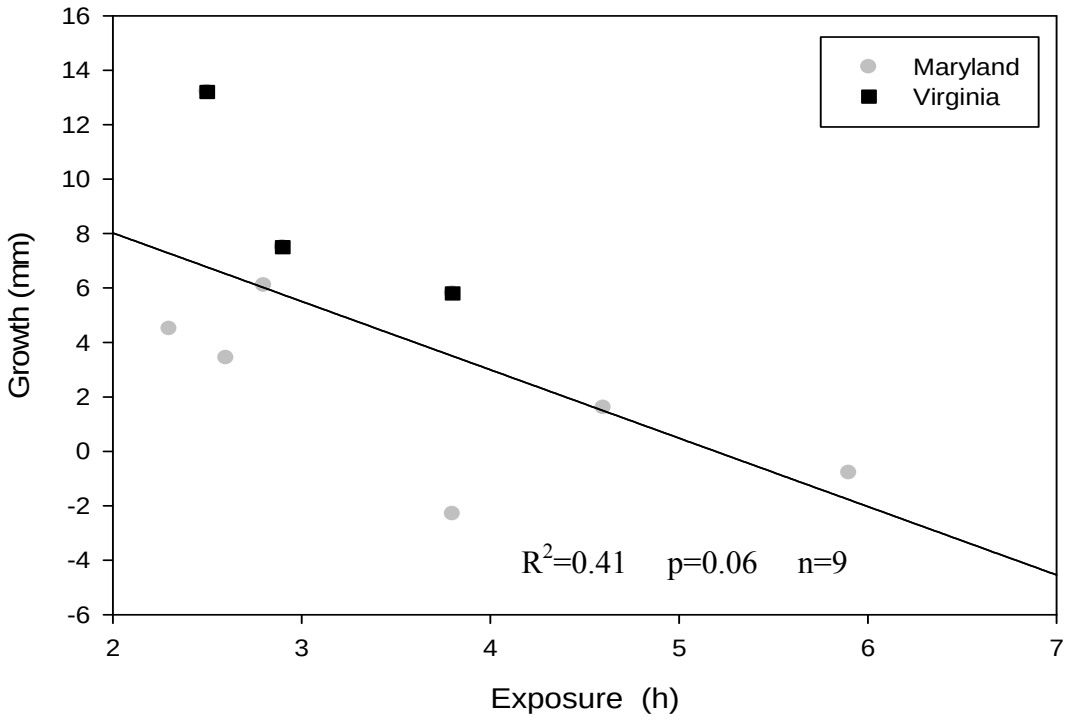


Figure 11. Growth of high and mid intertidal initially uninfected oysters at Maryland and Virginia sites by duration of intertidal exposure. Results of regression analyses presented.

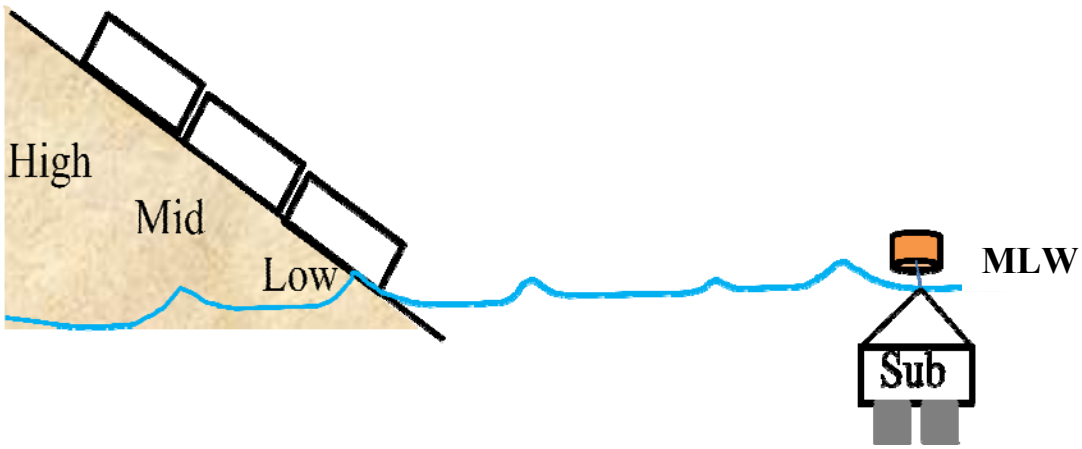


Figure 12. General experimental set-up of all tidal height treatments.

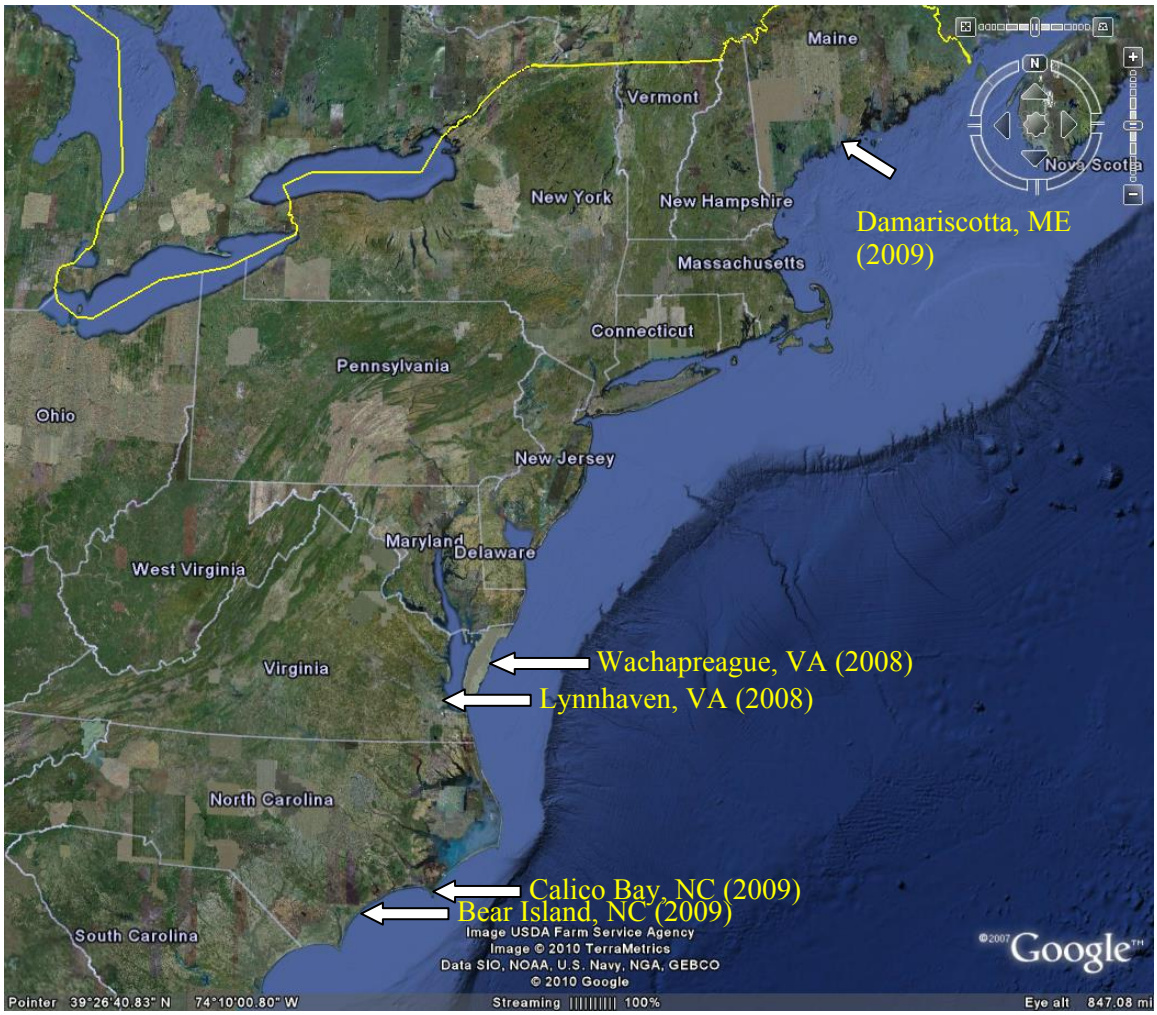


Figure 13. Map of showing location of field survey sites where wild oysters were collected in 2008 & 2009.

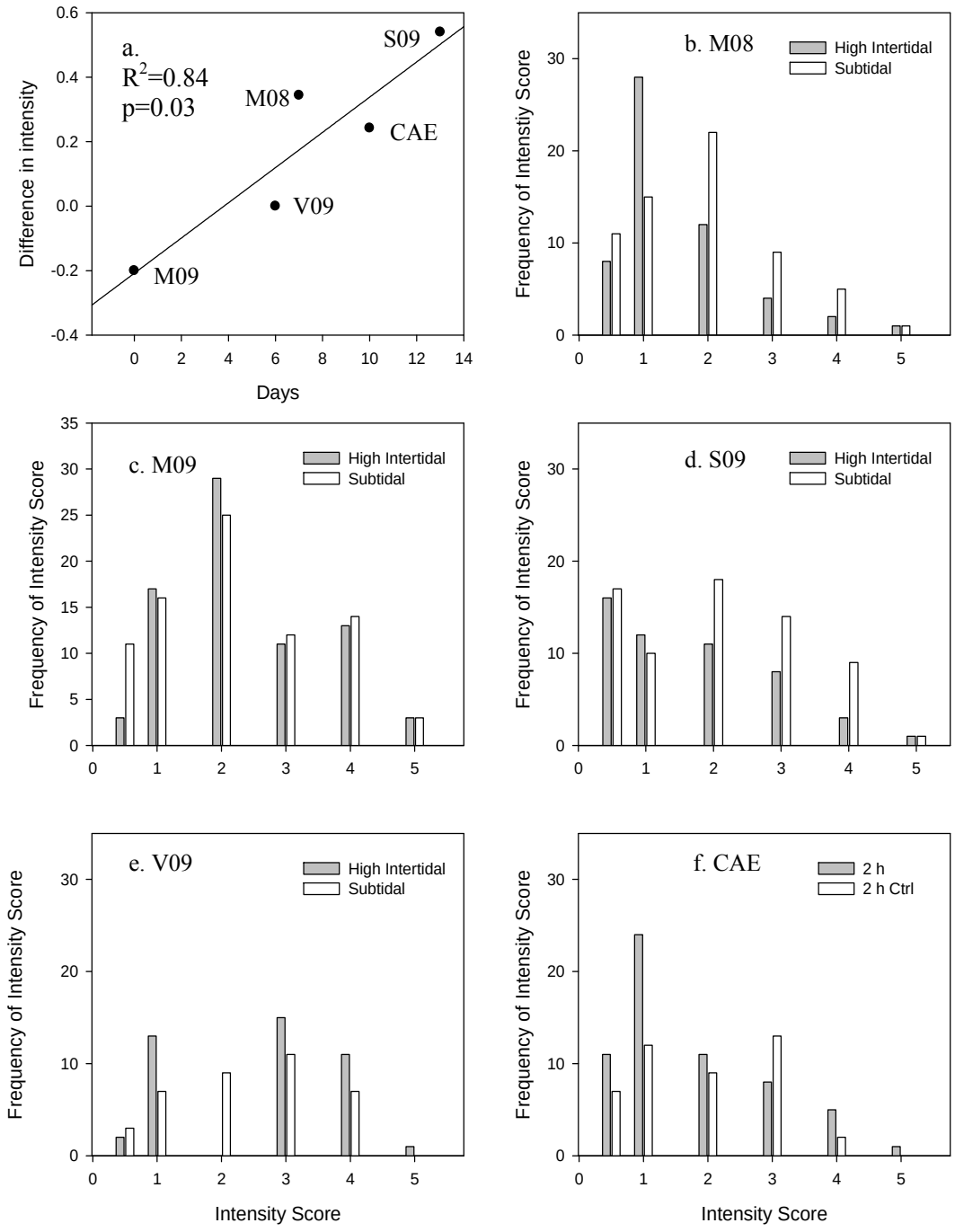
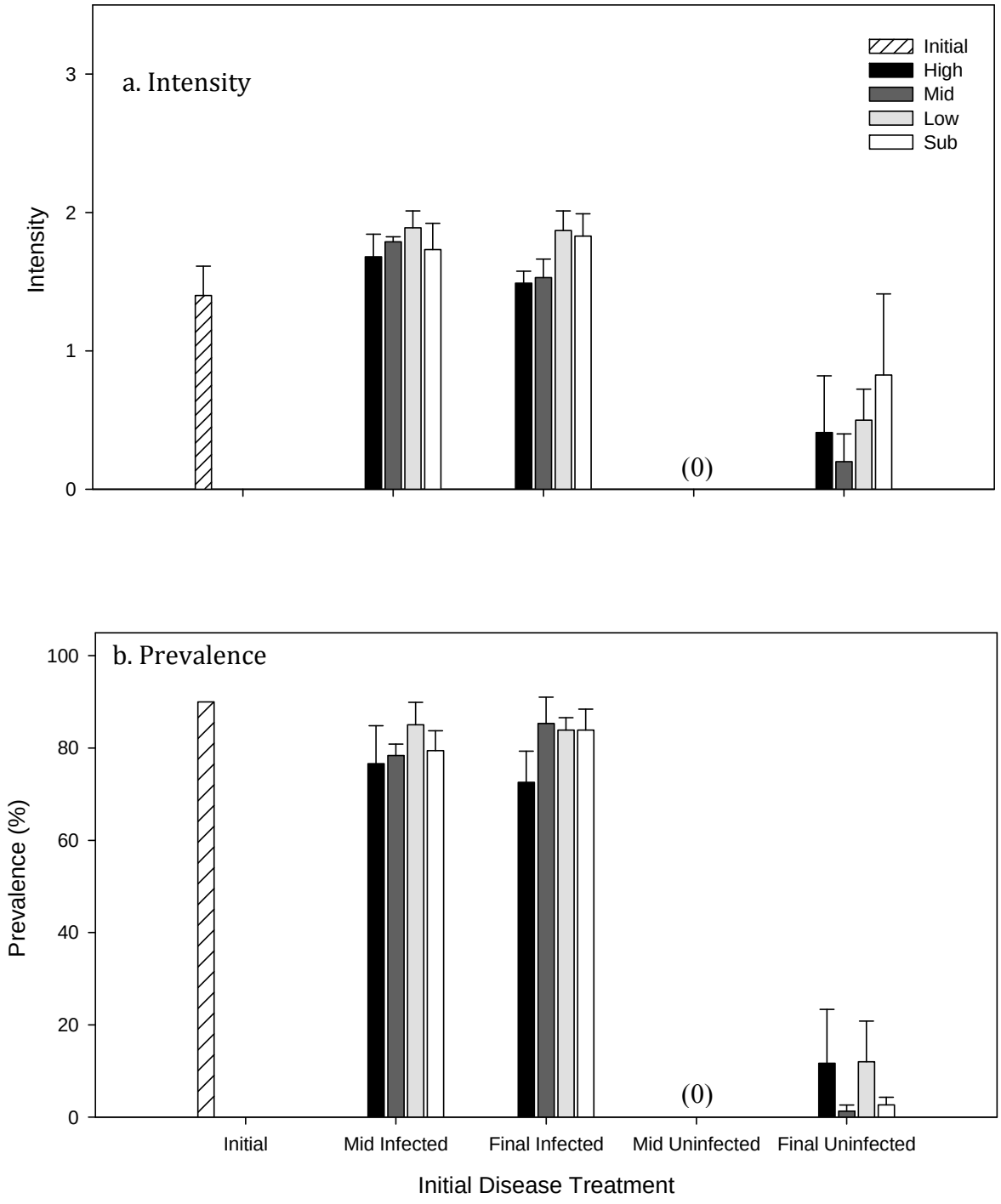
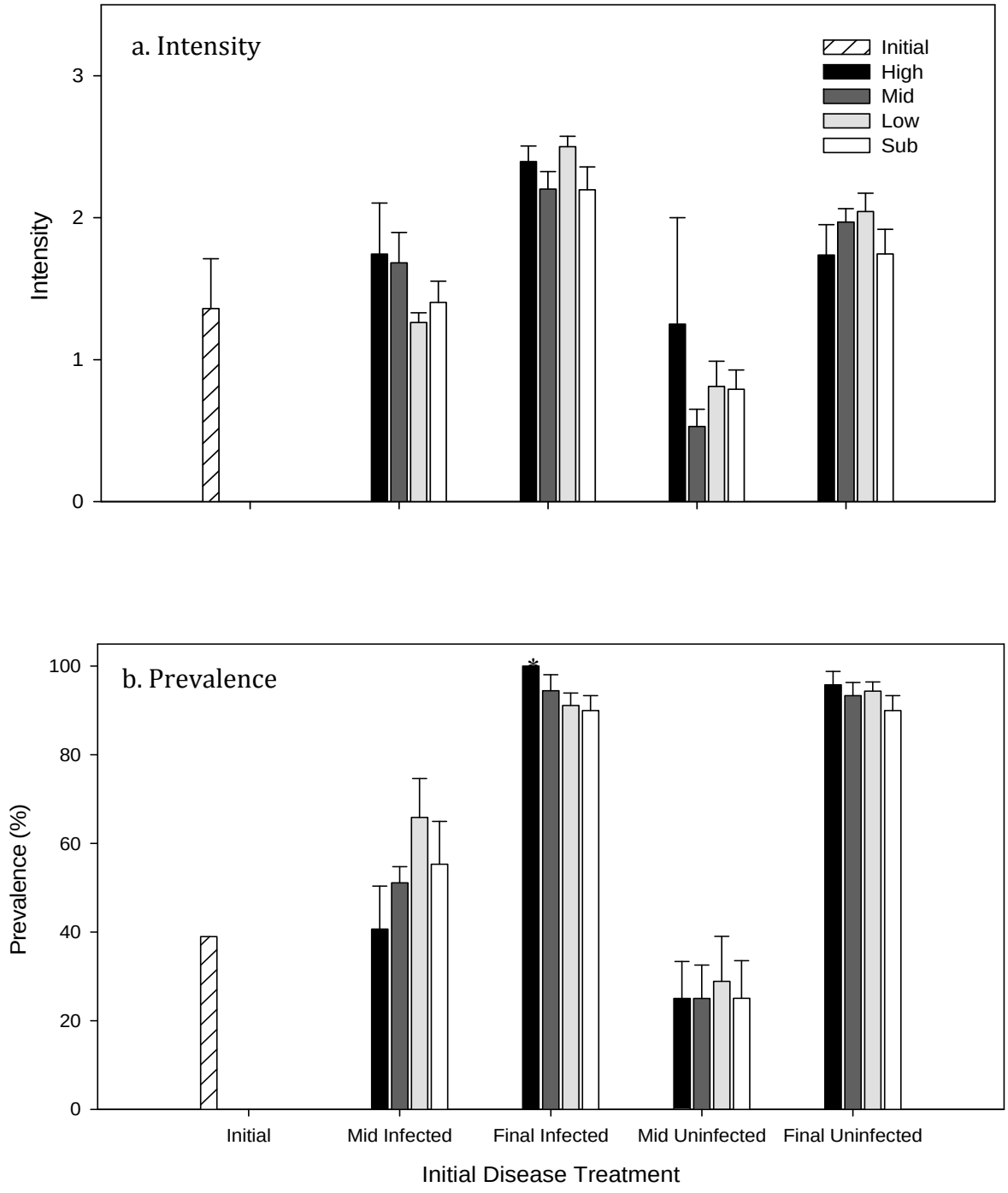


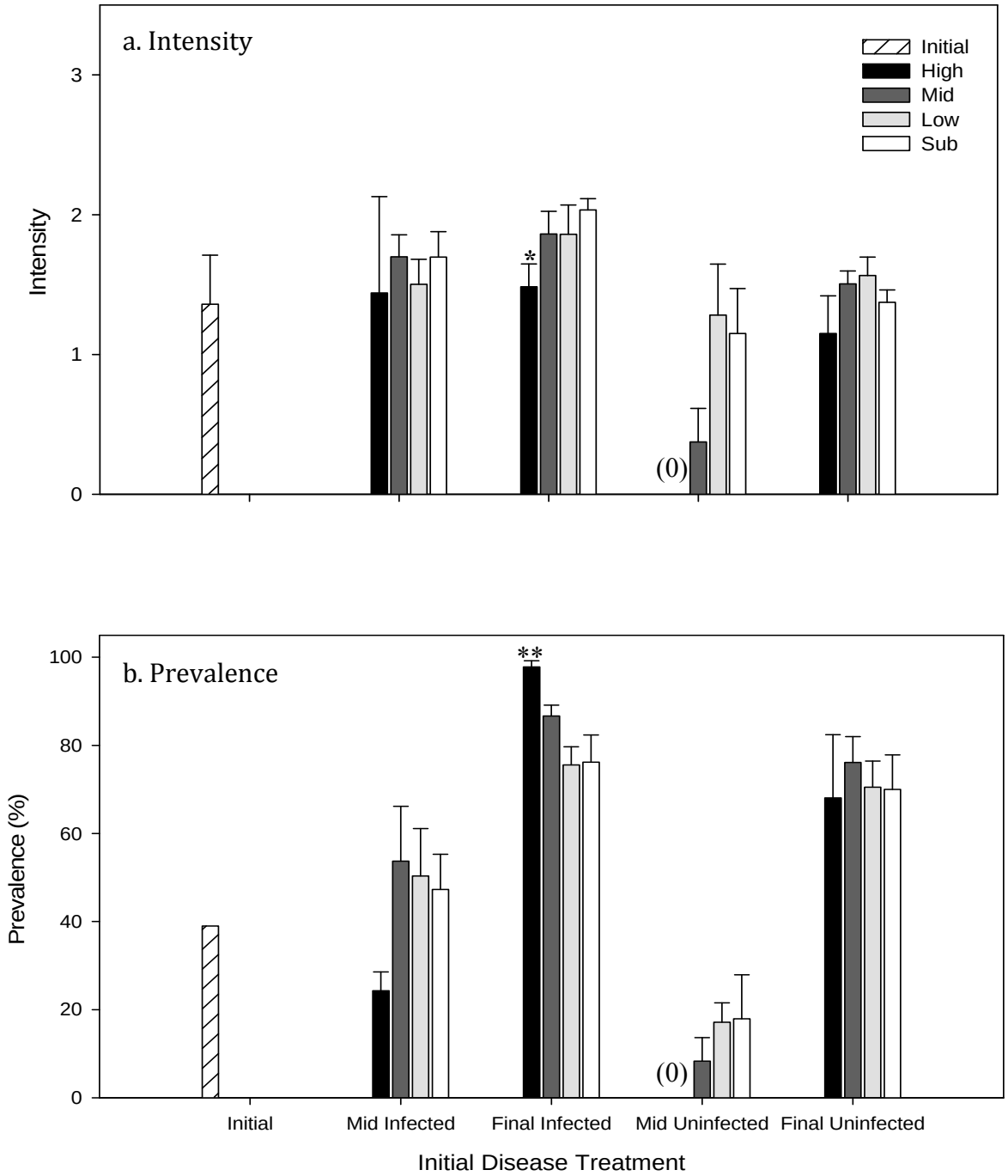
Figure 14a to 14f. a. Difference in subtidal and high intertidal intensity (subtidal – high intertidal) in relation to the minimum number of days intertidal oysters exposed to temperature fluctuations of ≥15°C. **b. – f.** Frequency of each infection intensity score in high intertidal and subtidal initially infected oysters for all field experiments and the controlled air-exposure experiment. M08=MSUERC 2008, M09=MSUERC 2009, S09=SERC 2009, V09=VIMS 2009, CAE= Controlled Air-Exposure Experiment, 2h d^{-1}.



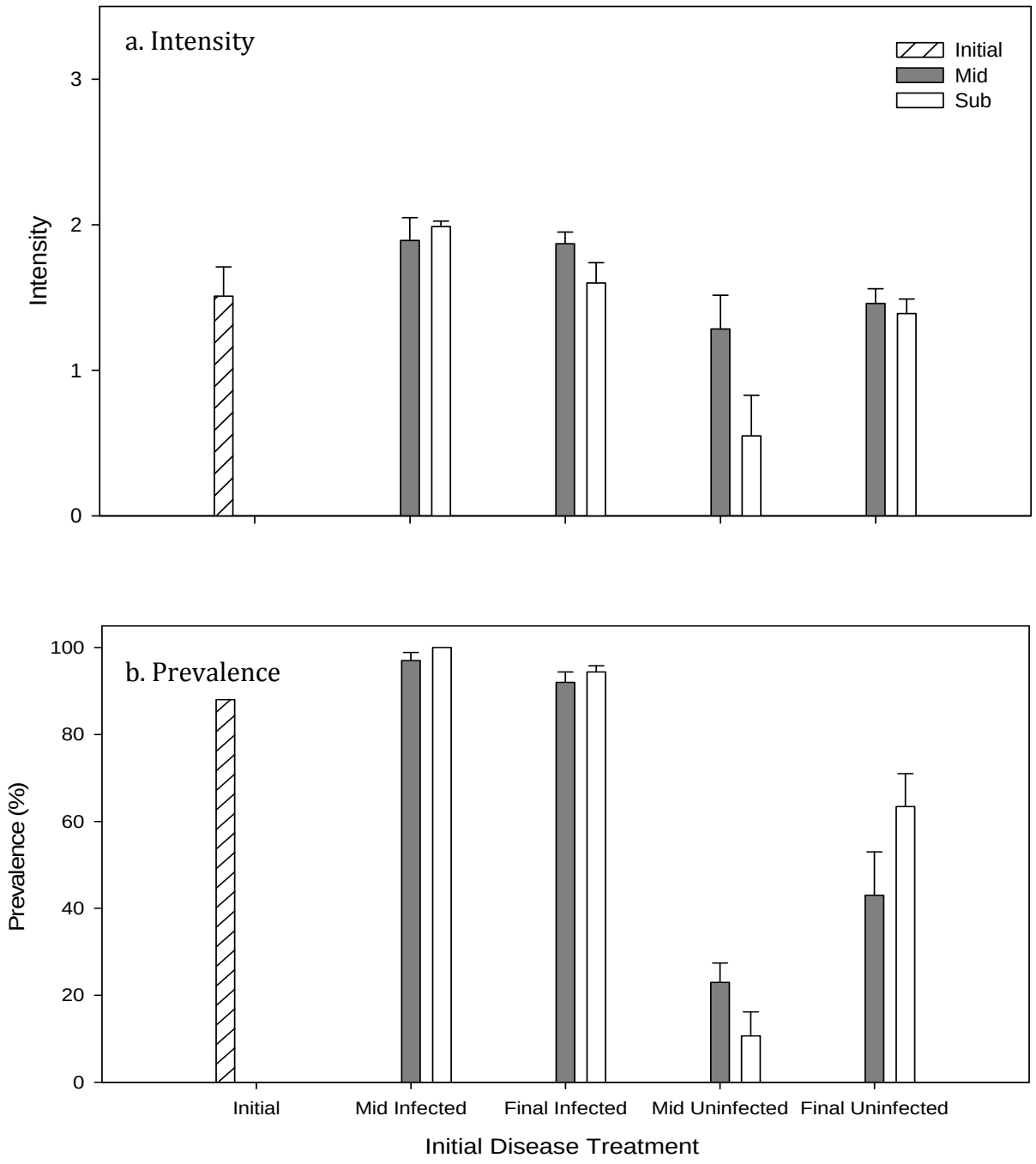
Figures 15a & 15b. *P. marinus* **a.** Intensity **b.** Prevalence for each tidal height treatment for Initially Uninfected and Initially Infected oysters at MSUERC in 2008. Each bar represents tidal height treatment mean ± SE (n≤90). There were no significant differences between tidal height treatments. (0) indicates no *P. marinus* infections were found during the mid-study sampling.



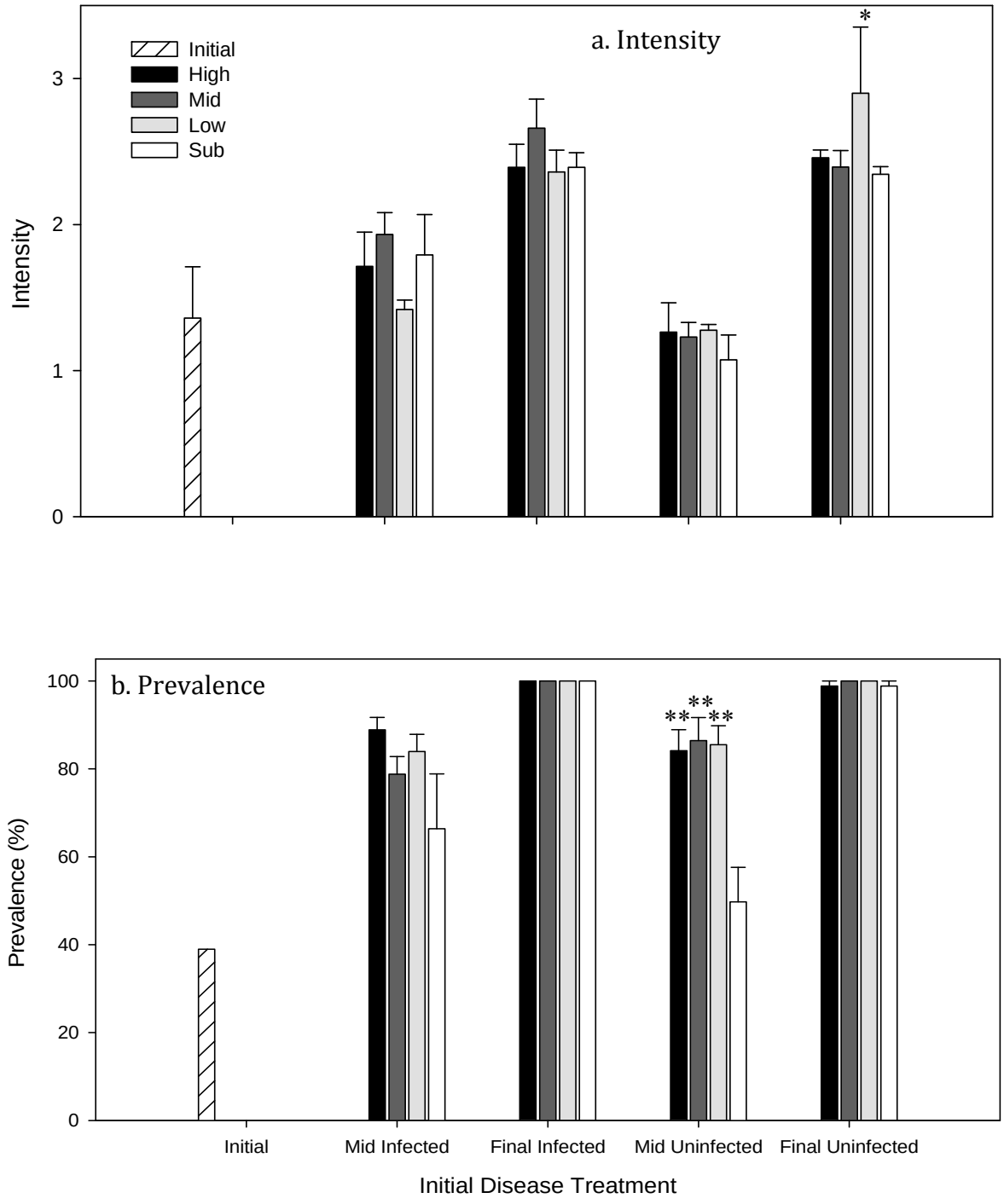
Figures 16a & 16b. *P. marinus* **a.** Intensity **b.** Prevalence for each tidal height treatment for Initially Uninfected and Initially Infected oysters at MSUERC in 2009. Each bar represents tidal height treatment mean ± SE (n≤90). * indicates intertidal treatment significantly different from the subtidal treatment (0.01≤p≤0.05).



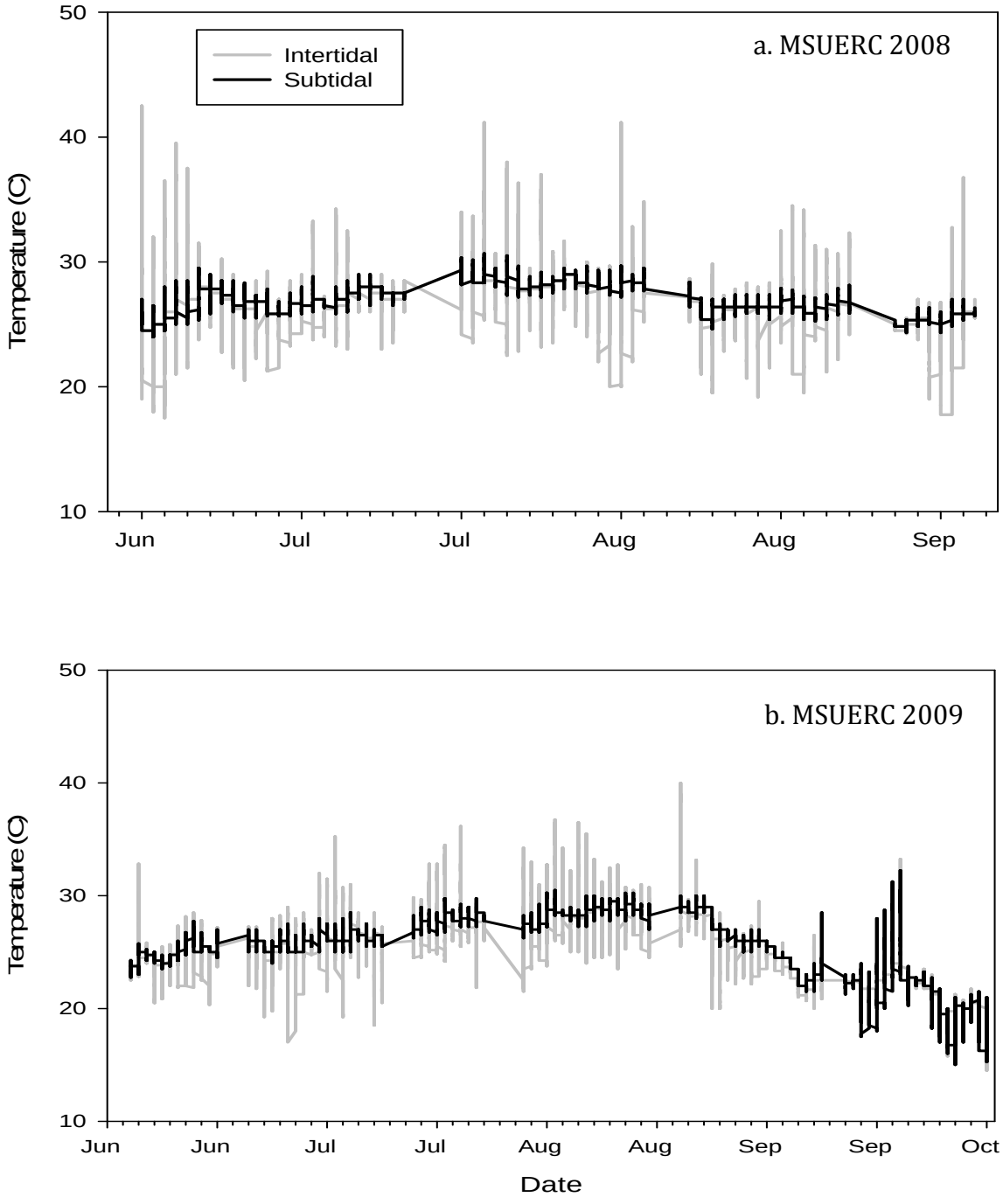
Figures 17a & 17b. *P. marinus* **a.** Intensity **b.** Prevalence for each tidal height treatment for Initially Uninfected and Initially Infected oysters at SERC in 2009. Each bar represents tidal height treatment mean ± SE (n≤90). (0) indicates no *P. marinus* infections were found during the mid-study sampling. * indicates intertidal treatment significantly different from the subtidal treatment (0.01≤p≤0.05). ** indicates intertidal treatment significantly different from the subtidal treatment (0.0001≤p≤0.01).



Figures 18a & 18b. *P. marinus* **a.** Intensity **b.** Prevalence for each tidal height treatment for Initially Uninfected and Initially Infected oysters at VIMS-ESL in 2008. Each bar represents tidal height treatment mean ± SE (n≤90). There were no significant differences between tidal heights.



Figures 19a & 19b. *P. marinus* **a.** Intensity **b.** Prevalence for each tidal height treatment for Initially Uninfected and Initially Infected oysters at VIMS in 2009. Each bar represents tidal height treatment mean ± SE (n≤90). * indicates intertidal treatment significantly different from the subtidal treatment.** indicates intertidal treatment significantly different from the subtidal treatment (0.0001≤p≤0.01).



Figures 20a & 20b. Temperatures from mid-intertidal and subtidal mimics at **a.** MSUERC in 2008 **b.** MSUERC in 2009.

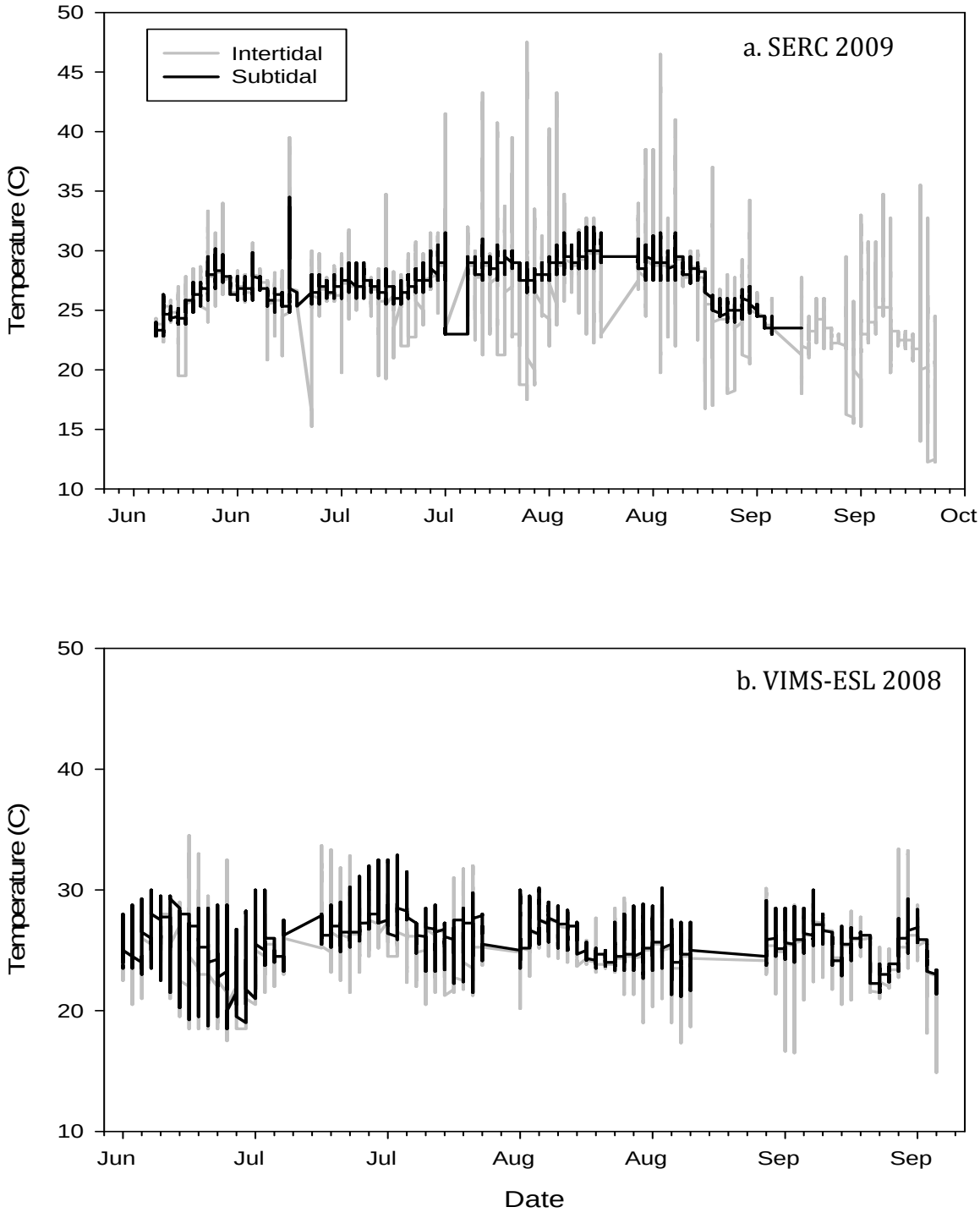


Figure 21a & 21b. Temperatures from mid-intertidal and subtidal mimics at **a.** SERC in 2009 **b.** VIMS-ESL in 2008.

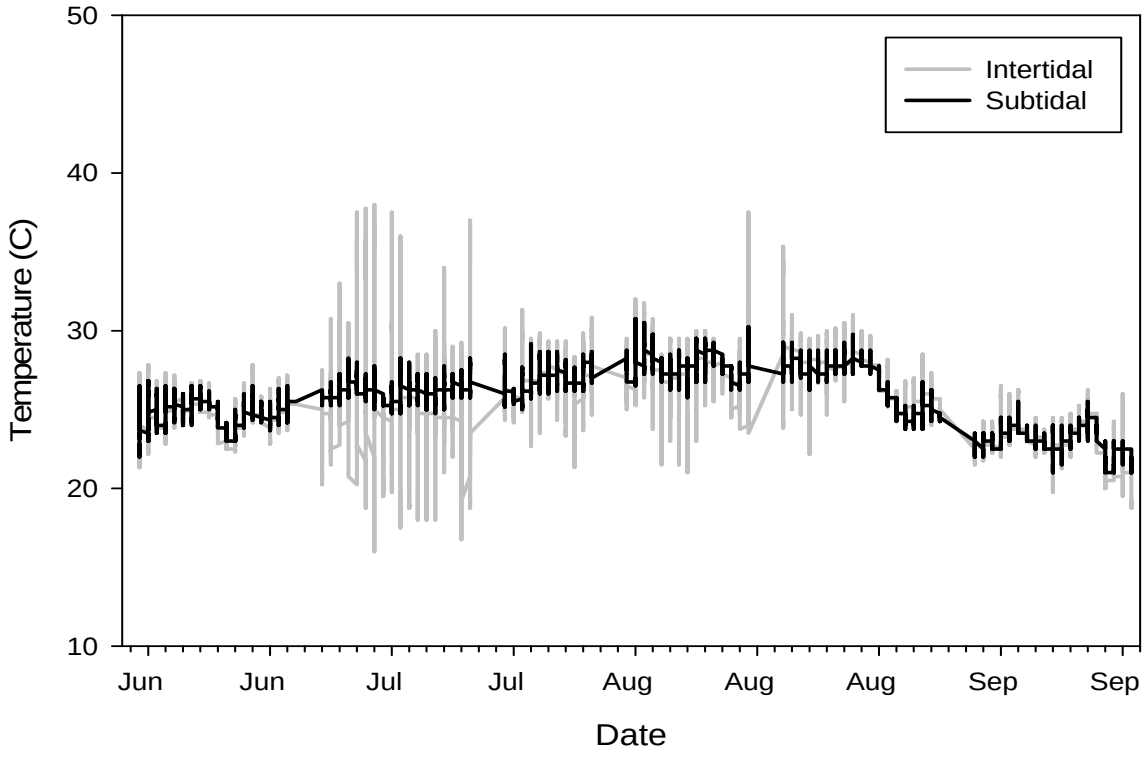
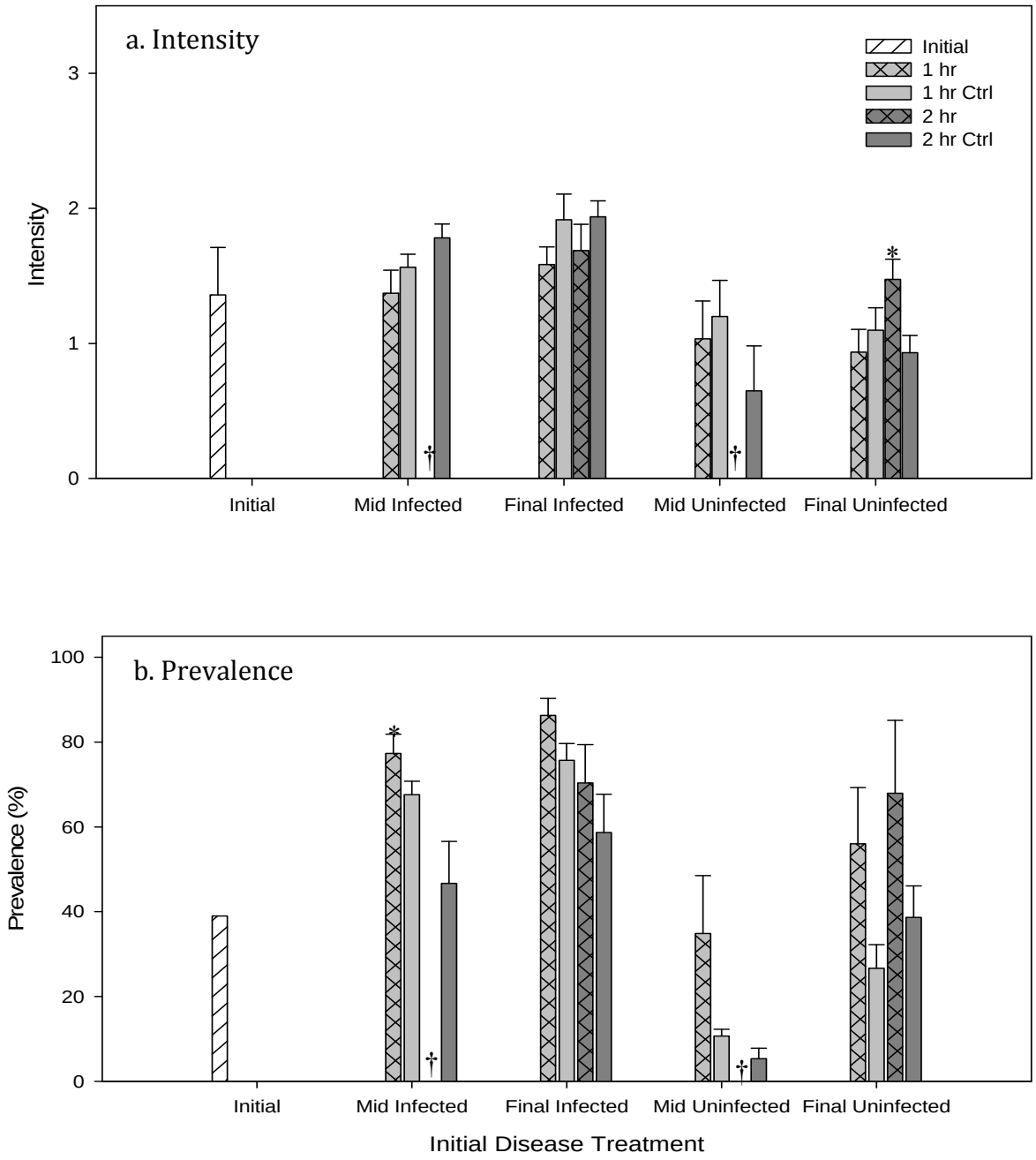
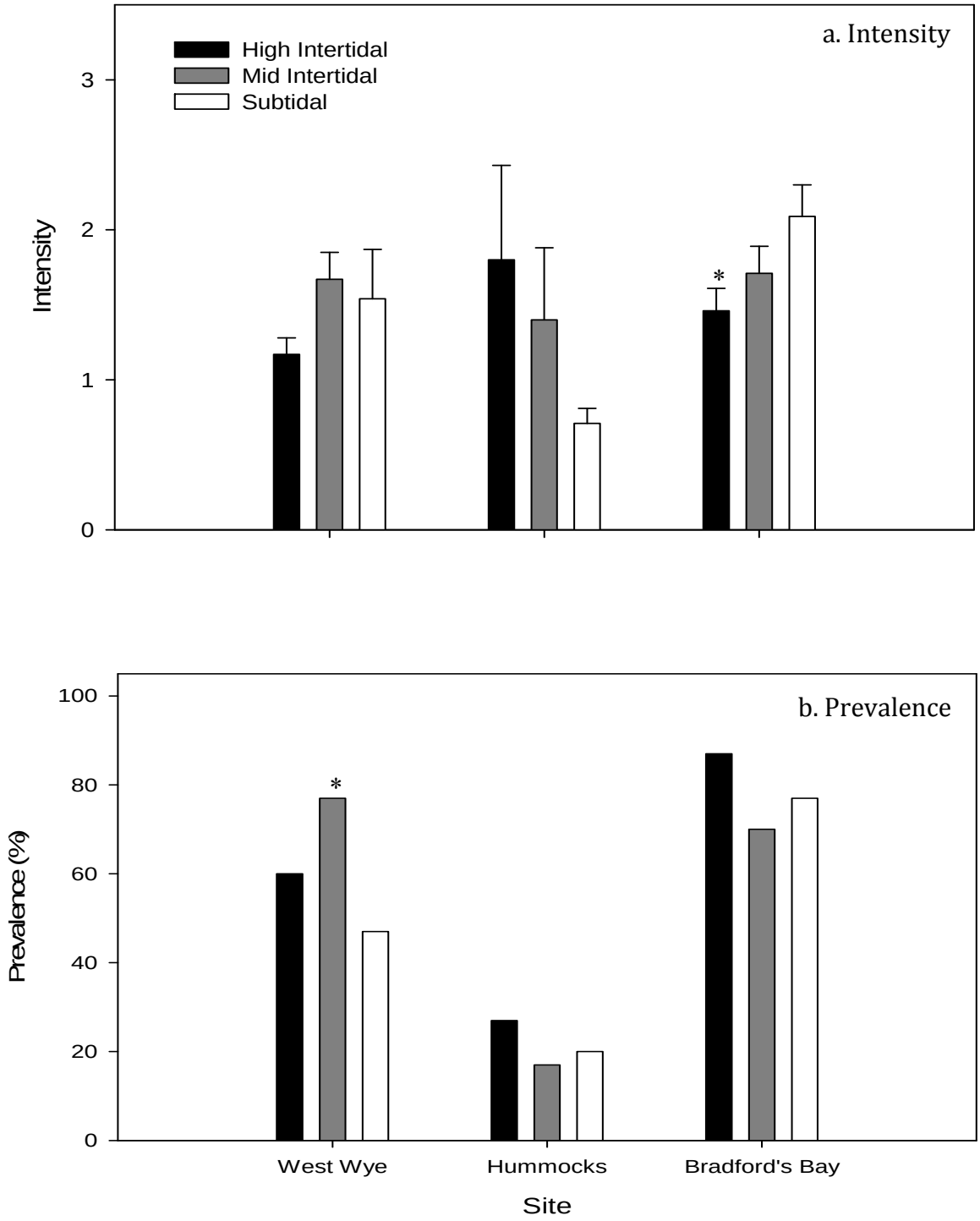


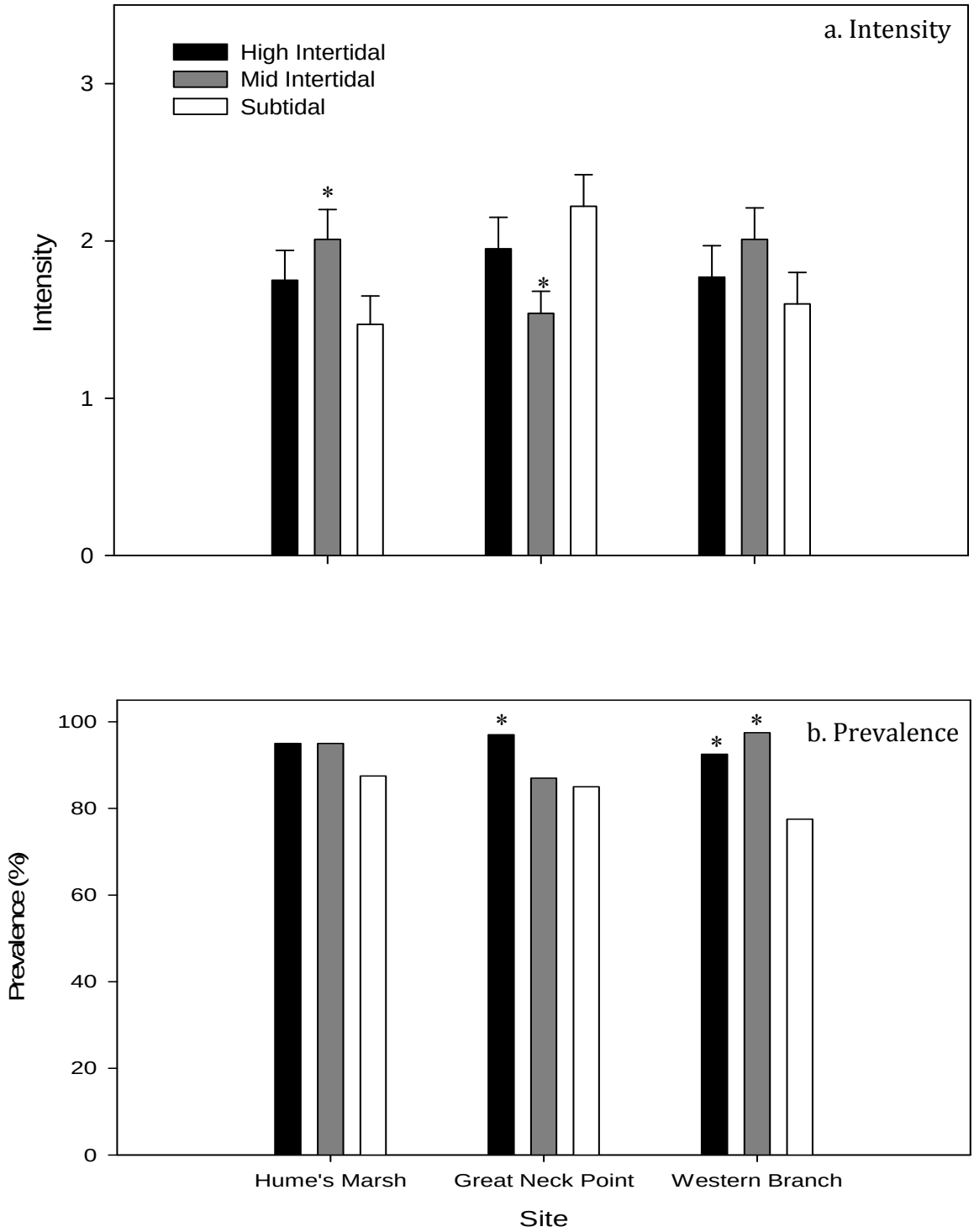
Figure 22. Temperatures from mid-intertidal and subtidal mimics at VIMS in 2009.



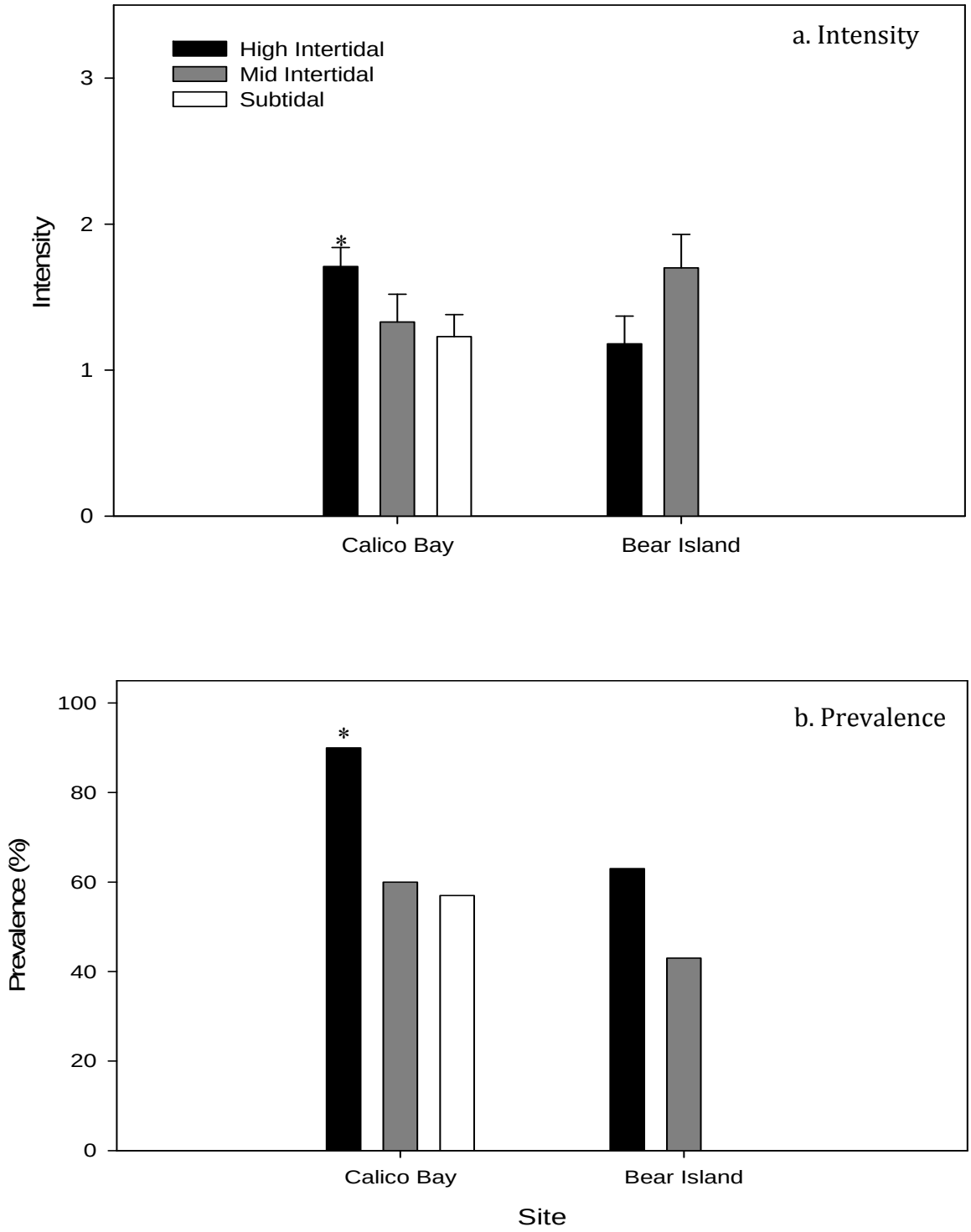
Figures 23a & 23b. *P. marinus* **a.** Intensity **b.** Prevalence for each treatment for Initially Uninfected and Initially Infected oysters in the Controlled Air Exposure Experiment in 2009. Each bar represents air-exposure treatment mean ± SE (n≤75). * indicates intertidal treatment significantly different from the subtidal treatment. † indicates no oysters sampled for disease.



Figures 24a & 24b. *P. marinus* **a.** Intensity **b.** Percent Prevalence for each tidal height sampled at Wachapreague in 2008. Each bar represents tidal height mean ± SE (n=30). * indicates intertidal height significantly different from subtidal.



Figures 25a & 25b. *P. marinus* **a.** Intensity **b.** Percent Prevalence for each tidal height sampled at Lynnhaven in 2008. Each bar represents tidal height mean ± SE (n=40). * indicates intertidal height significantly different from subtidal.



Figures 26a & 26b. *P. marinus* **a.** Intensity **b.** Percent Prevalence for each tidal height sampled in North Carolina in 2009. Each bar represents tidal height mean ± SE (n=30). * indicates intertidal height significantly different from subtidal.

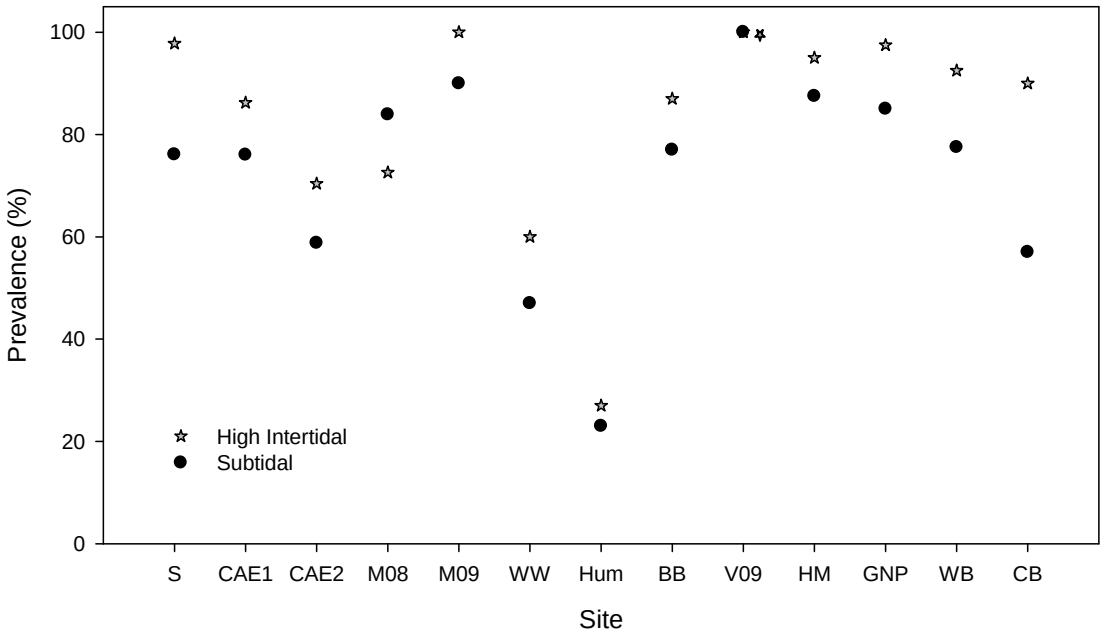


Figure 27. *P. marinus* prevalence in the high intertidal and subtidal at all field experiment and field survey sites from North to South. Tidal height data are offset for clarity along the y-axis for V09 because both tidal heights had same prevalence. S=SERC; CAE=Controlled Air-Exposure Experiment 1 and 2 h d^{-1} treatments; M08=MSUERC 2008; M09=MSUERC 2009; WW= West Wye; Hum=Hummocks; BB=Bradford's Bay; V09= VIMS 2009; HM=Hume's Marsh; GNP= Great Beck Point; WB=Western Branch; CB=Calico Bay.

APPENDIX 1

a. Description and examples of calculating exposure based on mid intertidal and subtidal mimic data

Duration of exposure was considered to be the time from which the intertidal and subtidal temperatures recorded by the iButton data loggers diverged, differing by 1°C or more, until the intertidal temperature began to increase or decrease again after a peak (or low). On the graphs below (Fig. A2, A3, & A4), the light gray circles indicate the beginning of exposure and the black circles indicate the ending of exposure. Peaks during the middle of the day were preferentially used because there was generally a more obvious divergence between temperatures in intertidal and subtidal. Temperatures were recorded every 15 min and duration of exposure was expressed in h (Table A1). This process was done for approximately the same 11 d at each site (excluding dates with missing data due to replacements of mimics). The duration of exposure for each day included in calculations was averaged to estimate the mean exposure of the mid intertidal treatment at each site (Table A1).

To determine the duration of exposure for the high and low intertidal treatments the following procedure was used, with data from MSUERC 2008 used in examples below (see Fig. A1). A constant rate of tidal change was assumed though the rate of tidal change is usually fastest during middle of the tidal cycle. But, due to the unpredictable nature of the atmospherically driven tides in Chesapeake Bay, a constant rate was calculated to in order to get an estimate of the exposure duration in the high and low intertidal treatments.

Y_{TC}: rate of tidal change (m h^{-1})
t_M: duration of exposure for mid intertidal mimic (h), as described above
½ t_M: duration of exposure for ½ of tidal cycle prior to or after low tide
d_T: distance of tidal movement from mid intertidal exposure to low tide (Mean Low Water) (m)
c_D: distance water moves to expose next intertidal treatment (m)
t_W: time it takes to move distance to expose next intertidal treatment
t_H: estimated duration of exposure for the high intertidal treatment
t_L: estimated duration of exposure for the low intertidal treatment

1. The rate of tidal change (Y_{TC}) for each site was calculated using average tidal range data and estimated duration of a tidal cycle (from NOAA historical tide data).

Average tidal change (m)/duration of tidal cycle = Y_{TC}

2. The distance of tidal movement from mid intertidal exposure to low tide (MLW) was calculated as:

$Y_{TC} * ½ (t_M) = d_T$ $\quad$ 0.060 m h^{-1} * ½ (2.3 h) = 0.069 m

3. It was assumed that due to wind and water movement (and based on observation), most oysters within a cage were in the bottom half of the cage, so each cage was divided into 2 halves. From the middle of the mid intertidal cage to MLW was 3 cage halves. To find the distance the water needed to move to expose the low intertidal oysters, d_T was used.

2/3 (d_T) = c_D $\quad$ 2/3(0.069 m) =0.05 m

4. The distance water moves to expose the next intertidal treatment was converted to time it took for water to move that distance.

$c_D / Y_{TC} = t_W$ $\quad$ 0.05m / 0.06 m h^{-1} = 0.76 h

5. To determine the duration of exposure for the high and low intertidal treatments the duration of time to expose another treatment (e.g. 0.76 h) was either subtracted (low intertidal) or added (high intertidal) to the duration of exposure for the mid intertidal treatment and multiplied by 2 to get the exposure for the complete tidal cycle (ebb and flood tide).

$2(t_M - t_W) = t_L$ $\quad\quad$ 2(1.15 h – 0.76 h)=0.78 h

$2(t_M + t_W) = t_H$ $\quad\quad$ 2(1.15 h + 0.76 h)=1.91 h

There was variation among cages within sites due to varying slopes of each set of intertidal cages and not every intertidal cage received exactly the same amount of exposure duration. The exposure calculated for each intertidal treatment is an overall estimate for the site, without taking into account the small differences in slope between the sets of cages. Additionally, there was variation in exposure in intertidal treatments among sites due to differences in slope at deployment locations (rip-rap or beach) and differences in tidal ranges. The differences in slope among sites were not accounted for in the above calculations, but by using predicted tide data and tidal duration for each site some of the other variations (tidal range) were accounted for and allowed potential differences in exposure between sites to be identified. The above methods were used to find the high and low intertidal exposure at each of the other sites, with the exception of VIMS-ESL which only had a mid intertidal treatment (Table A1).

b. Calibration Study

To determine how recorded temperatures from deployed mimics compare to actual internal temperatures in live oysters, both mimics and live oysters containing iButtons were exposed to the same conditions. An incubator (Percival I36VLC8) was used to simulate temperatures that intertidal oysters may experience during an average tidal cycle. Mimics of 2 different size classes, small or initially uninfected (average shell height 61.6 mm) and large or initially infected (average shell height 76.6 mm), were used in both field experiments and this laboratory experiment, allowing for any differences in temperature between disease classes to be observed.

Each treatment (mimic or live oyster) had 3 replicates of each size class. Mimics were made ~48 hours prior to the experiment to allow the silicon to fully set. Live oysters had iButtons inserted ~1 hour prior to the beginning of the experiment to allow acclimation to the surroundings. The valves of the oysters were separated slightly using an oyster knife to allow the iButton to be inserted. Oysters that would not close completely after insertion were gently held closed by rubber bands. All oysters and mimics were initially placed in water at 20°C prior to temperature cycling. The incubator was set on a step-schedule of 2, 4 hour cycles starting and ending at 20°C, peaking at 40°C. Temperature increased or decreased every 35 min at 5°C intervals. During the 20°C portion of the cycle, oysters and mimics were placed in a water bath to simulate abbreviated subtidal or high tide emersion. When iButtons were retrieved from mimics at the end of the experiment, it was found that the silicon had not completely solidified. Recorded mimic temperatures differed from

oyster temperatures by ~5°C during the first cycle for both size classes, but were almost identical during the second cycle (Fig. A5a & A5b). It is likely that the silicon in the mimics continued to solidify during the experiment, and as temperatures were similar during the second cycle, I assumed that complete solidification of the silicon resulted in accurate temperature predictions from field mimics. To account for overestimated temperatures caused by non-solidified silicon, I did not use temperature data recorded during the first 3 days of each mimic deployment at each site.

There was also a difference of 1-2°C between temperatures from small and large oysters, with slightly higher temperatures recorded in small oysters. This is to be expected as heat transfer between an organism and its environment can be largely determined by the size of an organism (Helmuth 2000), and smaller organisms will heat more quickly than larger organisms.

Table A1. Summary of exposure durations from Fig. 1-5 (July 30th for all sites but VIMS, which has July 29th, 2009) and the overall mean exposure for each site, calculated as described above.

Site	Year	Beginning of Exposure Duration	End of Exposure Duration	Difference	Total Min (Diff*15)	Mean Exposure for Mid Intertidal (h ± SE), n=13
MSUERC	**2008**	50	66	16	240	2.3 (0.32)
MSUERC	**2009**	53	68	15	225	2.8 (0.26)
SERC	**2009**	40	62	22	330	3.6 (0.36)
VIMS-ESL	**2008**	44	57	13	195	2.5 (0.31)
VIMS	**2009**	41	45	4	60	2.9 (0.40)

d_T : distance of tidal movement from mid intertidal exposure to low tide (Mean Low Water) (m)

c_D : distance water moves to expose next intertidal treatment (m)

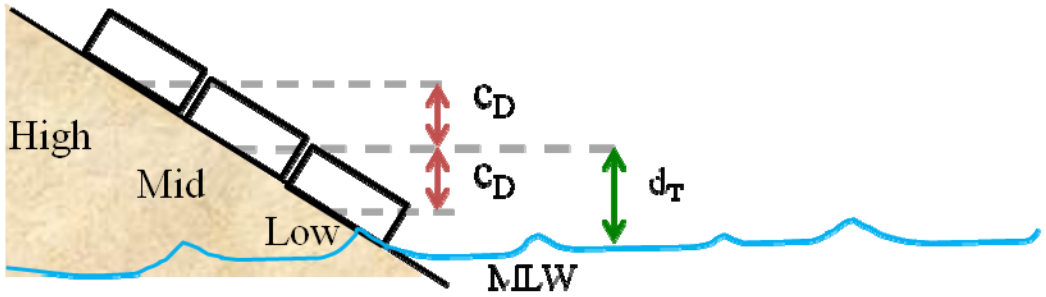
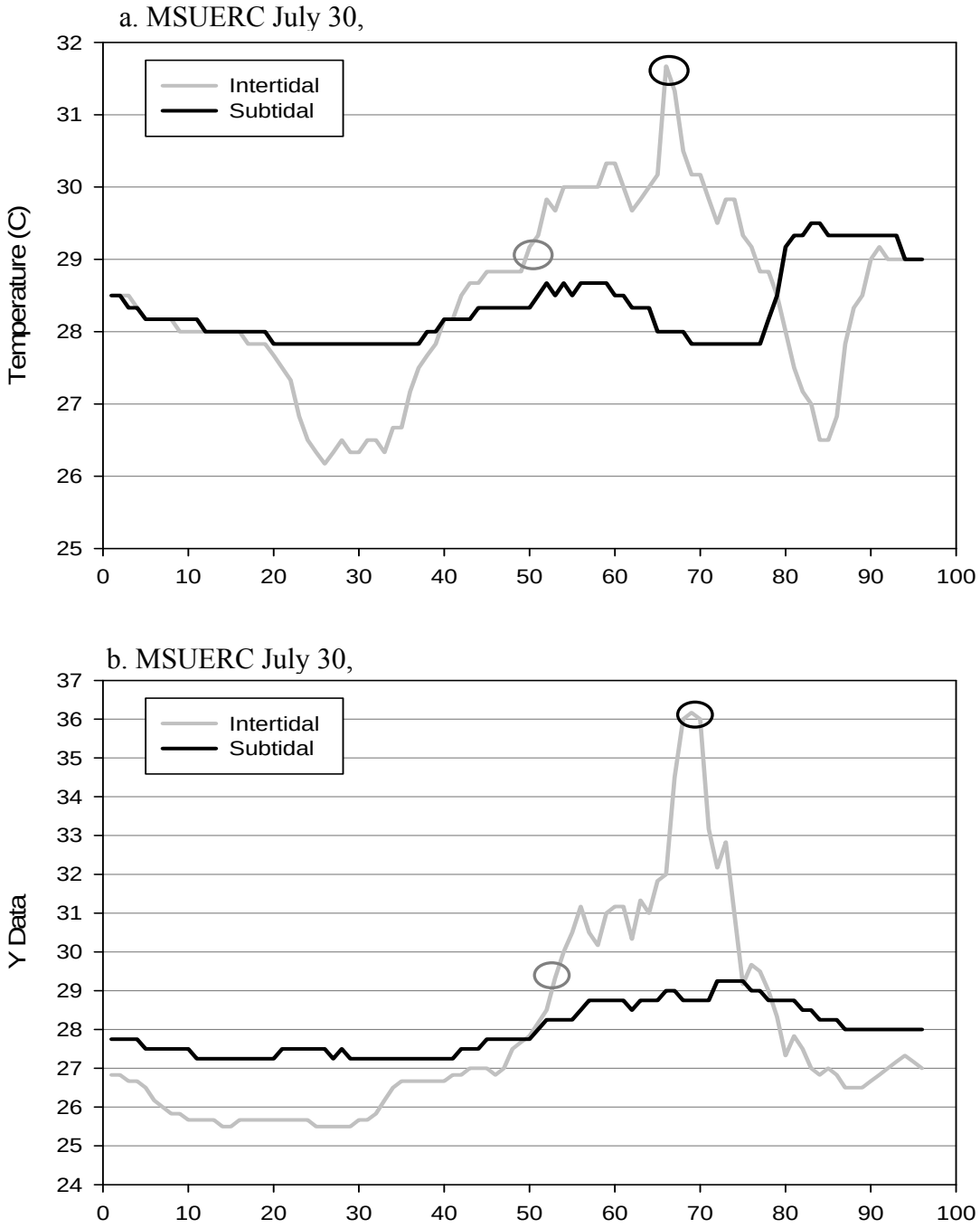
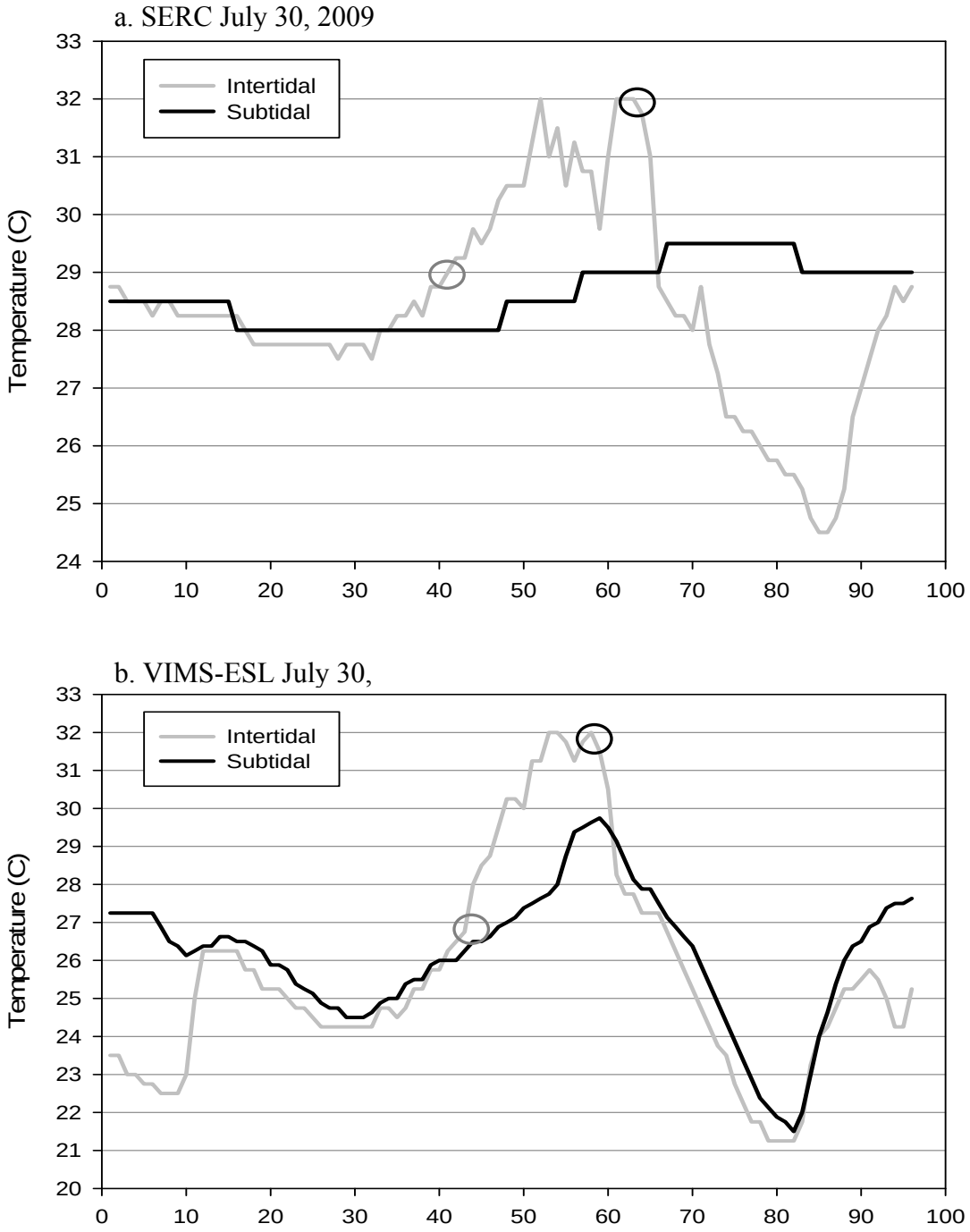


Figure A1. General experimental set-up of intertidal cages and reference distances for duration of exposure calculations.



Figures A2a & A2b. Intertidal and subtidal mimic data from **a.** MSUERC on July 30, 2008 **b.** MSUERC on July 30, 2009. The x-axis represents time in 15 min intervals (96 day^{-1}).



Figures A3a & A3b. Intertidal and subtidal mimic data from **a.** SERC on July 30, 2009 **b.** VIMS-ESL on July 30, 2008. The x-axis represents time in 15 min intervals (96 day^{-1}).

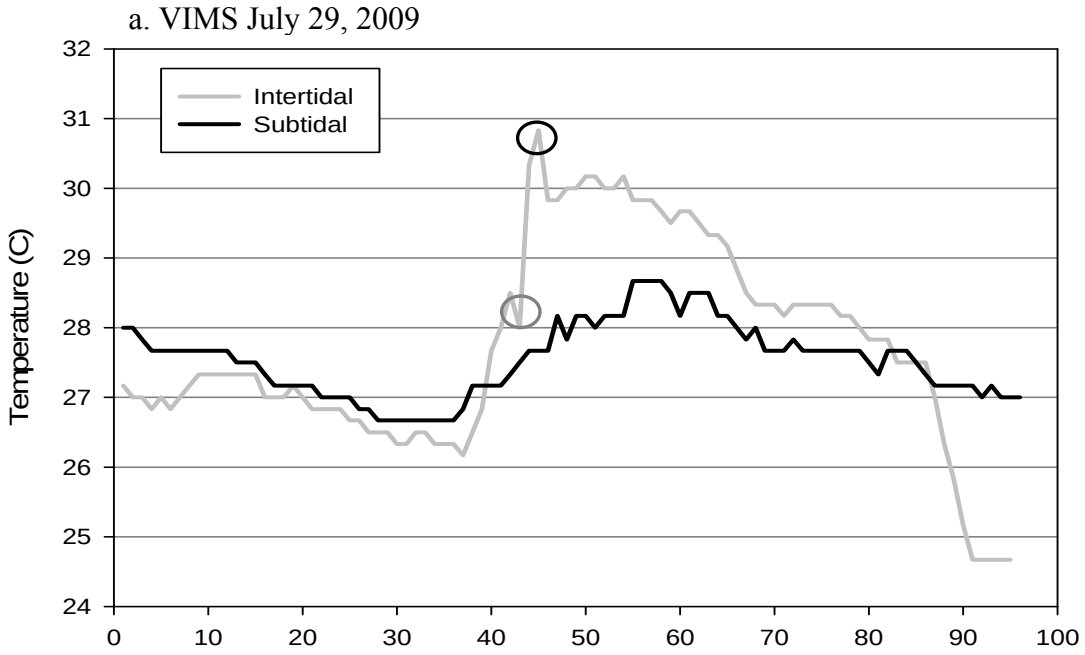


Figure A4a. Intertidal and subtidal mimic data from VIMS on July 29, 2009. The x-axis represents time in 15 min intervals (96 day^{-1}).

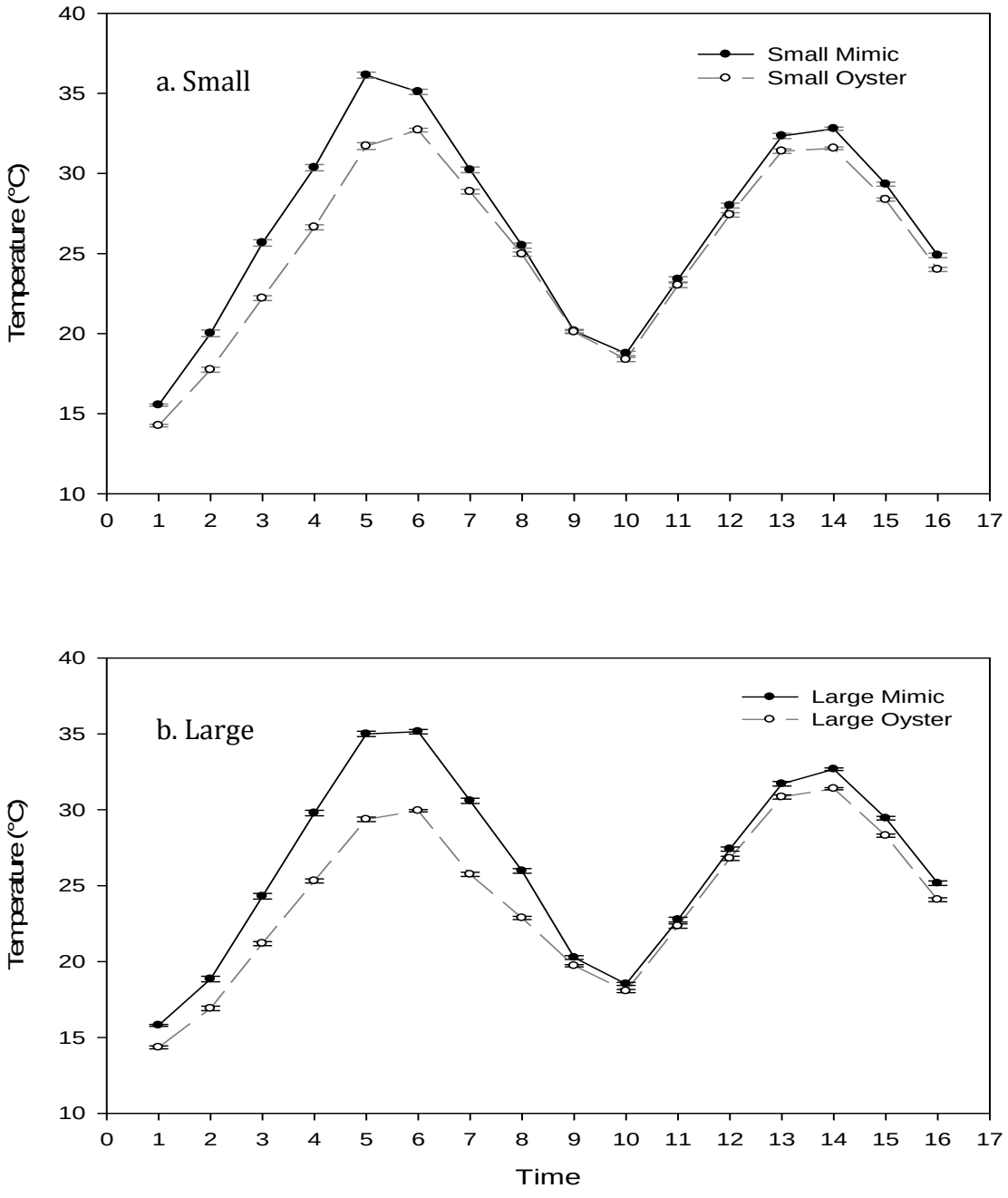


Figure A5a & A5b. Temperatures recorded during the calibration experiment by **a.** Small oysters and mimics **b.** Large oysters and mimics of similar size.

Literature Cited

Albright, B.W., Abbe, G.R., McCollough, C.B., Barker, L.S., Dungan, C.F. (2007). Growth and mortality of Dermo-disease-free juvenile oysters (*Crassostrea virginica*) at three salinity regimes in an enzootic area of Chesapeake Bay. *J. Shellfish. Res.* 26(2): 451-463.

Allen, S.M. & L.E. Burnett. (2008). The effects of intertidal air exposure on the respiratory physiology and the killing activity of hemocytes in the pacific oyster, *Crassostrea gigas* (Thunberg). *J. Exp. Mar. Biol. Ecol.* 357: 165-171.

Andrews, J.D. (1965). Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. *Chesap. Sci.* 6: 65-84.

Andrews, J.D. (1988). Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. *Am. Fish. Soc. Spec. Publ.* 18: 47-63.

Andrews, J.D. (1996). History of *Perkinsus marinus*, a pathogen of oysters in Chesapeake Bay 1950-1984. *J. Shellfish Res.*15(1): 13-16.

Andrews, J.D. & W.F. Hewett. (1957). Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters in Chesapeake Bay. *Ecol. Monogr.*27: 1-26.

Azevedo, C. (1989). Fine structure of *Perkinsus atlanticus* (n. sp. Apicomplexa. Perkinsea) parasite in the clam *Ruditapes decussates* from Portugal. *J. Parasitol.* 75: 627-635.

Bartol, I.K. & R. Mann. (1997). Small-scale settlement patterns of the oyster *Crassostrea virginica* on a constructed intertidal reef. *Bull. Mar. Sci.* 61(3): 881-897.

Bartol, I.K., Mann, R., Luckenbach, M. (1999). Growth and mortality of oysters on constructed intertidal reefs: effects of tidal height and substrate level. *J. Exp. Mar. Biol. Ecol.*237: 157-184.

Blackbourn, J., Bower, S.M., Meyer, G.R. (1998). *Perkinsus qugwadi* sp. Nov. (incertaesedis), a pathenogenic protozoan parasite of Japanese scallops, *Patinopecten yessoensis*, cultured in British Columbia, Canada. *Can. J. Zool.* 76(5): 942-953.

Boyd, J.N. & L.E. Burnett. (1999). Reactive oxygen intermediate production by oyster hemocytes exposed to hypoxia. *J. Exp. Biol.* 202: 3135-3143.

Burnett, L. E. 1997.The challenges of living in hypoxic and hypercapnic aquatic environments. *Am. Zool.* 37:633-640.

Burrell, Jr. V.G. (1982). Overview of the South Atlantic oyster industry. *World Mari. Soc. Spec. Publ.* no. 1: 125-127.

Burrell, V.G. (1986). Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic) – American Oyster. *US Fish Wld. S. Biol. Rep.* 82(11.57). U.S. Army Corps of Engineers TR EL-82-4.

Burrell, V.G., Manzi, J.J., Carson, W.Z. (1981). Growth and mortality of two types of seed oysters from the Wando River, South Carolina. *J. Shellfish Res*. 1: 1-7.

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

Burreson, E.M. & J.D. Andrews. (1988). Unusual intensification of Chesapeake Bay oyster diseases during recent drought conditions. Pages 799-802 in Oceans ’88 Proceedings. The IEEE Service Center, Piscataway, New Jersey.

Burreson, E.M. & L.M. Ragone Calvo. (1993). The effect of winter temperatures and spring salinity on *Perkinsus marinus* prevalence and intensity: a laboratory experiment (abstract). *J. Shellfish Res.* 12: 125

Burreson, E.M. & L.M. Ragone Calvo. (1996). Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay with emphasis on data since 1985.*J. Shellfish Res.* 15(1): 17-34.

Bushek, D., Ford, S.E., Allen, S.K. (1994). Evaluation of methods using Ray’s Fluid Thioglycollate Medium for diagnosis of *Perkinsus marinus* infection in the Eastern oysters, *Crassostrea virginica*. *Annu. Rev. Fish Dis*. 4: 201-217

Bushek, D., Allen, S.K., Alcox, K.A., Gustafson, R., Ford, S.E. (1994a). Dose response of the eastern oysters, *Crassostrea virginica*, to cultured cells of *Perkinsus marinus,* the agent of Dermo disease. *J. Shellfish. Res.* 13: 313

Butler, P.A. (1953) Oyster growth as affected by latitudinal temperature gradients. *Commer. Fish. Rev.* 15(6): 7-12.

Caceres-Martinez, J., Vasquez-Yeomans, R., Padilla-Lardizabal, G., del Rio Portilla, M.A. (2008). *Perkinsus marinus* in pleasure oyster *Crassostrea corteziensis* from Nayarit, Pacific coast of Mexico. *J. Invertebr.Pathol.*99: 66-73.

Casas, S.M., Grau, A., Reece, K.S., Apakupakul, K., Azevedo, C., Villalba, A. (2004). *Perkinsus mediterraneus* n. sp., a protistan parasite of the European flat oyster *Ostrea edulis* from the Balearic Islands, Mediterranean Sea. *Dis. Aquat. Org.* 58: 231-244.

Capone, M., Grizzle, R., Mathieson, A.C., Odell, J. (2008). Intertidal oysters in Northern New England. *Northeastern Nat.* 15(2): 209-214.

Cerco, C.F. & M.R. Noel. (2007). Can oyster restoration reverse cultural eutrophication in Chesapeake Bay? *Estuaries Coasts* 30: 331-343.

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

Coen, L.D., Brumbaugh, R.D., Bushek, D., Grizzle, R., Luckenbach, M.W., Posey, M.H., Powers, S.P., Tolley, S.G. (2007). Ecosystem services related to oyster restoration. *Mar. Ecol. Prog. Ser.* 341: 303-307.

Crosby, M.P., Roberts, C.F., Kenny, P.D. (1991). Effects of immersion time and tidal position on *in situ* growth rates of naturally settled eastern oysters, *Crassostrea virginica*. *J. Shellfish Res.*10 (1): 95-103.

Davenport, J. & T.M. Wong. (1986). Responses of the Blood Cockle *Anadara granosa* (L.) (Bivalvia: Arcidea) to Salinity, Hypoxia and Aerial Exposure. *Aquaculture* 55: 151-162.

Dame, R.F. (1972). The ecological energies of growth, respiration, and assimilation in the intertidal American oyster, *Crassostrea virginica. Mar. Biol.* 17: 243-250.

Dayton, P.K. (1971). Competition, Disturbance, and Community Organization: The provision and Subsequent Utilization of Space in a Rocky Intertidal Community. *Ecol. Monogr.* 41(4): 351-389.

Dittman, D.E. (1993). The quantitative effects of *Perkinsus marinus* on reproduction and condition in the eastern oyster, *Crassostrea virginica* (Abstract). *J. Shellfish Res.* 12: 127.

Dwyer, J.J. & L.E. Burnett. (1996). Acid-Base status of the oyster *Crassostrea virginica* in response to air exposure and to infections by *Perkinsus marinus. Biol. Bull.* 190: 139-147.

Ford, S.E. (1996). Range extension by the oyster parasite *Perkinsus marinus* into the northeastern United States: Response to climate change? *J. Shellfish Res.* 15(1): 45-56.

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

Ford, S.E. & M.R. Tripp "Diseases and Defense Mechanisms." The Eastern Oyster: *Crassostrea virginica*. Ed. Kennedy, V.S., Newell, R.I.E., and A.F. Eble. Maryland Sea Grant College, University of Maryland System, College Park 1996. 581-660.

Fulford, R.S., Breitburg, D.L., Newell, R.I.E., Kemp, W.M., Luckenbach, M. (2007). Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: A flexible modeling approach**.** *Mar. Ecol. Prog. Ser.* 336: 41-61.

Galtsoff, P.S. (1964). The American oyster *Crassostrea virginica* Gmelin. *Fish. Bull.*64: 1-480.

Goggin, C.L. & R.J.G. Lester. (1995). *Perkinsus,* a protistan parasite of abalone in Australia: A review. *Mar. Freshw. Res.* 48(3): 639-646.

Grizzle, R.E., Adams, J.R., Walters, L.J.(2002). Historical changes in intertidal oyster (*Crassostrea virginica*) reefs in a Florida lagoon potentially related to boating activities. *J. Shellfish Res*. 21:749-756.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D. (2002). Climate Warming and Disease Risks for Terrestrial and Marine Biota. *Science* 296: 2158-2162.

Heaven, C.S. & R.A. Scrosati. (2008). Benthic community composition across gradients of intertidal elevation, wave exposure, and ice scour in Atlantic Canada. *Mar. Ecol. Prog. Ser.* 369: 13-23.

Helmuth, B. (2002). How do we mean the environment? Linking intertidal thermal physiology and ecology through biophysics. *Integr. Comp. Biol.* 42: 837-845.

Hilbish, T.J. (1986). Growth trajectories of shell and soft tissue in Bivalves; seasonal variations in *Mytilus edulis*. *J. Exp. Mar. Biol. Ecol.* 96(2): 103-113.

Hidu, H. & H.H. Haskin. (1971). Setting of the American oysters related to environmental factors and larval behavior. *Proc. Natl. Shellfish. Assn.* 61: 35-50.

Hofstetter, R.P. (1977). Trends in population levels of the American oyster *Crassostrea virginica* Gmelin on public reefs in Galveston Bay, Texas. *Tx. Pk.Wild. Dept. Tech. Ser.* 24. 90 pp.

Hopkins, A. E. (1936). Adaptation of the feeding mechanism of the oyster (*Ostrea gigas*) to changes in salinity. *Bull. U.S. Bur. Fish.*48: 345-364.

Huang, S.-C. & R.I.E. Newell. (2002). Seasonal variations in the rates of aquatic and aerial respiration and ammonium excretion of the rubbed mussel, *Geukensia demissa* (Dwillyn). *J. Exp. Mar. Biol. Ecol.* 270: 241-255.

Ingle, R.M. & C.E. Dawson Jr. (1952). Growth of the American oyster, *Crassostrea virginica* (Gmelin) in Florida waters, *Bull. Mar. Sci. Gulf and Caribbean.*2: 393-404.

Ingle, R.M., Joyce, A.E., Quick, J.A., Morey, S.W. (1971).Basic considerations in the evaluation of thermal effluents in Florida. A preliminary investigation: The effect of elevated temperature on the American oyster *Crassostrea virginica* (Gmelin). *Florida Dept. Nat. Res. Prof. Pap. Ser.* 15: vii-viii.

Jordan, S.J. & J.M. Coakley (2004). Long-term projections of eastern oyster populations under various management scenarios. *J. Shellfish Res.*23 (1): 63-72.

Kennedy, V.S. (1996). The ecological role of the eastern oyster, *Crassostrea virginica*, with remarks on disease. *J. Shellfish Res.* 15:177-183.

Kennedy, V.S. and L.L. Breisch. (1981). Maryland's Oysters: Research and Management**.** 286 pp. University of Maryland Sea Grant Program, College Park Maryland.

Kenny, P.D., Michener, W.K., Allen, D. M. (1990). Spatial and temporal patterns of oysters settlement in a high salinity estuary. *J. Shellfish Res.* 9: 329-339.

Kim, Y. & E.N. Powell. (1998). Influence of climate change on interannual variation in population attributes of Gulf of Mexico oysters. *J. Shellfish Res.*17 (1): 265-274.

Krantz, G.E. & J.V. Chamberlain. (1978). Blue Crab predation on cultchless oyster spat. *Proc. Natl. Shellfish. Assn.* 68: 38-41.

Kraeuter, J.N., Ford, S., Cummings, M. (2007). Oyster growth analysis: A comparison of methods. *J. Shellfish Res.* 26(2): 479-491.

Lafferty, K.D. (2003). Is disease increasing or decreasing, and dies it impact or maintain biodiversity? *J. Parasitol.*89 (Suppl.): S101-S105.

Landers, W.S. & E.W. Rhodes Jr. (1970).Some factors influencing predation by the flatworm, *Stylochus ellipticus* (Girard), on oysters. *Chesap.Sci.* 15:55-60.

La Peyre, M.K., Casas, S.M., Villalba, A., La Peyre, J.F. (2008). Determination of the effects of temperature on viability, metabolic activity and proliferation of two *Perkinsus* species, and its significance to understanding seasonal cycles of perkinsosis. *Parasitology* 135: 505-519.

Lester, R.J.G. and G.H.G. Davis. 1981. A new *Perkinsus* species (Apicomplexa, Perkinsea) from the abalone, *Haliotis ruber*. *J.Invertebr.Pathol.***37**: 181–187.

Loosanoff, V.L. (1932). Observations on propagation of oysters in James and Corrotoman Rivers and the seaside of Virginia. *Va. Comm. Fish., Newport News, VA (USA)* pp. 1-45.

Loosanoff, V.L. (1965). The American or eastern oyster. *US Dept. of the Inter. Circ.* 205: 1-36.

Loosanoff, V.L. & C.A. Nomejko. (1946). Feeding of oysters in relation to tidal stages and to periods of light and darkness. *Biol. Bull.* 90: 244-264.

Loosanoff, V.L. & C.A. Nomejko. (1949). Growth of oysters, *O. virginica*, during different months. *Biol. Bull.* 97(1): 82-94.

Lutz, R.A., Hidu, H., Drobeck, K.G. (1970). Acute temperature increase as a stimulus to setting in the American oysters, *Crassostrea virginica* (Gmelin). *Proc. Natl. Shellfish. Assn.* 60: 68-71.

Mackin, J.G. (1956). *Democystidium marinum* and salinity. *Proc. Natl. Shellfish. Assn.* 50: 21-40.

Mackin, J.G. (1962). Oyster diseases caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publ. Inst. Marin. Sci. Univ. Tex.* 7: 132-229.

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

Malek Chapter 1. (2010). Effects of intertidal exposure on mortality and growth of the eastern oyster, *Crassostrea virginica*. M.S. Thesis, University of Maryland, College Park.

Malek Chapter 2. (2010). Effects of intertidal exposure on Dermo disease in the eastern oyster, *Crassostrea virginica*. M.S. Thesis, University of Maryland, College Park.

Mann, R. & E.N. Powell. (2007). Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. *J. Shellfish Res.* 26(4): 905-917.

McCollough, C.B., Albright, B.W., Abbe, G.R., Barker, L.S., Dungan, C.F. (2007). Acquisition and progression of *Perkinsus marinus* infections by specific-pathogen-free juvenile oysters (*Crassostrea virginica*) in a mesohaline Chesapeake Bay tributary. *J. Shellfish Res.* 26(2): 465-477.

McDougall, K. D. (1942). Sessile marine invertebrates of Beaufort NC. *Ecol. Monogr.* 13: 321-371.

McLaughlin, S.M., Tall, B.D., Shaheen, A., Elsayed, E.E., Fasal, M. (2000). Zoosporulation of a new *Perkinsus* species isolated from the gills of the softshell clam *Mya arenaria. Parasitology* 7: 115-122.

Menzel, R.W. & S.H. Hopkins (1955). The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. *J. Parasitol.* 41(4): 333-342.

Menzel, R.W. & S.H. Hopkins. (1956). Crabs as predators of oysters in Louisiana. *Proc. Natl. Shellfish. Assn.* 47: 177-184.

Meyer, D. L., E. C. Townsend, and G. W. Thayer. 1997. Stabilization and erosion control value of oyster cultch for intertidal marsh. *Restor. Ecol.* 5:93–99.

Milardo, C.S. (2001). The effects of environmental variables on the metabolism of the protozoan oyster parasite *Perkinsus marinus*. M.S. Thesis submitted to University of Charleston, South Carolina.

Mouritsen, K.N. & R. Poulin. (2002). Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* 124: S101-S112.

Newell, R.C. (1979). *Biology of intertidal animals*. Marine Ecological Surveys Favesham Kent UK.

Newell, R.I.E. (1988). Ecological Changes in Chesapeake Bay: Are They the Result of Overharvesting the American Oyster, *Crassostrea virginica*? In: Lynch M.P., Krome E.C. (eds). *Understanding the estuary*. Advances in Chesapeake Bay Research, Chesapeake Research Consortium Publ. 129, Gloucester Point, VA, p 536-546.

Newell, R.I.E., Alspach Jr., G.S., Kennedy, V.S., Jacobs, D. (2000). Mortality of newly metamorphosed eastern oysters (*Crassostrea virginica*) in mesohaline Chesapeake Bay. *Mar. Biol.* 136: 665-676.

NRC (National Research Council). 2004. Nonnative Oysters in the Chesapeake Bay. Washington DC: The National Academies Press.

O'Beirn, F.X., Dean, C.C., Walker, R.L. (1994). Prevalence of *Perkinsus marinus* in the Eastern oyster, *Crassostrea virginica*, in relation to tidal placement in a Georgia tidal creek. *Northeast Gulf Sci.* 13(2): 79-87.

Osman, R.W., Whitlatch, R.B., Zajac, R.N. (1989). Effects of resident species on recruitment into a community: larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 54: 61-73.

Park, K.I. & K.S. Choi (2001).Spatial distribution and infection intensity of the protozoan parasite *Perkinsus* sp. in the Manila clam *Ruditapes philippinarum* in Korea. *Aquaculture* 203: 9-22.

Park, K.I. Ngo, T.T.T. Choi, S-D., Cho, M. Choi, K-S. (2006). Occurrence of *Perkinsus olseni* in the Venus clam *Protothaca jedoensus* in Korean waters. *J. Invert. Path.* 93(2): 81-87.

Paynter, K.T. & E.M. Burreson. (1991). Effects of *Perkinsus marinus* infection in the Eastern oyster, *Crassostrea virginica*: II. Disease development and impact on growth rate at different salinities. *J. Shellfish Res.* 10(2): 425-431.

Potter, M.A. & B.J. Hill. (1982). Heat mortality in the Sydney rock oyster, *Saccostrea* (*Crassostrea*) *commercialis* and the effectiveness of some control methods. *Aquaculture* 29 (1-2): 101-108.

Potvin, C. & D.A. Roff. (1993). Distribution-free and robust statistical methods: viable alternatives to parametric statistics? *Ecology* 74(6): 1617-1628.

Powell, E.N., Klinck, J.M., Hofmann, E.E. (1996). Modeling disease oyster populations. II. Triggering mechanisms for *Perkinsus marinus* epizootics. *J. Shellfish Res.*15 (1): 141-165.

Ragone Calvo, L.M., R.L. Wetzel, E.M. Burreson. (2001). Development and verification of a model for the population dynamics of the protistan parasite *Perkinsus marinus* within its host, the eastern oyster, *Crassostrea virginica* in Chesapeake Bay. *J. Shellfish Res.* 20(1): 231-241.

Raimondi, P.T., Wilson, C.M., Ambrose, R.F., Engle, J.M., Minchinton, T.E. (2002). Continued declines of black abalone along the coast of California: are mass mortalities related to El Niño events? *Mar. Ecol. Prog. Ser.* 242: 143-152.

Ray, S.M. (1954). Biological studies of *Dermocystidium marinum,* a fungus parasite of oysters. Rice Institute Pamphlet Special Issue, November 1954.

Ray, S.M. (1966a). A review of the culture method for detecting *Dermocystidium marinum,* with suggested modifications and precautions. *Proc. Natl. Shellfish. Assn.* 54: 55-69.

Roegner, G. C. & R. Mann. (1990). Settlement patterns of *Crassostrea virginica* (Gmelin, 1791) larvae in relation to tidal zonation. *J. Shellfish Res.* 9(2): 341-346.

Roegner, G.C. & R. Mann. (1995). Early recruitment and growth of the American oyster *Crassostrea virginica* with respect to tidal zonation and season. *Mar. Ecol.Prog.Ser.*177: 91-101.

Rothschild, B.J., Ault, J.S., Goulletquer, P., Heral, M. (1994). Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. *Mar. Ecol. Prog. Ser.* 111: 29-39.

Schneider, K.R. & B. Helmuth. (2007). Spatial variability in habitat temperature may drive patterns of selection between an invasive and native mussel species. *Mar. Ecol. Prog. Ser.* 339: 157-167.

Shaw, W.N. (1966). The growth and mortality of seed oysters, *Crassostrea virginica*, from Broad Creek, Chesapeake Bay, MD., in high- and low salinity waters. *Proc. Natl. Shellfish. Assn.* 56: 59-63.

Shumway, S.E. (1996).“Natural Environmental Factors.” The Eastern Oyster: *Crassostrea virginica*. Ed. Kennedy, V.S., Newell, R.I.E., and A.F. Eble. Maryland Sea Grant College, University of Maryland System, College Park. 467-513.

Smith, G.F., Bruce, D.G., Roach, E.B., Hansen, A., Newell, R.I.E., McManus, A.M. (2005). Assessment of Recent Habitat Conditions of Eastern Oyster *Crassostrea virginica* Bars in mesohaline Chesapeake Bay. *North Am. J. Fish. Manage.*25: 1569-1590.

Smith, K.F., Sax., D.F., Lafferty, K.D. (2006) Evidence of the role of infection disease in species extinction and endangerment. *Conserv. Biol.* 20(5): 1349-1357.

Sokal, R.R. & F.J. Rohlf. (1995). *Biometry: the principles and practice of statistics in biological research* (3rd ed.). W.H. Freeman.

Soniat, T.M. & M.L. Koenig. (1982). The effects of parasitism by *Perkinsus marinus* on the free amino acid composition of *Crassostrea virginica* mantle tissue. *J. Shellfish Res.* 2: 25-28.

Soniat, T.M. & E.V. Kortright. (1998). Estimating time to critical levels of *Perkinsus marinus* in Eastern oysters, *Crassostrea virginica*. *J. Shellfish Res.*17 (4):1071-1080.

Soniat, T.M., Klinck, J.M., Powell, E.N., Hofmann, E.E. (2006). Understanding the success and failure of oyster populations: climactic cycles and *Perkinsus marinus. J. Shellfish Res.* 24: 83-93.

Tarnowski, M. (2008). Maryland Oyster Population Status Report: 2007 Fall Survey. Maryland Department of Natural Resources Shellfish Program & Sarbanes Cooperative Oxford Laboratory.

Taylor, J. & D. Bushek. (2008). Intertidal oyster reefs can persist and function in a temperate North American Atlantic estuary. *Mar. Ecol. Prog. Ser.* 361: 301-306.

Wethey, D.S. (1985). Catastrophe, extinction, and species diversity: a rocky intertidal example. *Ecology* 66(2): 445-456.

White, M.E. & E.A. Wilson. (1996). "Predators, Pests, and Competitors." The Eastern Oyster: *Crassostrea virginica*. Ed. Kennedy, V.S., Newell, R.I.E., and A.F. Eble. Maryland Sea Grant College, University of Maryland System, College Park. 559-579.

Wilson, E.A., Powell, E.N., Ray, S.M. (1988). The effect of the ectoparasite pyramidellid snail, *Boonea impressa*, on the growth and health of oysters, *Crassostrea virginica*, under field conditions. *Fish. Bull.* 86: 553-566.

Ybanez, C. (2007). Selective advantage of living in the intertidal zone: *Perkinsus marinus* (Dermo) and heat shock survival in Eastern oysters (*Crassostrea virginica*). M.S. Thesis submitted to Texas A & M University-Corpus Christi.

Zachary, A. & D.S. Haven. (1973). Survival and activity of the oyster drill *Urosalpinx cinerea* under conditions of fluctuating salinity. *Mar. Biol.* 22: 45-52