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Effects of air-exposure gradients on spatial infection patterns of *Perkinsus marinus* in the eastern oyster *Crassostrea virginica*

Jennafer C. Malek[1,2,*], Denise L. Breitburg[1]

[1]Smithsonian Environmental Research Center, PO Box 28, 647 Contees Wharf Road, Edgewater, MD 21037, USA

[2]*Present address:* Georgia Sea Grant, University of Georgia, 1180 E. Broad Street, Athens, GA 30602, USA

ABSTRACT: Spatial distributions of species can be shaped by factors such as parasites, mortality, and reproduction, all of which may be influenced by differences in physical factors along environmental gradients. In nearshore tidal waters, an elevational gradient in aerial exposure during low tide can shape the spatial distributions of benthic marine organisms. The eastern oyster *Crassostrea virginica* is an ecologically and economically important species that can dominate both subtidal and intertidal habitats along the east coast of the USA. Our goal was to determine whether prevalence and intensity of *Perkinsus marinus* (the causative agent of Dermo disease) infections vary along intertidal to subtidal gradients during summer. We used (1) field experiments conducted at 4 sites in the Chesapeake Bay and a Virginia coastal bay, (2) a controlled air-exposure experiment, and (3) field surveys from 7 sites ranging from Maine to North Carolina to test for effects of tidal exposure on infection. Results from our field surveys suggested that high intertidal oysters tend to have higher infection prevalence than subtidal oysters, but there was no effect on infection intensity. Field experiments rarely yielded significant effects of tidal exposure on infection prevalence and intensity. Overall, our study shows that exposure to air may not be a strong driver of infection patterns in this host–parasite system.

KEY WORDS: Intertidal exposure · Chesapeake Bay · Dermo disease · Perkinsiosis · Estuary · Epizootic · Host–parasite system

INTRODUCTION

There has been increasing recognition in the past several decades that parasites and pathogens play an important role in shaping community structure (Minchella & Scott 1991, Combes 1996, Mouritsen & Poulin 2002, 2010, Wood et al. 2007, Lafferty et al. 2008). Parasites can affect survival, reproduction, growth, and behavior of their host species (Kelsall & Prescott 1971, Anderson 1972, Mouritsen & Poulin 2005, Poulin 2013). These parasite effects may result in changes in host abundance and distribution, and the role of a host within its community. Such alterations in host populations are particularly important when host species serve as ecosystem engineers (Jones et al. 1997, Byers et al. 2006). These species affect community structure by shaping physical habitat and resource flow (Crain & Bertness 2006) and by modifying physical and biological conditions for other organisms (Hastings et al. 2007, Altieri et al. 2010). For example, in the Caribbean, coral diseases have caused severe reductions of important reef builders such as *Acropora palmata*, leading to reduced reef habitat and biodiversity, as well as decreased shoreline protection (Moberg & Folke 1999, McClanahan et al. 2002).

Host–parasite interactions can be influenced by environmental conditions. Variations in factors such

*Corresponding author: malekjc1@uga.edu

as temperature, salinity, and food availability can create latitudinal, elevational, spatial, or temporal environmental gradients that influence the occurrence and outcomes of these interspecific interactions (Mouritsen & Poulin 2005, Hobbs & Frisch 2010, Wolinska et al. 2011). Variation in parasite infection prevalence (proportion of infected individuals in a population) or intensity (abundance within an individual) has been observed along latitudinal, elevational, or other environmental gradients in amphibians (Kriger et al. 2007, Muths et al. 2008), birds (Zamora-Vilchis et al. 2012), trees (Wilds 1997), grasses (Seabloom et al. 2010), snails (Jokela & Lively 1995), and corals (Hobbs & Frisch 2010). However, patterns in parasite infection along these gradients differ among studies and systems. In systems dominated by engineering species, it is critical to comprehend how environmental gradients shape host–parasite interactions that can indirectly affect species dependent on the engineers.

The eastern oyster *Crassostrea virginica* is an ecologically and economically important engineer in coastal and estuarine environments along the Atlantic and Gulf of Mexico coasts of North America. This species creates 3-dimensional reefs that provide habitat for fish and invertebrates (Lenihan et al. 2001, Coen et al. 2007), reduce shoreline erosion (Meyer et al. 1997, Grizzle et al. 2002), and improve water quality by removing phytoplankton from the water column (Dame & Patten 1981, Newell et al. 2007). Oysters also support important fisheries throughout their range, but have suffered large declines in abundance due to a combination of overfishing, habitat destruction, and parasites (Andrews 1988, Lenihan & Peterson 1998, Jackson et al. 2001, Mackenzie 2007). In many portions of its range, *C. virginica* lives along an intertidal to subtidal environmental gradient (referred to herein as an 'exposure' gradient), but its tidal distribution varies with latitude. North and south of Chesapeake Bay, oysters live either both intertidally and subtidally (north, Gulf of Mexico; Kilgen & Douglas 1989), only intertidally (south), or only subtidally (north). Within Chesapeake Bay, oysters occur both intertidally and subtidally in the more saline Virginia region but only subtidally in the mesohaline Maryland region.

The protistan parasite *Perkinsus marinus* has caused extensive mass mortalities of *C. virginica* in the Chesapeake and Delaware Bays (Andrews 1988, 1996) and continues to be a concern for oyster recovery efforts, particularly in Chesapeake Bay (Mann & Powell 2007). Under preferred temperature and salinity conditions for *P. marinus* (25°C and 12 psu), epizootics can occur within 1 to 2 yr in a host population. The parasite spreads to the water and surrounding oysters through feces production (Bushek et al. 2002) and decaying, infected oyster tissue (Andrews & Hewatt 1957). Additionally, *P. marinus* can infect naïve oysters greater than 5 km from a known parasite source (McCollough et al. 2007), demonstrating wide-ranging transmission capability.

Along air-exposure gradients, oysters experience spatially varying durations and intensities of abiotic conditions. These conditions can result in extreme high or low internal temperatures, internal hypoxia (low oxygen), and hypercapnia (elevated CO_2) (Burnett 1997, Milardo 2001). As *P. marinus* lives within its host, the parasite can also be exposed to such extreme conditions. The effects of intertidal air exposure on the host and parasite could cause infections to vary spatially within oyster populations, depending on the duration, frequency, and severity of exposure conditions. Laboratory experiments have suggested that conditions during air exposure, specifically increased internal temperatures fluctuating by ≥15°C (25–40°C) and increased internal CO_2 levels (Dungan & Mamilton 1995, Milardo 2001), can reduce *P. marinus* growth and proliferation. Death of *P. marinus* hypnospores at 37°C when incubated in Ray's fluid thioglycollate medium (Ray 1954) also indicates that *P. marinus* is sensitive to the high internal temperatures sometimes reached by intertidal oysters. Thus, laboratory results suggest that intertidal habitats might serve as a refuge from the development of heavy infections.

In contrast, previous field studies have not found an effect of intertidal exposure on *P. marinus* infection prevalence or intensity (Burrell et al. 1984, Gibbons & Chu 1989, O'Beirn et al. 1994, Ybanez 2007). These studies were conducted mostly in high-salinity areas from South Carolina to Texas where *C. virginica* populations are primarily intertidal. However, the broad range of intertidal and subtidal habitats available were not tested, potentially limiting the ability to detect variations in *P. marinus* infections along the exposure gradient. The difference between laboratory and field results, and the variation in the tidal distribution of oysters in Chesapeake Bay, led us to further investigate the relationship between *C. virginica, P. marinus,* and the exposure gradient.

In this study, we asked: Do the prevalence and intensity of parasite infections vary spatially along environmental (air-exposure) gradients? Specifically, we wanted to know whether exposure gradients affect *P. marinus* infections in experimentally deployed and manipulated *C. virginica* and in wild

oyster populations. To address this, we used a combination of field and controlled-exposure experiments, and surveys of wild oyster populations. Spatial scales of our study ranged from a single site in Chesapeake Bay (0.1 km of shoreline) to the stretch of the Western Atlantic coastline from Maine to North Carolina, USA (~1500 km). Based on previous laboratory findings, we expected to see lower prevalence and intensity of *P. marinus* infections in oysters towards the peak of the exposure gradient (long durations of air exposure) compared to those in the subtidal.

MATERIALS AND METHODS

Field deployment experiments

To test for effects exposure gradients on *Perkinsus marinus* prevalence and intensity, we conducted large-scale field experiments in which oysters were deployed at 4 tidal heights with increasing durations of air-exposure during the summers of 2008 and 2009. We included 2 classes of oysters: nominally uninfected 1 yr old oysters to test for the prevalence and intensity of newly acquired *P. marinus* infections (referred to herein as initially uninfected: IU) and older oysters with initially moderate to high infection prevalence to test for prevalence and intensity of progressing *P. marinus* infections (referred to herein as initially infected: II). Experiments were deployed at 4 sites across Maryland (MD) and Virginia (VA) (Fig. 1a) that covered a range of temperatures, salinities, and proximities to oyster beds with documented *P. marinus* infections (Table 1).

We purchased all IU oysters (2008 and 2009) from Marinetics Inc., a Choptank River hatchery in MD that uses local, non-selectively bred (i.e. non-disease resistant) brood stock. In 2008, II oysters of various ages were collected near field sites. In 2009, we purchased 2 yr old II oysters from Marinetics. II oysters had average starting *P. marinus* prevalence of 90% in 2008 and 39% in 2009. Starting mean (±SE)

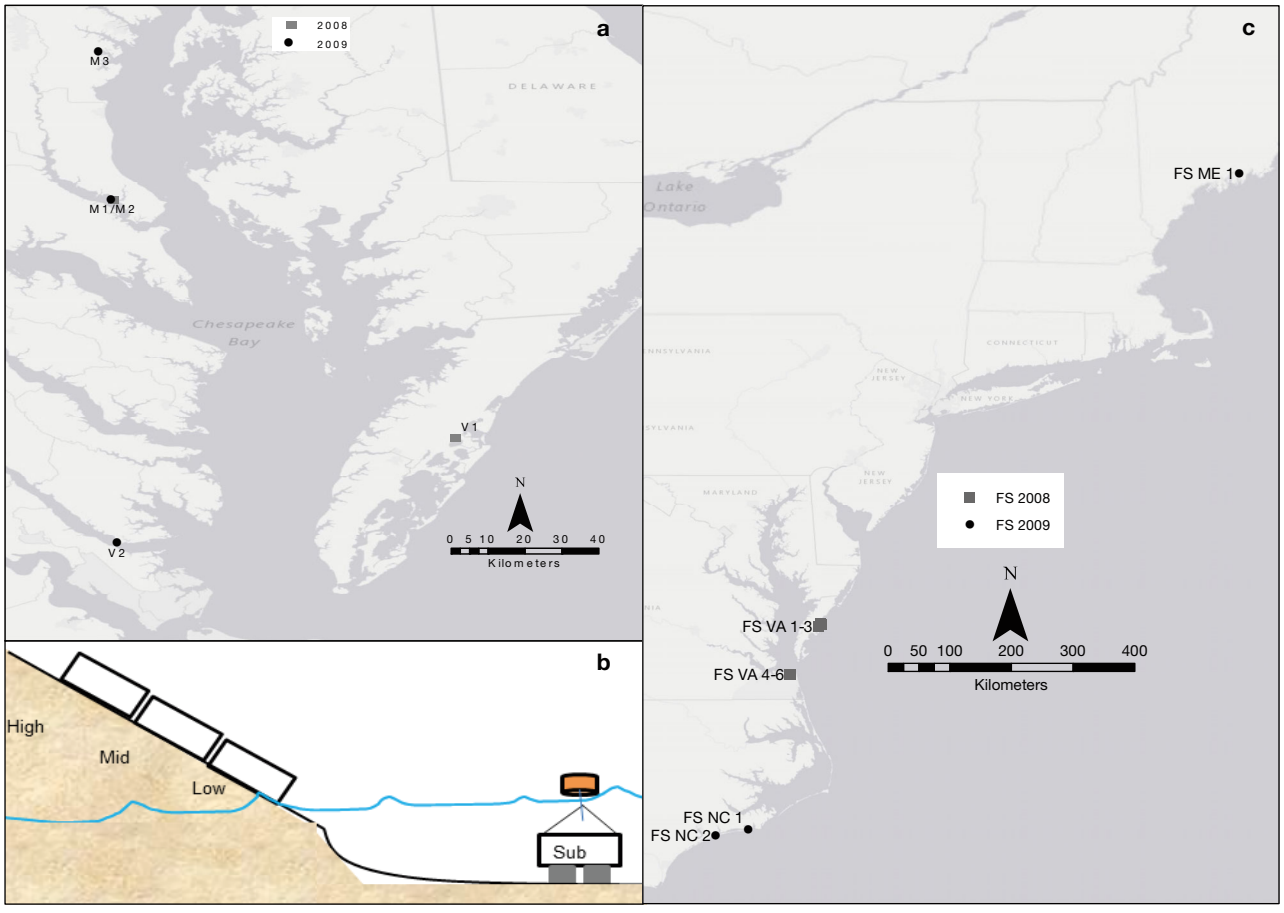

Fig. 1. (a) Field deployment experiment site locations in and around Chesapeake Bay, USA. (b) Field deployment experiment experimental setup of intertidal and subtidal cages at all sites except V1. (c) Field survey locations ranging from the Damariscotta River in Maine to Bear Island in North Carolina

Table 1. Summary of locations, dates, experiments, physical parameters, and number of eastern oysters *Crassostrea virginica* assessed for *Perkinsus marinus* infection at all field experiment and field survey sites. Physical data for each site were collected monthly from June to October using a YSI 85 meter. Temperature (*T*, °C) and salinity (PSU) data for M1/M2 are the averages for 2008 and 2009. Site abbreviations—ME: Maine; M: Maryland field deployment site; V: Virginia field deployment site; VA: Virginia field survey site; NC: North Carolina field survey site. Experiment abbreviations—FS: field survey; FDE: field deployment experiment; CE: controlled exposure experiment. Tidal height abbreviations—H: high intertidal; M: mid-intertidal; L: low intertidal; S: subtidal

Site ID	Latitude, longitude	Year(s) tested	Expt(s) run	*T*	PSU	Tidal heights assessed	Target n per tidal height
FS ME1	44° 00′ 33″ N, 69° 32′ 52″ W	2009	FS	21.1	29.0	H, M, S	30
M3	38° 53′ 10″ N, 76° 32′ 29″ W	2009	FDE, CE	17.8–29.9	7.9–14.2	H, M, L, S	30
M1/M2	38° 23′ 38″ N, 76° 30′ 40″ W	2008/2009	FDE	21.4–28	10–13	H, M, L, S	30
V1	37° 36′ 12″ N, 75° 40′ 39″ W	2008	FDE	22.8–26.3	26–34	M, S	30
FS VA1	37° 36′ 28″ N, 75° 37′ 46″ W	2008	FS	22.6	29.1	H, M, S	30
FS VA2	37° 37′ 12″ N, 75° 38′ 48″ W	2008	FS	23.5	29.2	H, M, S	30
FS VA3	37° 34′ 40″ N, 75° 40′ 47″ W	2008	FS	22.5	28.8	H, M, S	30
V2	37° 14′ 52″ N, 75° 30′ 00″ W	2009	FDE	24.6–31.3	18.7–20.2	H, M, L, S	30
FS VA4	36° 53′ 88″ N, 76° 05′ 29″ W	2008	FS	19.6	21.3	H, M, S	40
FS VA5	36° 53′ 74″ N, 76° 05′ 03″ W	2008	FS	20.0	20.7	H, M, S	40
FS VA6	36° 53′ 74″ N, 76° 05′ 45″ W	2008	FS	20.3	21.4	H, M, S	40
FS NC1	34° 43′ 21″ N, 76° 42′ 21″ W	2009	FS	25.3	30.7	H, M, S	30
FS NC2	34° 38′ 23″ N, 77° 08′ 21″ W	2009	FS	25.7	30.8	H, M	30

intensities for II oysters were 1.4 (±0.2, n = 82) and 1.3 (±0.3, n = 40) in 2008 and 2009, respectively, based on the Mackin scale (Mackin 1962) and including only infected individuals (see 'Infection assessment' below).

At each field site, we deployed subtidal oysters at 1 to 2 m depth during low tide in single cages that were continuously submerged and excluded most large predators. To capture a range of air-exposure durations for intertidal treatments, we placed oysters at 3 tidal heights at each MD site and at site V2 (Fig. 1a, Table 1). As there are no intertidal oysters in MD to gauge correct experimental placement, we created intertidal treatments using plastic oyster cages attached in series and deployed perpendicular to the shoreline across natural gradients (Fig. 1b). Although natural intertidal reefs did occur in the vicinity of site V2, none were close enough for positional comparison, so cages were deployed similar to MD sites. At site V1, there was a distinct tidal height where wild oysters occurred, so only 1 experimental intertidal treatment was used (comparable to the mid-intertidal treatment at other sites).

In 2008, we deployed IU and II in separate cages so that IU oysters acquired *P. marinus* infections from natural sources. We used 5 replicates of each tidal height and initial infection treatment at sites M1 and V1 (n = 45 oysters cage^{-1}; Fig. 1a, Table 1). Very low acquisition of new infections from natural sources in 2008 led us to combine the initial disease treatments in cages in 2009 to increase *P. marinus* transmission from II to IU oysters (n = 50 oysters of each initial disease treatment cage^{-1}). Each tidal height had 6 replicates of the combined infection treatments. We deployed oysters in early June in both years, and retrieved them in late September/early October after ~16 wk of field exposure. Destructive sampling to test for *P. marinus* infections (see 'Infection assessment' below) occurred immediately after retrieval (n ≈ 30 oysters at each tidal height for each initial disease treatment).

We used oyster mimics to approximate the durations of exposure for intertidal treatments (the amount of time an oyster was exposed to air, including partial exposure at the beginning and end of the tidal cycle) and to predict internal temperatures experienced by experimental oysters. Mimics consisted of a pair of silicone-filled oyster shells with an embedded temperature logger (iButton data loggers, Dallas Semiconductor; accuracy of ±0.5°C). Silicone has heat properties similar to bivalve tissue, resulting in temperature readings comparable to internal oyster temperatures (Helmuth 2002, Schneider & Helmuth 2007). We put mimics in mid-intertidal and subtidal cages, with 2 to 3 mimics per tidal height treatment at each site. With the temperature data, we calculated the approximate average duration of exposure for the mid-intertidal treatment at each site and then estimated the duration of exposure for high and low intertidal treatments (Table 2; see Malek 2010 for specific calculation details).

Controlled exposure experiment

To further test for effects of the duration of air exposure on *P. marinus* infections, we conducted an experiment in 2009 in which oysters were exposed to tightly controlled durations of air exposure (0, 1, 2 h of air exposure d^{-1}). We hung experimental oysters in cages from a dock at site M3, and all but subtidal controls were pulled out of the water for fixed periods of time 5 d wk^{-1} for 9 to 11 wk. We used 5 replicates of each exposure treatment, with both initial disease treatments combined in cages (n = 50 oysters $cage^{-1}$ for each initial disease treatment). Oyster mimics were used in all treatments for the duration of the experiment. At the end of the experimental period, 15 oysters of each initial disease treatment from each replicate cage were assessed for *P. marinus* infection (n = 75 for each initial disease treatment per exposure treatment).

Field surveys

To determine whether the effects of tidal exposure on *P. marinus* infections were similar in wild compared to experimental oysters, and to broaden the geographic scope of the study, we conducted field surveys of wild oyster populations in August of 2008 and 2009. We chose 9 sites from Maine to North Carolina (Fig. 1c, Table 1) that had natural intertidal to subtidal gradients. At each site, we collected oysters from 3 tidal heights: the highest point where oysters occurred (high intertidal), the central area where oysters were exposed during low tide (mid-intertidal), and below the low water mark at low tide (subtidal). We tested 30 to 40 oysters from each tidal height at each site for *P. marinus* infection prevalence and intensity (Table 1). No *P. marinus* infections were detected at site FS ME1, and no subtidal oysters could be collected at site FS NC2; these sites were therefore excluded from the analyses.

Infection assessment

We assessed *P. marinus* infection prevalence and intensity for all experiments and surveys by collecting rectal tissue from oysters and using Ray's fluid thioglycollate method (RFTM) of tissue incubation and microscopic parasite detection (Ray 1954). Observed infections were scored based on the Mackin scale, a 5-point scale measuring the intensity of infection (0.5: very light; 1: light; 2: light–moderate; 3: moderate; 4: moderate–heavy; and 5: heavy). Prevalence was calculated as the percentage of individuals with infections out of the total number sampled, and mean intensity as the average intensity including individuals infected with *P. marinus* (i.e. Mackin scores of 0.5 to 5; Soniat et al. 2006). Both metrics were calculated for each tidal height and initial disease treatment in field deployment and controlled exposure experiments. Prevalence estimates were based on infections that were detectable using the RFTM and likely missed some very light or newly acquired infections (Bushek et al. 1994). Overall, this methodology allowed us to test larger numbers of oysters than would have been feasible with more expensive and time-intensive techniques.

Statistical analyses

We analyzed *P. marinus* prevalence as a binary response (0 = no *P. marinus* present, 1 = *P. marinus*

Table 2. Summary of oyster mimic data for all sites (M: Maryland sites; V: Virginia sites). Historic tidal data were obtained from http://tidesandcurrents.noaa.gov/ and averaged to get the mean tidal range at each site during the periods when oysters were deployed. The approximate durations of exposure for the high and low intertidal were estimated based on the mean mid-intertidal exposure calculated using temperature data from oyster mimics. For specific details on calculations of exposure durations, see Appendix 1 in Malek (2010). NA: Tidal height treatments were not included at experimental site

Year	Site	Mean (SE) tidal range (m)	Rate of tidal change (m h^{-1})	Approx. high intertidal exposure duration (h)	Mean (SE) mid-intertidal exposure duration (h)	Approx. low intertidal exposure duration (h)
2008	M1	0.37 (0.012)	0.06	3.82	2.3 (0.32)	0.78
2009	M2	0.36 (0.009)	0.06	4.64	2.8 (0.26)	0.96
2009	M3	0.28 (0.007)	0.04	5.95	3.6 (0.36)	1.25
2008	V1	1.18 (0.024)	0.2	NA	2.5 (0.31)	NA
2009	V2	0.70 (0.012)	0.11	3.8	2.9 (0.40)	2

present) for all experiments and surveys. For the field deployment and controlled exposure experiments, we analyzed prevalence using mixed effects logistic regression, including tidal height as a fixed effect and replicate as a random effect to account for numerous oysters being sampled from the same cage. For the field surveys, we used logistic regression models including tidal height as a fixed effect. As RFTM intensity scores were based on the ordinal classifications of the Mackin scale, we analyzed intensity data from field deployment and controlled exposure experiments with mixed effects ordinal regression models, including tidal height as a fixed effect and replicate as a random effect. Field survey intensity data were analyzed with ordinal regression models using tidal height as a fixed effect. In all models for all experiments, each intertidal treatment was compared to the subtidal treatment.

Prompted by lab results suggesting that temperature fluctuations of ≥15°C influence *P. marinus* growth and reproduction, we used linear regression to examine the relationship between the number of days when experimental II oysters experienced internal temperature fluctuations ≥15°C (based on oyster mimics in the mid-intertidal and assumed to be the same in the high intertidal) and the difference between subtidal and high intertidal *P. marinus* intensity. Analyses were performed with RStudio, version 0.98.1087 using R, version 3.2.0. Results of $p \leq 0.05$ are presented as significant; comparisons with $0.10 < p \leq 0.05$ are discussed as non-significant trends.

RESULTS

Field deployment experiments

Mimic data confirmed expectations that intertidal treatments would have higher maximum temperatures and larger daily temperature fluctuations than subtidal treatments at all sites (Table 2). Mid-intertidal oysters experienced internal temperatures greater than 35°C during some exposures at site V2 and temperatures greater than 40°C at MD sites. The duration of exposure for intertidal treatments ranged from 0.5 to 5.95 h across sites (see Table 2 for site-specific details).

Mixed effects logistic regression indicated that tidal height was not a consistently significant predictor of *Perkinsus marinus* prevalence in IU or II oysters (Fig. 2a,b). Of the 26 possible comparisons of prevalence in intertidal to subtidal oysters within the 5 sites and 2 initial disease groups, only 1 was statistically significant ($p \leq 0.05$). Prevalence in II oysters was higher in the high intertidal than in the subtidal at site M3 (Fig. 2b, Table 3). We also observed several non-significant trends (i.e. $0.10 < p \leq 0.05$). Prevalence in II oysters tended to be higher in the high intertidal than the subtidal at site M1 and higher in the mid-intertidal than in the subtidal at site M3. No other comparisons were significant or showed non-significant trends.

Results of mixed effects ordinal regression indicated that tidal height was also not a consistently significant predictor of *P. marinus* infection intensity in IU or II oysters (Fig. 2c,d). The intensity of *P. marinus* infections was significantly higher in low intertidal than subtidal IU oysters at M2 and significantly lower in the high intertidal than the subtidal II oysters at M3 (Table 3). Additionally, infection intensity in II oysters tended to be lower in the mid-intertidal than in the subtidal at site V1, and higher in the low intertidal than in the subtidal at M2.

Effects of air exposure on *P. marinus* infection intensity were further evaluated by considering the prevalence of only moderate to heavy intensity infections (3–5 on the Mackin scale), which are more likely to be detrimental to the host than lighter, newly established infections (0.5–2 on the Mackin scale; see Dittman et al. 2001). Although there were some statistically significant differences between intertidal and subtidal treatments, the patterns were inconsistent. Subtidal oysters had significantly greater prevalence of moderate to heavy infection intensities than intertidal oysters at some sites, but significantly lower prevalence at others (Table 4). Thus, this metric also indicated that tidal height was not a consistent predictor of *P. marinus* infection intensity.

Controlled exposure experiment

Oysters in the 1 and 2 h d^{-1} exposure treatments experienced daily temperature fluctuations similar to low and mid-intertidal treatments, respectively, from field deployment experiments. Mixed effects logistic regression indicated that the prevalence of *P. marinus* infections was significantly higher in the 1 h d^{-1} treatment than in the subtidal control for both IU and II oysters (Fig. 3a, Table 5). There was also significantly higher prevalence in the 2 h d^{-1} treatment than in the subtidal control for IU oysters (Table 5). As with the field deployment experiments, there was no consistent effect of exposure duration on the

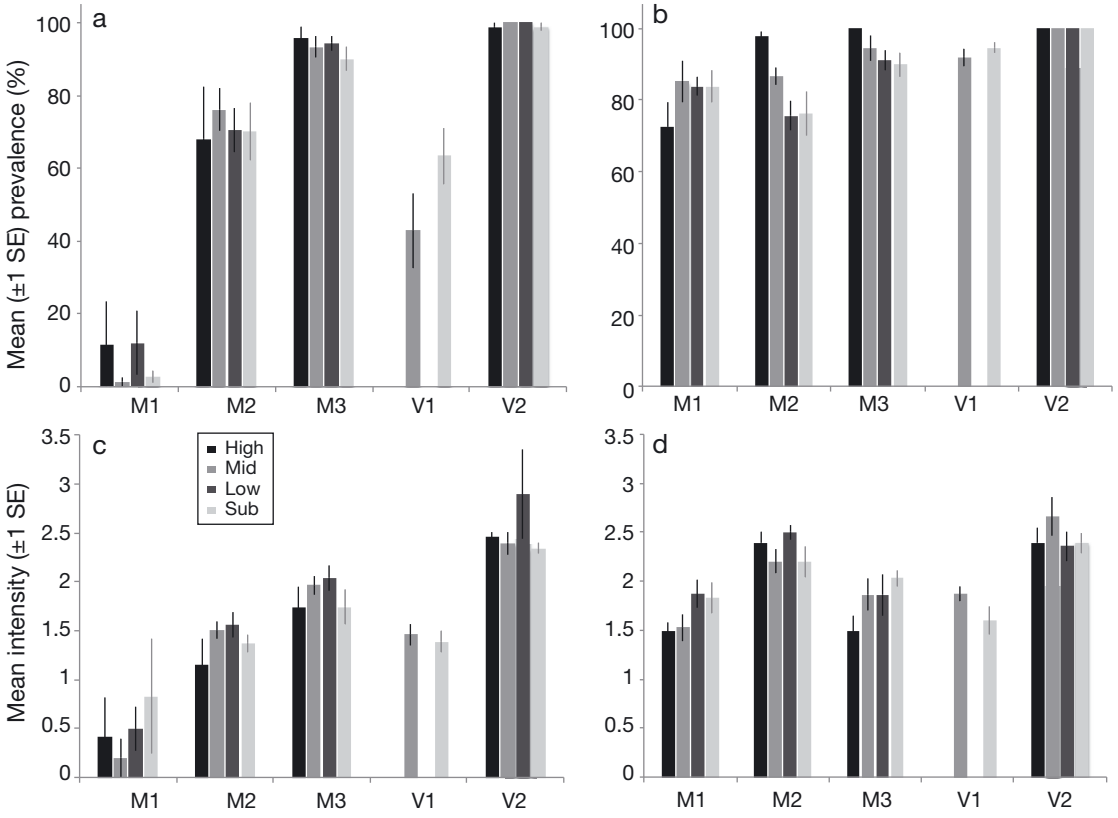

Fig. 2. *Perkinsus marinus* infection (a) prevalence in initially uninfected (IU) eastern oysters *Crassostrea virginica*, (b) prevalence in initially infected (II) oysters, (c) mean intensity in IU oysters, and (d) mean intensity in II oysters by site for the field deployment experiments. SE was calculated using replicates for each tidal height treatment. For prevalence, n ≈150–180 for each tidal height and initial disease treatment at each site. Mean intensity was calculated using only infected individuals, so the sample size for each tidal height and initial disease treatment at each site varied greatly, ranging from n = 5–180

intensity of *P. marinus* infections. Intensity was significantly higher in the 2 h d^{-1} treatment compared to the subtidal control in IU oysters (Fig. 3b). In II oysters, intensity was significantly lower in the 1 h d^{-1} treatment compared to the subtidal control (Table 5) and tended to be lower in the 2 d^{-1} treatment compared to subtidal control. Evaluation of moderate to heavy intensity infections suggested similar inconsistencies in the effect of air exposure for both IU and II oysters (Table 4).

Table 3. Results of logistic and ordinal mixed effects regression for *Perkinsus marinus* infection prevalence and intensity for each initial disease treatment in the field deployment experiments. Each site was analyzed separately. Individual models were run for each initial disease treatment, including tidal height treatment as a fixed effect and replicate as a random effect. Direction indicates whether *P. marinus* infection was higher or lower in the subtidal compared to intertidal treatments. IU: initially uninfected treatment; II: initially infected treatment. Only significant predictors ($p \leq 0.05$) are reported; all other models yielded non-significant trends ($0.05 \leq p \leq 0.10$; discussed in the 'Results' section) or non-significant results

Site/initial disease treatment	Source	Estimate	SE	p(*z*)	Direction	Variance	SD
Prevalence							
M3, II	High vs. Sub	2.149	0.769	0.01	High > Sub	0.058	0.241
Intensity							
M2, IU	Low vs. Sub	0.566	0.290	0.05	Low > Sub	0.177	0.421
M3, II	High vs. Sub	−0.6618	0.339	0.05	High < Sub	0.014	0.120

Table 4. Results of logistic regression models for prevalence of light-moderate intensity *Perkinsus marinus* infections in field deployment experiments, the controlled exposure experiment, and field surveys. Each site was analyzed separately. Individual models were run for each initial disease treatment for the field and controlled exposure experiments, including tidal height treatment as a fixed effect and replicate as a random effect. Direction indicates whether *P. marinus* infection was higher or lower in the subtidal compared to intertidal treatments. IU: initially uninfected treatment; II: initially infected treatment. Only significant predictors ($p \leq 0.05$) are reported; all other models yielded non-significant trends ($0.05 \leq p \leq 0.10$) or non-significant results

Site/initial disease treatment	Source	Estimate	SE	p(*z*)	Direction	Variance	SD
Field deployment experiment							
M1, II	High vs. Sub	−1.076	0.352	<0.01	High < Sub		
	Mid vs. Sub	−0.792	0.341	0.02	Mid < Sub	0.015	0.122
M2, IU	Low vs. Sub	0.690	0.311	0.03	Low > Sub	0.123	0.350
M2, II	High vs. Sub	0.749	0.346	0.03	High > Sub	0.006	0.076
Controlled exposure experiment							
IU	2 h vs. Ctrl	1.260	0.4515	0.01	2 h < Ctrl	0.063	0.250
Field surveys							
VA1	Mid vs. Sub	1.466	0.6537	0.03	Mid > Sub		
VA6	Mid vs. Sub	1.070	0.475	0.02	Mid > Sub		

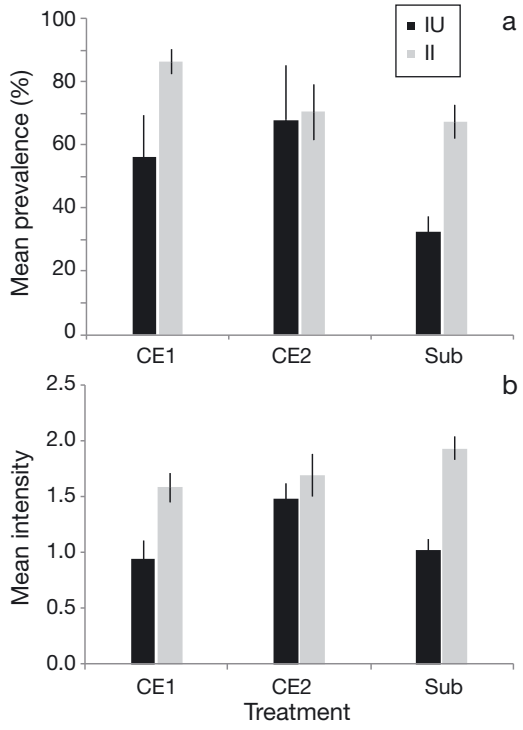

Fig. 3. *Perkinsus marinus* infection mean (±SE) (a) prevalence and (b) intensity for initially uninfected (IU) and initially infected (II) eastern oysters *Crassostrea virginica* by tidal exposure treatment in the controlled exposure experiment (CE1: controlled exposure to air for 1 h d^{-1}; CE2: controlled exposure to air for 2 h d^{-1}; Sub: subtidal). SE was calculated using replicates for each tidal exposure treatment. For prevalence, $n \approx 75$ for each exposure and initial infection treatment. Intensity was calculated using only infected individuals, so sample size for each tidal height and initial disease treatment varied greatly (n = 50–115)

Relationship between temperature and infection status

Mean *P. marinus* infection intensity in II oysters tended to be higher in the subtidal compared to the high intertidal at most sites, so we subtracted high intertidal from subtidal intensity. Regression analyses using data from the field deployment and controlled exposure experiments indicated that the difference in intensity increased with increasing numbers of days with temperature fluctuations ≥15°C ($R^2 = 0.84$; $p = 0.026$). However, when only light–moderate to heavy infections (2–5 on the Mackin scale) were considered, we found no effect of frequent large temperature fluctuations on the difference in *P. marinus* infection intensity between subtidal and high intertidal II oysters ($R^2 = 0.0007$; $p = 0.97$). This suggests that large temperature fluctuations are more likely to influence newly acquired, light to moderate *P. marinus* infections in high intertidal and subtidal habitats.

Field surveys

Prevalence of *P. marinus* infections tended to be higher in high intertidal compared to subtidal oysters at all 7 sites included in analyses (Fig. 4a). Most of these trends were not significant at individual sites, but there was a significant overall pattern of higher prevalence in high intertidal than in subtidal oysters across sites ($p \leq 0.05$,Wilcoxon signed rank

Table 5. Results of mixed effects logistic and ordinal regression models analyzing *Perkinsus marinus* infection prevalence and intensity for each initial disease treatment in the controlled air exposure experiment. Individual models were run for each initial disease treatment, including tidal height treatment as a fixed effect and replicate as a random effect. Direction indicates whether *P. marinus* infection was higher or lower in the subtidal control. IU: initially uninfected treatment; II: initially infected treatment. Only significant predictors ($p \leq 0.05$) are reported; all other models yielded non-significant trends ($0.05 \leq p \leq 0.10$) or non-significant results

Initial disease treatment	Source	Estimate	SE	p(*z*)	Direction	Variance	SD
Prevalence							
IU	1 h vs. Ctrl	0.970	0.291	<0.01	1 h > Ctrl		
	2 h vs. Ctrl	0.906	0.304	<0.01	2 h > Ctrl	0.025	0.159
II	1 h vs. Ctrl	1.166	0.386	<0.01	1 h > Ctrl	0.137	0.370
Intensity							
IU	2 h vs. Ctrl	1.120	0.430	0.01	2 h > Ctrl	0.014	0.1197
II	1 h vs. Ctrl	−0.568	0.288	0.05	1 h < Ctrl	< 0.001	< 0.001

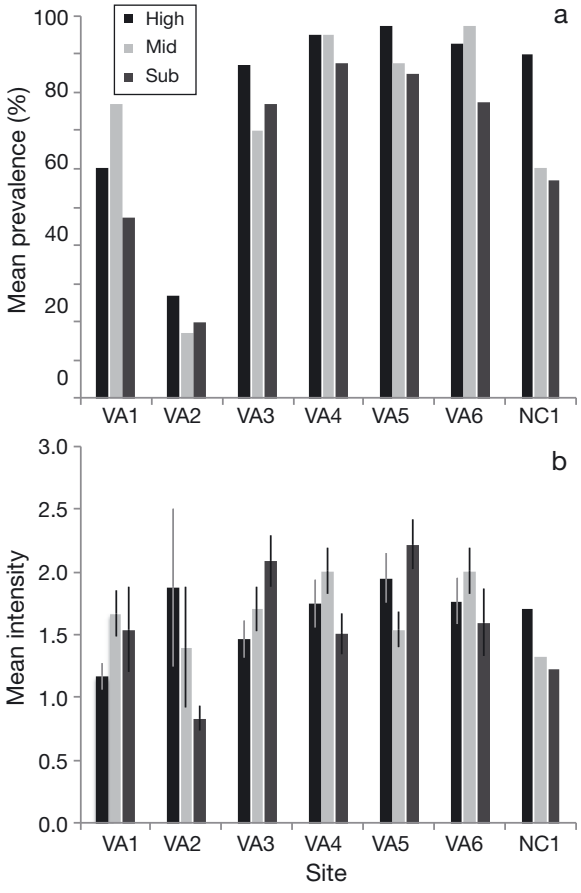

Fig. 4. *Perkinsus marinus* infection mean (a) prevalence and (b) intensity (± SE) by tidal height (high intertidal, mid-intertidal, and subtidal) at each field survey site. Because prevalence was calculated using all individuals from a given tidal height, we were unable to calculate and include SE. SE for mean intensity was calculated using individual eastern oysters *Crassostrea virginica* as replicates. For prevalence, n ≈ 30–40 for each tidal height at each site. Mean intensity was calculated using only infected individuals, so the sample size for each tidal height at each site varied greatly (n = 3–28)

analyses). Logistic regression indicated that prevalence was significantly higher in the high intertidal than in the subtidal at 1 site and significantly higher in the mid-intertidal than in the subtidal at 1 site in VA (Table 6). We also found that prevalence tended to be higher in the high intertidal at 1 site (VA5) and higher in both the high and mid-intertidal at another site (VA6).

Patterns of *P. marinus* intensity were more variable than patterns of prevalence (Fig. 4b, Table 6). Ordinal regression indicated that of the 3 sites with significant differences between tidal heights, 1 had the highest intensity in the subtidal and 2 had the highest intensity in the intertidal (Fig. 4b). Similar to our experiments, evaluation of moderate to heavy intensity infections indicated no consistent pattern in intensity among tidal heights (Table 4).

Table 6. Results of logistic and ordinal regression models analyzing *Perkinsus marinus* infection prevalence and intensity in field surveys. Separate models were run for each survey site including tidal height as a fixed effect. Direction indicates whether *P. marinus* infection was higher or lower in the subtidal compared to intertidal heights. Only significant predictors ($p \leq 0.05$) are reported; all other models yielded non-significant trends ($0.05 \leq p \leq 0.10$) or non-significant results

Site	Source	Estimate	SE	p(*z*)	Direction
Prevalence					
VA1	Mid vs. Sub	1.323	0.566	0.02	Mid > Sub
NC1	High vs. Sub	1.891	0.712	0.01	High > Sub
Intensity					
VA3	High vs. Sub	−1.263	0.543	0.02	Mid < Sub
VA4	Mid vs. Sub	−1.026	0.438	0.02	Mid > Sub
VA5	Mid vs. Sub	0.890	0.433	0.04	High > Sub

DISCUSSION

Physical and biological factors change substantially along the intertidal to subtidal exposure gradient. The abundance and distribution of many species are altered from the high intertidal peak of the exposure gradient, where physical factors tend to be the most extreme, to the subtidal bottom, where biological factors often have a more influential role (Menge 1976). Species living along this gradient are subject to spatial variation in the severity of temperature fluctuations, desiccation, extreme seasonal conditions such as ice, and interspecific interactions including herbivory, competition, and parasitism. These physical and biological patterns, in combination with the varied tidal distribution of *Crassostrea virginica* in Chesapeake Bay led us to ask whether tidal exposure gradients drive spatial variation of *Perkinsus marinus* infections in *C. virginica*.

We hypothesized that we would find lower prevalence and intensity of *P. marinus* infections towards the peak of the gradient compared to the subtidal, as *P. marinus* growth and proliferation have been shown to slow under simulated conditions characteristic of intertidal habitats (Milardo 2001). However, across the 3 different experimental approaches employed, we did not find a consistent relationship between position along the tidal exposure gradient and the prevalence or intensity of *P. marinus* infections. The weak pattern that we found for prevalence, although far from conclusive, was in the opposite direction than we had initially predicted. Data from our field surveys suggested that high intertidal oysters may be more susceptible to acquiring *P. marinus* infections, as prevalence was higher in high intertidal than subtidal oysters in 7 of 7 sites sampled (overall pattern across sites, $p \leq 0.05$). However, this pattern was statistically significant at only 1 individual site. Our controlled exposure experiment results also suggested a pattern of higher prevalence in air-exposed oysters, with significantly higher prevalence in both air-exposure treatments than in the subtidal control for IU oysters and in the 1 h d^{-1} exposure treatment for II oysters (Table 5a).

Spatial patterns in *P. marinus* infection intensity were even more variable than patterns in prevalence. Our analyses of the effect of the number of days with large temperature fluctuations on the difference between mean intensity in the subtidal and high intertidal for moderate to heavy infections also suggested no strong effect of the exposure gradient on *P. marinus* intensity. Thus, our findings are in agreement with previous field studies (Burrell et al. 1984, O'Beirn et al. 1994, Ybanez 2007). However, we are left with the question of why, despite the substantial environmental differences along the exposure gradient, there is no consistent spatial variation of infection between intertidal and subtidal habitats.

Some effects of intertidal exposure would seem to make oysters more susceptible to *P. marinus* infection, while others could help protect against the parasite. Oysters experience internal hypoxia and hypercapnia during intertidal exposure at low tide. These conditions can lead to hemolymph acidification (Burnett 1997, Milardo 2001) and suppressed cell lysis by host hemocytes, potentially increasing host susceptibility to infection (Allen & Burnett 2008). In the laboratory, production of reactive oxygen intermediates (ROIs), an important oyster defense mechanism, can be reduced by 66% in *C. virginica* under hypoxic conditions (Boyd & Burnett 1999). This change in ROI production could translate into reduced resistance to infection when oysters experience internal hypoxia in the field. However, Keppel (2014) found that diel-cycling oxygen and pH conditions can stimulate some hemocyte responses in ways that can counter several of the documented negative effects of intertidal exposure. He also found that these conditions may provide mechanisms that increase the acquisition of *P. marinus* infections. Ultimately, intertidal oysters may be at a physiological disadvantage when challenged by *P. marinus*, though it is unclear what the net balance of these various factors is on hemocyte function and host immune response overall.

Air exposure also prevents oysters from filtering water and disposing of waste or undigested particles within the shell cavity. Upon consumption, nonmotile *P. marinus* trophozoites passively enter into oyster hemocytes through phagocytosis (Tasumi & Vasta 2007). Once ingested, trophozoites reduce some hemocyte functions, such as reactive oxygen species production (Hégaret et al. 2003, Tasumi & Vasta 2007), and utilize the hemocyte as a mode of locomotion throughout the host (Smolowitz 2013). Thus, periods of intertidal exposure may increase the chance of *P. marinus* trophozoites using oyster hemocytes as a means to proliferate within the host. This could potentially result in higher acquisition of *P. marinus* infections in intertidal oysters, leading to spatially differing infection prevalence along exposure gradients.

Subtidal oysters may not experience the same environmental challenges as intertidal oysters. However, when oysters are able to filter undisturbed in the subtidal, they are exposed to waterborne parasites and

pathogens for a greater proportion of the day. The net balance between parasite exposure risk and lower exposure to physical factors that can reduce immune response is unclear, but could influence the susceptibly of subtidal oysters to acquisition and intensification of *P. marinus* infections.

Three previous field studies have addressed the topic of *P. marinus* infections in adult oysters across a range of tidal exposures in various manners and have all the same result as the current study. Burrell et al. (1984) found differences in *P. marinus* infection between sites with differing salinity in South Carolina, and Ybanez (2007) found higher survival of intertidal oysters in the Gulf of Mexico, but none of the studies found an effect of tidal height on infection (O'Beirn et al. 1994). Between these studies and our own, the issue of spatial variation in *P. marinus* has been addressed across a wide spectrum of tidal ranges, water temperatures, and salinities, but there is no consistent evidence that tidal exposure gives either the host or the parasite an advantage over the other.

There are several possible explanations as to why field studies have not found the somewhat intuitive results of either higher parasite prevalence in intertidal compared to subtidal oysters, or the laboratory-suggested result of lower intensity of intertidal infections (Milardo 2001). First, numerous factors may favor either the host or the parasite. However, the net balance of these factors may shift across tidal heights and be influenced by a wide range of local factors. Factors such as host immune response and ambient parasite abundance may vary greatly between geographic areas and tidal heights. These factors combined with environmental conditions (i.e. temperature) that could favor the host, the parasite, or both, may be driving the spatial patterns of infection observed in the field.

A second possibility is that the conditions necessary to cause lower infection intensities in the intertidal (primarily large temperature fluctuations and high respiratory CO_2; Milardo 2001) may not occur often enough or for long enough periods of time for an effect to be seen in the field. According to our mimic data, there were only a handful of days in our study (the exact number differed by site; see Malek 2010) in which intertidal temperatures had fluctuations comparable to those that resulted in decreased *P. marinus* growth in the laboratory (Milardo 2001). Large temperature fluctuations over a longer time period may be required to produce consistent spatial differences in intensity that are measureable at the population level.

Parasite distributions are expanding and shifting in terrestrial and marine environments (Harvell et al. 1999, 2002, Ford & Chintala 2006, Bruno et al. 2007), and new environmental gradients may develop as a result of climate and other anthropogenically driven changes. Therefore, it is particularly important to better understand how parasites, and their relationship with environmental gradients, influence the spatial distribution and abundance of host species that are key ecosystem engineers. The effects of parasites on the abundance and distribution of species such as corals and oysters can have system-wide impacts. A solid comprehension of host–parasite interactions across existing environmental gradients may allow us to understand how future changes in the environment will influence host abundance and distribution, in turn helping to guide conservation, restoration, and fisheries management efforts.

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