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Predator effects on host–parasite interactions in the eastern oyster *Crassostrea virginica*

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ABSTRACT: Both parasitism and predation may strongly influence population dynamics and community structure separately or synergistically. Predator species can influence host–parasite interactions, either by preferentially feeding on infected (or uninfected) hosts — and thus altering parasite prevalence patterns — or by affecting host behavior in ways that increase host susceptibility to parasites. In this study, we tested if predators (the mud crab *Panopeus herbstii* and the blue crab *Callinectes sapidus*) influence interactions between the eastern oyster *Crassostrea virginica* and 2 of its most prevalent parasites, *Perkinsus marinus* and *Haplosporidium nelsoni*. Using a combination of field and laboratory experiments, we tested for predatory effects on the prevalence and intensity of parasite infections and on oyster immune response (phagocytic activity). Our results consistently demonstrated that crabs do not influence parasite infections in oysters at either individual or population levels. Thus, even though predators often have strong top-down direct and indirect effects on marine communities, we found their influence on host–parasite interactions to be minimal in this system.

KEY WORDS: Predator-mediated interactions · Healthy herd hypothesis · Predator spreaders · Behavioral modification

INTRODUCTION

Environmental factors such as temperature, salinity, and pollution shape host–parasite relationships by affecting hosts, parasites, and their interactions (e.g. Reisser & Forward 1991, Hoole 1997, Koprivnikar et al. 2007, Wolinska & King 2009, Studer et al. 2010, Marcogliese & Pietrock 2011, Studer & Poulin 2012). However, biological factors may also substantially affect these interactions (Grosholz 1992, Schmitz & Nudds 1994, Thieltges et al. 2008, Hall et al. 2009, Orlofske et al. 2012, Rohr et al. 2015). For example, it is well established that parasites can make hosts more vulnerable to predation and even competition (Hudson et al. 1992, Grosholz 1992, Lafferty & Morris 1996, Hudson & Greenman 1998, Joly & Messier 2004). Likewise, predation can shape prey abundance and demography (Hairston et al. 1960, Paine 1966, Connell 1970, Messier & Crête 1985), which potentially influences subsequent density- or size-dependent interaction of the prey with its parasites (Arneberg et al. 1998, Grutter & Poulin 1998).

Predators are capable of affecting host–parasite interactions in several ways. The 'healthy herd' hypothesis suggests that by preferentially removing infected hosts from a population, predators reduce parasite prevalence (the proportion of individuals infected in a population), transmission, and parasite epizootics (Packer et al. 2003, Hall et al. 2005), and consequently have positive effects on host populations. Alternatively, predators can increase parasite prevalence within a host population either through preferentially consuming uninfected prey or by acting as 'predator spreaders' that aid in parasite dispersal when infected hosts are consumed (Cáceres et al. 2009). Despite an increasing number of examples of these different predator effects, the outcomes on host–parasite dynamics are often context-dependent and remain poorly studied in marine systems (Laf-

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ferty 2004, Duffy et al. 2005, Johnson et al. 2006, Duffy 2007, Cáceres et al. 2009).

Predators can also influence prey–parasite interactions by affecting prey traits, such as their behavior, growth, and development, which in turn can affect the susceptibility of prey to infection (Decaestecker et al. 2002, Richards et al. 2010, Duffy et al. 2011, Stephenson et al. 2015). For example, predators may increase intraspecific interactions among prey species or cause hosts to spend more time in habitats with high parasite density (Decaestecker et al. 2002, Byers et al. 2015), both of which can increase parasite transmission (Stephenson et al. 2015). Shoaling behavior by female Trinidadian guppies in response to predators increases direct contact between individuals, leading to higher transmission of monogenean parasites (Richards et al. 2010). Additionally, predators can alter host feeding behavior, reducing their ability to grow, develop (Trussell et al. 2003), and perform other physiological functions (Rigby & Jokela 2000, Navarro et al. 2004). Changes in energy allocation in response to predators can suppress host immune responses and increase susceptibility to parasites (Rigby & Jokela 2000, Allen & Little 2011, Kerby et al. 2011, Janssens & Stoks 2013).

The eastern oyster *Crassostrea virginica* is an ecosystem engineer that builds biogenic reefs along the Atlantic and Gulf of Mexico coasts of the USA. The interstices of these reefs provide habitat for predators, including some that eat the oysters themselves and are capable of influencing prey abundance and traits (Grabowski 2004, Grabowski & Kimbro 2005, Newell et al. 2007, Grabowski et al. 2008, Johnson & Smee 2012). Grabowski (2004) and Grabowski & Kimbro (2005) found that the presence of toadfish *Opsanus tau* boosts juvenile oyster abundance by reducing predation by mud crabs (*Panopeus herbstii*). Additionally, direct exposure to various species of reef-dwelling mud crabs can elicit trait-based morphological responses such as altered resource allocation and differential shell strength in juvenile oysters (Newell et al. 2007, Johnson & Smee 2012).

Throughout their range, oysters are infected with 2 lethal parasites, *Perkinsus marinus* and *Haplosporidium nelsoni,* which can have devastating effects on oyster populations (Ford & Haskin 1982, Andrews 1984). Numerous physical factors have been identified as environmental drivers of infection patterns of these parasites (Keppel et al. 2015, authors' unpubl. data), with water temperature and salinity having the largest influence (Ford & Tripp 1996). In general, infections tend to intensify as temperature and salinity increase from winter into summer and the highest parasite-related mortality (and transmission of *P. marinus*) occurs in late summer/early fall when these physical conditions reach their peak (Ford & Tripp 1996). However, it is largely unknown whether biotic factors such as predators, either individually or interactively with abiotic factors, also influence these host–parasite interactions (White et al. 1987, Diamond 2012).

Many oyster predators function simultaneously as consumers of live oysters and scavengers of dead oyster tissue. Infective *P. marinus* hypnospores are released from dead, infected oyster tissue or excreted through pseudofeces (Bushek et al. 2002), but the specific transmission mechanism of *H. nelsoni* has not been identified (Burreson & Ford 2004). Once in the water column, *P. marinus* hypnospores can be consumed (filtered) by susceptible hosts (Ray 1954, Bushek et al. 2002). The transmission mechanisms of *P. marinus* may provide an avenue for oyster predators to affect parasite transmission between hosts. For example, Diamond (2012) observed in the laboratory that scavenger species (crabs, snails, fish) presented with shucked, infected oyster tissue increased the rate of *P. marinus* transmission to live oysters, presumably by suspending parasite spores into the water column that are then filtered by other oysters. If such predator effects on transmission translate to the field, predator influences on parasites may differ spatially based on local environmental conditions, such as salinity, that affect predator distributions (Menzel et al. 1966, White & Wilson 1996).

Predators may also affect oyster–parasite interactions indirectly via effects on oyster behavior, through reduction of water filtration if crabs induce oysters to filter less, which could decrease oyster exposure to waterborne parasites such as *P. marinus*. Alternatively, reduced filtration could lengthen the time that ingested particles remain in the host digestive tract and thus increase the probability that ingested parasites establish infection within oyster tissue. While closed (i.e. not filtering), oysters can experience hypoxic (low O_2) or hypercapnic (high CO_2) conditions within their shell cavity that cause a decrease in important immune defenses such as hemocyte activity and production of reactive oxygen intermediates (Boyd & Burnett 1999, Allen & Burnett 2008, Keppel 2014). Unfavorable conditions within the host (i.e. hypoxia and hypercapnia) or changes in energy allocation in response to indirect predator effects (Newell et al. 2007, Johnson & Smee 2012) could further suppress immune defenses in the oyster host, leading

to higher susceptibility to parasite infection. These proposed physiological mechanisms align with the general pattern reported in much of the parasite literature that higher stress in organisms often leads to enhanced parasite susceptibility (Lafferty & Kuris 1999, Lacoste et al. 2001).

To determine if predators affect oyster–parasite interactions, we performed a combination of field and laboratory studies using multiple predator species. We tested whether mud crabs *Panopeus herbstii* affect parasite prevalence, intensity, and transmission, and whether the crabs have indirect, non-lethal effects on oyster immune response (phagocytic activity) in the field. In the laboratory, we conducted both a prey choice test to determine if blue crabs *Callinectes sapidus* preferentially consume healthy or infected oysters, and a test to explore whether the non-lethal cues emitted by blue crabs influence the prevalence and intensity of *Perkinsus marinus* infections.

MATERIALS AND METHODS

Field experiment

To test for predator effects on parasite infections (*Perkinsus marinus* and *Haplosporidium nelsoni*) and host immune response in oysters under field conditions, we hand-collected large wild oyster clusters from Romerly Marsh Creek in the Wilmington River, Savannah, Georgia, USA (31° 55′ 21.78″ N, 80° 59′ 20.85″ W). We processed these larger clusters (combination of live oysters and dead shell) by breaking them into 200 to 400 g portions that were cleared of predators and excess shell. We then weighed the clusters, counted the number of oysters <25 mm, and measured oysters >25 mm from the umbo to the bill. Processed clusters were then randomly assigned to the experimental reefs described below.

We placed 15 of the processed oyster clusters in a Vexar-lined plastic milk crate (0.09 m^2) and sealed the tops with Vexar mesh lids to prevent immigration or emigration of predators. Lids were sewn on with nylon line to enable re-entry to crates throughout the experiment. At Romerly Marsh Creek we secured 16 crates flushed with sediment by anchoring each to an embedded cinder block. Experimental reefs were spaced 1 m apart along the creek bank in line with naturally occurring intertidal oyster reefs (~1.5 m above mean low water). We released mud crab predators within 8 randomly selected experimental reefs, and the remaining 8 reefs served as predator-free controls. We hand-collected mud crabs (carapace width range 20 to 40 mm, mean 28 mm) from oyster reefs adjacent to our deployment site and added them to the predator treatment cages at a density of 8 per 0.09 m^2 reef, a realistic density for southeastern US oyster reefs (McDonald 1982). To maintain treatment integrity, reefs were checked twice weekly to remove any predators (mainly immature mud crabs) that had entered control treatments and to replenish dead or missing mud crabs in predator treatments. Reefs were deployed for 17 wk in 2013 (June 21 to October 18) to cover the peaks in parasite prevalence and intensity that occur from late summer to early fall (Ford & Tripp 1996). At the conclusion of the experiment, we haphazardly selected 15 oysters from each reef (~1 per oyster cluster; shell height 68.7 ± 17.5 mm, mean ± SD) for simultaneous assessment of parasite infection and immune response.

We assessed *P. marinus* and *H. nelsoni* infections in oysters using PCR. Using sterile methods, we collected gill and mantle tissue from randomly selected oysters (35 to 125 mm) on each experimental reef after retrieval from the field and froze samples at −20°C until they were processed. Following the protocol developed by N. Stokes (pers. comm.) adapted from Gauthier et al. (2006), we used quantitative PCR (qPCR) to assess the probability and intensity of *P. marinus* infection. We determined the probability of *H. nelsoni* infection using common PCR (cPCR) adapting the methods described by Stokes et al. (1995) and Renault et al. (2000). Full methods for each protocol are reported in Supplement 1 at www.int-res.com/articles/suppl/m556p131_supp.pdf. Prior to initial deployment, we also sampled a subset of 48 oysters from the initial collection in June to determine the baseline parasite prevalence (*P. marinus* and *H. nelsoni*) and intensity (*P. marinus*) in our experimental population. Baseline data for *H. nelsoni* was collected with cPCR as described in Supplement 1; however, we used the Ray's fluid thioglycollate media (RFTM) method (see 'Prey choice' below) for *P. marinus*. We switched to the qPCR methodology for final measurements of *P. marinus* because it gave higher sensitivity to detect light infections, and thus also higher resolution on parasite incidence. Though the switch disallowed direct, formal comparisons between baseline and final prevalence and intensity for *P. marinus* infections, it did not affect any of the statistical analyses that were conducted on final values only (see below).

We used phagocytic activity of oyster hemocytes as a proxy for oyster immune response. We collected

hemolymph samples to assess phagocytic activity from each of the oysters tested for parasite infection. See Supplement 2 at www.int-res.com/articles/suppl/m556p131_supp.pdf for detailed methods (adapted from Goedken & DeGuise 2004, M. Levin pers. comm.). Briefly, we incubated hemolymph with fluorescent latex beads and analyzed the samples with a FACSCalibur flow cytometer to detect the total number of hemocytes that phagocytized beads and numbers of hemocytes that consumed specific numbers of beads. From these data, we calculated the proportion of granular oyster hemocytes (granulocytes) that had high phagocytic activity, i.e. consumed ≥3 fluorescent beads (foreign cells), and the mean number of beads consumed by all granulocytes in a sample.

To test for effects of predators on infection, we analyzed the probability of infection for each parasite as a binary response (0 = not infected, 1 = infected) and ran a separate third analysis for co-infection with both parasites, again as a binary response (0 = not infected or infected with only 1 parasite, 1 = infected with both parasites), using 3 separate mixed effects logistic regression models ('lme4' package in R). Predator treatment was included as a fixed effect and replicate as a random effect to account for multiple oysters being sampled from the same experimental reef. The effect of predator treatment on the intensity of *P. marinus* infections (number of *P. marinus* DNA copies detected in a host based on qPCR amplification) was analyzed using a generalized linear mixed model (GLMM), with predator treatment as a fixed effect and replicate as a random effect. Infection intensities were $\log_{10}$ transformed to account for the high variance among individuals and normalize the data (Gotelli & Ellison 2004).

To determine whether mud crabs and *P. marinus* infection affected phagocytic activity of oyster hemocytes, we initially analyzed the proportion of highly active cells and the mean number of beads consumed by granulocytes within individual oysters using 2 separate GLMMs that included predator treatment, *P. marinus* infection status (infected or not), and intensity of *P. marinus* infection as fixed effects and replicate as a random effect. Because infection status and intensity are potentially correlated, we ran model competitions and chose the most parsimonious models based on Akaike weights. To meet the assumptions of normality, we arcsine square root transformed the proportion of highly active cells, log (ln) transformed the mean number of beads consumed, and $\log_{10}$ transformed infection intensity data (Gotelli & Ellison 2004).

Laboratory experiments

Prey choice

To test if predators preferentially select oysters based on *P. marinus* infection status, we ran a series of prey choice trials using *Callinectes sapidus* in summer 2015. We focused on *P. marinus* in this and the following laboratory experiment as this parasite tends to be more prevalent in Georgia than *H. nelsoni* and we were able to assess both the prevalence and intensity of *P. marinus* infections (compared to only the prevalence of infection for *H. nelsoni*). Wild blue crabs and oysters were collected in Savannah, Georgia. A dozen additional crabs were purchased from a local Atlanta live seafood market that receives crabs from the Savannah area. All organisms were transported to the University of Georgia, Athens, and maintained in a 113.5 l mesocosm filled with artificial seawater (Instant Ocean aquarium salt dissolved in tap water, maintained at 24 psu and ~22°C). We aerated tanks with airstones and maintained an average dissolved oxygen of 4.0 to 6.0 mg l^{-1}. Crabs were held in separate cages and starved for a minimum of 2 d prior to use in choice trials.

We mixed 37.8 l of clean, aerated seawater in 113.5 l rectangular mesocosms and placed 2 oysters of approximately the same shell height 50 cm apart at one end of the tank (shell height range 45 to 60 mm, average 52.6 mm). A starved crab was released at the opposite end of the tank and monitored from an adjacent room via live-feed video (GoPro Hero 3 using GoPro app) until 1 oyster was selected. We defined selection as the crab visibly consuming tissue once it had cracked open the shell of an oyster. We immediately removed and dissected both oysters to assess the presence and intensity of *P. marinus* infection using the RFTM method (described below). If crabs did not commit to a specific oyster within 6 h, the trial was ended and oysters were removed from the tank. Crabs that did not consume an oyster after three 6 h trials were no longer used. We tested a total of 13 adult crabs of various size (carapace length ≥100 mm) and sex. Of these, only 6 individuals selected oysters and were included in the analyses.

We assessed oysters used in the experiment for *P. marinus* using the RFTM method (Ray 1954). Gill, mantle, and rectal tissue were collected from experimental oysters and incubated in RFTM in the dark at 28°C for 6 d. We then stained tissue samples with Lugol's iodine and viewed them microscopically for the presence of *P. marinus* hypnospores which stain blue-black. Intensity of infection was scored based

on the 6 point Mackin scale (Mackin 1962), ranging from light (0.5 to 1) to heavy (4 to 5) infections.

Of 79 total trials in which an oyster was selected, only 24 trials contained 1 infected and 1 uninfected oyster (1:1) that presented the crab with a 'choice' based on *P. marinus* infection status. Of the 6 crabs that were presented with a 1:1 choice, only 3 crabs had more than 2 successful 1:1 trials. Binomial tests indicated that the proportions of infected to uninfected oysters consumed by each of these 3 crabs were equal ($p \geq 0.05$) so we assumed homogeneity across the 6 crabs and included all of their 1:1 trials in our analysis (n = 24). Using a chi-square analysis, we tested if crabs exhibited a preference for oysters based on their *P. marinus* infection status. We also analyzed if there was a difference in the amount of time taken by a crab to select its prey as a function of the selected oyster's infection status using a generalized linear model (GLM). Lastly, for the 55 trials in which a crab was presented with 2 oysters of the same infection status, we analyzed if the time to selection differed when faced with 2 infected or 2 uninfected oysters. Again, we used a GLM including time as our response variable and the infection status of both oysters as our fixed effect.

Non-lethal predator effects

We conducted another mesocosm study using blue crabs in summer 2015 to test if a large predator that tends to consume many oysters and which provides strong physical cues exerts non-lethal effects on oysters that affect the prevalence and intensity of *P. marinus* infections. Study organisms were collected at the same time and site, and handled in the same manner as those used in the prey choice experiment. However, instead of individual oysters, we collected large oyster clusters. In the lab, we cleaned the clusters, removed dead shell, and broke them into ~200 to 400 g clusters that were stored in artificial seawater. Oysters were fed daily with a mixture of Shellfish Diet 1800 diluted in Milli-Q water (5:1200 ml for ~8×10^6 cells ml^{-1}) and we conducted water changes once a week for all experimental and holding tanks to limit nutrient and waste accumulation.

Three experimental treatments were used to test for indirect effects: an oyster-only control, a chemical cue treatment, and a combined chemical and tactile cue treatment. In each of twelve 113.5 l tanks (n = 4 tanks per treatment) we built a focal oyster reef in one half using twelve 200 to 400 g oyster clusters. We sacrificed 12 oysters from each experimental tank (1 per oyster cluster) prior to applying experimental treatments to get a baseline measure of the probability and intensity of *P. marinus* infection. A large wire mesh predator cage containing 5 additional oyster clusters was placed in the other half of the tank where the treatment factor was manipulated. The chemical cue treatment contained an adult *C. sapidus* confined in the predator cage that could spread cues of crushed food oysters and *P. marinus* spores to focal oysters as it ate. The combined chemical and tactile cue treatment also contained this confined crab, but additionally included a juvenile *C. sapidus* with its claws wrapped shut (Gorilla tape) on the focal oyster side of the tank to provide non-lethal tactile stimulus to focal oysters. Clusters in the predator cage were replaced during weekly water changes in all experimental tanks to ensure a continuous supply of food and opportunity for *P. marinus* exposure (as *P. marinus* released into the water would be removed during water changes). The oyster-only control treatment contained no predators. No crab mortality occurred during the course of the experiment and after 5 wk, we sacrificed 36 oysters per tank (3 per cluster) for parasite infection assessment (n ~144 per treatment). We assessed the probability and intensity of *P. marinus* infections using the previously described RFTM method.

Due to the initial heterogeneity in *P. marinus* infection prevalence between treatment tanks (proportion infected ranged from 0 to 0.33) detected through baseline sampling, we used the change in prevalence for each experimental tank as our response variable. We analyzed the change in prevalence with a GLM including predator treatment as a fixed effect. We did not adjust for initial infection intensity because initial prevalence was often low (1 to 2 oysters), which prevented us from being able to reliably estimate tank-specific intensities. We analyzed final intensity of individual oysters with a mixed effects Poisson regression model using predator treatment as a fixed effect and replicate as a random effect. All analyses were run in R version 3.2.0 (R Core Team 2015).

RESULTS

Field experiment

Mud crabs did not significantly affect the probability of infection by either *Perkinsus marinus* or *Haplosporidium nelsoni*, or the probability of co-infection by both parasites (Table 1, Fig. 1). As previously mentioned, we were unable to compare the initial

and final probability of *P. marinus* infection due to a change in assessment methods. However, using a comparison of RFTM and qPCR data from another study (J. C. Malek unpubl. data), we can infer that the proportion of infections detected with qPCR is ~25% higher than RFTM. Thus, in the current study, the proportion of infections detected by RFTM (0.22) would approximately translate to a baseline proportion of 0.27, which suggests that the number of oysters infected with *P. marinus* increased by ~325% over the course of the study. The proportion of oysters infected by *H. nelsoni* increased by ~250% from the initial population baseline (Fig. 1). The average final intensity of *P. marinus* infections (as measured by *P. marinus* DNA copies) was ~60% higher on control reefs than those with mud crab predators; however among-individual variation was very large, thus this difference in intensity was not statistically significant (Table 2, Fig. 2).

Mud crabs also did not affect phagocytic activity of oyster hemocytes. Although our model competitions indicated that the models including predator treatment and *P. marinus* infection status were the most parsimonious for both the number of highly active

Table 1. Effects of mud crabs on the presence of parasite infections in oysters in the field experiment. Results of mixed effects logistic regression analysis of predator treatment are shown for (a) probability of *Perkinsus marinus* infection, (b) probability of *Haplosporidium nelsoni* infection, and (c) probability of co-infection, with replicate included as a random effect. The reference predator treatment for analyses was 'no predator', with negative estimates indicating a decrease in the probability of infection with the addition of predators

(a) *P. marinus* prevalence by predator treatment				
Fixed effect	**Estimate**	**SE**	***z*-value**	**Pr(*z*)**
Predator treatment	−0.168	0.4129	−0.407	0.684
Random effect	**Variance**	**SD**		
Replicate	1.662	1.289		
(b) *H. nelsoni* prevalence by predator treatment				
Fixed effect	**Estimate**	**SE**	***z*-value**	**Pr(*z*)**
Predator treatment	−0.161	0.329	−0.489	0.625
Random effect	**Variance**	**SD**		
Replicate	1.68	0.410		
(c) Co-infection by predator treatment				
Fixed effect	**Estimate**	**SE**	***z*-value**	**Pr(*z*)**
Predator treatment	−0.120	0.344	−0.350	0.727
Random effect	**Variance**	**SD**		
Replicate	0.338	0.582		

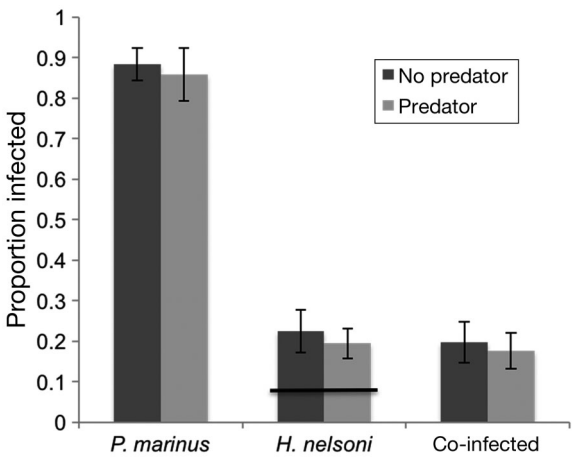

Fig. 1. Mean final proportion of *Perkinsus marinus* and *Haplosporidium nelsoni* infections in oysters and co-infected oysters by predator treatment (presence or absence of mud crabs) in the field experiment (n ~ 120 for each treatment). Error bars represent standard error calculated across replicates for each treatment. The solid black line represents the baseline proportion of oysters infected with *H. nelsoni*. Baseline data for *P. marinus* (and thus co-infection) was measured using the Ray's fluid thioglycollate media (RFTM) method. Because final infection data status was measured using qPCR, baseline and final data are not directly comparable

Table 2. Effects of mud crabs on the intensity of parasite infections in oysters in the field experiment. Results of GLMM analysis of *Perkinsus marinus* infection intensity by predator treatment with replicate as a random effect. The reference predator treatment for analyses was 'no predator', with negative estimates indicating a decrease in the intensity of *P. marinus* infection with the addition of predators

***P. marinus* intensity by predator treatment**				
Fixed effect	**Estimate**	**SE**	***t*-value**	**Pr(*t*)**
Predator treatment	−0.139	0.211	−0.657	0.511
Random effect	**Variance**	**SD**		
Replicate	0.245	0.495		

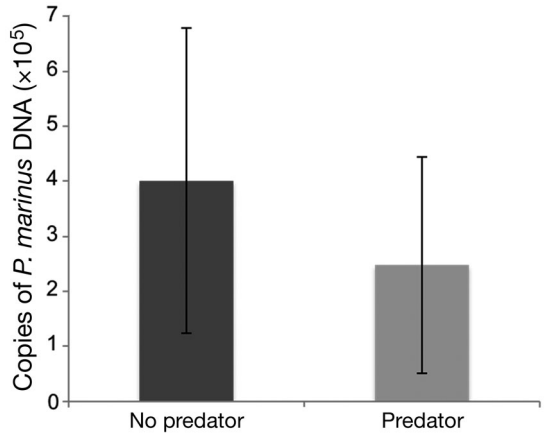

Fig. 2. Mean *Perkinsus marinus* infection intensity in oysters by predator treatment (presence or absence of mud crabs) as measured by qPCR (indexed by the number of copies of *P. marinus* DNA detected within a host sample) in the field experiment (n = 99 for 'no predator' treatment, n = 100 for 'predator' treatment). Error bars represent SE calculated using replicate for each treatment

cells (Akaike weight + [w_i] = 0.85) and the mean number of beads consumed (w_i = 0.996), neither of the independent variables were significant (Table 3). Both the proportion of highly active granulocytes (i.e. those that consumed ≥3 beads) and the mean number of beads consumed by granulocytes were similar between the control (proportion 0.34 ± 0.01, mean ± SE; number of beads 109 822 ± 1524) and predator (proportion 0.34 ± 0.01; number of beads 111 748 ± 1470) reefs (Table 3).

Laboratory experiments

Prey choice

Blue crabs did not have a preference for oysters based on *P. marinus* infection status (Table 4; χ^2 = 0.667, df = 1, p = 0.414). The time to selection did not differ based on infection status when crabs were presented with 1 infected and 1 uninfected oyster (GLM estimate 1.933, SE = 17.119, p = 0.910; average time to selection [±1 SE]: infected: 40.3 ± 11.6 min, n = 14, uninfected: 38.4 ± 12.5 min, n = 10). Also, the time to selection when presented with 2 oysters of the same infection status did not differ between infected and uninfected oysters (GLM estimate 2.100, SE = 13.774, p = 0.879; average time to selection ± 1 SE: infected: 41.6 ± 4.1 min, n = 5, uninfected: 39.5 ± 15.2 min, n = 50).

Table 3. Effects of mud crabs on phagocytic activity in oysters in the field experiment. Results of GLMM analysis of phagocytic activity by predator treatment, and *Perkinsus marinus* infection status for (a) the proportion of highly active hemocytes (i.e. those that consumed ≥3 beads) and (b) the mean number of beads consumed by the whole hemocyte population. The reference predator treatment for analyses was 'no predator', with negative estimates indicating a decrease in phagocytic activity with the addition of predators. The reference *P. marinus* infection status was uninfected. Thus, negative estimates indicated a decrease in phagocytic activity in the presence of infection

(a) Proportion of highly active cells				
Fixed effect	**Estimate**	**SE**	***t*-value**	**Pr(*t*)**
Predator treatment	<–0.001	0.046	–0.008	0.994
P. marinus infection status	–0.009	0.009	–1.008	0.313
Random effect	**Variance**	**SD**		
Replicate	<0.008	0.091		
(b) Mean number of beads consumed				
Fixed effect	**Estimate**	**SE**	***t*-value**	**Pr(*t*)**
Predator treatment	0.006	0.027	0.207	0.836
P. marinus infection status	0.002	0.009	0.217	0.828
Random effect	**Variance**	**SD**		
Replicate	0.003	0.052		

Non-lethal predator effects

We found that blue crabs had no effect on either the change in *P. marinus* infection prevalence or final infection intensity (Tables 5 & 6). The change in

Table 4. Results of laboratory prey choice experiments with blue crabs based on *Perkinsus marinus* infection status of oysters. Only crabs that had at least 1 successful trial where they were presented with 1 oyster infected by *P. marinus* and 1 uninfected oyster (1:1 trials) were included in statistical analyses

Crab ID	Number of trials where consumed oyster was:		Proportion infected chosen
	Infected	Uninfected	
A	6	4	0.60
B	3	3	0.50
C	2	1	0.67
D	1	1	0.5
E	0	1	0
F	2	0	1

Table 5. Non-lethal effects of blue crabs on the change in parasite infection prevalence in oysters in the laboratory experiment. Results of GLM analysis of the change in *Perkinsus marinus* prevalence (final minus initial) with predator treatment as a fixed effect. The reference treatment for analysis was the control treatment, with negative estimates indicating a decrease in the change in *P. marinus* infection prevalence with the addition of chemical and then chemical plus tactile predator cues

Difference in *P. marinus* infection prevalence				
Full model	**df**	**MS**	***F***	**Pr(*F*)**
Predator treatment	2	0.001	0.080	0.924
Fixed effect	**Estimate**	**SE**	***t*-value**	**Pr(*t*)**
Control vs. chemical cue	–0.020	0.80	–0.256	0.804
Control vs. chemical + tactile cue	–0.031	0.080	–0.393	0.703

Table 6. Non-lethal effects of blue crabs on parasite infection intensity in oysters in the laboratory experiment. Results of mixed effects Poisson regression of *Perkinsus marinus* infection intensity with predator treatment as a fixed effect and replicate as a random effect. The reference treatment for analysis was the control treatment, with negative estimates indicating a decrease in *P. marinus* infection intensity with the addition of chemical and then chemical plus tactile predator cues

***P. marinus* infection intensity**				
Full model	**df**	**χ^2**	**Pr(χ^2)**	
Predator treatment	2	0.172	0.918	
Fixed effect	**Estimate**	**SE**	***z*-value**	**Pr(*z*)**
Control vs. chemical cue	0.039	0.130	0.298	0.766
Control vs. chemical + tactile cue	0.055	0.134	0.410	0.682
Random effect	**Variance**	**SD**		
Replicate	<0.001	<0.001		

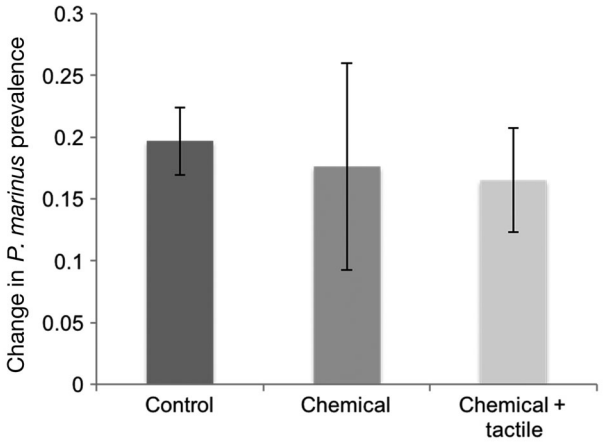

Fig. 3. Mean change in *Perkinsus marinus* prevalence in oysters within each replicate tank (final less initial) by non-consumptive predator treatment (control, chemical cue, chemical plus tactile cue) in the laboratory experiment (n = 4 for each treatment, with 36 oysters per replicate). Error bars represent SE calculated across replicate for each treatment

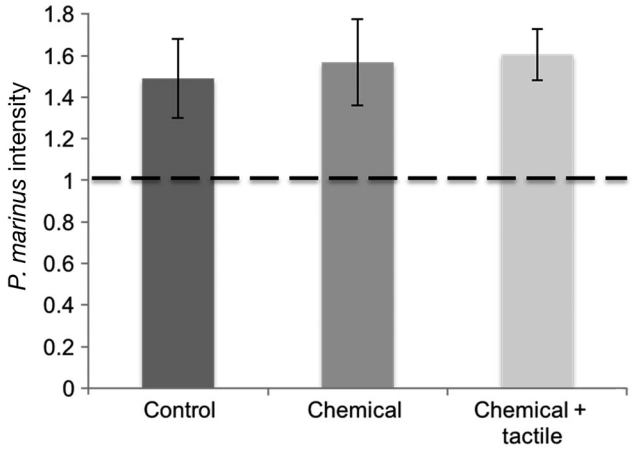

Fig. 4. Mean final *Perkinsus marinus* infection intensity in oysters within each replicate tank by predator treatment in the laboratory experiment (n = 40 for control, n = 64 for chemical cue treatment, n = 53 for chemical plus tactile cue treatment; only infected oysters were used in analysis). Error bars represent SE calculated across replicate for each treatment. The black dashed bar represents the baseline average intensity

prevalence (final vs. initial) was not different across all treatments (Fig. 3). Final mean infection intensities were similar across all treatments, falling between Mackin scores of 1 to 2 (Fig. 4), which are considered light to moderate infections (Mackin 1962).

DISCUSSION

Predators can have multiple effects on host–parasite interactions through changes in host abundance or traits (Werner & Peacor 2003, Pressier et al. 2005, Hall et al. 2009). Such effects of predators have been documented for oysters and other associated reef species (Grabowski 2004, Grabowski & Kimbro 2005, Grabowski et al. 2008, Johnson & Smee 2012). However, we found that the effects of crab predators on oysters do not extend to affect oysters' interactions with their parasites *Perkinsus marinus* and *Haplosporidium nelsoni*. Our combination of 3 field and laboratory experiments using multiple crab species suggests that predators do not shape individual or population level responses of hosts to parasites in this system.

We expected that mud and blue crabs could alter parasite prevalence or intensity if they differentially consume infected prey (Packer et al. 2003) or increased parasite transmission (Cáceres et al. 2009, Diamond 2012). Oyster mortality from predators was visually observed on our predator reefs in the field and in the predator cages in our laboratory experiment, indicating that crabs did consume oysters. However, the prey choice trials confirmed that blue crabs do not preferentially feed on oysters based on *P. marinus* infection status, and therefore do not promote a healthy herd or increase prevalence through selection of uninfected individuals. Our findings from the field showed no net difference in infection prevalence or intensity in the presence of predators, suggesting that mud crabs, similar to blue crabs, do not have an overall net effect on parasite prevalence, nor do they increase parasite transmission when they consume infected individuals and thus do not act as 'predator spreaders'.

Predators can indirectly affect hosts through changes in their behavior. As a sessile species, the primary behavior of oysters is filter feeding. Predators could hypothetically influence the filtration rate of oysters and the timing of when they feed. If mud crabs do reduce oyster feeding, we would expect that oysters exposed to mud crabs likely would have different *P. marinus* infection intensity or prevalence. However, we saw no indication of such effects on parasite infection in the field, suggesting that mud crabs do not affect the filter feeding behavior of oysters in a way that reduces or increases their exposure to parasites. This result prompted us to use larger blue crabs in the laboratory where oysters would be more inundated by non-lethal cues in a controlled environment, but we also saw no effects of blue crabs on oyster–parasite interactions. Our results corroborate studies by Byers et al. (2014) and Dodd (2015) who found that both mud and blue crabs

did not significantly affect chlorophyll *a* drawdown by oysters. In combination, these studies and our own suggest that, even with intensified exposure to non-lethal cues under controlled laboratory conditions, crab predators do not affect net oyster filtration in a manner that meaningfully affects host exposure to parasites.

As we recognize the ubiquity of parasites throughout natural systems, it is important for us to identify what factors influence how parasites interact with their hosts. There is growing empirical and theoretical evidence indicating that predators can significantly affect host–parasite interactions (Packer et al. 2003, Duffy et al. 2005, 2011, Hall et al. 2005) and it has even been observed that predator effects can result in parasite-driven trophic cascades that influence entire communities (Duffy 2007). However, we found that, in a coastal estuarine system that has well-documented examples of strong predator effects across a range of species, 2 prominent predators do not affect host–parasite interactions between the abundant host and 2 of its most lethal parasites. Although other abiotic environmental factors like salinity have been shown to affect oyster–parasite interactions, and other micropredators may play a role in parasite transmission (White et al. 1987), crab predation does not appear to play an important role in shaping these host–parasite interactions. Thus, our findings suggest that biotic factors may not play as large a role in shaping host–parasite dynamics in this system as abiotic factors. To our knowledge, this is one of the first multifaceted studies to examine predatory effects on host–parasite interactions in an ecosystem engineer in marine systems. We propose that examining the degree to which trophic interactions affect parasites is part of a larger important trend to recognize disease dynamics from a more holistic, community perspective.

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