COMMUNITY INTERACTIONS AND ENVIRONMENTAL DRIVERS OF HOST-PARASITE DYNAMICS IN AN ESTUARINE SYSTEM

by

ALYSSA-LOIS MADDEN GEHMAN

(Under the Direction of James E. Byers)

ABSTRACT

Not all hosts, communities or environments are equally hospitable for parasites. Direct and indirect interactions between parasites and their predators, competitors and the environment can influence variability in host exposure, susceptibility and subsequent infection, and these influences may vary across spatial scales. I evaluate abiotic and biotic drivers of parasite abundance and host-parasite dynamics utilizing the parasite *Loxothylacus panopaei*, which infects an oyster reef dwelling mud crab *Eurypanopeus depressus*. I conducted a survey of host, parasite, and predator abundance, parasite prevalence, and environmental characteristics of oyster reef communities from Florida to North Carolina. I found that water depth, predators and host characteristics were all positively correlated with the probability of infection within a reef. I further investigated whether the predatory crab *Callinectes sapidus* and other predators preferentially feed on *E. depressus* infected with *L. panopaei* and evaluated a mechanism behind prey choice. I

evaluated prey choice through mesocoms experiments, field tethering experiments and behavioral trials. I found that *C. sapidus* preferentially consumed infected *E. depressus* in the lab and this pattern was confirmed in the field. Contrary to expectations, I found that infected crabs ran faster in behavioral trials than uninfected *E. depressus*. Finally, I evaluated the influence of temperature on host and parasite survival and parasite reproduction. I quantified thermal response curves encompassing the thermal breadth of both host and parasite, and found a thermal mismatch in survival optima between infected and uninfected hosts. I then parameterized a physiologically based epidemiological model to predict the sensitivity of a host-parasite system to seasonal varying temperature and future climate change scenarios. I found that the model accurately recreates annual cycles and seasonality in this host-parasite system, and that the parasite is locally extirpated from the system under a 3ºC warming scenario. Together, these findings demonstrate the importance of community interactions and environmental drivers in driving the abundance and prevalence of *L. panopaei* infection in *E. depressus* at a regional, local scale and under climate warming scenarios.

INDEX WORDS: Disease Ecology, Marine Invertebrates, Host-Parasite Interactions, Predation, Climate Change, Rhizocephalans, *Loxothylacus panopaei*, *Eurypanopeus depressus*

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DEDICATION

I dedicate this dissertation to my grandparents:

Stephen and Lois Madden

and

Helen and Earl Gehman

Your hard work, dedication, joy and care for the world and the people in it remain an inspiration.

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CHAPTER 1

INTRODUCTION

Sensitive to heat

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Parasites exist within free-living organisms in our ecosystems, and by some estimates there are as many as four parasites per free-living host (Windsor 1998). Regardless of their exact relative abundance, parasites are increasingly recognized as influential members of ecological communities, contributing to community interactions and biodiversity (Wood and Johnson 2015). Parasite have a negative direct effect on their hosts by definition, and at the population level some parasite are able to regulate host dynamics (Dobson and Hudson 1992). At the community level, parasites can alter community composition through alteration of host feeding rates (Wood et al. 2007). At the ecosystem level parasite add to the complexity of foodwebs (Hechinger et al. 2011), and can make up a substantial proportion of the biomass within an ecosystem (Kuris et al. 2008).

While most free-living species are associated with at least one parasite, not all individuals within a species, or habitats within the species range will have parasites. As

with free-living organisms, we are finding that abiotic and biotic variables can drive the abundance of parasites within the environment (e.g. (Byers et al. 2013, Altman and Byers 2014)). First and foremost, for an obligate parasite to be found at a location, the host itself must be present – so often host abundance and density are strong drivers of parasite variability at larger scales (Chapter 2; Wilson et al. 1991). Abiotic factors such as temperature, pH or salinity may directly effect parasites through increased parasite reproduction or survival (Chapter 4; Lei and Poulin 2011, Araujo et al. 2015, Harland et al. 2015), or may indirectly affect parasites through alteration of host susceptibility (Parry and Pipe 2004, Harland et al. 2015). Finally parasites exist in their hosts within the context of their community, and predators and competitors can either enhance (Cáceres et al. 2009) or drive down (Chapter 3; Packer et al. 2003) parasite abundance.

Of particular interest is how parasite and their hosts will respond to the changing environment. Climate change will have many, varying effects on host-parasite interactions. A large body of research has suggested that in many cases, parasite and disease prevalence may increase as the climate warms (Porter et al. 1989, Harvell et al. 1999, Poulin 2006, Keesing et al. 2010, Levi et al. 2015, Araujo et al. 2015). Contradictorily, some parasites are actually more sensitive to higher temperatures and may be driven out of their host populations by increased temperatures (Chapter 4; Blanford and Thomas 1999, Blanford et al. 2003). Temperature is particularly important when considering parasites of ectothermic hosts. At the extreme, grasshoppers behaviorally manipulates body temperature to induce an 'environmental fever' to combat infection (Carruthers et al. 1992).

Organisms within ecological communities are being exposed to unprecedented changes in their abiotic environment (Karl et al. 2009, Doney et al. 2012). With a rapidly changing climate, investigation of how individual organisms are affected by temperature and how this will in turn affect the rest of the community is vital (Walther et al. 2009). Parasites have intimate interactions with their hosts, and interact with the community around them indirectly by modulating host response to climate and community interactions (Rohr et al. 2011). The collective body of this dissertation seeks to evaluate abiotic and biotic drivers of parasite abundance utilizing the parasite *Loxothylacus panopaei*, which infects an oyster reef dwelling mud crab *Eurypanopeus depressus*.

The four chapters of my dissertation are as follows:

Chapter 2: Predators, environment and host characteristics influence the probability of infection by an invasive parasite

This chapter evaluates large-scale abiotic and biotic drivers of parasite abundance. The methods consist of a predator survey for fish and the blue crab *Callinectes sapidus*, followed by an invertebrate survey quantifying *E. depressus*, *L. panopaei* infections and the habitat and competitors conducted from Florida to North Carolina in July and August, 2010. I demonstrate that at a regional scale there was a positive correlation between predators, including both fish and the blue crab *Callinectes sapidus* and probability of infection by *L. panopaei*, as is expected when looking at the relationship between mobile predators and less mobile prey. Host characteristics also were influential; infection prevalence increased with host density, but with an apparent limit around 35%, and infection increased with host size. Finally, the water depth over the reef (a potential proxy for recruitment) was also a positive predictor of infection.

Chapter 3: Non-native parasite enhances susceptibility of host to native predators

This chapter follows on results from the previous chapter and evaluates a local-scale mechanistic predator-host-parasite interaction. I conducted mesocosm studies in 2012, behavior studies in 2013 and field tethering studies conducted in 2014. At the local scale I found that *E. depressus* infected with *L. panopaei* were preferentially consumed, both in the mesocosms and in the field. Contrary to predictions, I found that *E. depressus* infected with *L. panopaei* ran faster in behavioral trials, suggesting that potentially fast movement may increase prey vulnerability in some way.

Chapter 4: Increased temperatures differentially impact parasites, freeing host from infection.

These chapter consists of two complimentary studies, and thus have been combined to evaluate the effect of temperature on host parasite dynamics. I use a combination of lab experiments conducted in 2014 and a stage-structured model designed for *L. panopaei* developed in 2015 to explore the effects of seasonality and climate change on host-parasite interactions. I demonstrate that while the thermal performance curve for survival of uninfected *E. depressus* is left-skewed, following expectations for an ectotherm, two different infection stages, the exposed virgin externa and infectious hosts have right-skewed survival and reproduction across temperature. This offset in thermal performance between the uninfected and infected host leads to seasonally driven variance parasite prevalence, characterized by a rising prevalence in witner and spring and by a falling prevalence in summer. When evaluated under simulated climate change scenarios, a simple increase in mean temperature by 3ºC is enough to remove the parasite from the population.

In sum, this dissertation demonstrates the importance of community interactions and environmental drivers in driving the abundance of *L. panopaei* infection in *E. depressus* at both a regional and local scale.

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CHAPTER 2

PREDATORS, ENVIRONMENT AND HOST CHARACTERISTICS INFLUENCE THE PROBABILITY OF INFECTION BY AN INVASIVE CASTRATING PARASITE[1]

[1] Gehman, A.M., J.H. Grabowski, A.R. Hughes, D.L. Kimbro, M.F. Piehler, and J.E. Byers. Submitted to *Oecologia*.

Abstract

Not all hosts, communities or environments are equally hospitable for parasites. Direct and indirect interactions between parasites and their predators, competitors and the environment can influence variability in host exposure, susceptibility and subsequent infection, and these influences may vary across spatial scales. To determine the relative influences of abiotic, biotic and host characteristics on probability of infection across both local and estuary scales, we surveyed the oyster reef-dwelling mud crab *Eurypanopeus depressus* and it's parasite *Loxothylacus panopaei*, an invasive castrating rhizocephalan, in a hierarchical design across >900 km of the southeastern USA. We quantified the density of hosts, predators of the parasite and host, the host's oyster reef habitat, and environmental variables that might affect the parasite either directly or indirectly on oyster reefs within 10 estuaries throughout this biogeographic range. Our analyses revealed that both between and within estuary-scale variation and host characteristics influenced *L. panopaei* prevalence. Several additional biotic and abiotic factors were positive predictors of infection, including predator abundance and the depth of water inundation over reefs at high tide. We demonstrate that in addition to host characteristics, biotic and abiotic community-level variables both serve as large-scale indicators of parasite dynamics.

Keywords: Parasite, Parasitic Castrators, Latitudinal Gradients, Infection Probability, Crustacea,

Introduction

Like most free-living organisms, parasites vary in abundance over space and time. Unlike free-living species though, parasites require the presence of a competent host,

increasing the number of external drivers that can affect variability in their abundance patterns. Hosts might vary in abundance, distribution and susceptibility, all of which affect the parasite's distribution and abundance (Hechinger and Lafferty 2005; Smith et al. 2007; Byers et al. 2008). Furthermore, even when competent hosts occur, not all communities or environments that are inhabited by hosts are equally habitable for parasites, since direct and indirect interactions between parasites and their predators, competitors, and the environment can influence the probability of a host population being infected (Pennings and Callaway 1996; Thieltges et al. 2009; Altman and Byers 2014). Here we aimed to evaluate determinants of parasite colonization and establishment that may operate at different scales.

A biological community can affect parasites either directly – during free-living life stages (Lafferty 2008; Johnson et al. 2010; Locke et al. 2014), or indirectly by influencing their hosts (Hudson et al. 1992; Wild et al. 2011). For example, predators can negatively impact a parasite through direct consumption of the free-living infective stages (Grutter 2002; Mouritsen and Poulin 2003; Lafferty 2008; Kaplan et al. 2009; Johnson et al. 2010). Additionally, the 'healthy herd' hypothesis suggests that predators can keep infection rates relatively low by selectively feeding on infected hosts (Packer et al. 2003; Hatcher et al. 2006). Predators may be an important factor that either enhances or reduces a host population's probability of infection.

The abiotic environment may also affect variability in parasite distribution. Environmental drivers can create "refuge" habitat at environmental extremes, where the host is able to survive and the parasite cannot (Li et al. 2010; Lei and Poulin 2011). For example, in marine systems low pH can reduce free-living parasite survival, resulting in

lower parasite diversity and richness (Marcogliese and Cone 1996), and many parasites are more vulnerable than their hosts to low salinity (Li et al. 2010; Lei and Poulin 2011; Studer and Poulin 2012). In addition, host habitat complexity and water movement might impact parasite distributions. For example, increased water flow may increase host exposure to water-borne parasites and pathogens, potentially increasing parasite recruitment and infection.

Broad-scale studies on parasite prevalence can be challenging, as many parasites are cryptic and infection can be difficult to detect. However, rhizocephalan barnacles that parasitize crustaceans are abundant, and their externally visible reproductive organ facilitates studies of rhizocephalan distributions across large geographic areas (Grosholz and Ruiz 1995; Alvarez et al. 2001; Chan et al. 2005; Sloan et al. 2010; Freeman et al. 2013; O'Shaughnessy et al. 2014). In several rhizocephalan systems, infection prevalence is quite variable between sites (Grosholz and Ruiz 1995; Alvarez et al. 2001; Chan et al. 2005; Sloan et al. 2010). Rhizocephalans have short larval durations (between 3 and 5 days) and there is some evidence that most larvae are locally dispersed (Grosholz and Ruiz 1995; Alvarez et al. 2001). While it has been hypothesized that variability in host susceptibility to infection can drive spatial variation in infection prevalence, there is currently limited data to support this hypothesis (Kruse et al. 2011, Grosholz 1995, Sloan 2010).

Loxothylacus panopaei is a castrating rhizocephalan barnacle parasite that infects the mud crab *Eurypanopeus depressus,* as well as several other mud crabs (Reinhard and Reischman 1958; Kruse et al. 2011). *Eurypanopeus depressus* is an abundant oyster reef-dwelling crab occurring in oyster reefs from the Gulf of Mexico to Massachusetts Bay.

Loxothyacus panopaei overlaps much of this range, but is native to the Gulf of Mexico and introduced to the US Atlantic coast from Long Island, New York to Cape Canaveral, Florida (Kruse and Hare 2007; Kruse et al. 2011; Freeman et al. 2013; Eash-Loucks et al. 2014; O'Shaughnessy et al. 2014). There are three genetic lineages of this parasite (Kruse et al. 2011), and *E. depressus* is infected by the ER lineage, which was first documented in North Carolina in 1983 and in Georgia and northeastern Florida in 2004/2005 (Kruse and Hare 2007; Eash-Loucks et al. 2014). Along the Atlantic coast, *E. depressus* is consumed by several fish species, as well as *Callinectes sapidus,* the commercially important blue crab. *Eurypanopeus depressus* utilizes the oyster reefs as a refuge from these highly mobile predators (Meyer 1994; Hulathduwa et al. 2011). While this is a relatively new invasion, all estuaries in the study are within a geographic range where the parasite had been documented for at least 5 years prior to the study. Prevalence of *L. panopaei* infections in *E. depressus* is spatially variable (Hines et al. 1997), making the parasite an excellent candidate for evaluating the potential influence of biotic and abiotic variables on its abundance.

Methods

To determine whether abiotic or biotic variables contribute to the probability of infection by *L. panopaei* in its invaded range, we conducted a detailed observational study. We conducted the survey within a single week at replicate estuaries across >900km of the South Atlantic Bight. We collected a suite of community-level variables, including the density of hosts and the occurrence of predators of both the parasite and host. Additionally, we collected environmental variables that we hypothesized would affect oyster habitat, including oyster density and vertical relief of oyster beds. Together,

we evaluated whether environmental attributes, host demographics or predators of the host and parasite influence the probability of finding *E. depressus* infected with *L. panopaei*.

Field survey. We selected five oyster reefs at each of 10 estuaries from Florida to North Carolina, creating a hierarchically structured design (Fig. 2.1; Kimbro et al. 2014). Reefs were selected to limit certain influential variables. All reefs were intertidal, located on tidal creek banks near the mouth of an estuary, near *Spartina alterniflora* and had a summer salinity around 25ppt (Byers et al. 2015). We placed permanent markers on each reef in a 3m x 3m intertidal sampling area to enable repeated measurements. We conducted invertebrate surveys at all sites during 7 to 13-August-2010. On each reef, a single 0.25 m^2 quadrat was placed mid-reef, in the center of the markers. All oysters, dead shell and sediments to a depth of 10 cm were excavated from inside each quadrat. Samples were brought back to the lab, rinsed and sieved with a 1 mm mesh, and all infauna (i. e. invertebrates that live within the oyster reef) were placed in 10% formaldehyde for storage. All mud crabs were identified to species and examined under a dissecting scope for an externa, the external reproductive organ of *L. panopaei*. Host crab carapace width was also measured. There are two visibly distinguishable stages of externa, a non-reproductive virgin externa and a reproductively mature externa. We counted only crabs with mature infections; to constrain our estimate of infection probability to infectious individuals, we only included *L. panopaei* infections that were developed to reproductive maturity. Although multiple infections on a single host are possible in this system (O'Shaughnessy et al. 2014), none were found in this survey,

potentially because multiple infections would likely result in mortality in August when high temperatures are common (Gehman, *unpublished data*).

We quantified predators of small mud crabs (such as *E. depressus*), including fish and the blue crab *Callinectes sapidus*, on each reef by setting un-baited crab, minnow, and fish traps one to two weeks directly before parasite collection during 25 to 30-July-2010. Fish identified as xanthid crab predators based on gut contents (Grabowski, *unpublished data*) were categorized as "fish predators". These include *Arius felis, Bagre marinus, Pogonias cromis, Sciaenops ocellatus, Pomatomus saltatrix, Opsanus tau, Leiostomus xanthurus, Orthopristis chrysoptera, Lagodon rhomboides* and *Lutjanus griseus.* All other captured fish were quantified collectively as a separate category of 'other fish' to isolate the effect of predatory fish from that of shared habitat between fish and oyster reef communities. If shared habitat drove correlations with *L. panopaei* then both the 'fish predators' and the 'other fish' should have similar effects.

Other important predictor variables we quantified included *Panopeus herbstii* density, a large reef-dwelling mud crab competitor and predator of *E. depressus,* which we quantified m^{-2} during the infaunal invertebrate surveys. *Crassostrea virginica* recruitment, the number of live *C. virginica* m^{-2} and the physical characteristics of the oyster reef were previously quantified as described in (Byers et al. 2015). Temperature is often an important abiotic controlling factor that we wanted to capture in our analyses. Although temperature was measured at each estuary, this occurred only after the invertebrate survey in the fall of 2010, and as such was not explicitly included in this analysis. Temperature negatively correlates with latitude among the estuaries included in

this study (Byers et al. 2015), so the effect of temperature is implicitly accounted for in our inclusion of an estuary-level blocking factor (see below).

Statistical analysis

Multicollinearity. Statistical analysis was conducted in R (R Development Core Team 2010). All variables collected were evaluated for multicollinearity (Online Resource 1). Any variables with a correlation coefficient greater then 0.70 were evaluated to determine biological relevance in relation to the response variable, and only the most relevant predictors were maintained in the model. There was a strong correlation between live oysters m^{-2} and oyster recruitment, so we kept only live oysters in our models. Water depth was correlated with several environmental variables, such as reef slope. As such, water depth was kept in the dataset and considered a proxy for general water movement over the reef.

Probability of Infection. We evaluated the relationship between reef biotic and abiotic predictor variables and *E. depressus* infection probability by fitting a binomial generalized mixed effects model. The parasite response variable was infection status, i.e. the number of *E. depressus* with and without an *L. panopaei* externa (1 and 0) within each quadrat on each reef. We included standardized host density, host size, number of *Callinectes sapidus*, *Panopeus herbstii* density, number of 'fish predators', number of 'other fish', vertical relief, and water depth as fixed variables, and infection status (0 or 1) as the response variable (package lme4). We evaluated an exhaustive suite of models and used AICc for model selection to create a candidate set of models (ΔAICc<2; package MuMIn; Burnham and Anderson 2002). We accounted for the hierarchy of our design (10 estuaries with 5 reefs each) by including estuary as a random effect. Any unmeasured

driver of variability between the estuaries, such as temperature, was captured in the estuary random term. We examined models that included reef as a random variable and estuary and reef nested within estuary as random variables in comparative model runs. When reef was included as random variable (either nested or not), its inclusion raised the AICc (Online Resource 3) and did not change the significant variables maintained in the top models (Online Resource 2). With the philosophy of presenting the most parsimonious model, we did not include reef in the final model.

We standardized each of the predictor variables using the *scale* function, which subtracts the mean for each variable and then divides by the standard deviation. This standardization allowed for direct comparison of regression coefficients of each predictor variable. The relative variable importance (RVI) ranks all variables based on their frequency of occurrence in top models and was calculated from an exhaustive suite of models by summing the model weights over all models that included that variable (package MuMIn). To visualize the relationship between the fixed variables and infection status we used the function *visreg* (package visreg). We utilized several metrics of model fit, first evaluating the residuals of the model to the fitted model using *plotresid* in R to create a simulated quantile-quantile plot (package RVAideMemoire). For each of the top models, we calculated marginal and conditional pseudo-R^2 values using the function *r.squaredGLMM*, evaluating the fit of fixed effects and the fixed+random effects model respectively (package lme4; Nakagawa and Schielzeth 2013). To evaluate model accuracy at predicting infection status, we calculated area under the curve (AUC) for each of the top models using the function *auc* (package arm). In order to interpret the odds of finding an infected individual, we calculated the Odd's Ratios (OR) by

exponentiating the top model coefficients. The predictor variables are scaled, so the OR estimates the effect of one standard deviation change in the predictor variable.

Parasite density. To assess additional measures of parasite response, we evaluated parasite abundance, a parasite trait that is increasingly being recognized as important (Lagrue and Poulin 2015). We quantified parasite density as the number of *E. depressus* infected with *L. panopaei* m^{-2}. Parasite density reflects the absolute abundance of parasites in an area, allowing evaluation of the relationship between resource (host) density and consumer (parasite) density (Lagrue and Poulin 2015). To evaluate whether *E. depressus* density had an effect on maximum parasite density, we ran a quantile regression (package quantreg; Cade and Noon 2003; R Development Core Team 2010). We evaluated whether host density effected the maximum density of *L. panopaei*, using the upper (0.95) quantile (Cade and Noon 2003). *XY*-pair bootstrapping was used to evaluate the fit of the model across each quantile.

Results

Field Survey. Eurypanopeus depressus were present in all estuaries and were found at all but 2 of the 50 surveyed reefs, with a mean density of 120 crabs m^{-2} (Table 2.1). *L. panopaei* were also found in all estuaries, but only at 40 of the 50 oyster reefs surveyed, with a mean density of 44 infected crabs m^{-2} and an infection prevalence across all samples of 25.4% (Table 2.1). Infection prevalence of *L. panopaei* varied within and between estuaries (Fig. 2.1). Five out of the 10 estuaries surveyed were first reports of *L. panopaei* infection, filling in previous gaps in the geographic range of the parasite. Biological and physical predictor variables varied across the geographic range sampled

(Table 2.1) and patterns across estuaries are reported elsewhere, see (Kimbro et al. 2014; Byers et al. 2015).

Predictors of Parasite Abundance

Probability of Infection. All models within the candidate set (ΔAICc<2) were good fits to the data, with no patterns in the residuals and good levels of accuracy in prediction as calculated by AUC (Table 2.2). Fixed variables explained approximately 26% of the variance in the data, and the random variable estuary explained an additional 9% of the variance (as measured by the difference between the conditional and marginal pseudo R^2, Table 2.2). Including estuary as a random variable improved the model fit substantially (based on residuals) and as such was included in all models.

The best-fit model for probability of infection included water depth, host size, 'fish predators', blue crabs and estuary as a random variable (Table 2.2, Fig. 2.2). Host size had the highest RVI and was included in all the candidate models (Table 2.2), with the odds of a crab being infected increasing 195% for every 2mm increase in carapace width of a host (Table. 2.2, Fig. 2.2). Water depth had the second highest RVI and was also included in all the candidate models, with the odds of a crab being infected increasing 105% for every 0.1m increase in the depth of the water over the reef at high tide (Table 2.2, Fig. 2.2). 'Fish predator' presence had the third highest RVI and was included in all the candidate models, with the odds of a crab being infected increasing 41% for every 10 additional fish caught in the traps at that reef (Table 2.2, Fig. 2.2). Host density was included in all the candidate models but was not significant for either the best-fit model or the model average (Table 2.2 and Fig. 2.2). The presence of *C. sapidus* was also included in all of the candidate models, with the probability of infection

increasing 33% for every additional blue crab caught in the traps by the reef (Table 2.2 and Fig. 2.2). *P. herbstii*, *C. virginica*, 'other fish' and vertical relief were each included in one of the candidate models, however these variables were non-significant when included, had low RVI and minimal ß-coefficients, suggesting that they added very little to the interpretation. Among the candidate model set, the best-fit model had moderate support (weight=0.33) and all variables included had high RVI.

Parasite density. Host density was significantly and positively correlated with maximum densities of infected individuals across the range of host density evaluated in this study (95^{th} quantile, $y=8.77+0.35x$, $\beta=0.35$, $p>0.001$, Fig 3).

Discussion:

Our results reveal that host characteristics influence the probability of *L. panopaei* infection (Table 2.2, Fig. 2.2). Perhaps more interestingly, we found that environmental and biological community characteristics can also be used to predict infection prevalence. In particular, both water depth and predator abundance were associated with higher probability of *L. panopaei* infection (Table 2.2, Fig. 2.2). Most of our predictor variables were evaluated at the local level (Table 2.1), and as might be expected, our results reveal that local processes primarily corresponded with infection prevalence. However, even with our limited estuary-level resolution, water depth was revealed as a strong predictor of infection prevalence. Increasingly, it is recognized that such external (i.e., non-host) factors can influence parasite dynamics (Pennings and Callaway 1996; Thieltges et al. 2009; Altman and Byers 2014).

Predators can influence host-parasite interactions in multiple ways, with evidence that direct predation can decrease infection (e.g. Hudson et al. 1992). Alternatively,

consumption of an infected host can enhance transmission by spreading parasite propagules (e.g. Duffy et al. 2011). In addition to these consumptive pathways, predator avoidance behaviors can increase susceptibility of the host and thus enhance infection (e.g. Caceres et al. 2009). In our study, we found that the prevalence of infected *E. depressus* increased in the presence of host predators, including both predatory fish and blue crabs (Fig. 2.2C and D). When predators are mobile and the prey are not, a positive association between predators and prey across large scales is likely (Sih 1982). If predators prefer infected, potentially more vulnerable, *E. depressus,* then a positive correlation between predators and infected hosts could be driven by predator aggregation near areas of higher infection prevalence within each estuary (Rose and Leggett 1990; Sih 2005; Wieters et al. 2008). Although mobile within the context of an oyster reef, *E. depressus* is likely confined to a given reef due to desiccation stress and high off-reef predation rates (Grant and McDonald 1979), thus reducing the chances that infected *E. depressus* can emigrate from high abundances of their mobile predators. Alternatively, positive correlations between infection and predator occurrence could indicate collinear responses to an underlying environmental variable.

Many marine parasites maintain a free-living larval stage, and as such may be influenced by the same recruitment dynamics as their free-living hosts. Water depth is associated with increased circulation and water volume moving over the reef, among a wide variety of other variables. In oyster reef communities, water depth is a positive predictor of multiple parasites, including *L. panopaei* infection prevalence examined here, and also *Zaops ostreus*, a pea crab parasite of oysters (Byers et al. 2013). In the case of the pea crab, parasite recruitment strongly mirrored host recruitment. However, that

pairing may be less likely for *L. panopaei* and its host, as *L. panopaei* releases larvae throughout most of the year (Walker et al. 1992), and *E. depressus* only reproduces twice a year at most (McDonald 1982). The positive relationship between water depth and probability of infection (Fig. 2.2E) suggests that areas with more water moving over the reef are also experiencing higher recruitment of *L. panopaei* larvae.

Organism size can markedly affect biological interactions. For example, organism size can control refuge from natural enemies such as predators and parasites (Paine 1976; Walde et al. 1989). For parasites, larger hosts can confer the additional benefit of increased energy available for parasite reproduction (Poulin 2007). As has been documented before (Alvarez et al. 1995; Hines et al. 1997; Poulin and Hamilton 1997), *E. depressus* size was a positive predictor of infection, with larger individuals more likely to be infected then smaller individuals (Fig. 2.2A). This could simply be because larger hosts are older and have had longer time to accrue infection. Or the positive relationship between host size and parasite size may indicate that smaller hosts do not have enough energy to sustain the energy demands of the parasite (Alvarez et al. 1995). Although larger hosts release an externa after a single molt, there is some evidence that infected megalopae undergo several molts cycles before the parasite releases an externa (O'Brien and Skinner 1990; Alvarez 1993; Alvarez et al. 1995). Larger hosts produce substantially more parasite larvae (Alvarez 1993), and releasing the externa halts molting (O'Brien and Skinner 1990), thus it is possible that *L. panopaei* may be selecting larger hosts.

Theoretical models have long predicted that parasitoids and castrators should have a density-dependent response (Hassell and May 1973; Hassell 1985; Murdoch et al. 2005) and be able to regulate their host populations (Kuris 1974; Best et al. 2012).

Parasite density often increases with host density (Blower and Roughgarden 1989; Lafferty 1993; Sonnenholzner et al. 2011; Lagrue and Poulin 2015); however, rhizocephalans have yet to be examined in this way. We found some evidence that probability of *L. panopaei* infection increased with *E. depressus* density (Fig. 2.2B), yet the maximum *L. panopaei* density was more clearly related to *E. depressus* density (Fig. 2.3). Across host densities, the maximum density of *L. panopaei* is correlated with approximately 35% infection prevalence (Fig 2.3). This apparent 'asymptote' in *L. panopaei* infection density may arise for several reasons. For example, *L. panopaei* can have devastating impacts on its host populations, with abrupt decreases in host populations correlated with the invasion of the parasite (Andrews 1980; Eash-Loucks et al. 2014). The estuary furthest south within this survey overlaps spatially and temporally with a time when *E. depressus* populations were at a historically low density that began following the documented *L. panopaei* invasion (Eash-Loucks et al. 2014). It is possible that the apparent 35% limit indicates the upper limit that the population can sustain without severe consequences for the host population.

This work demonstrates that while host characteristics play a dominant role in determining parasite distribution, biological and physical variables also influence host-parasite dynamics. Although all estuaries in our study could sustain parasites, variation in predation pressure and variables that may reflect parasite recruitment resulted in some areas having higher infection rates than others. Of particular interest were the patterns indicating that predators may be facilitating parasite infection. Future work designed to evaluate the interactions among hosts, parasites and predators would illuminate a greater understanding of how these interactions structure ecological communities.

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Table 2.1 Descriptive statistics for biological and environmental variables measured across 50 oyster reefs sampled within 10 estuaries from Florida to North Carolina. Variables hypothesized to effect parasite prevalence were included, such as host density and size. *Eurypanopeus depressus* predators included *Callinectes sapidus* and 'predatory fish' a subset of fish confirmed to consume Xanthid crabs based on gut content data (Grabowski, *unpublished data*). All other fish were included to evaluate for indirect effects. *Panopeus herbstii* is considered a competitor. Habitat variables included the number of *Crossostrea virginica* (live oysters), the vertical relief of the oyster reef and water depth over the reef. Salinity was standardized for polyhaline values as much as possible during oyster reef selection.

Variable	Mean	SD	Min	Max	N	Unit
Infection Prevalence	25.4	20.8	0	100	1179	%
Loxothylacus panopaei density	44	32	0	100	50	infected crabs m^{-2}
Eurypanopeus depressus density	120	68	0	300	50	Crabs m^{-2}
Host Size	7.96	2.36	4.03	20.3	1179	mm carapace width
Panopeus herbstii density	40	28	0	128	50	Crabs m^{-2}
Callinectes sapidus abundance	0.4	1	0	7	50	Crabs $trap^{-1}$
'Predatory Fish' abundance	7	10	0	36	50	Fish $trap^{-1}$
'Other Fish' abundance	5	3	0	13	50	Fish gill net^{-1}
Crassostrea virginica density	3772	3340	56	12048	50	Oysters m^{-2}
Vertical Relief of oyster reef	3.82	2.12	0.25	11.25	50	cm
Avg Water depth over reef at high tide	0.61	0.12	0.21	0.79	10	m
Salinity	34.49	2.68	30.00	37.67	50	ppt

Table 2.2. The candidate model set (ΔAICc<2) for infection status on 50 reefs from 10 estuaries from North Carolina to Florida. Standardized β-coefficients were reported for predictor variables included in each model. Predictor variables that were significant in the model are bold. Relative variable importance (RVI) was calculated for each predictor variable, scaled from 0-1. A null model with estuary as a random variable was included. To account for model selection uncertainty model averaging was conducted on the candidate set models (A-E). Odds ratios (OR) and confidence intervals (2.5-97.5%) were reported for the component variables of the best-fitting model (A) according to AICc criteria. All variables are standardized, so the effect of each variable is comparable between predictor variables. Odds ratios are associated with a single standard deviation change in the predictor variable.

	Variance	β Coefficients													Pseudo R^2		
Model	Estuary	Intercept	Host size	Water Depth	Fish Predators'	Host density	*C. sapidus*	Other Fish'	*P. herbstii*	*C. virginica*	Vertical Relief	AICc	Δ AICc	Weight	Marginal	Conditional	AUC
A	0.45	**-1.32**	**1.08**	**0.72**	**0.34**	0.23	**0.29**					1108.20	0.00	0.33	0.26	0.35	0.83
B	0.40	**-1.32**	**1.07**	**0.67**	**0.33**	**0.24**	0.25	0.11				1109.20	0.93	0.21	0.27	0.34	0.83
C	0.45	**-1.31**	**1.08**	**0.73**	**0.34**	**0.24**	**0.28**				-0.07	1109.70	1.43	0.16	0.26	0.35	0.83
D	0.43	**-1.33**	**1.08**	**0.71**	**0.32**	0.22	**0.28**		0.06			1109.80	1.56	0.15	0.26	0.35	0.83
E	0.44	**-1.32**	**1.08**	**0.72**	**0.34**	0.23	**0.29**			-0.02		1109.90	1.72	0.14	0.26	0.35	0.83
Full	0.39	**-1.33**	**1.08**	**0.66**	**0.30**	0.24	**0.25**	0.13	0.08	0.01	-0.04	1114.10	5.92	0.02	0.27	0.35	0.83
Null	0.43	-1.14										1292.50	184.20	0.00	0.00	0.12	0.67
Average		**-1.33**	**1.08**	**0.71**	**0.34**	0.20	**0.27**	0.03	0.01	0.00	-0.01						
RVI			1.00	0.89	0.88	0.74	0.71	0.43	0.37	0.30	0.29						
OR			2.95	2.06	1.41	1.25	1.33										
2.5-97.5%			2.48-3.55	1.58-3.19	1.09-1.82	0.99-1.59	1.05-1.70										

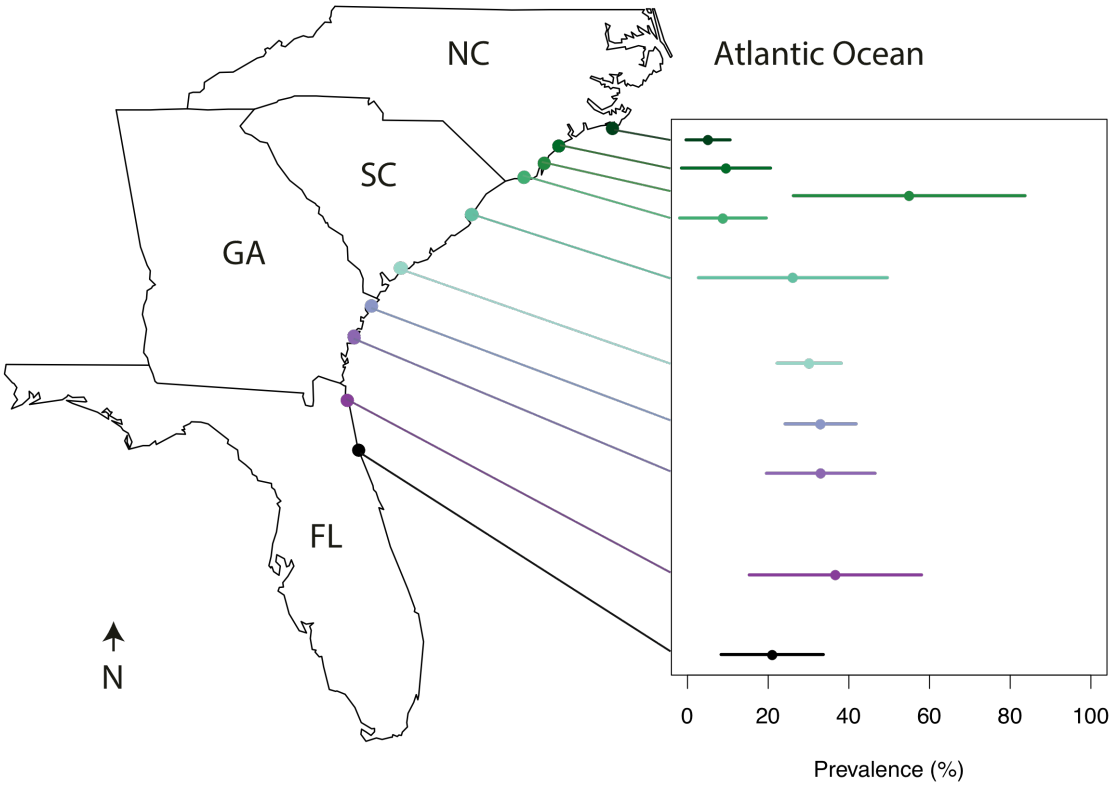

Fig 2.1 Map of the South Atlantic Bight indicating the average prevalence (± SD) of *L. panopaei* infection in *E. depressus* across five reefs sampled in each of 10 estuaries (for additional information see; (Byers et al. 2015).

Fig 2.2 Probability of infection as a function of each predictor variable included in the top model (model A in Table 2). 95% confidence intervals were calculated only considering the fixed effects (grey shading). Vertical hash marks along the x-axis indicate the spread of the data points used to fit the model. Differences in the number of hash marks between variables reflect the scale and resolution of each measurement; for example, water depth has fewer hash marks because there was only one measurement per estuary. All predictor variables are standardized so that the effects of the variables are readily comparable among each other, with every unit change associated with a single standard deviation change in the predictor variable. A. Standardized host size measure as carapace width (mm), B. Standardized host density measured as number of *E. depressus* m^{-2}, C. Standardized relative abundance of fish predators of small mud crabs (family Xanthidae), D. Standardized relative abundance of *Callinectes sapidus,* E. Standardized water depth over oyster reef (m).

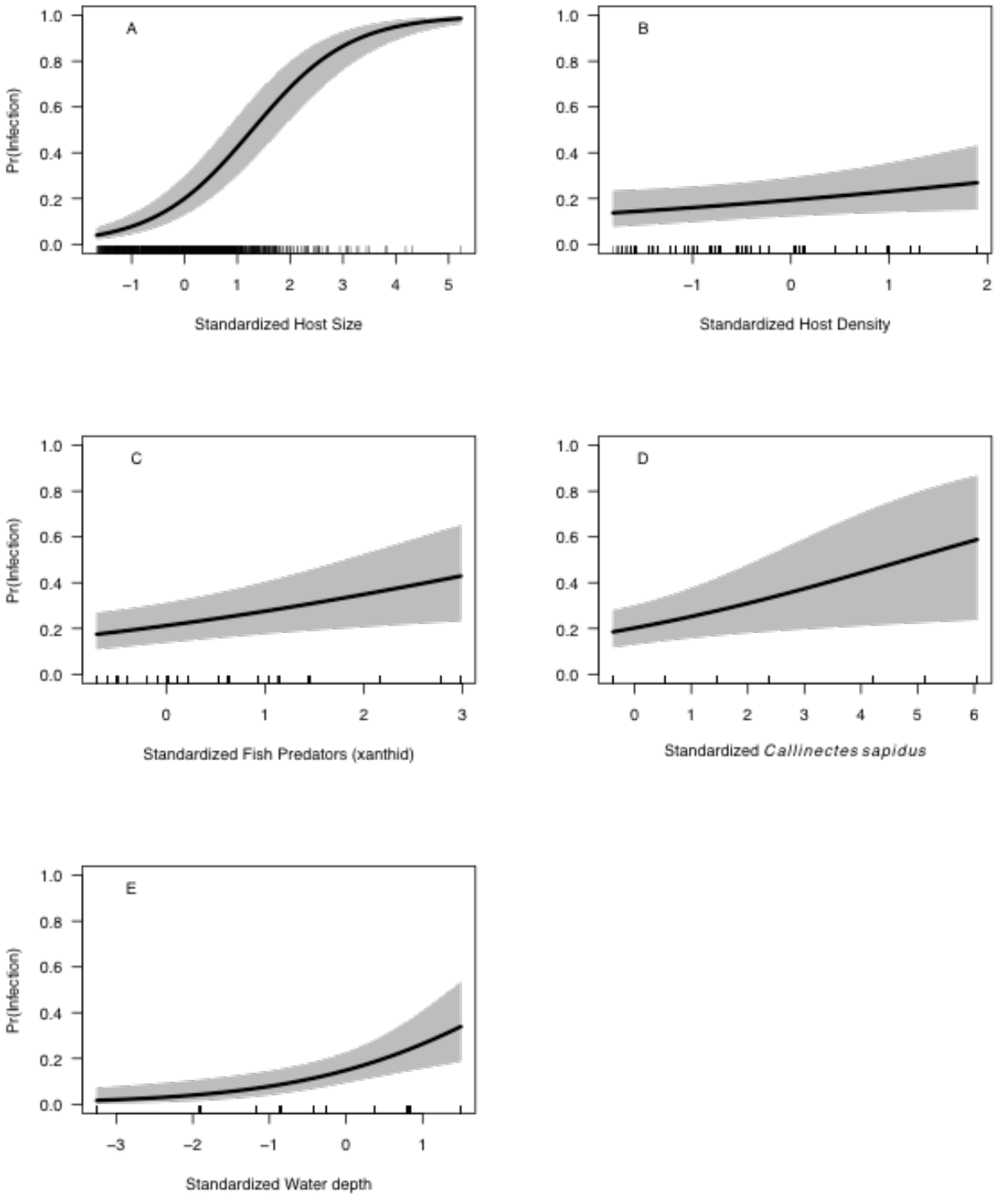
A
Pr(Infection)
Standardized Host Size
B
Standardized Host Density
C
Standardized Fish Predators (xanthid)
D
Standardized Callinectes sapidus
E
Standardized Water depth

Figure 2.2

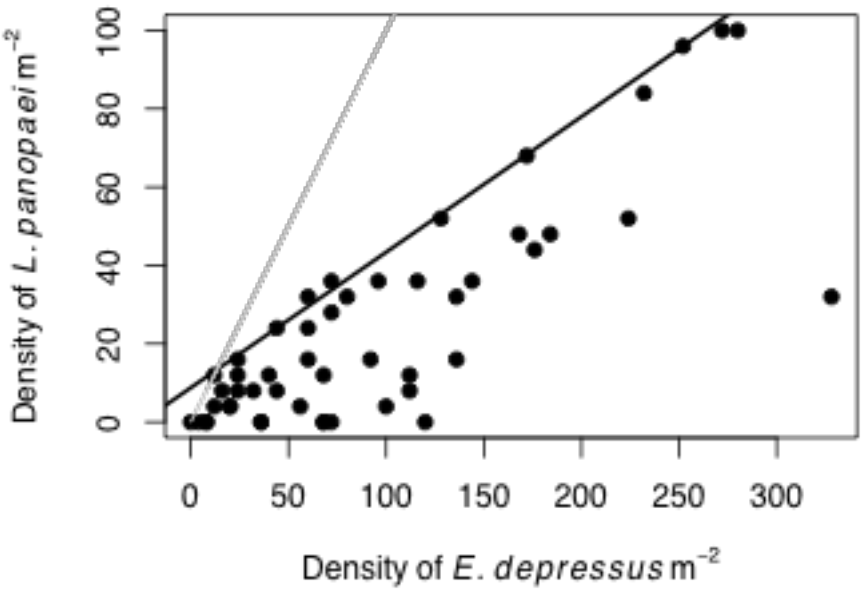

Fig 2.3 The relationship of parasite (*L. panoaei)* density, as a subset of the total host (*E. depressus)* density. The grey line indicates the 1:1 line, which is the maximum possible density of *L. panopaei* if all crabs are infected (100% prevalence). The black solid line indicates the 0.95 quantile regression line, indicating the upper limit of infected individuals.

CHAPTER 3

NON NATIVE PARASITE ENHANCES SUSCEPTIBILITY OF HOST TO NATIVE PREDATORS[2]

[2] Gehman, A.M., and J.E. Byers. To be submitted to *Oecologia*.

Summary

1. Parasites often alter host physiology and behavior, which may enhance predation risk for infected hosts. Higher consumption of parasitized prey can in turn lead to a less parasitized prey population (the healthy herd hypothesis).
2. *Loxothylacus panopaei* is a non-native castrating barnacle parasite on the mud crab *Eurypanopeus depressus* along the Atlantic coast. We investigated whether the predatory crab *Callinectes sapidus* and other predators preferentially feed on *E. depressus* infected with *L. panopaei* and evaluated a mechanism behind prey choice.
3. We evaluated prey choice through mesocosm experiments and a field tethering experiment. Through behavioral trials we evaluated whether *L. panopaei* affects *E. depressus* escape speed, making infected prey more susceptible to predator attack.
4. We found that *C. sapidus* preferentially consumed infected *E. depressus* 3 to 1 over visibly uninfected *E. depressus* in the mesocosm experiments. Similarly, infected *E. depressus* were consumed 1.4 to 1 over uninfected conspecifics in field tethering trials. Contrary to our expectations, infected *E. depressus* ran faster during laboratory trials than uninfected *E. depressus*, suggesting that quick movement may not decrease predation risk, and seems instead to make prey more vulnerable.
5. Ultimately, the preferential consumption of *L. panopaei* infected prey by *C. sapidus* suggests that the predatory crab can lower *L. panopaei* abundance within

the local host population, potentially providing a biotic defense against this invasive parasite.

Key-words Disease ecology, host-parasite, predator-prey, introduced species, marine invertebrate, rhizocephalan, parasitism.

Introduction

With the increase in movement of species around the globe, through vectors such as shipping and aquaculture, we are experiencing an era of new species interactions, including invasions of novel parasites and diseases (Mack *et al.* 2000; Levine & D'Antonio 2003). Invasive parasites, and more broadly emerging infectious diseases, can have devastating effects on new hosts, depressing host populations to low levels (Garner *et al.* 2006; Dunn & Hatcher 2015). This is likely due to the limited defenses of native populations against novel parasites, leaving the population largely vulnerable (Hatcher, Dick & Dunn 2012). However, native predators may help to mollify the influence of invasive parasites on prey populations if they target parasitized prey. Although biotic resistance, such as preferential predation on invasive species, is known to protect communities from free-living invasive species (Byers 2002; deRivera *et al.* 2005), the role of predators to affect invasive parasites in this manner has yet to be elucidated (Dunn et al. 2012).

Selective consumption of infected prey by predators can occur if infected hosts exhibit physiological or behavioral changes that increase their vulnerability (e.g. Lafferty & Morris 1996). For example, nematode infected red grouse release more scent, leading to higher predation rates on infected individuals (Hudson, Dobson & Newborn 1992).

Theory suggests that predators should be able to limit disease transmission within populations (Hethcote *et al.* 2004; Wild *et al.* 2011; Chakraborty *et al.* 2015). For example the 'healthy herd' hypothesis suggests that predators dampen disease transmission, particularly when predators selectively feed on infected prey (Packer et al. 2003). However, preferential feeding on infected hosts does not always lead to decreased infection prevalence (Duffy 2007). For example if predator avoidance behavior enhances susceptibility there can be increased prevalence even with strong preferential feeding on infected individuals (Duffy *et al.* 2011; Welch & Harwood 2011). Additionally, if the act of predation increases disease transmission (e.g. "predator spreader" hypothesis) then preferential predation on infected individuals can lead to increased infection prevalence (Cáceres, Knight & Hall 2009). While preferential feeding on infected hosts has been documented for a range of systems (Duffy *et al.* 2005; Duffy 2007; Cáceres *et al.* 2009; Krkosek *et al.* 2011), preferential feeding on native hosts infected by an invasive parasite has not been documented (Dunn et al. 2012).

Invasive parasites may be particularly likely to enhance vulnerability of their hosts to native predators. Coevolution between native hosts and their parasites is expected to evolve towards an optimum level of virulence, with the relative cost of increased host mortality balanced with the benefit of increased reproductive output (Price 1980; Anderson & May 1982). The naïve host theory suggests that novel infections may lead to the parasite overexploiting their new hosts (Cruz, Manolis & Wiley 1985; Fassbinder-Orth, Barak & Brown 2013; Lymbery *et al.* 2014), which may leave both host and parasite vulnerable to predators. In fact, for the invasive parasites with available comparative measures of pathogenic effects, 85% showed increased pathogenicity in

naïve hosts (Lymbery et al. 2014). Pathogenicity may lead to disease behavior (e.g. lethargy) and increased vulnerability to predators. Even when the parasite has a shared coevolutionary history with the host, parasite range expansion into previously uninfected areas of the host range can lead to overexploitation of the naïve host population (e.g. Cudmore *et al.* 2010).

The United States Atlantic coast populations of the mud crab *Eurypanopeus depressus* are invaded by a castrating Rhizocephalan barnacle parasite, *Loxothylacus panopaei* (Reinhard & Reischman 1958; Kruse, Hare & Hines 2011). Rhizocephalans often induce behavioral changes in their hosts, including decreasing infected host feeding rates (O'Shaughnessy, Harding & Burge 2014; Toscano, Newsome & Griffen 2014), and inducing movement of the host to risky positions to increase the spread of the parasite offspring (Hoeg 1995). These behavioral alterations are also likely to enhance predation risk in their hosts. *L. panopaei* is a native parasite of *E. depressus* in the Gulf of Mexico and was introduced to the Chesapeake Bay in the 1960s in association with oyster aquaculture (Van Engel et al. 1966). The parasite has since expanded its range north to Long Island, New York and south to Cape Canaveral Florida, and was first reported in Georgia 2004/2005 (Kruse & Hare 2007; Kruse *et al.* 2011; Freeman, Blakeslee & Fowler 2013; Eash-Loucks, Kimball & Petrinec 2014). *E. depressus* population density decreased following *L. panopaei* invasion in some regions (Andrews 1980; Eash-Loucks *et al.* 2014), and infection prevalence in the invaded range is markedly higher than that found in the native range (Hines, Alvarez & Reed 1997; Kruse & Hare 2007). *E. depressus* utilizes oyster reefs as a refuge from predators (Meyer 1994; Hulathduwa *et al.* 2011), which include several fish and crab species (e.g. the blue crab *Callinectes*

sapidus). In this study we evaluate (A) whether the blue crab *C. sapidus* preferentially feeds on *E. depressus* infected with *L. panopaei*; (B) whether *E. depressus* infected with *L. panopaei* are more vulnerable to predation in the field; and (C) whether *L. panopaei* impedes *E. depressus* movement, possibly increasing the vulnerability of both host and parasite to predators.

Materials and methods

Collection Techniques

For all experiments described below, we collected *E. depressus* and *C. sapidus* from Wassaw Sound, near Priests Landing, Savannah, GA (31°57'46.6"N, 81°00'47.9"W). We collected *C. sapidus* using small fish traps baited with frozen chicken. To collect *E. depressus* we collected oyster clumps at low tide and brought them back to the lab. In the lab we removed mud and broke oyster clumps apart in a mesh basket to find *E. depressus*. We collected healthy and infected *E. depressus* immediately prior to each experiment; 27-29 June 2012 and 16-19 July 2012 for experiment (A), 21 July and 6 August 2014 for experiment (B), and 7 February 2013 for experiment (C).

We considered a host 'infected' if it was bearing a mature externa (the external reproductive organ of the parasite, Fig. 3.1), and considered it "healthy' if it was non-externa bearing. There is an internal phase of infection, so some crabs labeled healthy were likely internally infected with an incipient infection, making any difference found between healthy and infected individuals in our study a likely conservative estimation of the parasite's effect. Throughout the manuscript we will refer to a visibly healthy *E. depressus* as a 'healthy host' and an *E. depressus* visibly infected with *L. panopaei* as an 'infected host'. For all experiments, attempts were made to keep host size similar,

however the size differences between healthy and infected hosts reflect those observed in the field (Table 3.1, Gehman et al, unpublished data). Prior to each experiment we kept infected and healthy hosts in separate flow-through seawater tables to prevent parasite transmission.

A. Do predatory crabs preferentially consume infected hosts over healthy hosts (mesocosm study)? To quantify consumption of infected and healthy *E. depressus* by predatory *C. sapidus*, we placed 5 healthy and 5 infected *E. depressus* hosts into a 19L tank and exposed them to *C. sapidus*. For habitat, each tank contained a single layer of sun bleached local oyster shell and its own separate delivery of flowing seawater which was kept at ~19L/half hour throughout the trial. We allowed *E. depressus* to settle for half an hour and then we placed a single *C. sapidus* predator (80-90mm carapace width) in each tank. Light-dark cycles were set to 15 hours of dark and 9 hours of light. We ran each trial for 7 days and monitored them twice a day, once just prior to turning off the lights and once immediately after the lights turned on. For staging purposes, we conducted the full experiment twice, over two consecutive time blocks with 10 replicate tanks in each block (n=20 *C. sapidus* for the whole experiment).

At each monitoring point we removed the predatory crabs from the tanks and placed them in a separate tank with seawater. We carefully examined the contents of each tank, removing each oyster shell one at a time to examine them for presence of the healthy and infected hosts. We recorded the number that were alive, dead, and missing, and removed the dead. We considered crabs consumed if they were missing. We considered crabs dead if they did not move in response to direct stimulus (only two crabs were recorded in this category over the course of the experiment). To keep the

availability of healthy and infected prey even across time, we brought the total number of available prey back to 5 healthy and 5 infected hosts at each monitoring point (twice a day). After tabulating we allowed the infected and healthy hosts to acclimate for a half an hour before returning the same predator to each tank. We quantified the cumulative number of infected and healthy hosts consumed by each predator over the course of the full 7-day experiment.

We performed individual χ^2 tests on each replicate predator to compare the preference of each crab for healthy or infected hosts. To determine whether crab preference was homogenous among replicates we conducted an I^2 analysis, which describes the proportion of the predator preference estimate that is driven by heterogeneity between individuals (Higgins & Thompson 2002). The I^2 was relatively low within each time block and similar between each time block (time block one I^2= 44, time block two I^2 = 47). Therefore the two time blocks were combined together for an analysis to test the null hypothesis of no preference by predatory crabs for the infected or healthy hosts. We used R 3.1.3 for this and all subsequent statistical analysis (R Development Core Team 2015).

B. Do predators preferentially consume infected hosts over healthy hosts (field study)?

To evaluate whether infected hosts were preferentially consumed over healthy hosts by a full suite of ambient predators in the field, we tethered healthy and infected hosts to a 30.5 cm long PVC pole with a 25.4 cm monofilament line. The tether line was then glued to the back of healthy and infected hosts carapace with Loclite® super glue (Fig. 3.2). Glue was allowed to dry for approximately 5 minutes. To assure that all host crabs were

satisfactorily attached to their tethers, host crabs were kept for 24 hours in the flow-through system prior to placement in the field.

During low tide we secured the PVC poles at 0.5m intervals within a mid-intertidal oyster reef at Priests Landing, near the collection site of the crabs. To account for the effect of location, we alternated healthy and infected crabs systematically along the reef. We placed approximately 20 infected and 20 healthy crabs in the field for each of 5 trials (n=93 healthy and n=95 infected hosts), blocked by date. Each trial lasted ~12 hours, starting on a late afternoon low tide and ending the following low tide. Although tethering limited host movement, crabs were still able to move into the oyster reef to hide from predators (Fig 3.2A and B). At collection we noted mortality, which was quantified by loss of crabs, as well as recovery of crab remnants (Fig 3.2D). We conducted trials on two adjacent reefs. We tested for the effect of blocking by reef and found no effect, so we pooled the results from the two reefs together for all statistical analysis. Because infected and healthy crabs were different sizes we explicitly included size to test the effect of size on predation risk. We fit a generalized linear mixed effects model with a binomial response (dead or alive), date of tethering trial as the random effect and size and infection status and their interaction as fixed effects.

C. Are infected hosts slower than healthy hosts? To test whether infected hosts were slower than healthy hosts, as a possible factor contributing to differential consumption, we conducted movement experiments in the lab. We quantified the time for a crab to move down an exposed runway to the end. To induce linear movement by each crab we created a standardized exposed runway, composed of a PVC tube cut in half lengthwise, which was submerged into artificial seawater in a container. To examine whether

exposure distance altered escape behavior, we ran two separate movement experiments, differing only in the length of runway available. Thus, for the first trial the runway was 220mm from the center point to either end and for the second trial the runway was 94mm. For all experimental runs we hand placed a single individual in the middle of the runway and recorded its movements and the time it took to reach the end of the runway with a camera suspended above the tank. We ran 20 healthy and 16 infected hosts along the long runway, and 19 healthy hosts and 10 infected hosts individually along the short runway. We analyzed each runway length experiment separately using an ANOVA, testing the effect of infection status on speed (mm/s) for each experiment.

Results

A. Do predatory crabs preferentially consume infected hosts over healthy hosts (mesocosm study)? Predatory crabs consumed three times as many infected hosts on average than healthy hosts (Fig. 3.3, χ^2=33.3, p<<0.001, I^2=47.2). Every one of the *C. sapidus* individuals consumed more infected *E. depressus* than uninfected, with the diet of each *C. sapidus* averaging 75% *E. depressus* infected with *L. panopaei* (± 1.7, SD). Overall, 120 infected hosts were consumed through the course of this experiment, whereas only 36 healthy crabs were consumed.

B. Do predators preferentially consume the infected hosts over the healthy host (field study)? Infected hosts were 43% less likely to survive a single high tide cycle than healthy hosts (Fig. 3.4, odds ratio=0.43, p=0.02). The interaction between host size and infection status was non-significant (β=-0.01, p=0.97) so it was removed from the analysis. Across both treatments, smaller host crabs were less likely to survive a single high tide cycle, and the odds of survival increased 173% for every 1.1mm increase in

carapace width (Fig. 3.4, odds ratio =1.73, p=0.002). Therefore, even after controlling for the effect of size there remained an effect of infection prevalence on survival.

C. Are infected hosts slower than healthy hosts? Regardless of runway length, infected hosts moved approximately twice as fast to the end of the PVC tube than healthy hosts in both experiments (long runway, F=8.5, df=1, MS=1323, p = 0.006, short runway, F=14.9, df=1, MS= 2565, p = 0.001, Fig. 5).

Discussion

Parasitic infection by *L. panopaei* enhances predator consumption of *E. depressus* by up to 3-fold in mesocosms (Fig. 3.3) and 1.4 to 1 in the field (Fig. 3.4). Contrary to our hypothesis, the enhanced consumption of infected hosts is not seemingly due to lower escape speeds. We had surmised that either increased drag from the externa on the infected crab's abdomen or infection-induced lethargy would slow the infected host. Rather, our experiment designed to isolate this behavioral mechanism showed that infected crabs were twice as fast (Fig. 3.5), yet still more vulnerable to predators. Thus heightened vulnerability to consumption of infected hosts occurs in spite of, or because of, faster movement. Indeed, it is possible that quick movement could increase the visibility (e.g. Hemmi & Pfeil 2010), or other sensory cues (e.g. mechanosensory (Schwalbe, Bassett & Webb 2012)), of the prey to their predators

The differentially high consumptive pressure on infected hosts suggests the healthy herd mechanism could be acting within this system. Evaluated at a mechanistic level, infected crabs are being differentially removed from the environment by predators over their healthy counterparts (Fig. 3.3). However, across estuaries there is a positive relationship between predator abundance and infection prevalence (Gehman et. al.

unpublished data), which superficially might lead to the opposite conclusion (i.e. that predators preferentially consume uninfected hosts). Given the preferential consumption of infected individuals that we document, this positive association at a large scale must be driven by other mechanisms. For example, the predator and prey may be positively associated with a third environmental variable (e.g. ocean currents). Alternatively, the positive association between the predatory crab and its prey at a regional scale may indicate that *C. sapidus* are moving to areas within the estuary with higher infection prevalence to take advantage of easy prey items (Sih 1982; 2005; Wieters *et al.* 2008).

Prey size is an important factor for prey survival, with many prey reaching a refuge from predators at larger sizes e.g. (McLennan *et al.* 2004). In our study, host carapace width significantly increased host survival and had a similar positive effect on infected and healthy hosts (Fig. 3.4). However, for any given carapace width, infected crabs have lower survival than healthy crabs (Fig. 3.4); a trend that holds across all carapace widths as signified by the non-significant interaction of infection status and carapace width. Size determines dominance in many crab species (Somers & Nel 1998; Shervette & Perry 2004) and refuge dominance is important for crab survival (Beck 1995; Shervette & Perry 2004; Hulathduwa *et al.* 2011). Therefore increased survival with size suggests that larger *E. depressus,* regardless of infection status, are able to dominate refuge use over their smaller counterparts, which may account for the strong effect of increasing size on survival.

The predator used in our mesocosm trials, the blue crab *C. sapidus,* is a voracious predator, and this is not the first time it has been implicated as biological resistance to invasion. *C. sapidus* preferentially consumes *Carcinus maenus*, the invasive European

green crab, and high predation rates have likely inhibited the southern spread of *C. maenus* (deRivera et al. 2005). The preferential feeding on infected *E. depressus* by *C. sapidus* we found in this study suggests that this predator has the potential to provide a biotic defense against this invasive parasite. Specifically, the preferential feeding on *L. panopaei*-infected *E. depressus* by *C. sapidus* suggests that the predators may be able to help limit the spread of this invasive parasite. *L. panopaei* infection prevalence varies substantially along the Atlantic coastline (Hines *et al.* 1997; Kruse & Hare 2007; Kruse *et al.* 2011; Freeman *et al.* 2013), and variation in predation pressure between sites may affect infection prevalence. In this era of increased biological invasions that in turn increase novel species interactions (Mack *et al.* 2000; Levine & D'Antonio 2003), there is a great need to identify how novel species will interact with the community they are invading. The results from this project help to highlight the importance of understanding how interactions between organisms, such as predation by predators, can likely influence the prevalence and abundance that novel parasites are able to achieve.

Data Accessibility

Data will be archived on Dryad prior to publication.

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Table 3.1 Mean (SD) size of the healthy *E. depressus* and *E. depressus* infected by *L. panopaei* that we offered to *C. sapidus* for consumption (A) and were tethered in the field (B) to test for the effect of parasite infection status on infected and healthy host vulnerability to predators.

	Infected host - mm	Healthy host - mm
Mesocosm	9.78 (1.35)	7.78 (1.13)
Field	9.96 (1.04)	9.52 (1.08)

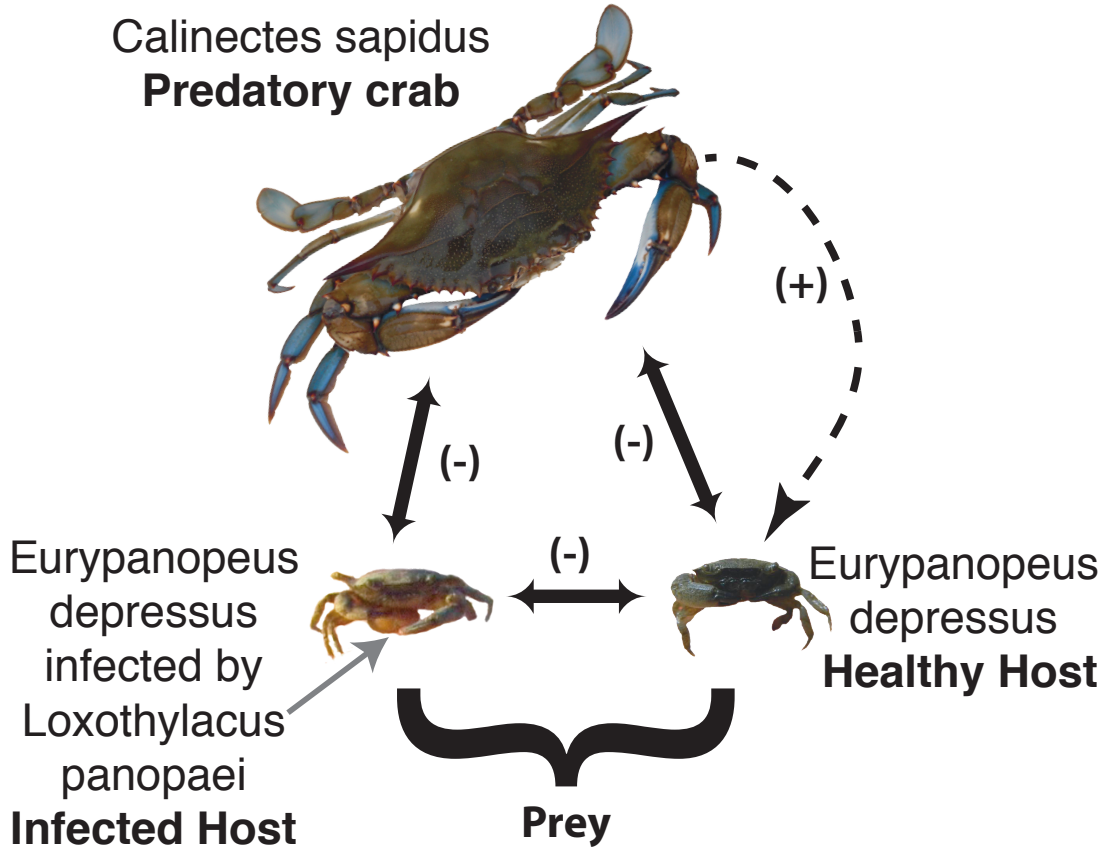

Figure 3.1 Conceptual diagram of experimental system, indicating the directions of interactions hypothesized between the species. Bold lettering indicates the terminology used throughout the manuscript for each of the biological entities. Grey arrow indicates the visible *L. panopaei* externa on the abdomen of an infected host.

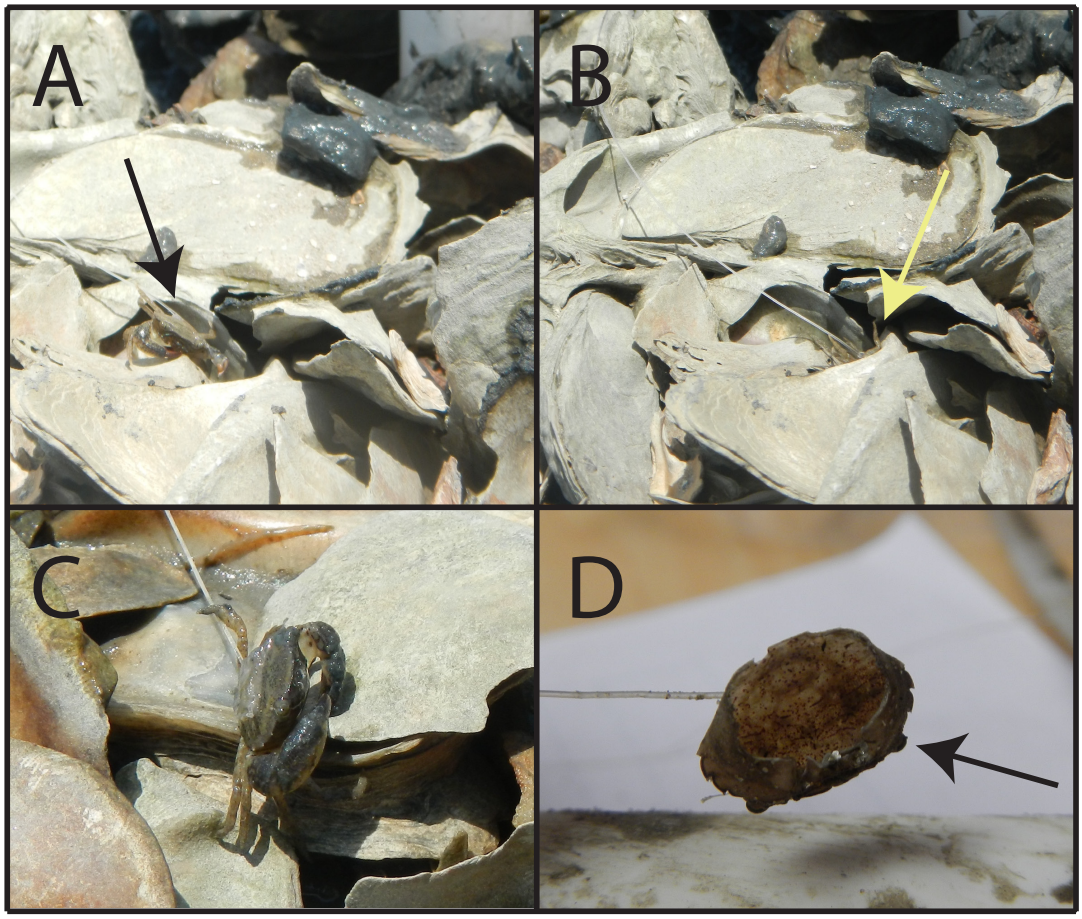

Figure 3.2 *Eurypanopeus depressus* tethered in an oyster reef at Priest Landing, Savannah, GA for a field predation trial. A. An *E. depressus* on its tether moving across the oyster reef (black arrow), B. towards a crevice within an oyster clump to hide inside (yellow arrow), demonstrating that *E. depressus* on the tethers were able to hide from predators. C. A live *E. depressus* shortly after being placed in the field. D. The empty carapace of a partially consumed *E. depressus* still on tether. The eyestalks (black arrow) remain with the carapace, indicating that the crab did not molt.

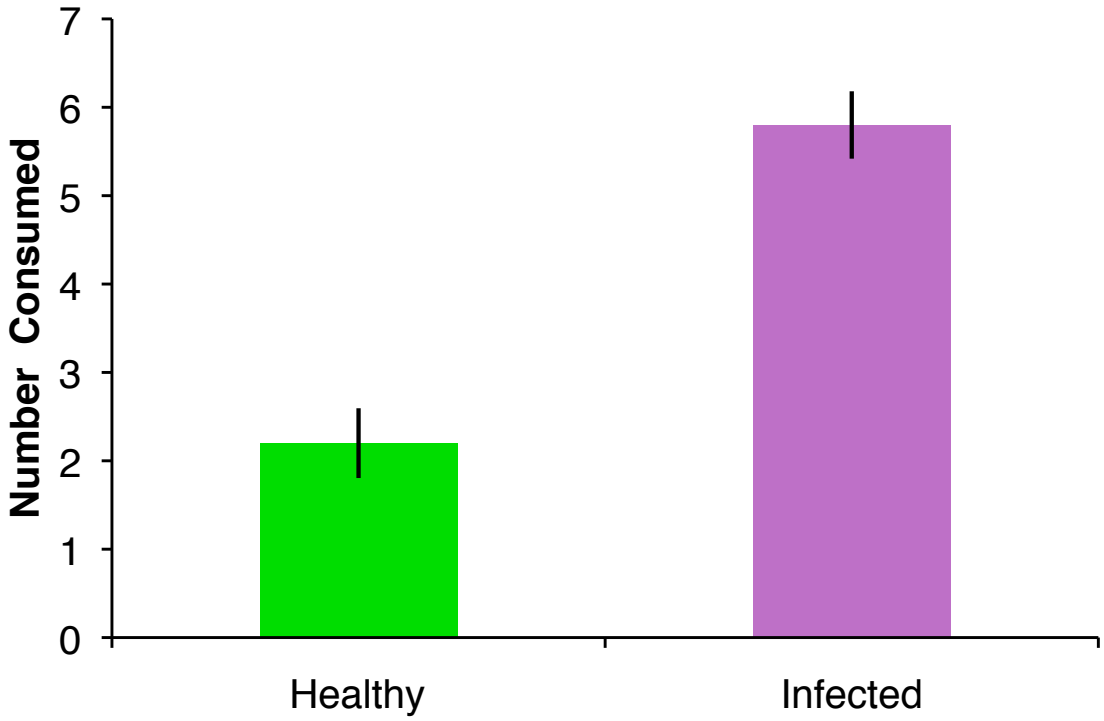

Figure 3.3 The average number of healthy (green) and infected (purple) E. depressus consumed by each predatory C. sapidus (n=20) over a weeklong mesocom trial. The error bars depict 1 SE. The diet of each C. sapidus averaged 75% (± 1.7 SD) E. depressus infected with L. panopaei.

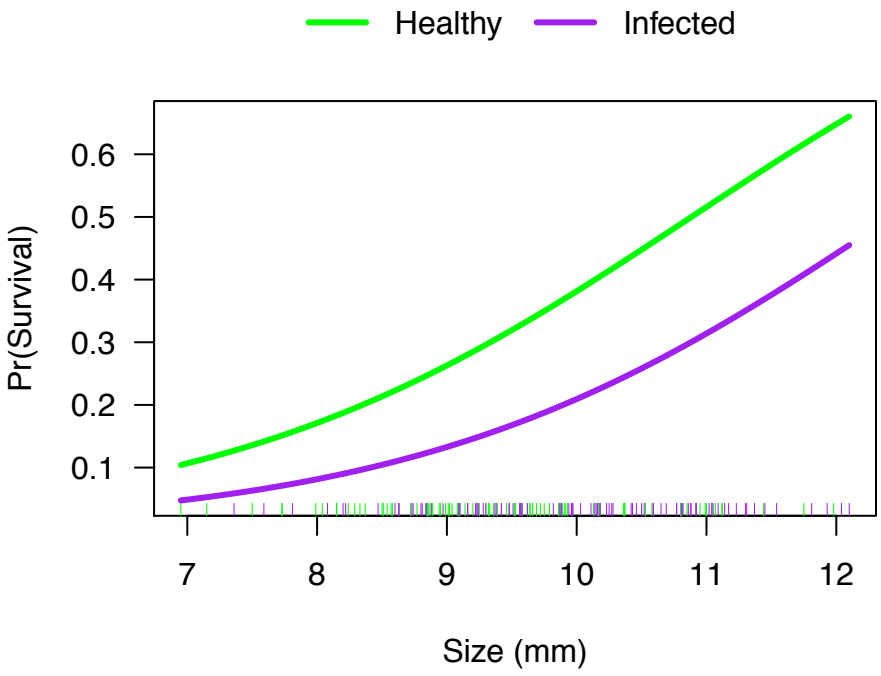

Figure 3.4 The probability of surviving a single high tide cycle for healthy *E. depressus* (green) and *E. depressus* infected with *L. panopaei* (purple), when tethered on an oyster reef. Probability curves represent the results from a logistic regression on survival with size, and rug marks along the x-axis indicate the spread of data across size.

Figure 3.5 Speed of healthy *E. depressus* (green) and infected *E. depressus* (purple) running 220mm (long runway) and 94mm (short runway) along a PVC tube. Error bars are standard errors and the number of replicates in each study are listed along the x-axis.

CHAPTER 4

INCREASED TEMPERATURES DIFFERENTIALLY AFFECT INVASIVE PARASITE, FREEING HOST FROM INFECTION[3]

[3] Gehman, A.M., R.J. Hall and J.E. Byers. To be submitted to *Science*.

Abstract

Hosts and parasites are coupled systems, but each can respond differently to changes in environmental variables like temperature. Temperature influences the physiology of hosts and parasites' thermal responses, differentially shift with climate change has been widely speculated on, but combined empirical and modeling work is critically needed to predict parasite response to climate change. Here we explicitly quantify thermal response curves encompassing the thermal breadth of the host and parasite, yielding an unprecedented dataset for a marine system. We found a thermal mismatch in survival optima between infected and uninfected hosts, with infected host demonstrating unexpected thermal performance optima at lower temperatures then the uninfected host. We parameterized a physiologically based epidemiological model to predict the sensitivity of this host-parasite system to future climate warming scenarios. The model accurately predicted the observed annual cycles and seasonality in the host-parasite system. Under plausible warming scenarios, parasite prevalence declined with summer temperature, with parasite local extinction predicted given mean 3º C warming. This work suggests that hosts might benefit from seasonal mismatch in thermal optima for host, and further highlights the need to explicitly include host and parasite thermal performance curves to predict infection responses to climate change.

Introduction, Results and Discussion

Environmental change fundamentally affects individual animals, and individual response to climate alters how species interact with each other. These changes in species interactions are the key link between climate effects on individuals and the responses of

communities. The intimate association between parasites and their host make them well suited for evaluating how environmental change alters species interactions. For example, warming in temperate regions is predicted to increase the distribution and activity of ectothermic vectors of human pathogens, which coupled with an increase in vector and parasite development rates might result in higher transmission potential (LaDeau et al. 2015, Ren et al. 2016). Together, the increase in vector borne diseases as the climate changes has led some to argue that a warmer world will be a sicker world (Harvell et al. 2002). Although, the generality of this hypothesis is under debate (Rohr et al. 2011, Altizer et al. 2013), since temperature can differentially influence within-host and between-host processes in non-additive ways, making the net effect of warming difficult to predict. For example, greater parasite reproduction with temperature may not change parasite dynamics if increased temperature also enhances parasite induced mortality in the host (Anderson and May 1979). Empirical data are limited, even in the best studied human disease systems (Mordecai et al. 2013, LaDeau et al. 2015), and a comprehensive analysis of the effects of temperature on multiple stages of infection within a single host has not yet been accomplished.

Temperature is one of the most important factors affecting the performance of ectotherms, with practically all physiological and behavioral attributes sensitive to temperature (Dell et al. 2011, Amarasekare and Sifuentes 2012). Ectothermic response to temperature is predictable, and can be described by thermal performance curves (Huey and Kingsolver 1989). For many species, development does not occur below a lower threshold temperature (T_{min}), increases to an optimum (T_{opt}) and rapidly decreases above the optimum to zero (T_{max}). Despite extensive knowledge of these physiological

responses, studies that consider temperature effects on host-parasite interactions focus on increasing relationship prior to T_{opt}, but ignore the non-linear responses to temperature on parasite development and reproduction (but see (Molnár et al. 2012, Mordecai et al. 2013)). Parasites of ectothermic hosts are likely have the same non-linear rate responses to temperature as their free-living hosts, and empirical evidence to explore this pattern is crucially needed to predict population dynamic responses to warming (Rohr et al. 2011, Altizer et al. 2013).

We test the predictions that hosts and parasites survival and parasite reproduction will respond non-linearly to temperature, and that host survival and parasite production might be optimized at different temperatures. We focus on a host-parasite system that exhibits seasonal variation in infection prevalence (O'Shaughnessy et al. 2014b), and whose biogeographic spread might be limited by high summer temperatures (Kruse et al. 2011). *Eurypanopeus depressus* is an abundant oyster reef-dwelling crab that is infected with an invasive rhizocephalan parasite, *Loxothylacus panopaei*. Parasites are directly transmitted with a free-living stage that recruits to hosts (detailed lifecycle in supporting online material; SOM). There are two distinct stages of *L. panopaei* infection: exposed hosts, which are infected but the parasite is non-reproductive, and infected hosts, in which the parasite is reproductive and releasing infective stages. We conducted laboratory experiments in Savannah, GA, in which we manipulated temperature and quantified thermal performance curves by subjecting uninfected hosts (susceptible) and two infection stages (exposed and infected; Figure 4.1) to temperatures encompassing the observed thermal breadth of the host and parasite in coastal Georgia.

Consistent with findings in other ectotherms (Huey and Stevenson 1979, Dell et al. 2011)), susceptible host mortality had a non-linear relationship with temperature, with a T_{min} = 4.05 ºC, T_{max} = 33.85 ºC and $T_{opt} \approx$ 20 ºC (Figure 4.1, Table S4.3). The parasitized hosts had a lower T_{opt} for survival, whether the parasite was actively reproductive (infected) or not (exposed), with a $T_{opt} \approx$ 10 ºC (Figure 4.1, Table S4.3). Parasite with active reproduction (infected) had a reduced thermal breadth at the upper limit, with the T_{max} = 31.9 ºC for parasite survival and T_{max} = 30.75 for parasite reproduction (Figure 4.1). Parasite reproduction was also contracted at the lower limit, with a T_{min} = 9.88 and reproduction $T_{opt} \approx$ 15 ºC (Figure 4.1, Table S4.3). Together the parasitized hosts had optimal performance at lower temperatures than that of the uninfected hosts. The offset in T_{opt} between uninfected and infected hosts reveals that parasites can drive a change in host thermal performance, thus causing a mismatch in the thermal optima and tolerance for parasites versus their hosts.

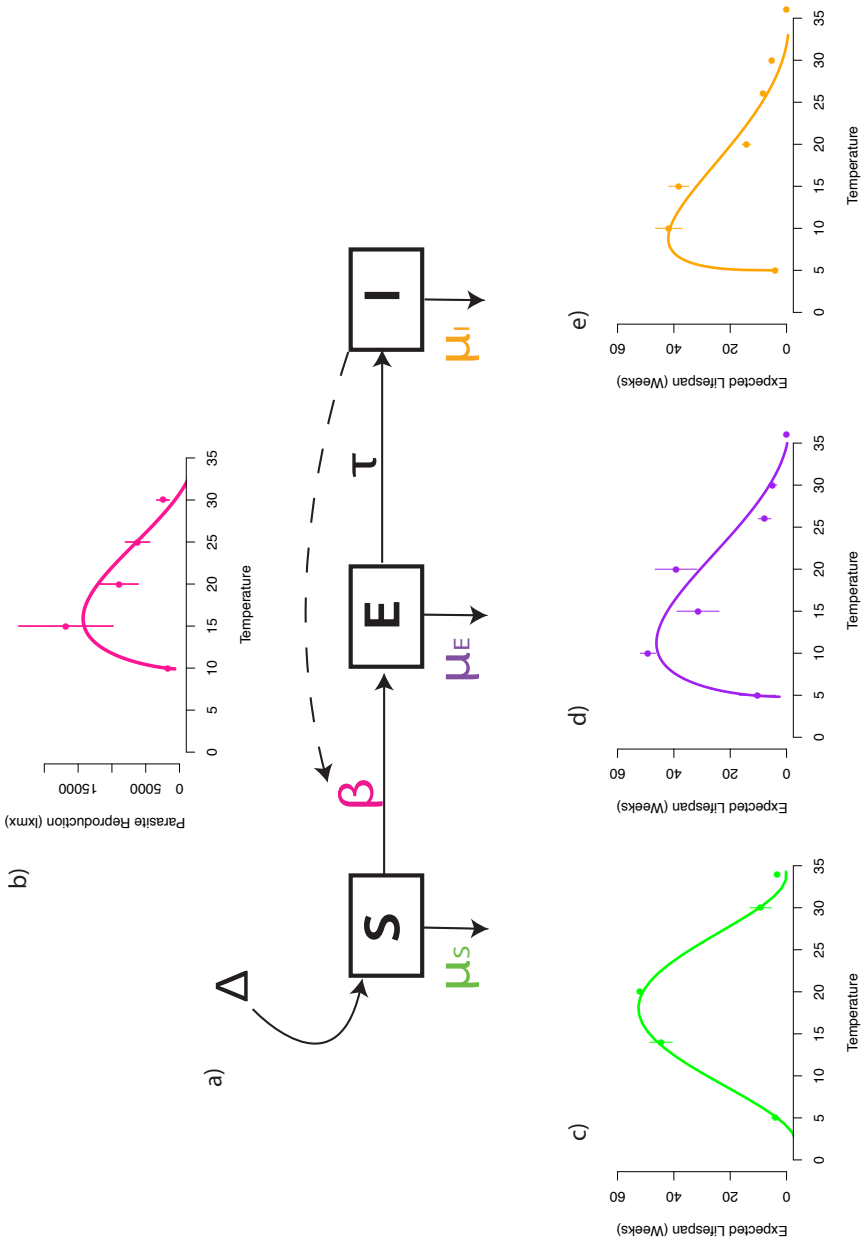

Figure 4.1. a) A model for *L. panopaei* transmission in *E. depressus* built in a Susceptible – Exposed – Infected framework. Susceptible hosts, S, are recruited at rate at a rate Δ, become infected at per capita rate β*S*I, where the transmission rate β is assumed proportional to larval parasite production, and die at per capita rate μ_s (μ=1/Expected weekly survival, c-d). Exposed hosts become infectious at a rate τ. The exposed and infected categories have mortality rates of μ_E, and μ_I, respectively. Model parameters labeled in color are temperature

dependent, and are parameterized based thermal performance curves fit to experimental measurements of survival and parasite reproduction. b) Thermal performance curves for parasite transmission and c-e) survival across multiple stages of infection including c) susceptible, d) exposed and e) infected hosts. The shape of parasite transmission in response to temperature was estimated from the sum of age specific parasite larval production calculated from 200 days of parasite reproduction (details in SOM). Expected weekly survival was calculated from Kaplein-Meier survival curves with estimate of survival at each temperature (details in SOM).

We investigated the ecological consequences of seasonal mismatch in thermal performance for host and parasite by developing a compartmental model of host infection (Anderson and May 1982), where parasite transmission and host survival are temperature dependent (Figure 4.1). To describe the thermal environment of our host and parasite we calculated mean weekly temperatures from a Georgia Coastal Ecosystems (GCE) buoy that measured temperature at a depth of ~1m (Figure 4.2A), the approximate depth of water found over an oyster reef in Georgia (Di Iorio 2012, 2013, 2014a, 2014b, Byers et al. 2015), and thus the thermal environment experienced by *E. depressus* and *L. panopaei*. The model dynamics rapidly converged on an annual seasonally-influenced cycle of parasite prevalence (Figure 4.2D). Consistent with field observations (O'Shaughnessy et al. 2014b), the model predicted that parasite prevalence would increase in the winter, and decrease in spring and early summer (Figure 4.2D). The drop in prevalence in the summer is driven by a combination of decreased parasite transmission (Figure 4.2G) and greater infected host mortality (Figure 4.2J) with increasing temperature (Figure 4.2A).

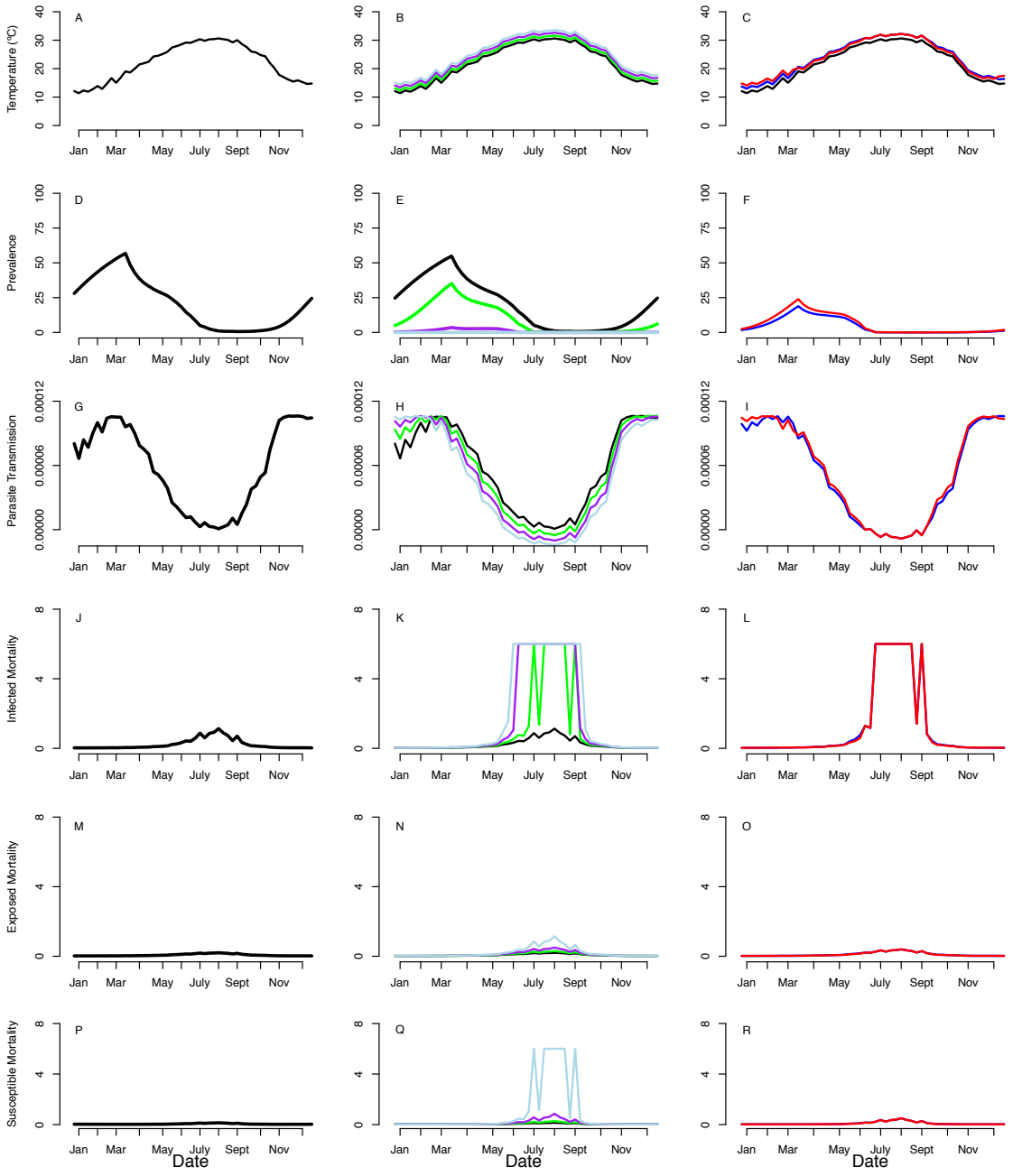

Figure 4.2. A: Temperatures used to drive seasonality in the host-parasite model, with the mean water temperature from the Georgia Coastal Ecosystem Long Term Ecological Research station 10 averaged weekly from 2011 to 2014 B: mean +1 (solid green), mean +2 (solid purple), mean +3 (solid light blue). C: Temperature increased based on hindcast from actual temperature change in the southeast from 1970-2008 (Karl et al. 2009), with the mean +1.6 (solid blue) and

seasonally varying increases. D-F: Model predictions of seasonal prevalence. Temperature dependant model parameters, including parasite transmission, β (G-I), infected host mortality (J-L), exposed host mortality (M-O) and susceptible host mortality (P-R).

Model exploration showed that parameters were behaving in a biologically realistic manner. We evaluated the minimum number of infected individuals within a single year and found that infection was maintained by >1 infected or exposed individual at all times throughout the season. To evaluate how robust the prediction of seasonality was to a range of parameters values, we conducted sensitivity analyses (SOM). Sensitivity analysis revealed that peak and mean prevalence, but not minimum prevalence, increase with transmission rate, β (Figure S4.10), and only weakly depend on the seasonal pattern of host recruitment, Δ (Figure S4.11) and latent period, τ (Figure S4.13). The seasonal pattern of prevalence is robust to variation in parameter values, and the timing of the seasonal peak in prevalence is most strongly influenced the timing of the host recruitment peak (Figure S4.11).

To evaluate potential effects of climate change on host-parasite interactions in ectothermic hosts, we ran model simulations at a range of increasing temperatures, from the GCE mean +1, +2 and +3ºC (Christensen et al. 2007). We began each simulation with infection prevalence around 30%, giving the parasite the best possible chance to maintain infection through the season. Host-parasite cycling persisted with decreasing amplitude in the model at +1 and +2º C (Figure 4.3N), and the parasite was driven out of the system within a single season at mean +3ºC (Figure 4.3N). As we started the model with higher prevalence than is predicted under current scenarios (or observed in the field, (O'Shaughnessy et al. 2014a)), the estimate of parasite local extinction with a mean increase of +3 ºC are conservative. Parasite extirpation is driven by warming induced

exacerbation of the seasonal drop in prevalence, specifically by greater infected host mortality (Figure 4.2K) and decreased parasite transmission (Figure 4.2H).

In the Southeastern US, the increase in temperature as a result of climate change in more intense in the winter months (Karl et al. 2009), and is not expected to increase evenly across seasons in the future (Christensen et al. 2007). Thus, to explore the effects of seasonally varying temperature, we used a simple hindcast of actual change in temperature in the southeast from 1970-2008. We compared the effects of actual mean temperature change and seasonally dependent temperature change (Figure 4.2C) on host-parasite dynamics (details in SOM; (Karl et al. 2009). The model predicted slightly higher maximum infection prevalence (+5%) and slight offsets in seasonality with seasonally dependant temperature change (Figure 4.3O).

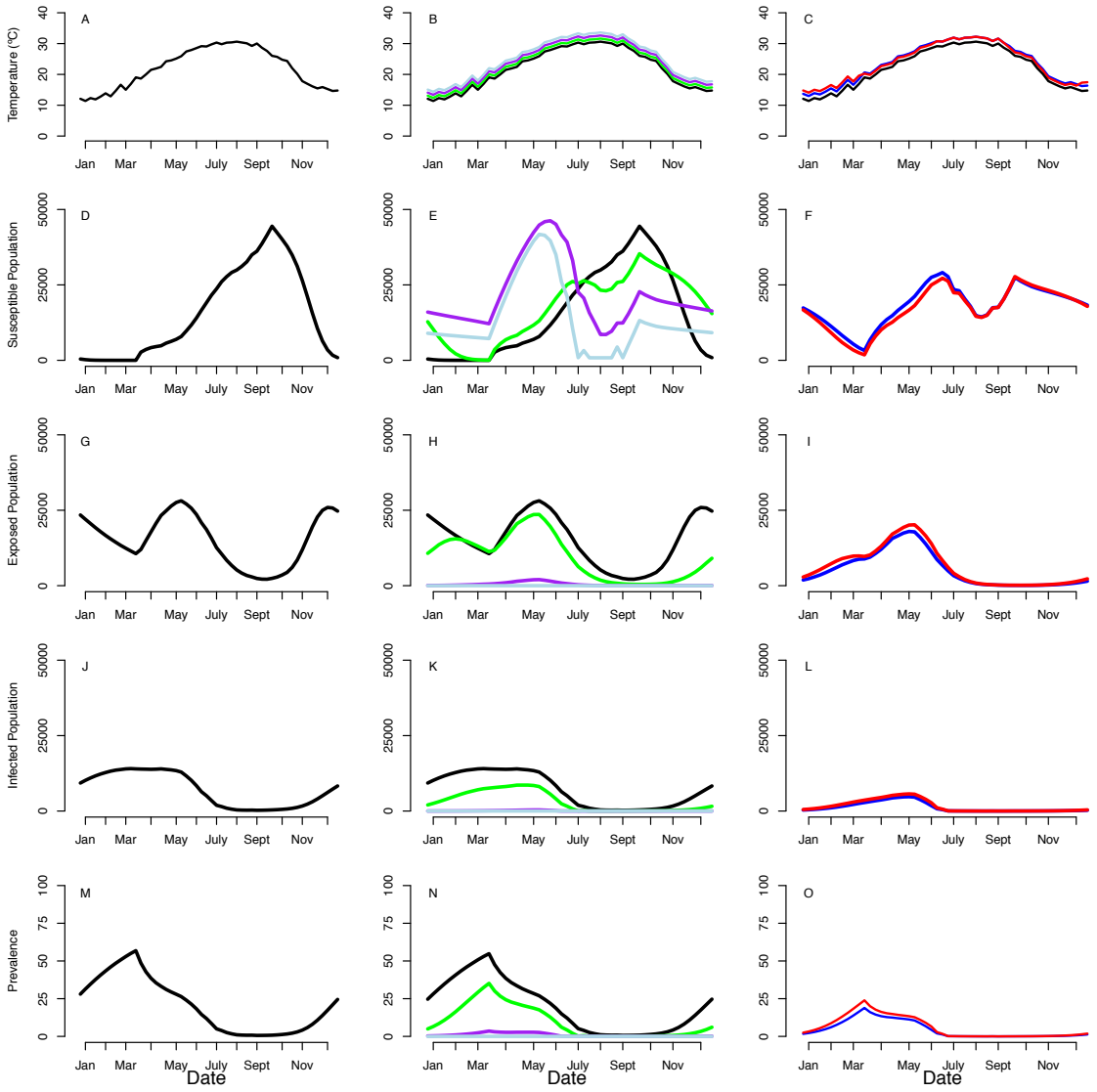

Figure 4.3. A: Temperatures used to drive seasonality in the host-parasite model, with the mean water temperature from the Georgia Coastal Ecosystem Long Term Ecological Research station 10 averaged weekly from 2011 to 2014 B: mean +1 (green), mean +2 (purple), mean +3 (light blue). C: Temperature increased based on hindcast from actual temperature change in the southeast from 1970-2008 (Karl et al. 2009), with the mean +1.6 (dark blue) and seasonally varying increases (red). Model predictions of population size of susceptible hosts (D-F), exposed hosts (G-I) and infected hosts (J-L), and prediction of seasonal prevalence (M-O).

This work highlights the consequences of parasite driven changes to host thermal performance for parasite persistence. If this thermal mismatch in survival between uninfected and infected hosts represents a general ectothermic host-parasite response, then increasing temperature may accelerate the population decline of parasites, leading to unexpected biodiversity loss. The differential in uninfected and infected host thermal performance, particularly of survival, may have been missed in other studies that focused on temperature effects on parasite transmission traits (Anderson and May 1979), such as reproduction and infection probability (Morley and Lewis 2014, Paull et al. 2015), or focused on survival of uninfected hosts only (Mordecai et al. 2013, Johnson et al. 2015). There is a suggestion of generality from trematode systems – where there is a consistent negative relationship between infected host mortality and temperature across a range of temperatures likely within the thermal optima of the host (Paull et al. 2015). Even if the thermal performance mismatch is not a generalized response, the inclusion of the non-linear effect of temperature on parasites will likely increase the accuracy of predictions of host-parasite interactions at higher temperatures (Mordecai et al. 2013). Non-linearity of response can account for the preponderance of studies suggesting an increase in disease transmission if those studies have been conducted in areas where measurements were taken only on the rising side of the non-linear response (Figure 4.2). Indeed, in this study if we were to only analyze the relationship between infection prevalence and temperature from January-March we would assume a positive effect of temperature on transmission (Figure 4.2).

The prediction that increased temperature could drive down disease can be beneficial – for example, in temperate climates increased temperature increases the

spread of mosquito borne disease, such as malaria, but in hotter areas of the tropics there may see a decrease in disease with increasing temperature (Mordecai et al. 2013). On the other hand, parasites are also important members of communities and an influential aspect of biodiversity, and their removal can have unintended (and often undetected) consequences on the predators or prey of their hosts (Wood and Johnson 2015). Parasites can alter host dynamics (Figure S7, (Hudson and Greenman 1998)), are themselves prey (Kaplan et al. 2009), and can enhance transfer of energy to higher trophic levels through manipulation of their hosts (Lafferty and Morris 1996). In fact, predation rates on *L. panopaei* infected *E. depressus* are three times that of uninfected hosts, suggesting that removal of this parasite from the system will alter prey availability (Gehman and Byers, in review). Our model results corroborate recent regional scale observations from the field that demonstrate that prevalence is limited in its southern range (e.g. absent in southern Florida), and early indication that the parasite may be expanding its range further north up the Atlantic coast of the US.

In sum, this work reveals a thermal mismatch in performance between uninfected and infected hosts that leads to the prediction of local extirpation of the parasite under moderate climate change scenarios. If the thermal mismatch found here can be generalized for ectothermic host-parasite interactions at high temperature, then this may cause hitherto unforeseen emergent effects on community and ecosystem dynamics.

Supplemental Online Material

Methods

System and Field Collection

Host Natural History

Eurypanopeus depressus is an abundant oyster reef-dwelling crab occurring from the Gulf of Mexico to Massachusetts Bay. Temperature varies considerably across this range, and within individual sites seasonally. As an ecotherm, *E. depressus* body temperature can be expected to conform to the surrounding water temperature (Willmer, Stone and Johnson text). Refuge within the oyster reef ameliorates desiccation stress and protects them from predation (Grant and McDonald 1979, Hulathduwa et al. 2007). While there is a great range of oyster reef size and shape, the average reef found in Chatham County, GA is ~200m^2 (Corely and Harris, Harris *personal communication*). Average density of *E. depressus* is 120 adult m^{-2} (4-20mm carapace width) and 140 juvenile m^{-2} (<4mm carapace width, Gehman et. al. in review).

The mean expected longevity of adult *E. depressus* (from their first larval release) is 2.2 year, with a maximum life span of 3.5 years (McDonald 1982). Female *E. depressus* in South Carolina have two broods per year, with two peaks of ovigerous reproduction, with the first peak in April-May and the second peak late August-September, however ovigerous females can be found any time between March and October (McDonald 1982). Interestingly, larval recruitment is year-round in south Florida, but had similar peaks late spring and summer (Tolley et al. 2006), with decreased reproduction correlated with decreased salinity associated with the wet season (Miner et al. 2013). Further north recruitment seems to come in a single pulse, from July to

October (although this study measured all xanthid recruitment and only collected in the spring for a single year;(Jones and Epifanio 1995)). Eurypanopeus *depressus* has 4 zoeal stages and one megalopal stage (under laboratory conditions; (Costlow and Bookhout 1961). Larval behavior suggests that *E. depressus* recruitment is retained within estuaries, with larvae migrating towards the surface during flood tide and towards the bottom at ebb tide (Sulkin 1984, Epifanio and Tilburg 2008). While recruitment is likely relatively closed within an estuary, reefs within the estuary are likely experiencing open recruitment from within the estuary.

Parasite Natural History

Loxothylacus panopaei is a castrating rhizocephalan barnacle parasite that infects the mud crab *Eurypanopeus depressus,* as well as several other mud crabs (Reinhard and Reischman 1958; Kruse et al. 2011). *Loxothylacus panopaei* is native to the Gulf of Mexico and introduced to the US Atlantic coast from Long Island, New York to Cape Canaveral, Florida. Genetic work suggests that there are three lineages of this parasite, and only the ER lineage is invasive along the US Atlantic coast (Kruse et al. 2011). *Eurypanopeus depressus* is infected by the ER lineage, which was first documented in North Carolina in 1983 and in Georgia and northeastern Florida in 2004/2005 (Kruse and Hare 2007; Eash-Loucks et al. 2014). The Harris mud crab *Rithropanopeus harrisi* is also infected by the ER lineage, and could serve as a resevoir host for this parasite, however it is generally found in lower salinities and has not been document near where this study took place (Prezant et al. 2002)(personal observation). While *L. panopaei* is present within the Gulf of Mexico and in Northern Florida, it has not been reported in Southeastern Florida, suggesting that the higher temperatures in Southeastern Florida

may represent poor conditions for the parasite and be limiting spread (Kruse et al. 2011). At a local level, there is evidence that temperature may drive seasonal patterns in parasite infection, with infection prevalence increasing in the spring, followed by a marked decrease in infection prevalence mid-summer, correlated with temperatures reaching above 25° C (O'Shaughnessy et al. 2014b). Together these observations suggest that temperature may be driving changes *L. panopaei* infection prevalence.

Rhizocephalans have direct transmission, and the adult parasite resides within its crab host and produces larvae, lending itself for quantification of reproductive output. *Loxothylacus panopaei* larvae are planktonic and lecithotrophic (non-feeding), take 2-5 days to become infectious and can be male or female (Walker et al. 1992, Walker 2001). Larval sex dictates it's role within the transmission cycle; female larvae find and infect susceptible hosts, form an internal infection (interna) and then release a virgin externa (Glenner 2001). Exposed hosts are infected, but non-reproductive and the time to release of the virgin externa can take from 18-57 days (Alvarez et al. 1995). For the parasite to become reproductive, and thus infectious, a male larvae (or two) must recruit to the virgin externa after which the ovary develops to reproductive maturity (Hoeg 1995). The time between male larval colonization of an exposed host and reaching parasite reproductive maturity can take from 18-22 days (Alvarez et al. 1995).

Collection

To create thermal performance curves for multiple stages of parasite infection, we collected crabs from a single location from Romerly Marsh Creek, Savannah, GA (31°55'15.5"N 80°59'13.0"W) from 9th March to 23rd April, 2014. We collected a single cohort of infected individuals to standardize infection age, as age of infection may

determine parasite reproduction. Previous observation by A. Gehman suggested that in Georgia there is semi-synchronized release of virgin externa correlated with the seasonal increase in ambient water temperature above ~20°C (*personal observation*). To collect a cohort of approximately the same age *L. panopaei* infections, we isolated crabs not exhibiting external infections, held them at ambient temperature, fed them ad libidum with frozen brine shrimp and monitored for evidence of externa release. New infections were first noted 24th April 2014, and we began the experiment on May 13th*;* thus infections used for the experiment were all less then a month old when the experiment began.

Internal infection is highly prevalent in the spring months, an ideal time for collecting multiple stages of parasite infection, however for that reason it was also a very difficult time to collect uninfected crabs. Additionally, as noted above, the lack of manifested externa does not guarantee that crabs are uninfected. *Loxothylacus panopaei* causes complete sterilization, so if a female is ovigerous then it is not currently infected (Alvarez et al. 1995, Hoeg 1995). In addition, *L. panopaei* feminizes its host, so *E. depressus* that have strong morphologically male characteristics are less likely to be infected. To take advantage of these traits and increase the chances of collecting uninfected hosts, we collected ovigerous females and male *E. depressus*. For the logistical reasons described above, the uninfected *E. depressus* collection began a few months after the start of the infected crab experiment. Collection was conducted from the 3rd of July and the 4th of September 2014, and collected crabs were maintained filtered seawater in individual jars at ambient temperature and monitored for any evidence of

infection. A few males exhibited virgin externa, but no formerly ovigerous females ever exhibited externa. The experiment started on the 28th of September 2014.

Lab Experiments

Experimental set up

Our objective for this study was to evaluate the effect of temperature on host-parasite survival and parasite reproduction by creating thermal performance curves for the infected and uninfected host. To achieve this goal we subjected two stages of infected hosts (exposed/non-reproductive infection and infectious infections) and uninfected hosts to a range of temperatures that encompass the full range experienced in the field (Figure 2A). Seven coolers (Igloo 45L, 65 x 36 x 26 cm) were set up in a light and temperature controlled room, with ambient air temperature set to 15.5° C and a 12:12 light-cycle (8am to 8pm). To maintain a consistent water temperature within each cooler each cooler was maintained as a water bath with a pump in each cooler to keep the water well mixed. To keep crabs isolated but at the same temperature we placed 25 glass jars (30 mL spice jars, 56.6mm diameter, Freund 5023B06-B) within each cooler, placed on a porous shelf 130mm above the bottom of the cooler (Figure S4.1). We kept crabs in 30μm-filtered seawater to maintain the non-mated state of the exposed infected crabs (individuals with virgin externa), and to ensure that all parasite reproduction was produced by the experimental crabs. Water was changed every other day from 13 May to 24 June 2014, every four days from 24 June to 12 December 2014 and once a week from 12 December 2014 to 11 March 2015. To maintain the oxygen availability, each jar was fit with an air stone connected to an aerator.

Figure S4.1. Experimental setup, with 20 jars each containing an *E. depressus*, fit with an airstone connected to an aerator. To maintain a constant temperature for the whole cooler we placed a single pump head next to an aquarium heater that was isolated some distance from the experimental jars.

To calculate a thermal response curve for host-parasite survival and parasite reproduction rates we maintained cooler temperature treatments at 5°C intervals from 5-35°C (Table S4.1). We used chillers (RTE-111 and RTE-140) to cool water for the 5° and 10° C treatments. The 15º cooler was kept at ambient. We used a single aquarium heater (Marine land submersible heater, 25W) to heat the 20° and 25° C treatments and two aquarium heaters for the 30° and 35° C treatments. To monitor the temperature treatments we placed an ibutton into a single jar in each cooler with a temperature measurement every 10 minutes, and took the temperatures once daily with a hydrolab (brand). To keep temperature constant in each jar during water changes we used aquarium heaters to raise filtered seawater to the exact temperature of the water bath before adding it into the crab jars in each treatment. Water changes were conducted one at a time, so that crabs were only outside of their temperature treatments for ~1 minute for each water change. To control for any effect of processing order we randomized the order in which water was changed for each water change.

Table S4.1. Average temperature and standard deviation for each cooler over the length of the experiment.

Experiment	Temperature (ºC)	SD (ºC)
Exposed/Infected	5	0.13
Exposed/Infected	10	0.17
Exposed/Infected	15	0.85
Exposed/Infected	20	0.59
Exposed/Infected	26	0.48
Exposed/Infected	30	0.55
Exposed/Infected	36	1.27
Susceptible	5	0.07
Susceptible	14	0.62
Susceptible	20	0.59
Susceptible	30	0.27
Susceptible	34	2.09

Infection Experiment (Exposed and Infected)

The exposed and infected survival experiment ran for 312 days (including 11 days of acclimation). We randomly distributed ~20 infected crabs among the seven temperature treatments, with 13-14 crabs with mature infections (infected) per treatment, and 6-7 crabs with virgin externa (exposed) per temperature. Because size has an effect on parasite reproduction we measured the carapace width and limited the range of included crabs be to 6.5-14.7 mm carapace width. We verified that crabs of different carapace width had been randomly distributed between the treatments (Figure S4.2).

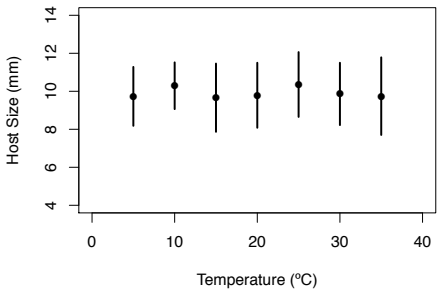

Figure S4.2. The mean and SD of host size for each of the temperature treatments.

The ambient temperature at the beginning of the experiment was ~20º C and we started all temperature treatments at that temperature. We acclimated the *E. depressus* to their experimental treatments over the following 10 days, adjusting cooler temperatures by 2°C per day. The 2ºC temperature change was gradual, as we changed the setting of the water bath and let the bath slowly increase to the new temperature. Water changes were conducted throughout the acclimation period, however we only analyze results starting after this transition period when the crabs had been at experimental temperature for a full day (day 11, May 25th 2014).

We monitored crabs at each water change. At each monitoring we checked for crab and parasite mortality and parasite larval release. Nauplii and cyprids are visible to the naked eye, and we tested our detection limit and found that we could correctly identify empty jars. In a dilution detection test we detected nauplii down to ~5 nauplii per jar. We changed the water and fed the *E. depressus* the equivalent of one brine shrimp per day. In jars where we detected a release, or we were not confident of lack of release, we collected all larvae released by pouring out the water through a 30 μm mesh, then refilling the jar and again filtering the water through the mesh. Crabs remained within their jars throughout water changes. We then rinsed the contents on the mesh into a scintillation vial with ~15mL of 90% ethanol and stored for later quantification.

We quantified the number of larvae released using a $1mm^3$ gridded sedgewick rafter slide (model# 1801-G20). We homogenized samples, collected 1mL of sample and placed it into the counting chamber. We quantified all larvae present (cyprids, nauplii and eggs). We quantified the remaining volume of the sample in a graduated cylinder. Total number of larvae released was calculated by multiplying the number of larvae calculated in 1μL by the total volume of the sample.

Uninfected Experiment (Susceptible)

Because of limitations (difficulty) in the collection of uninfected crabs we used fewer temperature treatments, but still captured the limits of the thermal curve (Table S4.1). An additional five coolers were set up to run the uninfected *E. depressus* experiment. Crabs were acclimated as described above, except that ambient water temperature was closer to 25ºC, and acclimation was done from that temperature. At each monitoring point we checked for crab mortality and any evidence of infection. We did not find evidence of infection for any individual included in the susceptible category. The uninfected – susceptible- experiment ran for 169 days (including 11 days of acclimation time).

Analysis

To get estimates of the effect of temperature on survival for each of the treatments we conducted survival analysis across temperature for the uninfected (susceptible), exposed (virgin externa) and infected *E. depressus*. We evaluated each infection status category individually using Kaplan-Meier survival analysis with the R package *survival*. This and all subsequent analyses and simulations were conducted using R (R Development Core Team 2015). We calculated the restricted mean survival time (RMST) at each temperature, which allowed us to estimate the expected weekly survival across

treatments, even if experiments were not run for an equal length (Royston and Parmar 2011)(Hosmer and Lemeshow, pg. 53:57). Additionally RMST does not require proportional response of all treatments to temperature and can accommodate censored data – e.g. crabs that hadn't died by the end of the experiment or were removed for other reasons besides mortality (Hosmer and Lemeshow). We calculated RMST by estimating the area under the curve up to time t*, which we set to 365 days, which allowed us evaluate the expected number of weeks each infection category would be expected to experience out of a year.

To evaluate the shape of the effect of temperature on host and parasite performance traits we fit functions relating performance to temperature. We fit three functions to evaluate whether the relationship was quadratic (symmetrical) or left or right skewed in response to temperature. The Briere function can capture the non-linear effects of temperature on ectotherm performance however it is limited to the right-skewed patterns often evident in ectotherm response to temperature (Briere et al. 1999). To evaluate whether responses were right- or left-skewed, we flipped and reversed the Briere to create a modified Briere function that could fit a left-skewed pattern. We evaluated whether there was any evidence for either skew using a quadratic function. To evaluate the best fit for temperature dependence for each variable we fit a quadratic, Briere and a modified Briere function to each infection class. We evaluated model fit through AICc.

Model Development

We investigated the ecological consequences of the thermal performance mismatch for host and parasite found in our experiments through a simple compartmental model. We used this model to describe seasonal disease dynamics of an *E. depressus* population

within a single oyster reef. The host population was categorized according to *L. panopaei* infection status, such that S represents the abundance of uninfected, susceptible *E. depressus*, E represents the abundance of infected but non-reproductive *E. depressus* (i.e. individuals with internal infections or virgin externa) and I represents infectious *E. depressus* (i.e. those with externa producing parasite larvae). Pelagic free-living infective stages of female and male parasite larvae are denoted by W_f and W_m respectively.

In the absence of the parasite, the crab population experiences recruitment at a rate Δ and per capita mortality at a rate μ_S. When the parasite is introduced into the system, susceptible hosts are infected at a per capita rate that is a function of the abundance of free-living female larvae (W_f). Exposed hosts die at rate μ_E and move to the infectious class depending on time to development of a virgin externa ($1/\tau$) and the probability of encountering male larvae, πW_m. Infectious individuals die at rate μ_I, and release infected female and male larvae at respective rates λ_f and λ_m. Pelagic larval mortality is considered equal (and high) for both sexes and occurs at rate μ_w. Therefore parasite transmission can be described by the following system of equations:

$$\frac{dS}{dt} = \Delta - \mu_S S - f(W_f)S \qquad \text{Eq 1}$$

$$\frac{dE}{dt} = f(W_f)S - \mu_E E - \pi(W_m)\tau E \qquad \text{Eq 2}$$

$$\frac{dI}{dt} = \pi(W_m)\tau E - \mu_I I \qquad \text{Eq 3}$$

$$\frac{dW_m}{dt} = \lambda_m I - \mu_W W_m \qquad \text{Eq 4}$$

$$\frac{dW_f}{dt} = \lambda_f I - \mu_W W_f$$ Eq 5

Female larvae must find a host, evade the host immune system and form an interna, whereas male larvae are left with the relatively simple task of fertilizing an infected host (which is presumably attempting to attract a mate). As such, we assumed that male larvae were not the limiting factor for transmission, and if a female larva infects a susceptible host and moves into the exposed category, a male larva will fertilize it. Through this assumption we restrict the temperature limitation on the parasite to the female colonization rate – so that if the temperature is such that female larvae are being produced and colonizing then we assume that temperature will be acceptable for the male larvae colonization and externa development.

We make the following (plausible) simplifying assumptions to reduce model complexity and better match experimental data:

Since larval parasite mortality is high relative to host mortality (i.e. $\mu_w >> \mu_s$), we can make a the following quasi-equilibrium approximation:

$$W = \frac{\lambda_W I}{\mu_W}$$ Eq 6

If we further assume that host infection rate scales linearly with larval parasite density, the transmission rate can we expressed as βSI, where the transmission rate β, is the product of the contact rate C, the probability of infection given contact (π_f), and the number of female larvae produced by one infectious host during the expected lifespan of a larva ($\frac{\lambda_f}{\mu_w}$) :

$$\beta = \frac{\pi_f C \lambda_f}{\mu_W}$$ Eq 7

Finally, as there are many more limiting steps for female infection, including evading the host immune system and developing the interna (Hoeg 1995), we assume that male parasite larvae are never limiting relative to the abundance of female larvae, so that all virgin externa are fertilized with probability $\pi_m W_m = 1$.

Under these assumptions, the model simplifies to:

$$\frac{dS}{dt} = \Delta - \mu_S S - \beta SI$$ Eq 8

$$\frac{dE}{dt} = \beta SI - \mu_E E - \tau E$$ Eq 9

$$\frac{dI}{dt} = \tau E - \mu_I I$$ Eq 10

Parameterization

To explore how temperature affects host-parasite interactions we allowed several model parameters to vary with temperature, including mortality rates for susceptible (S), exposed (E) and infected (I) host and parasite reproduction rate (β). To parameterize temperature dependence for the mortality of each class and parasite reproduction we incorporate the thermal performance curves created in the laboratory experiments. Mortality rate, μ is estimated by the inverse of the expected weekly survival calculated above, and through the use of functional fits to the survival data we are able to let μ vary dependent on temperature (Figure 4.2). Similarly, the reproduction rate β is set by the reproductive output calculated above and varies with temperature (Figure 4.2).

The remaining model parameters were set based on previous literature (Gehman et al; (Alvarez et al. 1995)) and are described in Table S4.2. While not strictly temperature dependent, we accounted for seasonality (and likely temperature dependence) of host reproduction by limiting recruitment to March-October. Host recruitment rate was set based on the number of juveniles found m^{-2} in a survey conducted in 2010 (Gehman et al. in review). While it seems probable that τ, development from exposed to infectious is also temperature dependent; there is no evidence to support this relationship either in the literature or from our experiments. The reported variance in time from exposed to infectious is relatively short (18-22 days), and the time to development is shorter than transitions in the model, so we chose a constant τ across temperature.

Table S4.2. Description of the parameters used for the model.

Term	Definition	Dependence	Support	Units	Estimate
Δ	Host recruitment	Seasonal	Estimated from literature (Gehman et al)	Number of juvenile recruits	5000
μ_s	Susceptible mortality	Temperature	Fig. 1	Weekly mortality	Briere Function
β	Transmission (susceptible to exposed)	Temperature	Fig. 1	# of larvae produced	Modified Briere Function
τ	Parasite development (exposed to infected)	Constant	Estimated from literature (Alvarez et al. 1995)	Weekly	0.05
μ_E	Exposed host mortality	Temperature	Fig. 1	Weekly mortality	Modified Briere Function
μ_I	Infected host mortality	Temperature	Fig. 1	Weekly mortality	Modified Briere Function

Sensitivity Analysis

We ran sensitivity analyses for all parameters that were parameterized using literature citations to evaluate the consequences for model output. This included Δ, τ and β. Although β was estimated from our laboratory experiment, the simplifications made in the model development mean that β represents multiple stages of infection, and we scaled β to match field levels of transmission.

Δ: We evaluated the sensitivity of the model to changes in host recruitment patterns by altering the length and structure of Δ on the maximum, mean and minimum parasite prevalence and total host population size predicted by the model. We explored the effect of length of transmission, from yearlong host reproduction to a single month of reproduction. In addition we evaluated the effect of assuming constant recruitment from April-October by implementing a break in reproduction in July and August, with breaks in reproduction ranging from one month to four months (Figure S4.3).

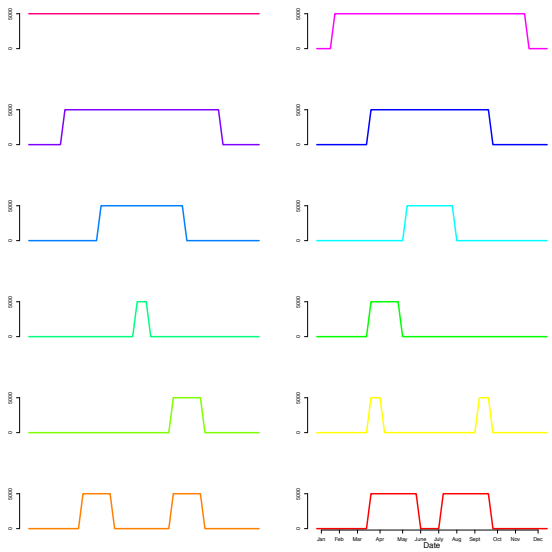

Fig S4.3. Various lengths and timing of host recruitment, used to evaluate the effect of host recruitment on infection dynamics (i.e. sensitivity analysis of Δ).

β: Because scaling was required to account for a variety of unmeasured aspects of transmission (e.g. parasite larval mortality and recruitment rates) we evaluated the models sensitivity to changes in β by running the model with -100% to 500% of the set β (Figure S4.4).

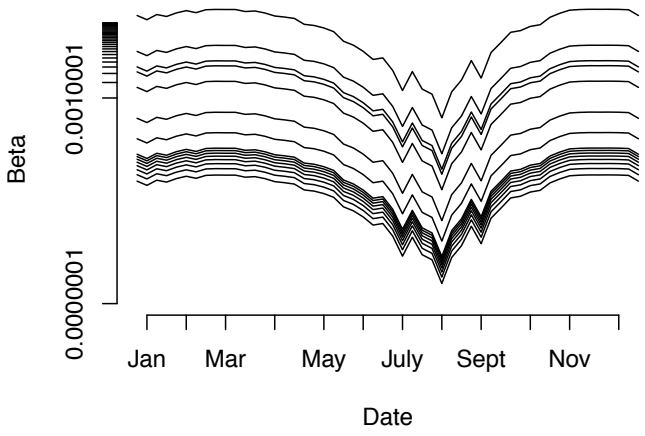

Figure S4.4. Sensitivity analysis evaluating the effect of changed β, from -100% to 500%, on a log scale.

τ: As with beta, we varied τ by orders of magnitude to explore the effect of our assumption on the model outcomes (from a little over a day to transition from exposed to infected to over 1000 days; 0.001, 0.005, 0.009, 0.01, 0.02, 0.03, 0.04, 0.05, 0.09, 0.1, 0.5, 0.9)

Seasonality and Climate Change Simulations

Parasites in the infected class release larvae approximately once a week (Walker et al. 1992), so we ran our model at a weekly time step. To account for seasonal variability in temperature we used mean weekly temperatures from 2010-2014 collected by the Georgia Coastal Ecosystem Long Term Ecological Research program ((Di Iorio 2012,

2013, 2014a, 2014b); Figure 4.2A). To best approximate the temperature experienced on a submerged oyster reef (the mud crabs habitat), data was acquired from GCE station 10, a buoy located on a creek on Sapelo Island, GA at a depth of ~1m (approximately the depth of water found over an oyster reef in Georgia (Di Iorio 2012, 2013, 2014a, 2014b, Byers et al. 2015)). We downloaded mean daily temperature and translated those to mean weekly temperatures averaged across 4 years, from 2010 to 2014. This level of averaging necessarily limits the maximum and minimum temperature experienced. However, the mean is relatively representative of the actual temperature experienced in this system because temperature is fairly stable, with the daily standard deviation of temperature between 0 and 1.7 for all four years (Di Iorio 2012, 2013, 2014a, 2014b).

For each week of the model we updated the mortality rates and parasite reproductive rates by plugging the GCE temperature data into the Briere function for host/parasite response to temperature and updating the model. We evaluated the change in abundance in each class and the change in prevalence over the year.

Model simulations were run until equilibrium was reached. Patterns were considered at equilibrium when infection prevalence for each week varied less than 0.001% between years and populations by week were equal to the nearest one crab between years. Models were evaluated for evidence of the infected population dropping below one individual and any model with less then one infected individual at any point within the annual cycle was considered not capable of cycling (as transmission by less than one individual is not biologically possible).

We evaluated the effect of climate change through five different temperature simulations. First we simply evaluated the effect of the mean+1, +2 and +3ºC across all

weeks in the year ((Sears et al. 2011); Figure 4.2B). Finally we evaluated the effect of seasonally dependant climate change by taking hindcast seasonal change in temperature in the southeast from 1970-2008 ((Karl et al. 2009); Figure 4.2C). The mean observed change was 1.6ºC, and this was compared to the season specific change; winter 2.7ºC, spring 1.2ºC, summer 1.6ºC and fall 1.1ºC.

Results

Experimental Results

For the infected individuals there was 100% mortality within the trial for five of the temperatures (5º, 20º, 25º, 30º and 35ºC), 50% mortality at 15ºC and 25% mortality at 10ºC. For the exposed (virgin externa) individuals there was 100% mortality in three of the temperatures (25º, 30º and 35º C), with a single individual that was censored in the 5ºC treatment (Figure S4.5). There was 25% mortality in the 20º C temperature treatment, 66% mortality in the 15º C temperature treatment and 86% survival in the 10ºC treatment (Figure S4.5). For logistical reasons the susceptible host survival experiment was run for 159 days and there was 100% mortality in the extreme temperature treatments (5º, 30º and 35º C), and only 16% mortality at 15ºC and no mortality at 20ºC (Fig. S4.5).

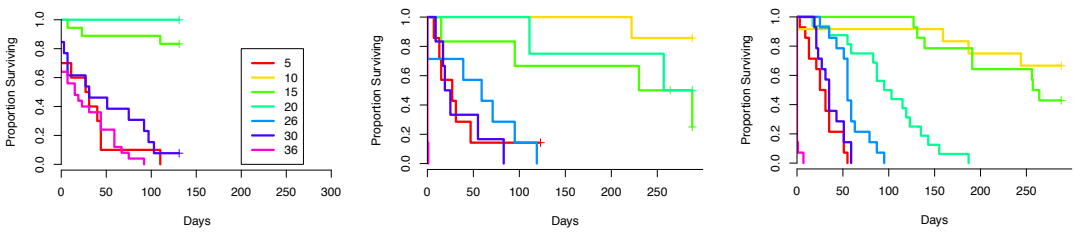

Figure S5. Kaplan-Meier survival analysis evaluating the effect of temperature on host survival for susceptible hosts (left), exposed hosts (middle) and infected hosts (right).

Expected weekly survival (used in the model) was evaluated by calculating the area under the curve for survival at each temperature.

Susceptible survival had a non-linear response to temperature and the best described by a Briere Function (Figure 4.1b-e, Table S4.3). Exposed and infected survival also had a non-linear response to temperature, however the best fitting function was the modified Briere, accounting for the right-skew in the survival response (Figure 4.1c, Table S4.3). Parasite reproduction also varied non-linearly with temperature and was best described by a modified Briere (Figure 4.1b, Table S4.3).

Table S4.3. The best fit function, the thermal minima (T0) and maxima (TM) and the AIC for that function. For comparison the AIC score for the same data with the response evaluated as a quadratic function of temperature is provided.

Infection status	Response	Function	T0	TM	AIC	Quad AIC
Susceptible	Survival	Briere	4.05	33.85	27.52	40.35
Exposed	Survival	Modified Briere	4.81	34.63	52.73	61.92
Infected	Survival	Modified Briere	4.99	31.9	44.8	61.92
Infected	Reproduction	Modified Briere	9.87	30.75	98.29	104.09

Model Results:

Disease Free: The disease free model shows seasonality, driven by host recruitment and host mortality rates (Figure S4.6). Comparison of host population between the disease-

free model and the model with infection reveals why pulsed reproduction is important for consistency of model results found when varying Δ. It appears that without fall recruitment the infection levels in the spring and early summer will drive the host population to 0.

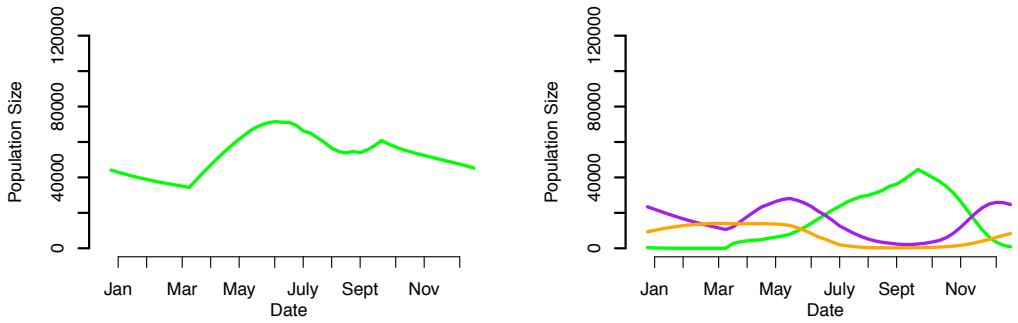

Figure S4.6. The susceptible (green) population over a single year in the model run without disease (left). The susceptible, exposed (purple) and infected (orange) populations over a single year in a model run to equilibrium with disease introduced in the population.

Model Simulations:

Model simulations reached a repeating pattern of infection quickly, after 5 annual cycles (Figure S4.7). The number of infected individuals increased with water temperature from October through mid-May, followed by a decrease in infected individuals when temperatures rise above ~25ºC (Fig. 4.3J). The number of exposed individuals has a double peak through the season, with the second peak likely driven by the onset of susceptible host recruitment in March (Fig. 4.3G and Fig. S4.12). Exposed individuals also show a marked decrease in population size at temperatures above 25ºC, however the

trough of the response is higher, suggesting that exposed hosts are maintaining infection in the population through the hot summer months (Fig. 4.3G).

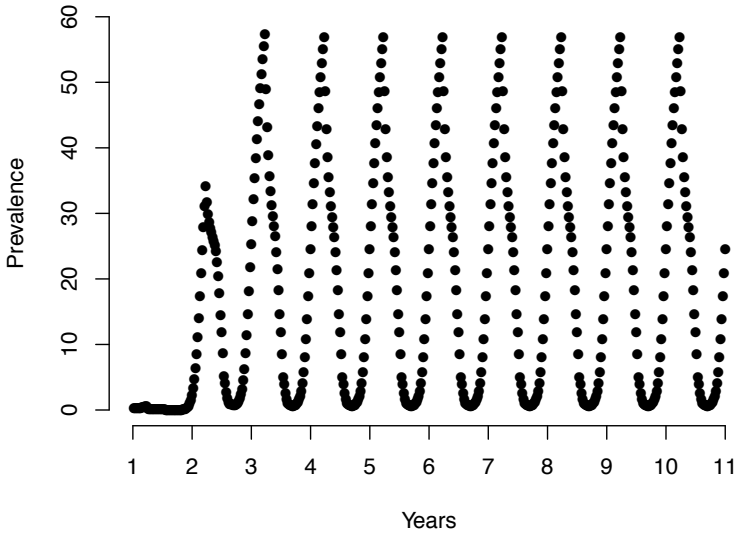

Figure S4.7. Prevalence over years.

Climate Change

Model Sensitivity Results:

β: The model was sensitive to changes in β, particularly lowering of β (Figure S4.8 and S4.9). With ~40% reduction in β the parasite fails to invade the population and infection prevalence stays at 0%. However, even with a 300% increase in β there is only a slight increase in the maximum and mean prevalence, and the result that high temperatures limit reproduction in July and August remains robust to changes in β (Figure S4.8 and S4.9).

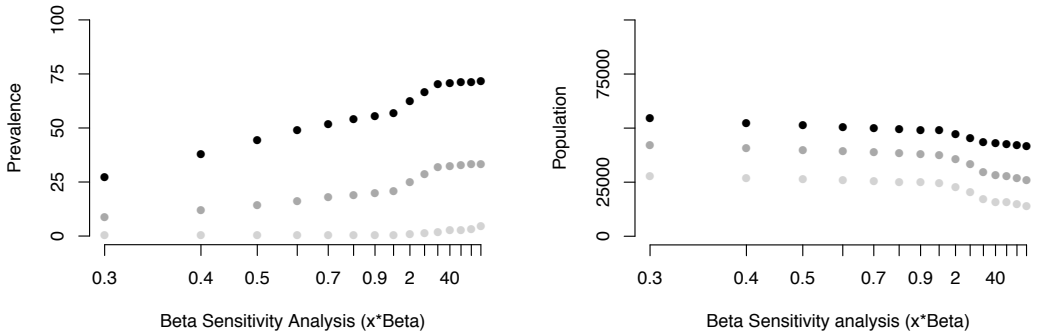

Figure S4.8. Maximum (black), mean (grey) and minimum (light grey) prevalence (left) and population (right) size predicted over a year by the model with β varying from -100% to 500% (Figure S4.4). Sensitivity analysis demonstrated an upper limit to infection prevalence (left) and relatively stable total population size in response to changing β (right).

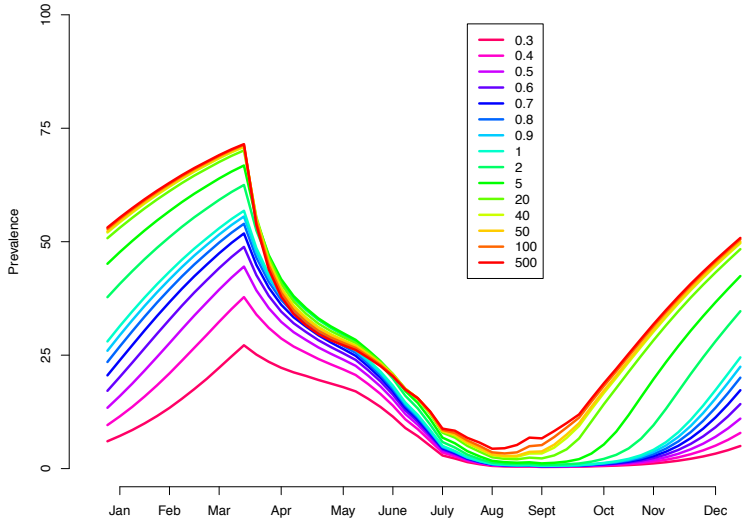

Figure S4.9. Sensitivity analysis of β, evaluating the effect of increasing β on the prediction of seasonality in the model, e.g. does increasing the parasite transmission up to 500x change the prediction that infection prevalence increases in the spring and decreases

through the summer (Figure S4.4)? The figure illustrates a single year after the model has reached equilibrium (year 10).

Δ: Maximum prevalence is particularly robust to changes in host recruitment, with only minor changes in the maximum prevalence as long as the number of months that the host is recruiting is above 5 months of a year, or there is recruitment both at the beginning and end of the summer months (Figure S4.11). However the model was sensitive to the timing of host recruitment, with the timing of peak prevalence varying depending on the timing of the onset of recruitment (Figure S4.10).

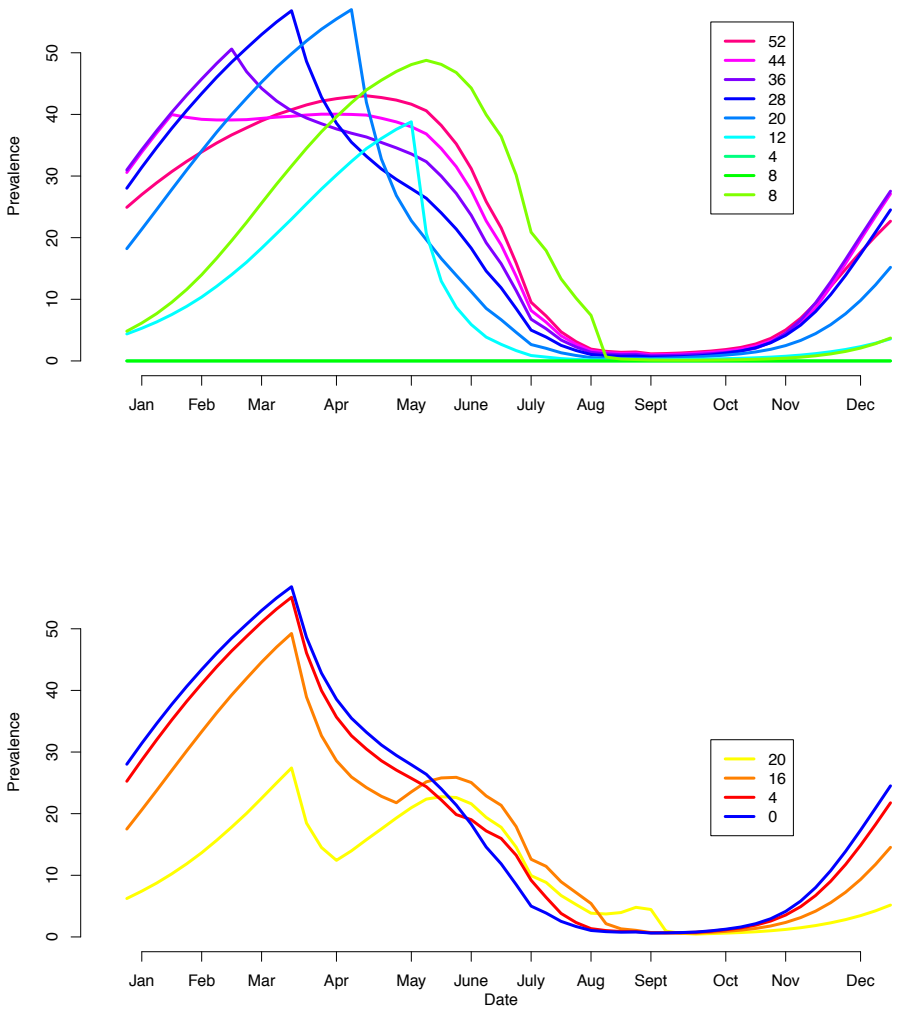

Figure S4.10. The effect of length of susceptible host recruitment on seasonality in the model ranging from full year long reproduction (52) to only 4 weeks in the middle of the summer, and two examples of 8 weeks of recruitment either in the Spring or Fall (top; Figure S4.3). An additional sensitivity analysis was run to evaluate the effect of limiting summer recruitment (bottom), with a 20-week gap in recruitment, a 16 week gap in recruitment and a 4 week gap in recruitment. In both graphs the blue line indicates the recruitment patterns used for model runs. The figure illustrates a single year after the model has reached equilibrium (year 10).

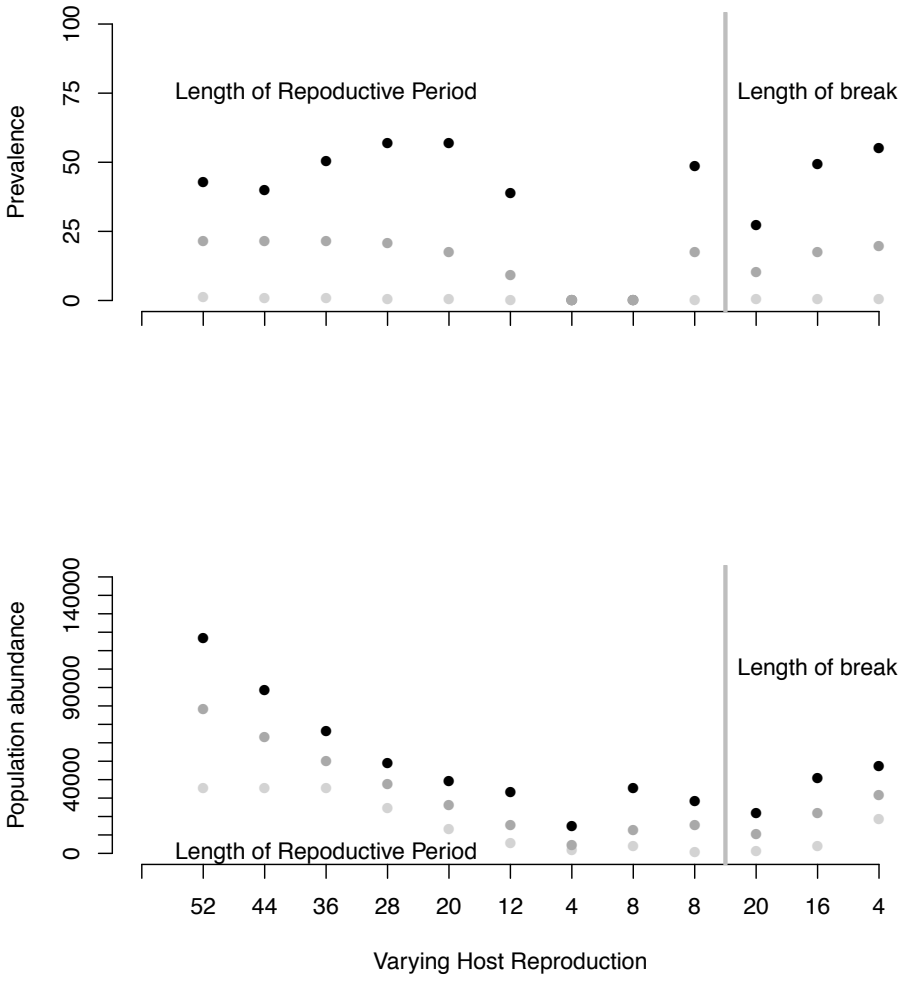

Figure S4.11. Maximum (black), minimum (light grey) and mean (grey) prevalence (top) and total population size (bottom) in response to changes in Δ. Variance in host recruitment treatments are best described in Figure S4.3. To the left of the grey line are varying lengths of the host recruitment down to 4 months (centered in the summer) and then two 8 month recruitment periods set on either side of the season (spring/fall recruitment). To the right of the line are Δ with two peaks in recruitment on each side of the summer, with varying lengths of the break in recruitment during the summer.

τ: Variance in tau changed the maximum prevalence within a season, however the patter of seasonality was robust to estimates of the transition taking between 10 and 500 days.

If exposed individuals were able to move into the infected class in less then 10 days then the shape of peak changes, and if the exposed individuals take more then 500 days to move between exposed and infectious then the parasite would be driven out of the population (Figure S4.12).

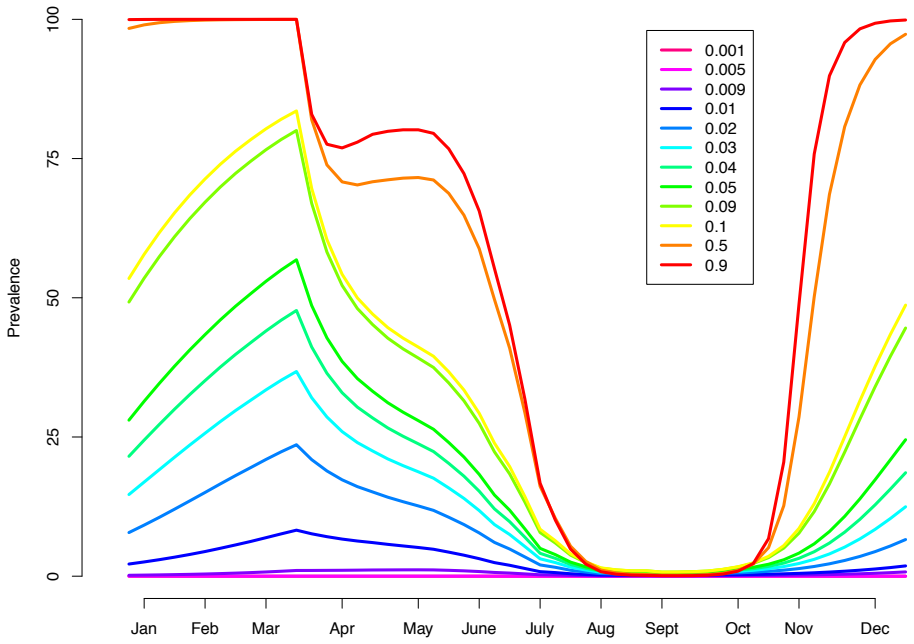

Figure S4.12. Predicted prevalence over the year in response to changes in tau (the model run is set to tau=0.05, colored in green).

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CHAPTER 5

CONCLUSION

The outcomes of species interactions are context dependent; whether the predator captures its prey (Sanford et al. 2014), the effect of a grazers consumption (Ruesink 1998), and whether a parasite infects its host (Blanford et al. 2003). This dissertation explored the abiotic and biotic drivers that influence the outcomes of host-parasite interactions, across large-scales (Chapter 1), mechanistically evaluating predator-prey-parasite interactions within an estuary (Chapter 2) and exploring climate change impacts on host-parasite dynamics (Chapter 3 and 4).

At a large-scale I demonstrated that a variety of mechanisms influence *E. depressus* probability of infection by *L. panopaei*. The results of this work provide insight into factors that should be considered when attempting to manage and control parasite populations. For example, we found that high water movement over a reef was correlated with higher probability of infection, suggesting that it is important to consider the rate of 'recruitment' of parasite propagules to an area (Chapter 2). I also found evidence to support density dependence in this system, with infection prevalence increasing with host density. We explored the relationship between parasite and host density further, demonstrating that there appears to be an upper limit around 35% (Chapter 2). A next step will be to explore if this is an emergent property of host-parasite

interactions (e.g. do all density dependant systems have limits?) or does finding a limit indicate something unique about that host-parasite interaction (e.g. that a parasite is regulating the host population).

Both through large-scale patterns and local mechanistic studies, I expanded the healthy herd hypothesis into invasion ecology, demonstrating that native predators preferentially consume an invasive parasite (Chapter 3). The outcome predator-prey-parasite interactions are far from determined, with field evidence of predators enhancing (Cáceres et al. 2009, Hawlena et al. 2010), suppressing (Packer et al. 2003, Lafferty 2015) and having no effect on parasites (Duffy 2007, Rohr et al. 2015). This is the first work to explore the effect of predators on invasive parasites, and it will be important to see if the range of interactions between invading parasite and predators is as wide as that seen in native predator-prey-parasite interactions.

Finally, through the combination of lab experiments and epidemiological modeling I was able to expand ideas of ecophysiology, thermal ecology and disease ecology to a marine host-parasite interaction, exploring the effects of temperature on host-parasite dynamics. While thermal ecology has begun to be explored in the debate on how host-parasite interactions will respond to climate change (Blanford and Thomas 1999, Molnár et al. 2012, Mordecai et al. 2013), empirical evidence lacks far behind (Altizer et al. 2013). By considering the host and multiple stages of the parasites performance response across the full range of temperatures experience in the field, it is possible to both explain why at some points there is a clear, positive relationship between increased temperature and disease, and yet at the highest temperatures we are also seeing a loss of disease (Chapter 4).

In Chapter 4, I elucidated new patterns of thermal response. External stressors (either biotic or abiotic) might decrease the thermal breadth, the temperature range over which an organism has positive performance, or decrease the thermal optima of an organism. I found that there was a decrease in the thermal breadth of infected host survival, and I also saw a shift in the thermal survival optima to substantially below that of the uninfected host. The consequences of this offset in performance are dire for the parasite, with infected hosts unable to survive simulated climate change scenarios of over a 3ºC increase in temperature (Chapter 4). It has yet to be seen whether this mismatch in performance between infected and uninfected hosts is a general trend. The shape and response of ectothermic hosts to temperature has been long evaluated and shows large-scale generalities (Huey and Kingsolver 1989, Angilletta et al. 2002), and it will be exciting to see if and how generalities can be applied across interacting species.

Through this dissertation I have demonstrated that both abiotic and biotic drivers affect host-parasite interactions. I have used a single host-parasite system to explore and expand on ideas from across the field of ecology, from invasion, to disease, to thermal and physiological ecology. Through combining fields I have found new host-parasite patterns, from a potential upper limit to infection prevalence based on host density, to an offset in thermal performance between infected and healthy hosts. Future work exploring the generalities of these patterns will elucidate whether these are unique to this host-parasite pairing, or whether they represent new emergent patterns of host-parasite ecology.

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