The Effects of Parasites on Coastal Marsh Ecosystem Structure and Functioning

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in
Marine Science and Conservation in the Graduate School
of Duke University

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ABSTRACT

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Abstract

Recent experiments and comparative surveys in Southern US salt marshes revealed that a common larval trematode parasite, *Parorchis acanthus*, generated a trophic cascade that protected foundational marsh plants (*Spartina alterniflora*) from drought-associated overgrazing by suppressing the per capita grazing impacts of its host, the marsh periwinkle (*Littoraria irrorata*). While it is clear that parasites can play a positive role in mediating marsh ecosystem response to disturbance, there is still little known about the context dependency of this interaction, the role of definitive avian hosts in regulating parasite prevalence, and whether other commonly-occurring parasites may also modify processes that underpin ecosystem stability. The purpose of this project was to extend the current understanding of the roles played by parasites and their hosts in mediating marsh ecosystem stability. A field manipulation of *Littoraria* density in which infection prevalence with *Parorchis acanthus* was held at a constant value revealed that these parasites yielded positive impacts on *Spartina* aboveground biomass at middling densities of snails, but the positive effects of parasites were negligible at both low, and high snail densities. Surveys of drought-impacted marshes revealed that birds – the definitive hosts for trematode that infect *Littoraria* – congregated within die-off areas and that increased bird usage of die-off areas was associated with increased trematode parasitism in snails within grazer fronts, decreased

per capita grazing rates of snails, and proportionate decreases in ecosystem die-off rate. Multi-site bird exclusion and mechanistic field studies experimentally confirmed that birds increased ecosystem resistance to drought-driven die-off by acting as the dispersive vectors for parasites that suppress *Littoraria* grazing. Finally, we explored how the trematode *Cercaria opaca* in ribbed mussels (*Geukensia demissa*) influenced the facultative mutualism between *Guekensia and Spartina* – an interaction that underlies marsh ecosystem resilience to drought-associated die-off. A field manipulation using experimentally infected mussels revealed that mutualistic benefits to *Spartina* decreased with increasing infection intensity in mussels. Subsequent mechanistic experiments demonstrated that increasing infection with *C. opaca* decreased mussel biodeposit production, the functional trait underlying mutualistic benefits to *Spartina.* Additionally, increasing parasite load was associated with decreased strength of both shells and byssal attachments, potentially explaining the relatively higher predation on heavily infected mussels in our field study. A survey of five North Carolina salt marshes revealed that infection intensity in mussels increased with proximity to die-off areas, indicating that *C. opaca* could influence marsh recovery following die-off events. Taken together, these results underscore the importance of parasitism's influence on Southern salt marsh ecosystem stability and more generally show that parasites can be major arbiters of community structure and functioning.

Dedication

This dissertation is dedicated to my grandmother, Patsy Rudisill. I got my smarts from her.

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1. Introduction

It has long been recognized that biotic interactions can regulate ecological communities. Early experimental ecologists focused on the structuring role of negative interactions, such as competition and predation (Hrbáček et al. 1961, Hrbáček, 1962, MacArthur 1958, Connell 1961, Paine 1966). More recent studies have revealed a similarly important role played by positive species interactions (e.g., mutualism, facilitation, and facilitation cascades) in structuring communities (Bertness & Grosholz 1985, Bertness & Hacker 1994, Hacker & Bertness 1995, Cruz-Angón & Greenberg 2005, Altieri et al. 2007, Angelini et al. 2015). While parasitism is a ubiquitous negative species interaction and represents the most common feeding strategy on the planet (Price 1980, Rhode 1982, Windsor 1998, Dobson 2008), the role of parasitism in ecosystems has only recently begun to be explored.

Despite their small size, parasites can represent a sizable swath of biological diversity in ecosystems with nearly all metazoan species acting as host to at least one (and often more) parasite species (Dobson 2008). Parasite biomass can also be substantial in ecosystems and sometimes rivals or even exceeds that of top-predators (Kuris et al. 2008). A significant body of research has demonstrated the ability of parasites to wield powerful control over host populations directly by reducing host densities or reproductive capacity, or indirectly through powerful or subtle changes in the behavior of their hosts (Holmes & Bethel 1972, Dobson 1988, Moore & Gotelli, 1990, Lafferty and

Morris 1996, Moore 2002, Toscano et al. 2014, Soghigian et al. 2017). If a host species is numerically dominant or otherwise ecologically influential, parasite regulation of host density or behavior may have large-scale, indirect effects on ecosystem structure and functioning (Wood et al. 2006, Sato et al. 2012).

There is a growing body of evidence that demonstrates the ability of parasites to play roles that were previously only associated with macro-consumers in ecosystems; for example, parasites can influence species coexistence by modifying the outcome of competition and predation (Hatcher et al. 2006, 2008), alter ecosystem energy budgets and nutrient cycling (Johnson et al. 2010, Kuris et al. 2008, Dick et al. 2010), act as ecosystem engineers (Renaud et al. 1998, Mouritsen & Poulin 2010), increase ecosystem resistance to disturbance (Morton & Silliman 2020), and control community structure and function in natural habitats (Thomas et al. 1998, Wood et al. 2006, Hernandez & Sukhdeo 2008, Holdo et al. 2009, Hatcher and Dunn 2011, Sato et al. 2012, Sánchez et al. 2016). Despite increasing recognition that parasites, like macro-consumers, can powerfully regulate communities, there is still relatively little known about the conditions under which parasites are important agents in influencing ecosystem patterns and processes. Additionally, because parasites can regulate communities, understanding what aspects of biological communities regulate their establishment, prevalence, and intensity is critically important.

Recent experiments and comparative surveys in Southern US salt marshes demonstrated that the larval trematode parasite, *Parorchis acanthus*, generated a trophic cascade that protected the foundational marsh cordgrass (Spartina alterniflora) from drought-associated overgrazing by suppressing the per capita grazing impacts of its host, the marsh periwinkle (*Littoraria irrorata*) (Morton 2018, Morton & Silliman 2020). While parasites can play a powerful positive role in mediating marsh ecosystem response to disturbance, there is still little known about the context dependency of this interaction, the role of definitive avian hosts in regulating parasite prevalence, and whether or not other parasites in this system may also modify processes that underpin ecosystem stability.

2. Grazer host density mediates the ability of parasites to protect foundational plants from overgrazing

2.1 Abstract

Like many top-consumers, parasites can regulate feeding of their prey via trait-mediated means. If parasites modify the feeding behavior of ecologically important grazers, they may have cascading effects on the structure and functioning of whole plant communities. The extent to which parasites can influence plant communities in this way is largely dependent on the strength of their behavioral alteration, their prevalence in host grazers, and the density of those hosts. Recent experiments and comparative surveys in southern USA salt

marshes revealed that common larval trematode parasites, by suppressing the per capita grazing impacts of the marsh periwinkle (*Littoraria irrorata*), generated a trophic cascade that protected foundational marsh plants from drought-associated overgrazing. Here, we conducted a field manipulation wherein we modified grazer host density while holding infection prevalence constant at an ecologically relevant level (20%) to determine whether the indirect, facilitative effects of parasites on marsh plants varied with the density of grazers. We found that parasites had significant positive impacts on marsh net primary productivity at moderate densities of snails (80 snails/ 0.7 m^2), but that the positive effects of parasites were negligible at both low and high snail densities. Our results confirm the findings of previous studies that parasites can protect marsh plants from overgrazing at sufficiently high prevalence, but show that their ability to do so depends on host density.

2.2 Introduction

A sizable body of research has been devoted to the role of top predators in organizing communities and increasing ecosystem stability through trait-mediated or density-mediated control of herbivorous prey populations. Though small, parasites can also regulate grazers populations and trigger trophic cascades, with powerful ramifications for the structure and functioning of natural communities (Wood et al. 2007,

Buck & Ripple 2017, Morton & Silliman 2020). In fact, because parasites can initiate trait-mediated indirect effects via both consumptive and nonconsumptive pathways, they may be even more likely than predators to trigger trait-mediated trophic cascades (Buck & Ripple 2017, Buck 2019). Unlike predators, however, parasites only attack one host per life stage, meaning that the potential for parasites to exert trait-mediated influence on ecosystems is largely a function of the number of infected hosts (Buck 2019). The extent to which parasites alter the structure and functioning of ecological communities through cascading trait-mediated indirect effects necessarily depends on the myriad processes that underlie disease transmission, the specifics of the host traits being modified, and the ecological context in which those traits are expressed. While there are many well-publicized examples of parasites inducing dramatic changes to the behavior or appearance of their host species, most changes to host traits take the form of subtle alterations to behaviors like feeding and locomotion. Any detectable cascading effects as a result of these subtle changes may only emerge when conditions favor high infection prevalence/intensity. However, if the ecological influence of host behavior scales with host density, the effects of parasites at a given prevalence may not be detectable at high host densities when the aggregate ecological impacts of host behavior drown out the aggregate effects of parasites or at low host densities where the ecological impacts of host behavior is negligible.

In southern USA salt marshes, the keystone gastropod, *Littoraria irrorata* (hereafter referred to as snails or grazers) can form high-density consumer fronts (~100–1000 snails/m^2) in response to sublethal drought stress that weakens plant defenses (Silliman et al. 2005). High densities of grazing snails can yield cascading vegetation loss even after the initiating drought stress has passed, converting large swaths of highly productive marsh into relatively unproductive mudflat habitats (Silliman et al. 2005, 2013). In this system, parasitism by larval digenean trematodes can be prevalent among snails within these consumer fronts (>30%), likely because the mudflat areas generated by overgrazing are attractive to birds, which are definitive hosts of these trematodes (Morton 2018, Morton & Silliman 2020, Sharp & Angelini 2020). Previous experiments and observational studies revealed that one common trematode, *Parorchis acanthus*, can increase plant ecosystem resistance to die-off from drought-associated overgrazing by reducing per capita grazing rates of snails (Morton 2018, Morton and Silliman 2020).

Both infection prevalence (0% - >40%) and density (50 - 2000 snails/m^2) of adult snails vary considerably within marsh die-off areas throughout the southeastern USA. Thus, the ability of trematode parasites to confer ecosystem resistance to snail overgrazing is likely highly context dependent. Previous field manipulations of *P. acanthus* prevalence found that infection prevalence was roughly proportionate to reductions in the top-down impacts of grazers on marsh plants (Morton & Silliman 2020). Consistent with this, multi-site surveys found that the magnitude of grazer-

induced damage (radular wounds) to plants along die-off borders decreased with increased prevalence of infection. Damage also increased with snail density (Morton & Silliman 2020). These results suggest that the ability of parasites to ameliorate stress and slow the rate of die-off expansion is likely dependent on both infection prevalence and grazer density, but the latter has yet to be experimentally tested. Therefore, a more robust understanding of how this parasite operates as an agent of ecosystem resistance in this system necessitates determining at what levels of host density parasites are likely to generate cascading trait-mediated impacts.

Here, we experimentally determine at which host densities parasites effectively ameliorate snail impacts on marsh vegetation via trait-mediated indirect effects. We did so by manipulating the density of grazer hosts in the field while holding infection prevalence constant at an intermediate value. We predicted that at high grazer densities, any ameliorating effects of parasitism on marsh plants would be overwhelmed by the magnitude of snail grazing. At low grazer densities, we predicted that parasitism would not lead to meaningful reductions in snail grazing that would translate to positive effects on plant growth and reproduction. We anticipated that parasites would be most effective at increasing ecosystem resistance to overgrazing at moderate snail densities where grazers' top-down impacts on marsh vegetation just begin to emerge.

2.3 Methods

To determine how trematode infection influences marsh productivity at different levels of grazer host density, we conducted a field manipulation where we modified grazer host density while keeping infection prevalence constant. In June 2016 we established 0.7 m^2 caged plots in a structurally homogenous swath of intermediate *Spartina* marsh within the Hoop Pole Creek Clean Water Reserve in Atlantic Beach, North Carolina, USA (34°42'25.12" N, 76°45'1.14" W). The site was characterized by a relatively uniform elevation, very low snail densities (< 1 adult snail per m^2) and minimal visible signs of snail grazing.

Snails used in the experiment were collected from a marsh die-off area where snails were abundant and infection prevalence was known to be high (Morton & Silliman 2020). Collected adult snails (shell length > 15mm) were transported back to the lab where their infection status was determined using a previously described cercariae shedding method that produces no false-positives (Morton 2018, Morton & Silliman 2020). We marked the shell of each infected snail with a red dot using a non-toxic, water resistant paint pen while uninfected snails were marked with a blue dot (Henry & Jarne 2007). Snails were kept in separate aquaria and provisioned with damp *Spartina* wrack for ~3 weeks until they were deployed in the field.

Roofless cages (0.7 × 0.7 × 1 m) were constructed from untreated wooden posts and galvanized hardware mesh. A stripe of copper tape was applied to the inner base of

each cage, just above the sediment, to discourage snail escapes. Caged plots were spaced at least 1-m apart to assure independence of replicates - a design confirmed from past studies (Silliman & Zieman 2001, Silliman & Bertness 2002, Morton & Silliman 2020) Cages were buried 10-cm into the substrate to prevent snail and mud crab migration in and out of cages, and to inhibit belowground connections between plants inside and outside of the cages. Each plot was assigned to one of 8 snail density treatments (20, 40, 50, 60, 70, 80, 90, and 100 snails/per 0.7-m^2) and one of two parasite addition treatments (0% and 20% infection prevalence). This resulted in 16 total treatments (n = 4 replicates per treatment). The selected range of snail densities was meant to capture the full range of adult snail densities observed within marsh die-off areas at this site. The 20% infection prevalence used in the experiment reflected the average naturally occurring summertime infection prevalence value for snails within local die-off areas (Morton & Silliman 2020). Four uncaged plots marked at the corners with colored PVC flags and four partial cages with one open side served as cage controls.

Before the beginning of the experiment, we removed any mud crabs and snails from cages. We took measurements of several marsh characteristics in all plots at the beginning of the experiment. We counted all *Spartina* stems and measured the heights of ten randomly selected stems in each plot. Random selection of stems was accomplished by tossing a plastic dowel into plots and measuring the first 10 stems touching the dowel. We constructed a height-to-biomass regression by collecting 30 *Spartina* stems of

varying sizes from the marsh directly adjacent to our experimental site. The stems were washed, their length measured, and were dried at 70 ˚C until they reached a constant weight. We used the resulting height-to-biomass regression to estimate standing *Spartina* biomass in each plot. We also counted all juvenile (< 0.5-cm) and adult fiddler crab burrows at both the beginning and end of the experiment because these organisms are known to influence *Spartina* growth by oxygenating the sediment through burrowing (Bertness 1985, McGraith et al. 2003, Daleo et al. 2007, Angelini & Silliman 2012, Gittman & Keller 2013).

Infected and uninfected snails within each plot were counted twice weekly and replaced as necessary to maintain the assigned snail density and infection prevalence treatments for the 3-month duration of the experiment. During monitoring, any predatory mud crabs found within plots were removed and their burrows plugged with marsh sediment to discourage successful re-occupation.

We took final metrics of marsh vegetation structure 12 weeks after the beginning of the experiment in September 2016. All snails were removed and dissected to confirm infection status. Because snail grazing had dramatically reduced stem densities in many plots, we were able to measure all stems within each plot to generate final plot biomass estimates by calculating and summing individual biomass estimates. Net primary productivity (NPP) was calculated as the difference in initial biomass estimates and final biomass estimates for each plot (g biomass/m^2/month. Belowground biomass cores (15-

cm diameter x 25-cm height) were also taken from the center of plots. Cores were washed and root material separated from rhizomes. Roots and rhizomes were dried to a constant weight at 70 °C and weighed.

We examined the influence of parasitism, snail density, and the interaction of these factors on NPP and final belowground biomass using multiple linear regression. Because we predicted that the ameliorating effects of parasitism would emerge at middling densities, we conducted planned comparisons between infected and uninfected treatments at each level of snail density using multiple t-tests with Bonferroni correction to control the familywise error rate. Root and rhizome data were log-transformed to meet the test assumptions of linear models prior to analysis. All statistical analyses were conducted in R version 4.0.2 (Team 2013).

2.4 Results

Initial plot estimated plant biomass was not significantly different among treatments ($p = 0.057$, one-way ANOVA). Additionally, there were no significant differences in the densities of fiddler crab burrows, plot elevation, and porewater salinity among treatments ($p>0.6$ at least, one-way ANOVA, for all response variables).

The final mean infection prevalence for each treatment did not differ from initial assigned conditions. No false negatives were observed when snails were dissected following the end of the experiment. The mean weekly deviation in snail density in those treatments where they had been added never exceeded 10% for any plot.

Exogenous snails very rarely found their way into experimental plots and were removed during weekly snail counts (<1 snail per plot per week). Exogenous snails discovered within plots were typically below the size threshold (shell height <10mm) associated with active grazing (Silliman & Bertness 2002). On average, less than one mud crab per week was removed from caged snail exclusion controls.

There were no significant differences in any final plot metrics between caged controls and uncaged plots, indicating no caging artifacts ($p>0.07$, paired t-test, for all response variables).

Net primary productivity decreased with increasing snail density (Figure 1, $p = 2.2\times10^{-16}$, Student's t-test). Parasite presence at a 20% prevalence was associated with significant increases in net primary production (Figure 1, $p = 7.29\times10^{-7}$, Student's t-test There was a significant interaction between infection and snail density (Figure 1, $p = 0.037$, Student's t-test). Parasites shifted the host density threshold at which net primary production became negative from 64 snails to 85 snails per 0.7 m^2 (Figure 1). At snail densities between 20 to 70 individuals per 0.7 m^2, parasitism had no significant effect on plant growth (two-sample t-test, $p > 0.82$, all cases). In contrast, parasitism significantly increased plant growth relative to uninfected controls at 80 snails per 0.7 m^2 ($p = 0.044$, two-sample t-test). At snail densities between 90 and 100 individuals per 0.7 m^2, parasitism had no significant effect on plant growth ($p > 0.63$, two-sample t-test).

There was no effect of parasitism, snail density, or the interaction of these factors on *Spartina* root biomass or rhizome biomass ($p > 0.15$, all cases, Student's t-test). Likewise, planned comparisons of root and rhizome biomass between infected and uninfected treatments at each level of snail density revealed no significant effects ($p > 0.92$, all cases, two-sample t-test).

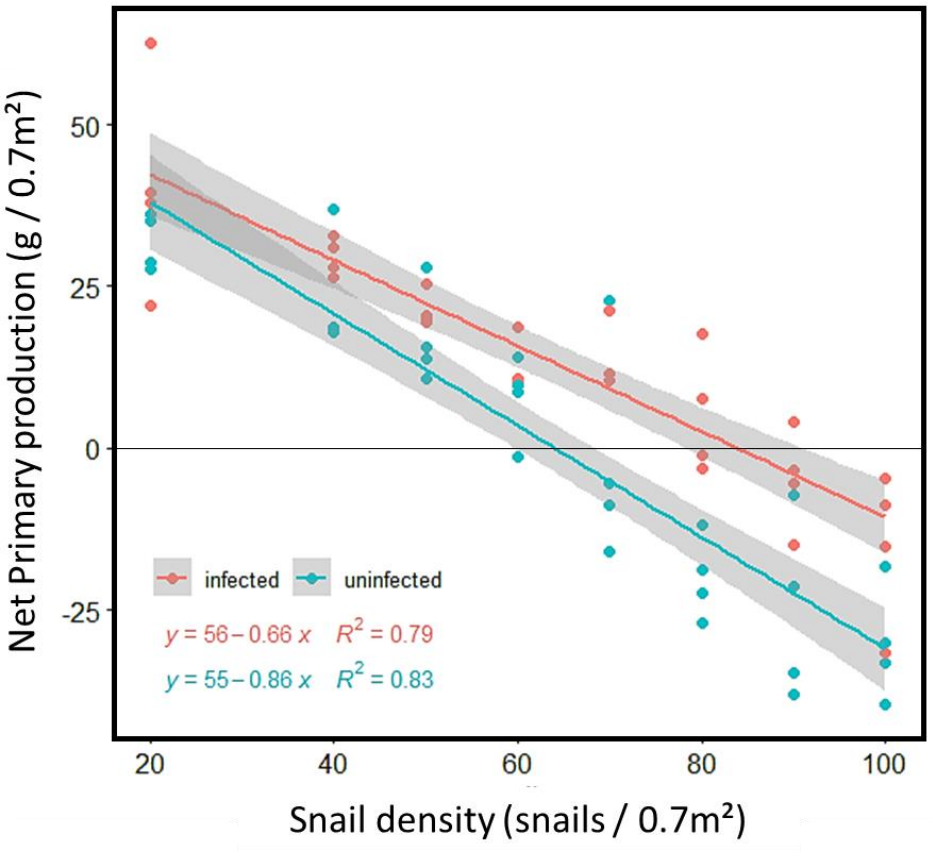

Figure 1: Relationship between *Littoraria* density and *Spartina* net primary productivity in plots with uninfected snails and plots with 20% of snails infected with *Parorchis*. Shaded areas are 95% confidence intervals.

2.5 Discussion

Our field manipulation of grazer host density and trematode parasite presence confirmed the findings of previous studies that *Parorchis acanthus* can protect foundational plants by generating a trait-mediated trophic cascade, and revealed that the emergence of these ameliorating effects occurs at particular levels of grazer host density. Parasites protected foundational plants at intermediate snail densities but not low or high densities. Our results underscore the context-dependent nature of trophic facilitation by parasites and inform a more robust understanding of when and where parasitism may promote ecosystem resistance to overgrazing in this system.

At an average infection prevalence, the trait-mediated impacts of trematode parasitism on snail grazing reduced the grazer density threshold at which there was a net loss of *Spartina* aboveground biomass by nearly 25% (Figure 1). Additionally, the ameliorating trait-mediated indirect effects of *P. acanthus* on marsh aboveground growth emerged at intermediate densities of hosts (80 snails/0.7m^2 or ~163 snails/m^2). At low grazer host densities, parasite reduction of per-capita grazing rates did not translate to detectable increases in aboveground plant biomass, likely because the top-down impacts of these snails only begin to emerge at densities of 60 – 144 snails per m^2 (Silliman & Zieman 2001, Silliman & Bertness 2002). At higher densities (90 – 100 snails/0.7 m^2 or ~183 – 204 snails/m^2), the negative impacts of snail grazing on *Spartina* overwhelmed any positive impacts of parasite behavior modification at the chosen prevalence. Thus, at a

given prevalence, there is likely to be a snail density range over which the *P. acanthus* can generate strong trait-mediated indirect effects.

While our results indicate that the ameliorating trait-mediated impacts of parasitism by *P. acanthus* are mediated by grazer host density, the particular density at which parasites have the potential to positively impact marsh plants is likely highly variable. For instance, a previous study in this system revealed that parasitism by *Parorchis acanthus* yielded significant reductions in snail grazing that translated to cascading effects on aboveground biomass at 10% and 30% infection prevalence and snail densities of ~204 snails/m^2 (Morton & Silliman 2020). While we found linear relationships between *Littoraria* density and grazing pressure in the present study, previous work has shown that this functional relationship can be linear (Silliman & Zieman 2001), logarithmic (Atkins et al. 2015), or exponential (Renzi & Silliman 2021) depending on the specific context. This variation is likely mediated by local differences in abiotic stress, nutrient regime, vegetation characteristics, predation risk, benthic productivity, snail size structure (Atkins et al. 2015), and the strength of positive species interactions that enhance *Spartina* growth (Bertness & Miller 1984, Bertness 1985, Gittman & Keller 2013, Angelini et al. 2015, Renzi et al. 2019).

Previous investigations in this system found that trait-mediated reductions in the top-down impacts of grazers on *Spartina* biomass by *Parorchis acanthus* was roughly proportionate to the level of infection prevalence (Morton 2018, Morton & Silliman 2020,

Morton et al. 2021). However, the magnitude of the effect of trematodes on per capita snail grazing may vary with host condition, environmental factors or host genetics (Leung et al. 2010, Thomas et al. 2011). Additionally, different parasite species may yield different trait-mediated indirect effects depending on the specifics of how they impact host physiology and modify snail feeding. The trematode species used in this study, *Parorchis acanthus*, was chosen for its relative abundance at marsh die-off areas within our study site, but there are at least four trematode species that infect *Littoraria*. For instance, the trematode *Cercariae opaca* (Holliman 1961) also uses *Littoraria* as a first intermediate host and can be locally prevalent (>40%) in certain marsh die-off sites and produces similar negative impacts on host per capita grazing as *P. acanthus* (Morton et al., *in review*). While infections with *P. acanthus* are common at many marsh die-off sites, the trematode component community at a given site is variable, likely owing to local variation in definitive host diversity (Byers et al. 2008, Altman & Byers 2014). Thus, the relative diversity and abundance of different trematodes at a given site is likely to influence the density threshold at which snails exert strong control over *Spartina* growth.

The effects of both grazer density and parasitism on aboveground production were clear from our field experiment, but we detected no significant effect of either of these factors on *Spartina* root or rhizome biomass. Belowground growth of marsh plants can be affected through direct consumption by herbivores, including snow geese (Smith & Odum 1981) and burrowing crabs (Coverdale et al. 2012, Gittman & Keller 2013, Daleo

et al. 2015). While *Littoraria* does not directly graze on belowground plant tissues, snail grazing could ultimately have indirect impacts on *Spartina* belowground biomass through wholesale destruction of *Spartina* aboveground tissues. However, removal of *Spartina* roots and rhizomes is largely mediated by processes of microbial decay that likely operate on timescales greater than the duration of our experiment (Benner et al. 1987, Blum 1993, Blum & Christian 2013). How grazers and their parasites mediate *Spartina* belowground biomass requires future investigation.

Trematode infection prevalence in *Littoraria* shows great spatial variation at both local and regional scales (Rossiter 2013, Morton & Silliman 2020). While many of the specific processes that mediate infection prevalence in *Littoraria* have yet to be experimentally tested, snail size, spatial variation in definitive host (bird) density and tidal flooding regime appear to be important determinants (Morton & Silliman 2020). Snail density itself may mediate infection dynamics in the field. Large densities of grazer hosts could result in lower individual encounter rates with mobile trematode miracidium, diluting infection risk and depressing prevalence (Mooring & Hart 1992, Buck & Lutterschmidt 2017). To date, observational and manipulative studies in this system have examined both parasite infection dynamics and parasite trait-mediated indirect effects across a fairly narrow range of host densities. However, large consumer fronts that can generate cascading ecosystem loss can exceed 1000 snails/m^2. Examining

trematode infection dynamics across a wider range of *Littoraria* densities is necessary to determine whether dilution effects mediate snail infection at high densities.

2.6 Conclusions

Parasite alteration of host behavior, whether it be manipulation, an antiparasitic tactic of the host, or merely a byproduct of infection, is likely exceedingly common, though we still know perishingly little about how different parasites affect their hosts – even ecologically influential ones (Poulin 1995, Morton 2018, Buck 2019). Additionally, the ecological reach of parasites through trait-mediated indirect effects is not reducible to the prevalence of behavior-altering parasites in a host population. Our results underscore the importance of host density as one determinant of the ecological reach of parasite-induced trait-mediated indirect effects, however there are numerous factors that may ultimately influence such effects. Host populations, behaviors, and the environments in which those hosts exert influence through their behavior can vary widely in space and time. Fully incorporating parasites into our understanding of how ecosystems work necessitates that we not only quantify the often subtle ways that parasites alter the behavior of hosts, but that we determine under what conditions these alterations can add up to have a wider ecological influence.

3. Birds Increase Ecosystem Resistance to Climactic Stress by Leaving Parasites Behind

3.1 Abstract

Predators regulate many plant ecosystems by consuming and/or eliciting fear in grazers. As definitive hosts for parasites, predators may also control plant ecosystems via parasite dispersion if the intermediate hosts to which parasites are dispersed are influential herbivores. In salt marshes of the southern U.S.A., extreme drought and runaway consumption by extensive fronts of grazing snails interact to generate large-scale vegetation die-off. Surveys of drought-impacted marshes revealed that birds congregate within die-off areas and that increased bird usage of die-off areas is associated with increased trematode parasitism in snails within grazer fronts, decreased per capita grazing rates of snails, and proportionate decreases in ecosystem die-off rate. Using multi-site bird exclusion and mechanistic studies, we experimentally demonstrated that birds increase ecosystem resistance to drought-driven die-off by dispersing parasites that suppress grazing of herbivorous snails. Together, these results show that highly mobile predators can indirectly enhance ecosystem resistance to climatic disturbance by spreading ecologically influential parasites.

3.2 Introduction

Empirical studies and meta-analyses have revealed that predators play key roles in shaping the structure, function, and stability of many plant ecosystems (Ripple et al.

2014). Evidence from both terrestrial and aquatic systems shows that loss of top predators can trigger trophic cascades that lead to state changes in plant ecosystem structure and function (Estes & Palmisano 1974, Carpenter et al. 1985, Silliman & Bertness 2002, Myers et al. 2007, Estes et al. 2011, Rosenblatt et al. 2013, Bertness et al. 2014). Other studies have shown that predators confer increased resistance to biological invasions and physical stressors, including those associated with climate change (Wilmers & Post 2006, Wilmers et al. 2006, Wallach et al. 2010, Mittelbach et al. 2019). The primary mechanisms by which predators are thought to have such powerful effects in these ecosystems is by consuming and/or generating fear in keystone grazers, which in turn has strong impacts on plant community biomass and dynamics.

Predators also play a critical role in influencing the spread of disease (e.g., bacteria, viruses, and helminths) by regulating the abundance or behavior of vectors, or by acting as vectors themselves (Ostfeld & Holt 2004, Moore et al. 2010, Tholt et al. 2018, Taggart 2019). These disease agents are increasingly recognized as being able to impact higher levels of ecological organization: they can influence species coexistence (17), modify the outcome of competition and predation (Hatcher et al. 2006, 2008), alter ecosystem energy budgets and nutrient cycling (Kuris et al. 2008, Johnson et al. 2010), act as ecosystem engineers (Poulin et al. 1998, Mouritsen & Poulin 2010), as well as control community structure, function, and stability in natural habitats (Wood et al. 2007, Hernandez & Sukhdeo 2008, Holdo et al. 2009, Sato et al. 2012, Sánchez et al. 2016,

Morton & Silliman 2020). Since virtually all predators act as intermediate or final hosts to parasites, predator regulation of parasite prevalence in keystone grazers could also be a common, but underappreciated, pathway of top-down control of plant ecosystem stability. If this control pathway occurs, it would mean that highly mobile predators, such as birds, may be able to exert strong, top-down control over plant ecosystems, despite spending relatively little time in an area.

Birds are common top-order consumers in many ecosystems and play a variety of important ecological roles (Sekercioglu 2006). By acting as hosts and dispersive agents for a variety of parasites (Schwan et al. 1983, Hechinger & Lafferty 2005, Brinkerhoff et al. 2011), birds can impact the spread and range expansion of disease agents and their vectors (Brinkerhoff et al. 2011). In southeastern U.S.A. salt marsh ecosystems, highly mobile predatory birds occur in locally abundant and diverse assemblages, feeding both within and on the edges of these important coastal wetlands (Craig & Beal 1992, Rush et al. 2009, Brown & Dinsmore 2019). Large-scale vegetation die-off in these marshes is generated by interactions between intense drought and runaway consumption by the plant-grazing snail, *Littoraria irrorata* (Silliman et al. 2005). When salt marshes die off, expanses of unvegetated mudflat are created within the salt marsh interior. High-density snail fronts then form on the border between die-off areas and healthy marsh, propelling continued ecosystem decline (36). Co-occurring in these same mudflats generated by die-off are locally elevated abundances of birds (Morton 2018, Morton &

Silliman 2020). Many of these birds act as terminal hosts for digenean trematode parasites that infect *Littoraria* as a first intermediate host (Coil & Heard, 1966; Heard, 2011; Morton, 2018). Because increased prevalence of trematode parasitism in these snails reduces their top-down impacts on marsh plants via a trait-mediated trophic cascade (Morton & Silliman 2020), increased bird usage of die-off areas could increase ecosystem resistance to intense drought by increasing trematode parasitism and consequently reduce the impact of snail fronts that actively expand marsh die-off.

To test for the impacts of bird presence on marsh ecosystem resistance to drought-driven die-off and to differentiate the relative importance of potential bird impact pathways (specifically nutrient addition, parasite addition, and soil aeration), we surveyed seven marsh sites in North Carolina experiencing active die-off, and set up bird exclusions and mechanistic experiments on the die-off/healthy marsh border at three of those sites. We hypothesized that: (I) mobile birds would be more abundant in marsh die-off areas, (II) high bird activity in these unvegetated areas would indirectly increase resistance in remaining marsh plants on the die-off border by generating a parasite-mediated trophic cascade, and (III) exclusion of birds and their parasites from actively expanding die-off borders would increase the rate of vegetation die-off.

3.3 Methods

3.3.1 Relative Bird Usage of Healthy and Disturbed Marsh

To quantify differences in relative bird usage of marsh die-off areas and healthy marsh stands, we deployed camera traps at seven marsh die-off sites in July 2017 (Hoop Pole Creek 34°42'19.33"N, 76°44'57.77"W and 34°42'17.7078", -76°44'52.3818" ; The Theodore Roosevelt Natural Area 34°41'49.09"N, 76°50'9.19"W; Pine Knoll Shores 34°41'56.40"N, 76°50'1.27"W; Gunning Hammock Island 34°40'20.9064", -76°30'5.0364"; Harkers Island 34°41'31.4808", -76°31'39.666"; Beaufort Town Creek 34°43'23.6526"; -76°39'20.9226"). Camera traps were mounted atop 3 m PVC posts, which were firmly embedded in the marsh substrate. Cameras were affixed at an angle so that they pointed downward at the marsh surface. A 4x4 m observation area within the viewshed of each camera was marked with flags. Four trap assemblages were deployed in both the die-off area interiors and the adjacent undisturbed marsh at each of the seven sites. Camera traps were set to take pictures if they sensed any motion within their viewshed. After a week, traps were collected and photographic data were extracted. The number and species of each bird within the marked observation area of each photograph was recorded for the week-long study period. To evaluate differences in bird usage, we used the lmerTest package (Kuznetsova et al. 2017) in R (Team 2013) to perform a linear mixed effect analysis with marsh type (healthy vs. die-off) as the fixed effect, with site as a random effect.

3.3.2 Survey of bird usage

To determine the relationship between bird usage, trematode parasite prevalence, and snail grazing impacts over a wide spatial scale, we surveyed the same seven salt marsh die-off areas (see above) during the summer of 2017, and quantified the relationship between usage by birds, prevalence of parasitism in snails, and rate of ecosystem loss. At each site, we established eight 1 m^2 plots along die-off borders such that the borderline between live marsh vegetation and un-vegetated mudflat ran through the middle of the plot. Plots were at least 5 m apart. Plot corners and borderline were marked with wooden stakes. Initial and final (after two months) plot border position, stem density, snail density, grazing intensity (length of radulation scars per stem), stem height, fiddler crab burrows, mud crab burrows, and ribbed mussels were quantified within each plot using previously described methodology (Angelini et al. 2015, Morton & Silliman 2020). Sediment redox measurements were taken at low tide at the center of each plot immediately before surveying began. Additionally, we determined the relative abundance of predatory crabs at each site by placing two baited, conventional crab traps at each site for a 48 hour period before the start of the photographic survey. At the end of the experiment, all snails within a plot were collected, measured, and dissected to determine infection status.

Daytime bird usage at each site was evaluated using time-lapse photography. Game cameras were positioned on 3 m PVC posts embedded in the marsh surface such

that their view shed encompassed the entire area of each die-off surveyed. Camera traps were programmed to take photographs and air temperature measurements every ten minutes during daylight hours for ten days. Photographs were subsequently analyzed to determine the number and species of each bird in each photograph. Total bird usage was quantified as the number of birds present on the estimated area of a die-off site per day (birds/ha/day).

To determine the relationships between birds, parasite infection prevalence, and the rate of ecosystem retreat at our seven surveyed marsh sites, we employed model selection and multimodal inference (Grueber et al. 2011). Using the lmerTest package in R, we constructed two global linear mixed effects models (Kuznetsova et al. 2017). The first focused on factors explaining trematode infection prevalence within plots at each site, while the second focused on factors explaining the rate of marsh ecosystem retreat. The first model included bird usage, snail size, snail density, total inundation time, and initial *Spartina* biomass, as predictor variables, and site as a random effect. The second model included parasite infection prevalence, air temperature, fiddler crab burrow density, total inundation time, initial *Spartina* biomass estimate, mussel density, average snail size, average snail density, and grazing scar length as predictor variables, and site as a random effect. Neither model included interactions, as we did not have strong *a priori* expectations for any. The chosen variables in each of the two global models were standardized on two standard deviations (Gelman 2008), and reflected all biologically

relevant variables identified through graphical data exploration. Before proceeding to model selection, we sequentially removed factors with the highest VIF values from each model until all were below a threshold value of 10 (Zuur et al. 2010). Model selection was performed for each model using the dredge function from the R package MuMIn (Barton 2019) to fit all possible permutations of each model. We then ranked the model pool for each global model using delta Akaike information criteria corrected for small sample sizes (ΔAICc) (Grueber et al. 2011). We selected a subset of the most informative non-nested models with a ΔAICc <4 for all chosen models (Table 2). For our first model, subsetting based on ΔAICc yielded only one candidate model. We report the results of this singular linear mixed effects model. For our second model, we used the model.avg function within the MuMIn package to carry out model averaging of our subsets of models and to obtain our final model (Zuur et al. 2010, Grueber et al. 2011). We report conditional model estimates (Grueber et al. 2011). For both models, we report parameter-specific *P*-values generated from *t*-tests via the Satterthwaite approximation.

3.3.3 Bird Exclusion Experiment

In order to experimentally test whether birds increase marsh ecosystem resistance to drought-induced overgrazing and marsh die-off, we experimentally excluded birds from actively expanding die-off borders in June 2016. We established 5 × 5 m^2 plots along three North Carolina marsh die-off borders where snails had formed consumer fronts (Hoop Pole Creek 34°42'19.33"N, 76°44'57.77"W; The Theodore

Roosevelt Natural Area 34°41'49.09"N, 76°50'9.19"W; Pine Knoll Shores 34°41'56.40"N, 76°50'1.27"W). The approximate size of die-off areas at each site was 0.2558 ha (Hoop Pole Creek), 0.16448 ha (Pine Knoll Shores), and 0.1122 ha (The Theodore Roosevelt Natural Area). Snail densities were determined by throwing ten replicate m^2 quadrats within fronts at each site and were (mean ± SD) 70.11 ± 10.87 snails/m^2 (Hoop Pole Creek), 111 ± 21.70 snails/m^2 (Pine Knoll Shores), and 121.44 ± 27.26 snails/m^2 (The Theodore Roosevelt Natural Area). Plots within each site were selected such that there was no significant difference in initial marsh structural characteristics (stem density, average stem height).

Plots were assigned to one of three treatments (n = 6 plots per treatment per site): bird exclusions, controls, and cage control plots. All plots consisted of 5 × 5 m^2 areas positioned on marsh die-off borders such that half of each plot was live, vegetated marsh and half unvegetated mudflat. In bird exclusion and control plots, 1-m^2 cages (constructed from 0.25 inch mesh galvanized hardware cloth stapled to wooden stakes) were positioned at the center of each 5 × 5 m^2 plot area. Exclusion plot areas were surrounded by a 5 × 5 × 1.5 m cage constructed from the same materials as the smaller cages they circumscribed. The buffer zone between the inner cage and the outer cage was meant to limit exposure of the contained snails, which were uninfected, to free-swimming trematode miracidia deposited in bird feces. The larger outer cage of exclusion treatments was raised above the marsh substrate by 10 cm so that blue crabs,

terrapins, and other small foraging marsh predators were allowed access but birds would be excluded. Additionally, nylon fishing line with pieces of reflective tape affixed to it was strung in a zig-zag pattern across the tops of plots to discourage birds from landing in exclusion plot interiors. Control plots had wooden posts at the four corners of the plot area surrounding the interior cage, but no caging material to allow access by birds and all other marsh predators. Cage controls consisted of wooden stakes marking the boundaries of interior and exterior plot areas, but no caging material. Roosting by birds on wooden posts in all replicates was discouraged by driving a single nail into the top of each post.

The borderline between live marsh vegetation and un-vegetated mudflat was marked in each 1 m^2 area at plot centers with plastic marker flags. All snails in each center plot were initially collected, taken back to the laboratory, and analyzed for infection using cercariae-shedding method proven to yield no false negatives (Morton 2018, Morton & Silliman 2020). Uninfected snails were re-introduced to plots at a standard density which approximated the average snail density observed along die-off borders at the three sites (~100 snails per m^2).

To determine the effects of bird exclusion on marsh plant health and ecosystem retreat, we initially took measurements of several marsh structural characteristics in all center plots, and did so for each additional month of the four month long experiment to determine the effects of bird exclusion on marsh plant health and ecosystem retreat.

Because the total length of snail radulations per stem is directly proportional to the magnitude of the negative effects of snail grazing on *Spartina* production, we enumerated the total length of radulations on ten randomly selected stems within each plot. Additionally, we determined live and dead stem densities and measured the heights of ten randomly selected stems in each plot (measured to the highest extent of live, green tissue). In both instances, stems were randomly selected by tossing small plastic dowel into the plot and then taking measurements of stems touching the dowel. Die-off border retreat was measured by determining the average distance between stems and the initially marked borderline using the five live stems closest to the initially marked borderline.

Because some birds plunge their beaks into the sediment when feeding on benthic invertebrates, we considered that this might facilitate plant growth by locally enhancing sediment oxygen availability. To account for this potential effect, we measured sediment redox potential (mV) within and directly outside of each experimental plot. Redox measurements were taken using a platinum electrode probe and a Fisher Scientific Accument double junction Ag/AgCl electrode (+200 mV correction added to the measured value), connected through a Fisher Scientific Accument PH/mV/C meter, model AP71 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). At low tide, probes were placed 10-cm into the sediment at the center of each plot and 4 meters outside the plot. Probes were inserted into the marsh

surface and left for 30 minutes to allow the sediment surrounding the probe to stabilize. We measured redox in all plots before the application of any treatments, one week following the installation of the experiment, and each month following installation until the end of the experiment.

We conducted model selection and multimodal inference, similar to what was carried out for our bird usage survey, to determine both the effects of bird exclusion on trematode infection prevalence and the effects of infection prevalence (and other plot characteristics) on the rate of ecosystem retreat. We used the LmerTest package in R to construct two global linear mixed effects models. The first focused on factors explaining trematode infection prevalence within experimental plots, and included treatment group and initial plot biomass estimate, as predictor variables, and marsh site as a random effect. The second global model focused on the rate of marsh ecosystem retreat. Our second model included trematode infection prevalence, grazing scar length, initial *Spartina* biomass estimate, soil salinity, mussel density, initial snail density, sediment, initial redox potential, fiddler crab burrow density as predictor variables and marsh site as a random effects. Neither model included interactions, as we did not have strong *a priori* expectations for any. The chosen variables in each of the two global models were standardized on two standard deviations and reflected all biologically relevant interactions with explanatory variables identified through graphical data exploration. We carried out the process of model selection and averaging to produce two final

models, following the same procedure as our bird exclusion experiment (Table 3). Similar to our multi-site bird survey, subsetting based on ΔAICc for our model of infection prevalence yielded only one candidate model, which we report.

Differences in sediment redox potential within and outside plots were determined using linear mixed effects models with treatment and position (within or outside of plots) as main effects, and site as a random effect.

3.3.4 Field test of the impacts of bird fecal inputs

At each die-off site where bird abundance surveys were performed, we estimated daily bird fecal inputs by performing replicate 10 × 2 m belt transects (n = 5 per site). Fecal matter observed in these belt transects was collected, dried at 70°C, and weighed. Mean fecal addition was calculated from belt transect values across sites as grams feces per m^2 per day.

We used values determined from belt transects to manipulate bird fecal inputs on the marsh surface along die-off borders. We first established caged plots along die-off borders at three sites (Hoop Pole Creek, Pine Knoll Shores, and Beaufort Town Creek, see above for coordinates), using the same methods from our manipulation of predator presence. We took initial measurements of plant stem height, stem density, and the density of fiddler crab burrows and mussels. All snails were initially removed and then replaced with uninfected snails (determined via the cercariae shedding method) at a density of 100 snail/m^2. Cages were assigned the following treatments: fecal addition at

ambient levels (0.14 g per m^2 per day), no feces added, and high addition (0.56 g per m^2 per day) which represented the maximum transect value observed across all sites. All treatments were replicated 6 times at each site.

At low tide, freshly deposited bird feces were collected from the die-off areas at the three experimental sites. Collected feces was heated in a drying oven to 100 °C for at least six hours to ensure the death of any trematode eggs. Pre-weighed quantities of dried, sterile feces were rehydrated to natural consistency and homogenized before being added to appropriate treatments. Feces were added to plots every week for three months (May-July 2017), and were added during the rising tide (when birds tend to be most active in these areas). To ensure that snails within all plots remained uninfected for the duration of the experiment, all snails in all plots were removed every month and replaced with new uninfected snails. At the end of the experimental period, die-off border retreat was measured in each plot. Grasses within each plot were then harvested, individually measured and their dry biomass was determined. Treatment differences in border retreat and marsh structural characteristics were analyzed using a linear mixed effects model.

3.4 Results and Discussion

Comparative time-lapse photographic surveys of bird usage of seven North Carolina marsh die-off areas and adjacent healthy marsh areas revealed that birds utilize marsh die-off areas almost four times more often than adjacent, undisturbed marsh

(Figure 2). On-the-ground surveys of these same sites also revealed strong, positive correlations between the level of bird usage of a die-off site and the prevalence of trematode parasitism in snails that formed grazer fronts on the die-off/healthy marsh border (Figure 3A, 4A). Correspondingly, the rate of plant ecosystem die-off decreased significantly with increasing parasite prevalence in keystone grazers (Figure 3B, 4B).

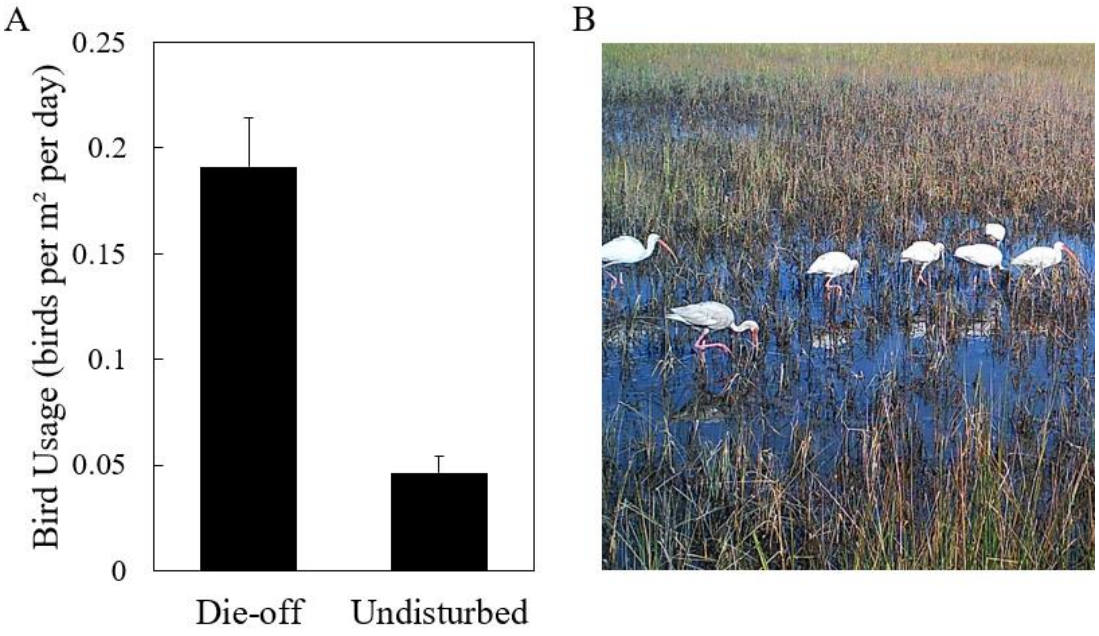

Figure 2: (A) Comparative usage of die-off and adjacent undisturbed marsh areas by birds. (B) photograph of white ibis foraging along a marsh die-off border. Data are means and standard error bars.

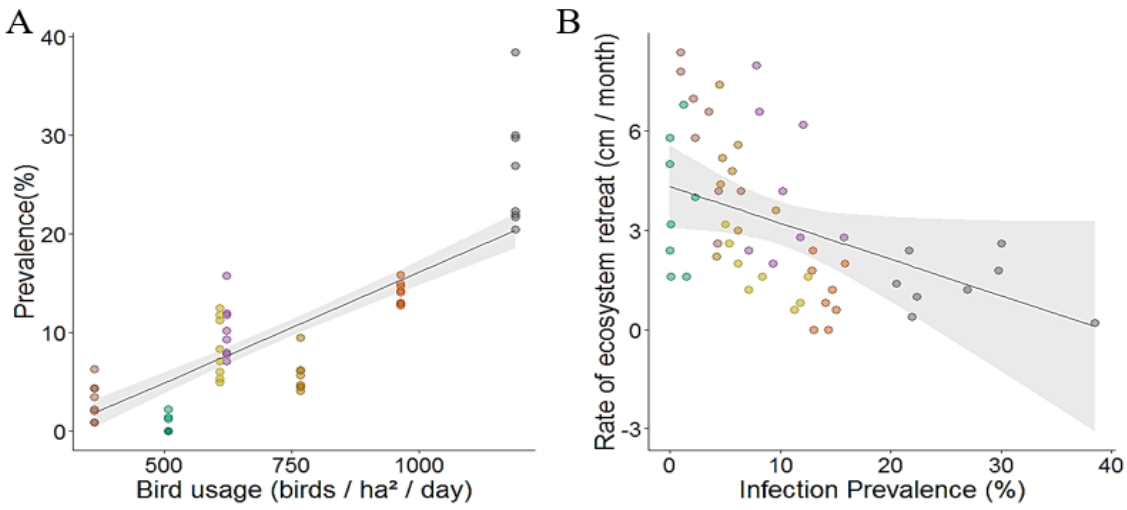

Figure 3: Marginal effects plots showing the relationship between (A) infection prevalence and bird usage and (B) infection prevalence and ecosystem retreat rate.Lines are predicted values holding all other variables at mean values; gray areas represent 95% confidence intervals. Differently colored points indicate site.

Experimental exclusion of birds from actively expanding marsh die-off borders at three separate die-off sites resulted in diminished trematode parasite infection prevalence in snails in the grazer fronts, and accelerated the rate of plant ecosystem loss compared to control plots (Figure 5A, 5C). Finally, experimental manipulation of bird fecal inputs and measurements of sediment redox potential inside and outside of bird exclusion plots indicate that birds did not have a detectable effect on die-off border vegetation growth through deposition of feces or aeration of sediments while feeding (Figure 7). Taken together, our results point to birds as important agents in maintaining marsh ecosystem resistance to combined drought and grazer-driven die-off by acting as dispersive agents for parasites that alter the behavior of potent grazers. These results underscore the importance of birds as mobile link organisms and suggest a new

functional role for predators–increasing ecosystem resistance to disturbance by dispersing parasites that generate powerful trophic cascades.

Common name	Scientific name	Total captures	Mean captures	SE
White ibis	*Eudocimus albus*	2875	410.7	±97.2
Wilson's plover	*Charadrius wilsonia*	1103	157.6	±102.2
Snowy egret	*Egrette thula*	600	85.7	±41.2
Tricolor heron	*Egretta tricolor*	357	51.0	±25.7
Red wing blackbird	*Aglaius phoniceus*	257	36.7	±31.0
Great egret	*Ardea alba*	99	14.1	±6.5
Great blue heron	*Ardea herodias*	83	11.9	±10.1
Herring gull	*Larus argentatus*	58	8.3	±3.9
Semipalmated sandpiper	*Calidris pusilla*	36	5.1	±4.9
Willet	*Tringa semipalmata*	21	3.0	±1.7
Sanderling	*Calidris alba*	21	3.0	±2.4
Clapper rail	*Rallus crepitans*	19	2.7	±1.2
Seaside sparrow	*Ammodramus marit*	12	1.7	±0.7
Glossy ibis	*Plegadis falcinellus*	6	0.9	±0.9
Unidentified		121	23.0	±8.3

Table 1: Bird community metrics from photographic surveys at seven marsh die-off sites.Of the 5708 total photographic captures of birds, more than 50% were white ibises (*Eudocimus albus*).

Our surveys of seven marshes demonstrated a strong relationship between bird usage, trematode infection prevalence, and the rate of ecosystem loss. Across all sites, a total of 5708 birds were photographed over the 10-day period (Table 1). Within marsh die-off areas, birds were most common while the marsh surface was in the early stages of flooding or nearly drained at low tide. The strongest predictors of parasite prevalence at surveyed sites were bird usage (Figure 3A, 4A, linear mixed effects model with parameter-specific *P*-values generated via the Satterthwaite approximation, $t = 13.25$, $P < 2e^{-16}$) and tidal inundation time (Figure 2A, $t = 6.84$, $P = 6.33e^{-9}$): parasite prevalence

increased with increasing usage of sites by birds and with inundation time (Figure 4A). Snail density, snail size, plot biomass, and fiddler crab burrow density were all included in the final averaged model but produced no significant effects on parasite prevalence (Figure 4A). The strongest predictors of the rate of ecosystem die-off (i.e., border movement) across and within the surveyed die-off sites were initial *Spartina* biomass (Figure 3B, 4B, $Z = 2.42$, $P = 0.016$), trematode infection prevalence (Figure 3B, $Z = 2.99$, $P = 0.00274$), average air temperature (Figure 3B, 4B, $Z = 2.713$, $P = 0.007$) and snail grazing scar length per stem (Figure 3B, $Z = 2.37$, $P = 0.017$). While increasing snail density has previously been associated with increased rate of ecosystem retreat (Silliman & Bertness 2002, Silliman et al. 2005), variation in average snail densities was relatively small across our seven study sites (mean ± SE: 90.34 ± 1.69 snails/m^2), likely resulting in it not being a significant predictor for cross-site variation in die-off rate. The observed significant, positive relationship of average air temperature on the rate of ecosystem retreat is likely related to soil drying, a common manifestation of drought that can lead to increased plant stress and grazer-induced damage (Silliman et al. 2005).

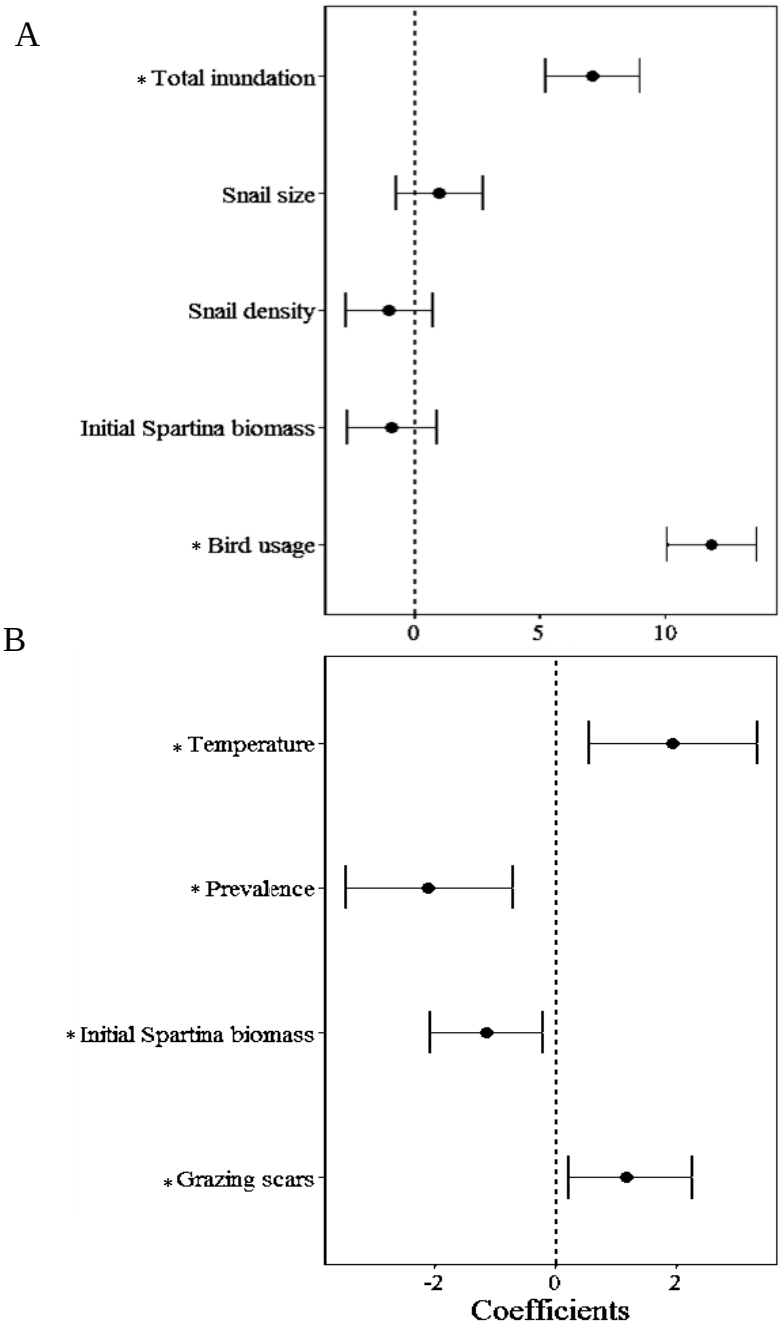

Figure 4: Coefficient estimates of generalized linear mixed-effects models for (A) parasite prevalence and (B) die-off border expansion from seven marsh die-off areas. Error bars are ±95% confidence intervals. Stars indicate estimates that are significantly different from zero at $P < 0.05$ (95% confidence intervals not crossing zero). Parameter estimates have been standardized on two standard deviations so that they are on a comparable

Intercept	Total inundation	Snail size	Mussel density	Grazing scars	Infection prevalence	Average temperature	Initial *Spartina* biomass	Fiddler crab burrows	Snail density	*df*	Log-likelihood	AICc	ΔAIC	Weight
3.24		-	-	1.19	-2.05	-	-1.19	-	-	6	-104.7	223.0	0	0.59
3.24		-	-	1.24	-	1.93	-1.07	-	-	6	-105.9	225.6	2.5	0.17
3.24		-	-	1.06	-1.99	-	-	-	-	5	-107.4	226.1	3.0	0.13
3.24		-	-	-	-2.55	-	-1.07	-	-	5	-107.6	226.3	3.3	0.11

Table 2: All candidate models used to generate our averaged model of die-of border retreat in our multi-site marsh survey. Predictors are standardized and all models included site as a random effect.

Experimental exclusion of birds from marsh die-off borders resulted in markedly diminished prevalence of trematode parasitism in initially uninfected snails in experimental plots relative to control plots, consistent with the well-established positive relationship between the incidence of parasitism in first intermediate hosts and the presence of final hosts (Hechinger & Lafferty 2005, Byers et al. 2008) (Figure 6A, linear mixed effects model with parameter-specific *P*-values via the Satterthwaite approximation, $t = -10.084$, $P < 1.33e^{-11}$). Parasitism was essentially nonexistent in snails within the center of the bird exclusion areas (mean prevalence ± SE: 0.11% ± 0.08), while previously uninfected snails in control plots displayed relatively higher infection prevalence after four months of exposure to birds in the field (mean ± SE: 10.25% ± 1.1) (Figure 5A). Initial *Spartina* biomass was included as a predictor variable in our final model, but did not significantly affect infection prevalence (Figure 5A, $t = 0.771$, $P > 0.4$).

Intercept	Grazing scars	Initial *Spartina* biomass	Initial mussel density	Salinity	Initial Snail Density	Trematode infection prevalence	*df*	Log-likelihood	AICc	ΔAIC	Weight
6.42	-	-2.2	0.95	-	-	-1.62	6	-63.8	142.4	0	0.54
6.42	-	-2.5	-	-	-	-1.56	5	-65.6	143.2	0.81	0.36
6.42	1.28	-2.7	-	-	-	-	5	-66.9	145.7	3.31	0.10

Table 3: All candidate models used to generate our averaged model of die-off border retreat in our bird exclusion experiment. Predictors are standardized and all models included site as a random effect.

Stark differences in trematode infection prevalence between exclusion and control plots translated to differences in the magnitude of grazer-induced damage to marsh plants and the rate of ecosystem die-off. When birds were excluded, ecosystem die-off rate increased by 40% on average (Figure 5C). The biggest predictors of ecosystem die-off rate were initial estimated plot biomass (Figure 5B, final averaged linear mixed effects model with parameter-specific *P*-values via the Satterthwaite approximation $Z = 4.141$, $P < 3.46e^{-5}$), parasite prevalence ($Z = 3.06$, $P = 0.00264$), and grazing intensity ($Z = 2.327$, $P = 0.01996$). The initial density of ribbed mussels, a known driver of marsh plant recovery (Angelini et al. 2016), was included as a model predictor, but did not significantly affect ecosystem die-off rate (Figure 6B).

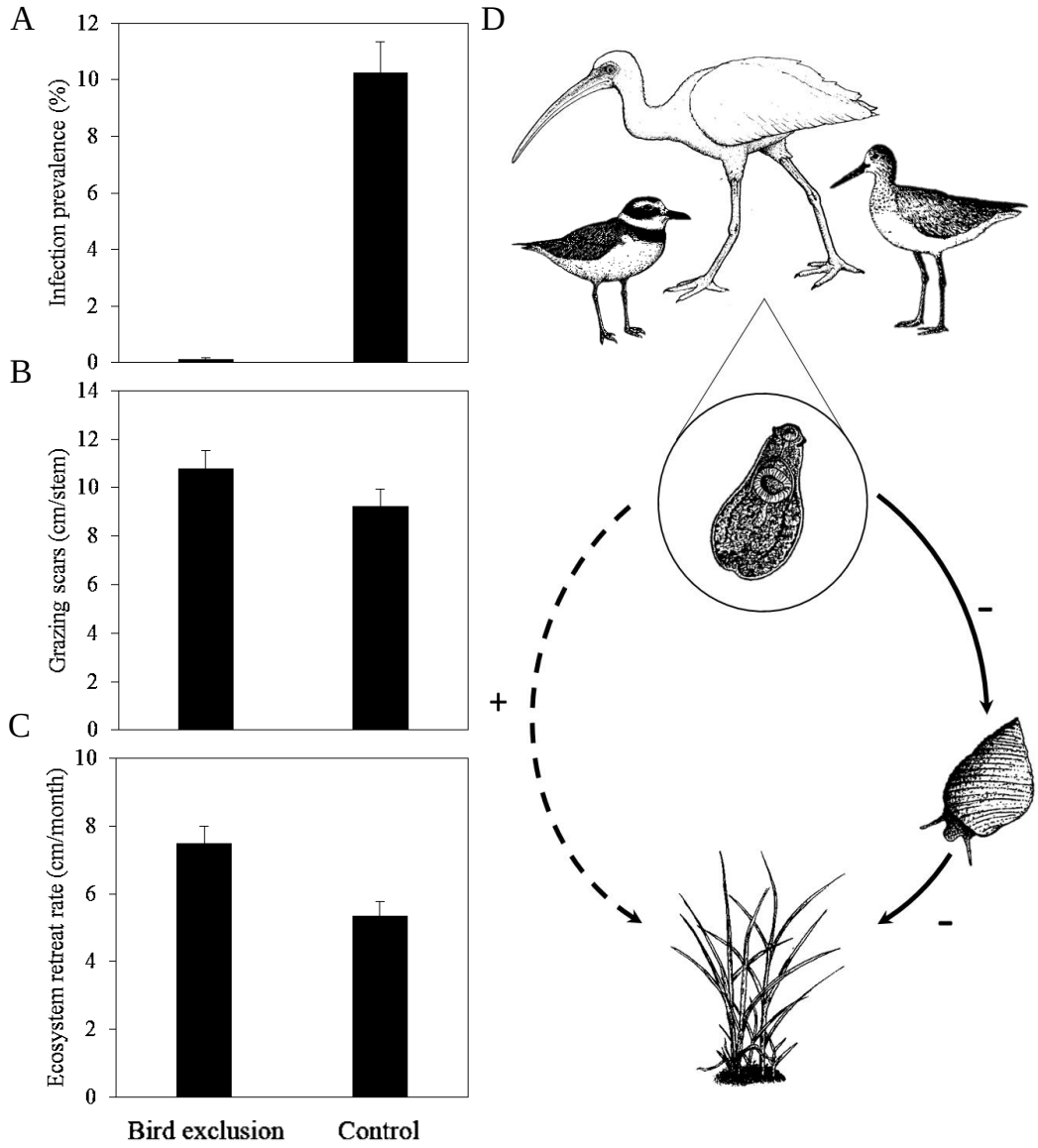

Figure 5: Effects of bird exclusion on (A) infection prevalence, (B) snail grazing scars, and (C) die-off expansion rate. (D) Conceptual diagram. Birds act as dispersive vectors for parasites that decrease per capita grazing impacts of snails, resulting in reduced rates of ecosystem decline at sufficiently high prevalence (Illustrations: Joseph P. Morton). Data are means and SE; n=18 treatment replicates.

Increasing infection prevalence and initial plot biomass were associated with significantly decreased rates of ecosystem loss (Figure 6A, Table 3). The most likely mechanism by which parasites slowed the rate of marsh die-off was by reducing the per

capita top-down effect of the infected snails on marsh plants to effectively zero (Figure 5D) (Morton 2018, Morton & Silliman 2020). The total length of snail grazing scars per stem was 17% higher in exclusion plots where parasitism was very near zero (Figure 5B, 6B), consistent with previous experimental studies that demonstrated a causal relationship between increased parasite prevalence in *Littoraria* and decreased grazing intensity of snails on marsh grasses (Morton 2018, Morton & Silliman 2020). The snail density used in our experiment (100 snails/m^2) represents a critical density threshold where the strong, top-down impacts of these grazers begins to emerge (Silliman & Zieman 2001, Silliman & Bertness 2002). Below ~60 snails/m^2, snails feed on standing dead grass, their preferred food, and rarely feed on live marsh grass (Silliman & Zieman 2001, Silliman & Bertness 2002).

Above ~60 snails/m^2, standing dead plant material becomes limiting and snails begin to graze on live marsh grass, promoting the growth of intertidal fungi on wounded plant tissues (Silliman & Bertness 2002, Silliman & Newell 2003). This dietary switch can double per capita grazing intensity and likely impact live grass (Silliman & Bertness 2002). Thus, seemingly small reductions in snail grazing on live plants at these critical densities (~60-144 snails/m^2) via parasite behavioral modification are likely to yield out-of-proportion top-down impacts (Morton 2018, Morton & Silliman 2020), a result we observed in our experiment.

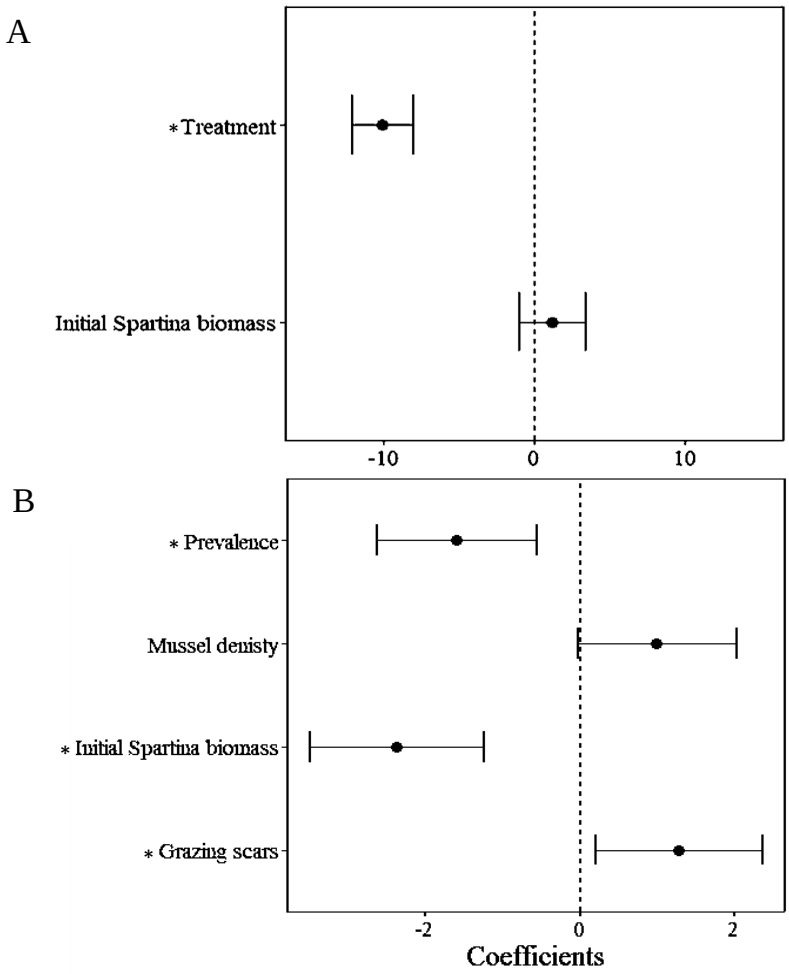

Figure 6: Coefficient estimates of generalized liner mixed-effects models for (A) parasite prevalence and (B) die-off border movement from our bird exclusion experiment. Error bars are ±95% confidence intervals. Stars indicate estimates that are significantly different from zero (95% confidence intervals not crossing zero). Parameter estimates have been standardized on standard deviations so that they are on a comparable scale.

While previous studies have focused on the effects of a single trematode species, *Parorchis acanthus*, on snail grazing and ecosystem resistance (Morton & Silliman, 2020), the current study suggests that other species likely have similar effects. In contrast to most areas surveyed where *Parorchis* was dominant, the prevalence of *Cercariae opaca* exceeded that of *Parorchis* at the Gunning Hammock marsh survey site, exceeding 40%

in some samples. Our survey study indicates that trematode infection across broad spatial scales generally decreases snail grazing pressure and the rate of ecosystem die-off, but we were unable to account for possible differences in the magnitude of behavioral impacts produced by different species.

The most represented bird species in our photographic survey were white ibises and Wilson's plovers, comprising 50% and 20% of all birds captured on film, respectively (Table 1). Both species are known to harbor trematodes that use *Littoraria* as a first intermediate host (Bush & Forrester, 1976; Coil & Heard, 1966), and thus may be important drivers of trematode infection in *Littoraria*, and consequently impact ecosystem resistance. Less-common birds observed in our survey harbor trematodes that use *Littoraria* as a first intermediate host as well (Heard, 2011); other observed species may also harbor similar trematodes, but there is little available data about their parasitic fauna. Thus, other bird species may also contribute to trematode infection in *Littoraria*. While white ibises have historically been relatively rare in the mid-Atlantic region, they have undergone an extensive northward range expansion over the past century, such that there are now breeding populations throughout coastal North Carolina and Virginia (Shields & Parnell 1983, Frederick et al. 1996). Disease and disease vector range expansions have been the topic of much study, especially as climate change is rapidly changing the biophysical parameters that dictate the geographic range, seasonality, and severity of many infectious diseases (Harvell et al. 2002). While disease and vector range

expansions are often predicted to have negative effects on populations and ecosystem structure and functioning (Cook et al. 1998), our results suggest that such range expansions could yield positive effects.

In other wetlands systems, birds have been shown to increase productivity of marsh macrophytes through allocthanous nutrient inputs and reworking the sediments (Dessborn et al. 2016, Jefferies 2019). Conversely, previous experiments in *Spartina*-dominated salt marshes have demonstrated that nutrient addition can increase snail grazing intensity and decrease plant aboveground biomass (Silliman & Zieman 2001). However, our manipulation of nutrient inputs from bird feces in the absence of parasitism did not significantly affect plant growth, the magnitude of snail grazing, or the rate of ecosystem retreat (linear mixed effects model with parameter-specific *P*-values generated via the Satterthwaite approximation, $P > 0.3$, all cases). We also considered that birds may facilitate plants by increasing soil redox potential; white ibis in particular commonly feed on benthic invertebrates by plunging their long beaks into the sediment. However, we found that redox potential at the depth of the rhizosphere did not differ significantly across treatments (linear mixed effects model with parameter-specific *P*-values generated via the Satterthwaite approximation, $t = -1.113$, $P = 0.27$), or inside and outside of bird exclusion areas (linear mixed effects model, $t = -1.075$, $P = 0.286$). Because feeding was concentrated when the marsh was just beginning to flood or nearly drained (i.e., there was a small amount of water on the marsh surface),

holes in the marsh substrate that were generated by foraging birds quickly closed after birds moved through (Morton pers. obs.). Under the right circumstances, birds may be able to influence plant growth by concentrated feeding and fecal deposition, but our study did not detect these effects, and instead found that parasitism spread by birds appeared to be the greatest factor in explaining their impacts on marsh vegetation resistance to die-off.

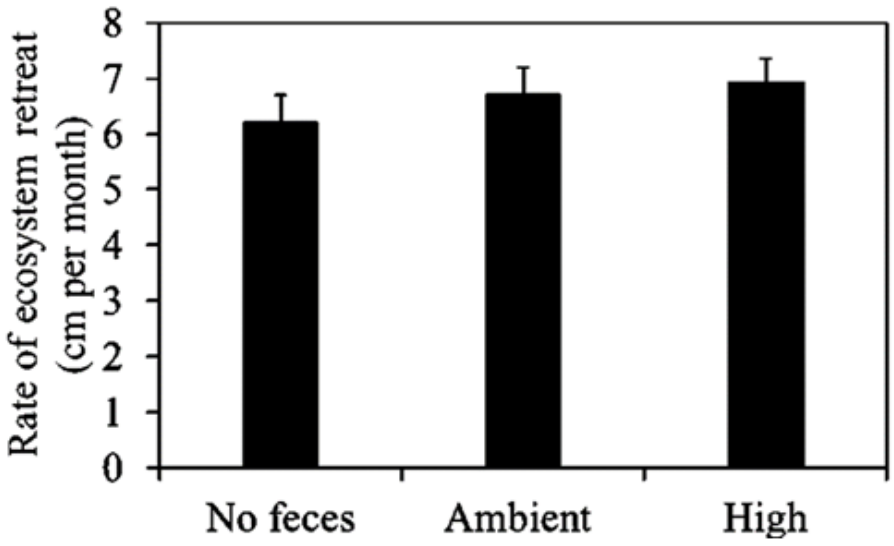

Figure 7: Average rate of ecosystem retreat at different levels of avian fecal inputs.Data are means and SE; n=18 treatment replicates.

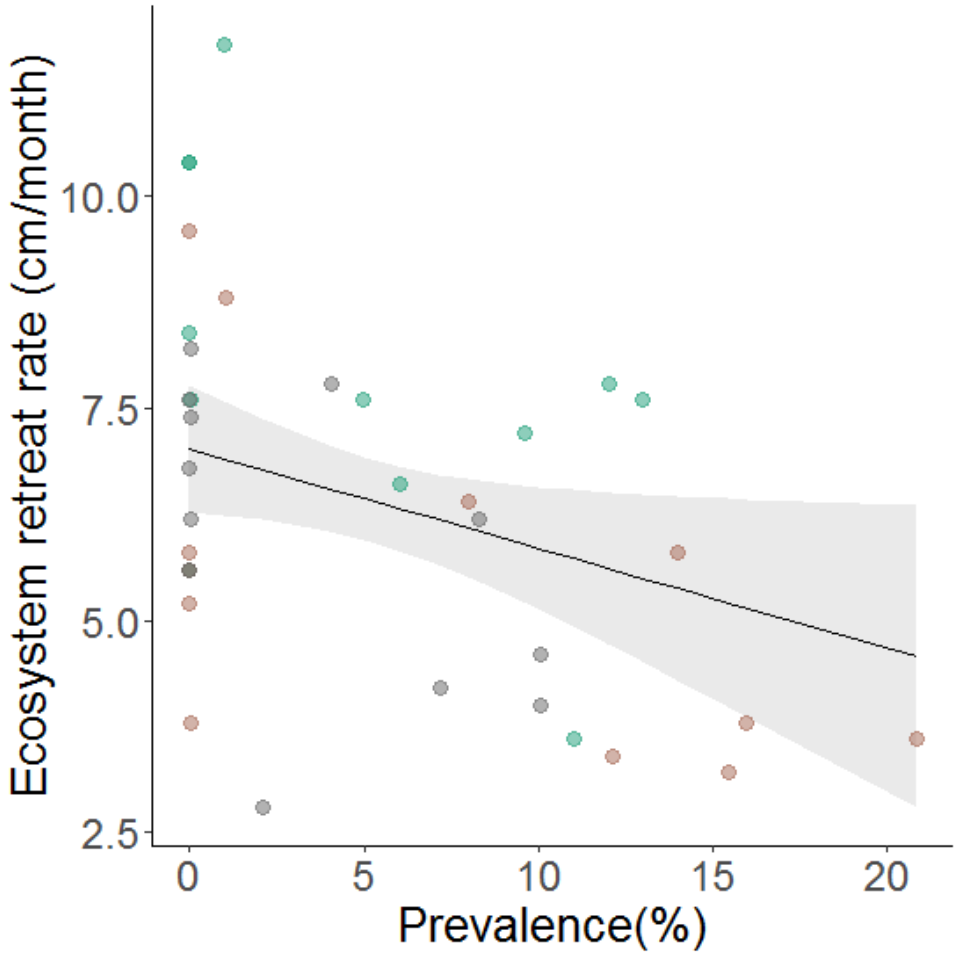

Figure 8: Plot of marginal effects of parasite infection prevalence and its predicted impact on ecosystem retreat rate from our bird exclusion experiment. Line is predicted ecosystem retreat rate holding all other variables at mean values. Shaded regions represent 95% confidence intervals. Differently colored points indicate site.

3.5 Conclusions

The determination that predators can regulate plant ecosystems through trophic control of the population numbers of their herbivorous prey has been a powerful contribution in the history of ecology (Hairston et al. 1960, Estes & Palmisano 1974, Paine 1980, Carpenter et al. 1985). The demonstration of the ability of predators to

indirectly control plant communities by altering the behavior of their herbivorous prey was an important extension of this idea. Studies across aquatic, terrestrial and marine ecosystems have shown that these trait-mediated indirect interactions generated by predators (TMIIs) can have important impacts on plant ecosystem structure, functioning, and dynamics (Werner & Peacor 2003). Like TMIIs, the novel, indirect mechanism of control over plant ecosystem stability described here means that predators can spend relatively short periods of time in an area while still altering herbivore traits and influencing their consumption. Unlike traditional TMIIs, parasite dispersal by predators can continue to regulate herbivory long after predators have left an area, provided that infection with parasites or parasite modification of herbivore traits persists. Thus, rather than predators altering the behavior of herbivores directly, they can do so indirectly by dispersing parasites that can have long-lasting consumptive or nonconsumptive trait-mediated indirect effects on herbivorous hosts (Buck 2019).

These results also underscore the importance of interspatial exchange via highly mobile predators. Mobile interspatial consumer links, like migratory birds, insects, fish, and mammals like bats and bears, are notable for connecting spatially and temporally disparate habitats, supporting essential functions, and playing key roles in determining the direction of ecosystem development after disturbance (Whelan et al. 2008, Lundberg & Moberg 2019). For example, birds and other mobile link organisms may contribute to ecosystem resilience by providing "external ecological memory," acting as vectors for

seeds, pollen, eggs, larvae, mycorrhiza, and nutrients through their trophic and nontrophic interactions with other species within a disturbed site (Lundberg & Moberg 2019). Our study expands the mechanisms by which mobile link organisms and predators in general support plant ecosystems following a disturbance–increasing resilience by dispersing parasites that negatively impact grazers. Additionally, it has long been acknowledged that long-distance dispersal of parasites and pathogens can regulate populations and communities, but in the majority of these cases, the spread of disease via mobile link vectors has a largely negative or destabilizing effect (Reed & Medical 2003, Morshed et al. 2005, Barros et al. 2018, Nekouei et al. 2018). Our results suggest that interspatial exchange of parasites by mobile links can yield positive outcomes for ecosystems being altered through disturbances associated with global change.

4. Parasitism Disrupts a Keystone Facultative Mutualism in a Coastal Ecosystem

4.1 Abstract

Mutualistic associations are pervasive and powerful interactions that can underpin the structure, functioning, and stability of entire ecosystems. Parasites may alter the traits or densities of mutualistic partners, destabilizing mutualistic associations. Despite the potentially wide-ranging consequences of such disruptions, no studies have directly manipulated parasite prevalence and/or intensity in a mutualistic partner, nor quantified the resulting community-level effects. Here, we investigated the effects of

parasitism by a common trematode (*Cercaria opaca*) on the strength of a keystone facultative mutualism between the marsh cordgrass, Spartina alterniflora, and the ribbed mussel, *Geukensia demissa*. The ecosystem consequences of this interaction are far-reaching – mussel mounds increase biodiversity, ecosystem functioning, and increase marsh ecosystem resilience to drought-induced die-off. A field manipulation using experimentally infected mussels revealed that mutualistic benefits of mussels to *Spartina* decreased with increasing infection intensity within mussels. Subsequent lab and field assays demonstrated that these observed changes are likely caused by parasites influencing per capita filtration and biodeposit production. Additionally, increased parasite load was associated with decreased strength of both shells and byssal attachments, potentially explaining the relatively higher predation on mussels from mounds with higher infection intensities in our field study. A survey of five North Carolina salt marshes demonstrated that infection with *C. opaca* was most common in mussels within marsh die-off areas and that infection intensity decreased with distance from disturbed areas. Because the *Geukensia-Spartina* facultative mutualism underpins marsh ecosystem resilience to drought-associated die-off, our results suggest that parasitism may be an important mediating force in ecosystem response to disturbance. Although this is the first experimental demonstration of parasites indirectly altering community structure and functioning by undermining an ecologically influential

mutualism, this relationship could be common given that parasites commonly infect influential secondary foundation species.

4.2 Introduction

Mutualisms, defined as interactions in which there are fitness benefits for both interacting partners, are ubiquitous in nature – it is likely that every species on earth participates in one or more mutualistic relationships (Bronstein 2001, 2009). Because mutualisms are generally acknowledged to be evolutionary responses to stressful or limiting conditions, mutualists may be particularly vulnerable to the loss of mutualistic partners or the disruption of mutualistic associations (Dunn et al. 2009, Aslan et al. 2013). Many mutualistic associations have powerful ramifications for higher levels of biological organization, and the disruption of such relationships may impact the persistence of populations, biodiversity, and ecosystem functioning and resilience (Bond 1994, Toby Kiers et al. 2010, Hale et al. 2011, Aslan et al. 2013).

Parasites can have strong effects at the organismal level, impacting growth, survivorship, reproductive capacity, and behavior. Such individual-level modifications can have indirect consequences for interspecific interactions, including mutualistic associations (Gehring & Whitham 1992, Gillespie & Adler 2013). In a highly-publicized example, declines in populations of honeybees and other native pollinators over the last 50 years have been at least partially attributed to a number of emerging parasites and pathogens that have been introduced around the world by human actions (Potts et al.

2010, Vanbergen & Initiative 2013). These declines in keystone pollinators have had negative impacts on both wild and cultivated plants that depend on their pollination services (Allen-Wardell et al. 1998). While it is well-established that parasites can significantly alter host densities or phenotypes, and that they thus have the *potential* to affect influential mutualistic relationships, there are few demonstrations of this (Gehring & Whitham 1992, Gillespie & Adler 2013). Furthermore, the studies that do exist rely on correlational evidence, making it difficult to tease out whether parasitism is a cause or consequence of observed changes to mutualistic interactions (Gehring & Whitham 1992). Currently, no studies have directly manipulated parasite prevalence and/or intensity in a mutualistic partner, nor quantified the resulting community-level effects. The paucity of such studies is likely due in part to the relative difficulty of experimentally infecting hosts or identifying naturally infected hosts in a non-destructive manner. However, we were able to leverage a system in which this is possible to examine.

In east coast U.S. salt marshes, the ribbed mussel, *Geukensia demissa*, participates in a facultative mutualism with the marsh cordgrass, *Spartina alterniflora* (Bertness 1984). *Geukensia* attach to the basal portion of *Spartina* stems with their byssal threads and deposit nutrient-rich biodeposits in the form of consolidated feces and pseudofeces (Bertness 1984, Smith & Frey 1985, Angelini et al. 2015). The presence of *Geukensia* mounds throughout higher-level marsh platforms stimulates the growth of *Spartina*, leading to increased above- and belowground biomass, and increased flowering

(Bertness 1984, Angelini et al. 2015, 2016). Mussels benefit from the shade provided by associated *Spartina*, which reduces thermal stress and increases mussel survival (Bertness 1984, Angelini et al. 2015, 2016). This facultative mutualism also enhances a number of important ecosystem functions including sediment infiltration rate, decomposition rate, and nitrogen removal through microbial denitrification and water filtration (Angelini et al. 2015, Bilkovic et al. 2017). The structured habitat provided by *Geukensia* mounds, as well changes to benthic algal production and sediment texture as a result of mussel biodeposition, enhances the local diversity and abundance of marsh macrofauna (Angelini et al. 2015, Derksen-Hooijberg et al. 2019). Additionally, recent mussel addition experiments and regional surveys have demonstrated the ability of this facultative mutualism to increase marsh ecosystem resilience to drought-induced vegetative die-off (Angelini et al. 2016).

In many marsh die-off areas, increased local abundance of avian definitive hosts leads to high prevalence of trematode parasitism in the keystone grazing snail, *Littoraria irrorata* (Morton 2018, Morton & Silliman 2020, Sharp & Angelini 2020). One trematode species, the renicolid *Cercaria opaca*, uses *Littoraria* as a first intermediate host, and *Littoraria* and *Geukensia* as a second intermediate (trophic) hosts (Morton et al., Holliman 1961, Epstein 1972, Wardle 1974). In areas around die-offs where infected snails are abundant, the metacercariae of *C. opaca* are ubiquitous in mussel hosts and infection intensity can be very high, with some individuals harboring thousands of metacercariae

within the tissues of the digestive gland (Figure 9, Morton pers. obs.). Given that infection prevalence and intensity can often be high and that this trematode preferentially encysts in the tissues of the digestive gland, *C. opaca* likely interferes with mussel feeding. Additionally, laboratory observations (Morton pers. obs.) suggest that highly infected mussels have thinner shells and weaker byssal attachment relative to uninfected conspecifics, potentially leaving them more vulnerable to predators. While infection by *C. opaca* appears to impact ecologically influential functional traits of mussels, the effects of trematode parasitism on the *Geukensia-Spartina* mutualism has yet to be determined.

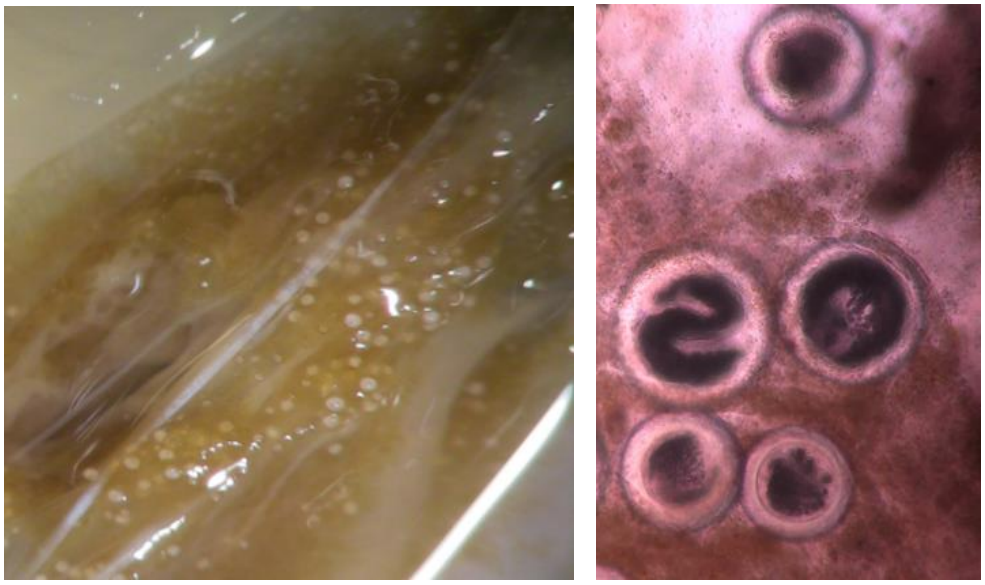

Figure 9: The digestive gland of *Geukensia demissa* infected with metacercariae of *Cercaria opaca* at 10x and 50x magnification, respectively. The pictured specimen was collected from a marsh die-off area on Core Banks, NC.

Here, we experimentally manipulated the average infection intensity of mussel mounds in the field and subsequently quantified changes to *Spartina* growth. Because the *Geukensia-Spartina* mutualism supports marsh biodiversity and numerous functions, we also monitored the effect of increased parasite intensity on macroinvertebrate abundances, marsh sediment elevation, and sediment redox. Because we found that increasing infection intensity impacted the mutualism, we examined the mechanisms by which such changes occurred using lab and field experiments to determine the per-capita effects of increasing parasite intensity on mussel biodeposit production, byssal attachment, and shell characteristics. Based on previous lab and field observations, we predicted that (1) increasing average parasite intensity in mussel mounds would decrease mutualistic benefits conferred to associated *Spartina*, by reducing nutrient-rich biodeposit production and/or increasing successful predation on mussels, and (2) that reductions in mutualistic benefits from mussels would negatively impact mound-associated community components and functions. In order to contextualize the ecological significance of our experimental findings, we also assessed spatial patterns of *C. opaca* infection in mussels from five North Carolina marshes containing die-off areas. We predicted that the intensity of infection by *C. opaca* would be highest in and around die-off areas, where trematode infection prevalence in snail first intermediate hosts is highest.

4.3 Methods

4.3.1 Field manipulation of *C.opaca* infection intensity

4.3.1.1 Acquiring infected *Littoraria*

From June to July, 2017, adult *Littoraria irrorata* were collected at low tide from Gunning Hammock Island, NC (34.672454 N, -76.501743 W), where infection with *Cercaria opaca* was known to be common. Collected snails were gently washed in filtered seawater and placed in small petri dishes. Each dish was filled entirely with filtered seawater (~30 mL) and sealed with a plastic lid such that the snail was completely immersed and unable to escape. Petri dishes with snails were allowed to sit at room temperature for 24-hrs. After this period, the contents of each dish were inspected under a microscope to determine the presence of shed cercariae (Morton 2018). Average incidence of trematode infection from 5 collections was 39.4% ± 10.8 (mean ± SE). For each infected individual, cercariae were identified to species (Stunkard & Cable 1932, Holliman 1961, Coil & Heard III 1966, Heard III 1968, Epstein 1972, Wardle 1974). Infection with *C. opaca* was most prevalent (36.7% ± 10.5). We only used snails that were either infected by *C. opaca*, or that were completely uninfected in our experiment. To distinguish between the two groups of snails, individuals infected with *C. opaca* were marked with a small dot of red paint, while uninfected individuals were marked with a dot of blue paint. Infected and uninfected snails were kept in separate aquaria and

provisioned with moistened *Spartina* wrack until used to experimentally infect *Geukensia*.

4.3.1.2 Experimental infection of *Geukensia*

We collected *Geukensia demissa* from an intertidal marsh on Radio Island, NC (34.723292 N, -76.680375 W) where adult gastropod first intermediate hosts were not present and where numerous previous dissections of mussels revealed no metacercariae in the tissues (Morton pers. obs.). Mussels were transported back to the lab where they were gently washed in filtered seawater to remove any excess mud from the exterior. Any debris attached to the byssus was clipped off with scissors. Mussels were then placed in circular glass dishes (diameter = 20 cm, height = 6 cm) in groups of eight. Each dish was filled with filtered seawater and equipped with a bubbler stone for aeration. A circular lid crafted from 0.635 cm vexar mesh was placed on top of each dish and held firmly in place with a small stone weight. Mussels were provisioned with Shellfish Diet 1800® every day (8 mL per dish per day) and the water within each dish was changed twice weekly. Dishes containing mussels were divided into the following treatments: (1) High exposure to infected gastropods (two infected snails per individual), (2) Medium exposure to infected gastropods (one infected snail per individual, and 1 uninfected snail), (3) No exposure, with mussels exposed to 2 uninfected snails per individual (all snails were confirmed uninfected via dissection at the end of the experimental infection period). If mussels died, they were removed from their dishes along with the

appropriate number of snails such that the snail to mussel ratio never changed. If any snails died, they were immediately replaced.

After 3 months, 20 mussels from each treatment group were selected at random and dissected to determine average infection intensity. At this time, mussels from the "high exposure" treatment had reached an average infection intensity comparable to that observed in field-collected specimens from local marsh die-off areas (1138 ± 111 metacercariae per individual). The medium exposure treatment had an average infection intensity that was roughly half of mussels in the high exposure group (632 ± 43 metacercariae per individual). No metacercariae were found in the tissues of mussels not exposed to snails.

4.3.1.3 Field manipulation

In October 2017, we established 32 permanent 0.5 m^2 plots in a structurally homogenous swath of intermediate *Spartina* marsh at Hoop Pole Creek, Atlantic Beach North Carolina (34.705288 N, -76.749650 W). The study site was characterized by very low snail densities (<1 snails/m^2) and scattered mussel mounds. Dissections of mussels (n = 30) and snails (n = 100) from the study area before the beginning of the experiment revealed no trematode infections. Plots were marked at two corners with 2-cm diameter PVC posts labeled with the individual number of each plot, and we selected plot areas without pre-existing mussels or mud crab burrows. After the establishment of experimental plots and before the application of treatments, we enumerated and

measured the heights of all live *Spartina* stems in each plot. Additionally, we counted the number mud crab (*Panopeus herbstii*) burrows, purple marsh crab (*Sesarma reticulatum*) burrows, and juvenile and adult fiddler crab (*Uca spp.)* burrows (adult burrows were 5 to 10 times wider than juvenile burrows) as a proxy for the abundance of these organisms (Macia et al. 2001, Angelini et al. 2015, Martínez-Soto & Johnson 2020). Additionally, we counted the number of adult and juvenile *Littoraria* in each plot (Angelini et al. 2015a). There were no statistically significant differences in any of these initially measured variables between treatment groups (ANOVA, $p > 0.23$, all cases).

Plots were assigned to the following infection intensity treatments, corresponding to the aforementioned mussel exposure treatments (n = 8 replicates per treatment): (1) highly infected mussels, (2) intermediately infected mussels, (3) uninfected mussels. We also had 8 plots with no mussels to control for the effect of mussel addition. Mussels from corresponding treatment groups were transported to the field in aerated, seawater-filled buckets, with mussels in each group placed in their own separately labeled bucket to avoid confusion. Thirty mussels from the same exposure treatment were arranged in circular mounds at the center of corresponding treatment plots. All mussels were inserted such that they protruded no more than 2 cm from the sediment. The length, width, and depth of each mussel was measured prior to being inserted into the sediment. A week after experimental setup, plots with mussels were examined to ensure that all mussels were alive and were firmly rooted in the sediment.

Any dislodged mussels were re-inserted into the sediment and any dead mussels were replaced with new mussels of the appropriate treatment group and the dimensions of these new mussels were recorded. We continued to enumerate all mussels in each plot every month for the year-long duration of the experiment (October 2017- October 2018).

To examine how differing infection intensity could modify the role of mussels in binding sediment and influencing vertical accretion of the marsh, three level rods were installed in each plot. Level rods consisted of 0.5 cm diameter PVC dowels driven vertically into the marsh until firmly embedded in the underlying clay (~0.75 m below the marsh surface). Each rod was cut so that it stuck out 15 cm above the marsh surface and was labeled with a permanent marker. The height of each level rod over the marsh surface was taken one week after their installation and every month afterward.

To determine whether parasite load could indirectly alter marsh sediment oxygen availability, we measured sediment redox potential (mV) within each plot. Redox measurements were taken using a platinum electrode probe and a Fisher Scientific Accument double junction Ag/AgCl electrode (+200 mV correction added to the measured value), connected through a Fisher Scientific Accument PH/mV/C meter, model AP71 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) (Gittman & Keller 2013). At absolute low tide, each electrode was placed 10cm into the mussel mound sediment near the center of each plot and left for 30 minutes to allow the sediment surrounding the probe to stabilize. We measured redox in all plots before the

sediments were disturbed by the application of various treatments. We measured redox again one week after mussels had been added to assigned plots and then subsequently every month following installation.

At the end of the experiment, we determined benthic Chlorophyll a (Chl-a) concentration as a proxy for algal biomass in each plot by taking five replicate cores (1.75 cm diameter) from the center of plots at low tide (Parsons et al. 1984, Howes & Teal 1994). Cores were placed on ice and transported back to the lab in opaque vials to be processed immediately. The top 2 mm of sediment were removed from each core and placed in labeled vials of 100% methanol for 48 hours to extract pigments. Vials wrapped in aluminum foil and stored in a -20°C freezer until processed. Prior to florometric analysis, vials were warmed to room temperature. Each vial was decanted into a 5 mL glass tube which was then placed in a calibrated Tuner 10-AU flourometer and the fluorescence was read (Parsons et al. 1984). Chlorophyll concentration was calculated as mg Chl-a per m^2.

At the end of the experiment, we enumerated all macroinvertebrates as before as well as all flowering *Spartina* in each plot. Following this, all live *Spartina* within each plot was harvested, washed, and dried for 3 weeks at 70°C before biomass was determined to the nearest 0.01 g. *Spartina* belowground biomass was determined by taking 25 cm deep sediment cores using a 6 cm diameter coring device (Bertness 1984). Cores were washed and sieved through a 2mm mesh sieve. Live roots and rhizomes

were separated out on the basis of rigidity and coloration (Bertness 1984), dried at 70°C for three weeks, and weighted to the nearest 0.01g.

Immediately prior to the collection of all mussels, five live individuals were selected from each mound and subjected to a biodeposit production assay using methods adopted from Smith and Frey (1985). Mussels were inserted into the sediment of a marsh area adjacent to the site of the field manipulation. Each mussel was surrounded with an apron made from a circular section of white sheet plastic (diameter = 20 cm). Aprons were pinned in place with galvanized lawn staples and marked with an identifying number using permanent marker that corresponded to each mussel's mound number and treatment group. Mussels at the apron's center began filter feeding and producing biodeposits as the high tide covered them. During the last ~30 minutes of tidal inundation when mussels were still covered with water, biodeposits were collected from each apron with a 50 mL pipet, returned to the laboratory, dried at 70°C for two weeks, and then weighted to the nearest 0.01 g. Biodeposit production rate was calculated as the net dry weight of collected biodeposits divided by tidal inundation time (5 hours). Following this assay, all mussels used in the experiment were collected, measured, and dissected to determine parasite infection intensity. Mussel meats were removed from the shell, gently torn apart with a scalpel, and then sandwiched between two panes of plexiglass so that metacercariae could be easily counted under a dissecting microscope.

Differences in treatment means for different response variables (final mussel density, aboveground biomass, belowground biomass, sediment elevation, macrofauna abundances, benthic Chl-a, sediment redox potential, flowering, and mussel mortality rate) were evaluated using one-way ANOVA followed by Tukey HSD tests for pairwise comparisons. Because the data did not meet the normality assumptions of ANOVA, differences in treatment means for mud crab burrow abundance were evaluated using a Kruskal-Wallace test, followed by Dunn's test of multiple comparisons with p-values corrected using Holm's method to control for the family-wise error rate.

4.3.2 Mechanisms underlying parasite disruption of the *Geukensia-Spartina* facultative mutualism

4.3.3 Effects of parasite load on mussel filter feeding

To determine how mussel filtration rate varied with parasite infection intensity, we subjected field-collected mussels to a laboratory filtration assay. In July 2017, mussels of roughly the same size (8.0 ± 0.05cm shell length) and initially unknown infection intensity were collected from mounds found in the vegetated marsh adjacent to a marsh die-off area on Gunning Hammock Island where infection with *Cercaria opaca* was common in snail first intermediate hosts.

Mussels were gently washed under filtered seawater and any material attached to their byssus was removed with scissors. Mussels were stored in a flowthrough tank for 48 hours prior to the beginning of the filtration assay. Forty 1.8 L glass jars that

served as experimental units were each filled with 500 mL of unfiltered seawater and aerated with an aquarium air stone. Immediately prior to the addition of mussels we took an initial 25 mL water subsample from each jar. Water subsamples were stored in numbered glass vials covered in foil and placed in a refrigerator prior to filtration. Following this, a single mussel was placed in each jar and allowed to filter feed. Eight aerated jars with no mussels acted as controls. We took subsequent 25 mL water subsamples each hour for the 3 hour duration of the experiment (Nagabushanam 1963, Jordan & Valiela 1982). At the end of the experiment, mussels were removed from jars and a final 250mL water sample was taken. Mussels were weighed and measured as with previous experiments, placed in individually labeled bags and stored in a freezer before being dissected to determine infection intensity. Immediately following the end of the experiment, all water subsamples were vacuum filtered onto Whatman 47 mm microfiber filters which were then transferred to 25 ml vials of methanol and placed in a freezer to extract pigments. After 48 hours, samples were removed from the freezer and warmed to room temperature before Chl-a content was analyzed using a calibrated Tuner 10-AU fluorometer (Parsons et al. 1984). Average filtration rate was calculated from hourly rates and expressed in units of mg Chl-a per hour.

Relationships between parasite load and filtration rate were evaluated using multiple linear regression with filtration rate as the dependent variable and infection intensity and mussel size (shell length) as predictor variables.

4.3.4 Effects of parasite load on mussel shell thickness strength

In order to determine the impact of trematode infection intensity on ribbed mussel shell strength, we conducted a laboratory experiment using field-collected mussels to determine the force required to cause structural failure of mussel shells. In October, 2018, we collected mussels (n = 50) from a marsh die-off area on Gunning Hammock Island. Mussels were stored in a flowthrough tank. To estimate the mass required to crush live mussels with different parasite loads, we constructed a crushing device based on a similar device used by Hughes (2016). The device consisted of a pivoting wooden arm that came to rest on a stationary wooden plat form that was bolted firmly to the edge of a tabletop. A piece of 10 × 7 × 1.5 cm steel plate was affixed one end of a 40 × 7 × 5 cm wooden platform such that the pivot-arm came to rest at its center. Another piece of steel plate was affixed to the edge of the pivot-arm that came to rest on the steel plate. A steel bolt affixed to the end of the pivot arm accommodated a free-hanging bucket to which weight (iron weights and water) could be added.

Mussels were retrieved from their holding tank one at a time to be tested. After determining each mussel's wet weight, shell length, width, and depth, individual mussels were then positioned at the center of the crushing platform such that the steel edge of the pivoting arm made contact with the mussel at its widest point. A bucket was hung on the end of the pivot-arm, and weight was gradually added to the bucket until the shell of the mussel reached the point of structural failure. The force required to crush

a mussel was calculated using the mass of the bucket and weights added to the weight of the pivot arm at rest. After being crushed, we dissected mussels and quantified infection intensity. We evaluated the relationship between parasite load and mussel shell strength using multiple linear regression with crush force (N) as the dependent variable and infection intensity and mussel size as predictors. Because the force required to crush mussels likely varies with mussel size, and because of the orientation in which mussels were crushed, we selected shell depth as the most relevant metric of mussel size to include in the model.

Because we were unable to take reliable measurements of mussel shell thickness after mussels were crushed, we used shell thickness metrics taken from mussels used in our lab biodeposition assay to determine how mussel shell thickness varied with *C. opaca* infection intensity. Using a Shahe digital micrometer, we measured the thickness of clean mussel shell halves (n = 50 mussels) to the nearest 0.001 mm at three points (lip, ventral surface, and dorsal surface) following the methods of Goss-Custard (2002). Measurements from each mussel shell half were averaged together to generate a shell thickness metric for each mussel. We evaluated the relationship between mussel shell thickness and parasite load using multiple linear regression with average shell thickness as the dependent variable infection intensity and mussel size (shell depth) as predictors.

4.3.5 Effects of parasite load on byssus formation and attachment strength

To determine how mussel byssus formation and byssal attachment strength varied with parasite infection intensity, we subjected field-collected mussels to a laboratory assay. Experimental units consisting of terra cotta flower pots (diameter = 11 cm, height = 11 cm) were filled with 500 mL of marsh sediment. The sediment used was excavated from a salt marsh adjacent to Pivers Island, Beaufort NC (34.71823 N, -76.67121 W) and thoroughly homogenized prior to adding it to each pot to ensure uniformity of grain size. A 20 cm long segment of dry bamboo (diameter = 1 cm) was inserted into the sediment in the center of each pot. Mussels (n = 60) of an initially unknown infection status were collected from a marsh die-off area within the Rachel Carson Estuarine Research Reserve (34.709133 N, -76.58506 W) in September 2020. Mussels were gently washed with filter seawater and any byssal threads protruding from the shell were clipped off. A single mussel was inserted vertically into the sediment adjacent to the bamboo segment such that it made contact with the interior margin of the mussel shell where the byssus emerges. Pots with mussels were placed in a flowthrough tank filled with filtered seawater. After a week, mussels were removed along with their corresponding bamboo, taking great care not to sever attached byssal threads. The length, width, and wet weight of each mussel was measured and the number of byssal threads attached to the bamboo post were counted under a dissecting microscope. Following this, the post connected to each mussel was secured to the edge of a table with

clamps and a small harness comprised of two adjustable loops of wire were affixed to each mussel, with one loop of wire wound around the mussel on either side of the byssus. A polystyrene cup was hung from the harness with string and water was added to the cup until the byssal attachments broke, fully separating the mussel from the bamboo. The force required to disconnect a mussel was calculated from the mass of the cup of water added to the weight of the harness and mussel. Following detachment, each mussel was dissected and parasite load quantified.

We evaluated the relationship between intensity of *C. opaca* infection and mussel byssal thread formation using multiple linear regression with byssal thread count as the dependent variable and infection intensity as dependent variables. We evaluated the relationship between mussel byssal attachment strength and parasite load using multiple linear regression, with the force required to dislodge mussels (N) as the dependent variable and infection intensity, number of attached byssal threads, and mussel size (wet weight) as predictors. Prior to these analyses, infection intensity was log-transformed and detachment force was square root-transformed to meet the normality of residuals assumption of linear regression.

4.4 Survey of infection intensity within and around die-off areas

In order to place our experimental findings in a spatial context and determine where parasitism in *Geukensia* may have the greatest impact at the landscape level, we assessed infection intensity of mussels within and outside of marsh die-off areas. In May

2018, we identified 5 marsh die-off areas along 40 km of the North Carolina coast where large grazing fronts of *Littoraria irrorata* had formed (Gunning Hammock Island 34.6726073°N, -76.4992263°W; Harkers Island 34.6920993°N, -76.5265395°W; Town Creek 34.7238264°N, -76.6585029°W; Carrot Island 34.709038°N, -76.6589893°W; and Hoop Pole Creek 34.7054731°N, -76.7491902°W). At each site, we haphazardly collected mussels along 50 m × 1 m transects along die-off borders, 25 m from the die-off edge, and 50 m from the edge (n = 25 mussels per transect). In order to determine the relationship between infection frequency in gastropod first intermediate hosts and infection intensity in mussels, we also haphazardly collected adult *Littoraria* along each transect (n = 100 snails per transect) and dissected them to determine the prevalence of infection with *C. opaca* within each transect. Mussels were weighed, measured and dissected to determine infection intensity using the previously described methods.

We used the lme4 package in R to conduct a linear mixed effects analysis to evaluate differences in infection intensity in mussels by distance from the die-off border. Die-off size and mussel size (length) were included as covariates and site was included as a random effect in our model. We approximated p-values for model factors using type II Wald χ^2 tests. We used the same approach to evaluate patterns of infection intensity in *Littoraria.* Die-off size and distance from the die-off border were included as predictor variables while site was included as a random effect.

4.5 Results

4.5.1 Field manipulation of parasite intensity in mussels

4.5.1.1 Final infection intensity of mussels

No metacercariae were found in the tissues of any mussels from the treatment group unexposed to infected snails. Mussels in the intermediate exposure group had an average of 671 ± 30 metacercariae in the tissues while mussels in the high exposure group had an average of 1093 ± 46 metacercariae (Figure 10). There were no differences in any metric of mussel size (length, width, depth, or wet biomass) between treatments (one-way ANOVA, $p > 0.9$, all cases).

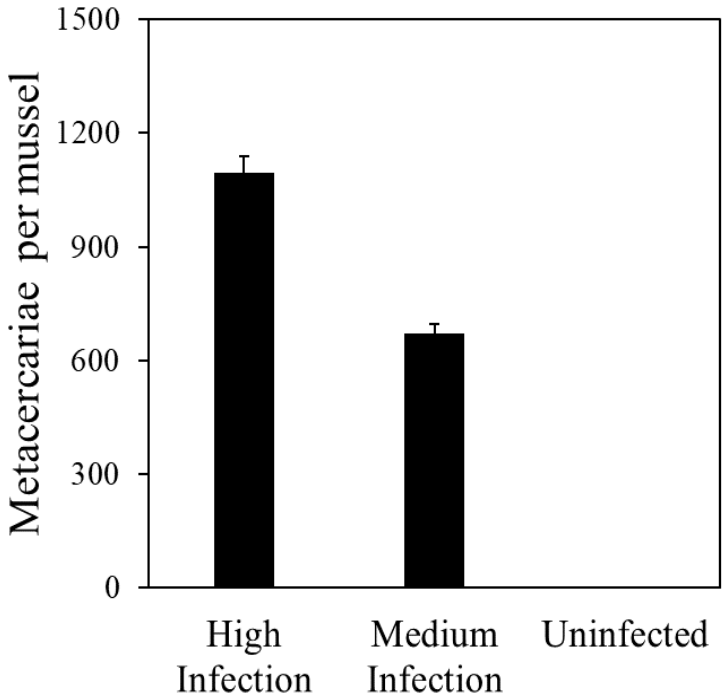

Figure 10: Final infection intensity of mussels from each treatment in our field manipulation of mussel infection intensity. Data are treatment averages (n = 94, 159, and 178 surviving mussels per treatment, respectively) with standard error bars.

4.5.1.2 Spartina aboveground biomass

The addition of uninfected mussels was associated with substantial, significant increases in *Spartina* aboveground biomass relative to plots without mussels, consistent with previous studies (Bertness 1984) (Figure 11). However, increasing parasite intensity yielded marked decreases in *Spartina* biomass. There was no significant difference between high infection plots and control plots where no mussels were present (Figure 11, Tukey HSD, $p = 0.929$). Mounds with a middling level of parasite intensity yielded almost twice the amount of plant biomass that plots without mussels did (Figure 11, Tukey HSD, $p = 0.0184$), but did not differ significantly from high infection plots (Figure 11. Tukey HSD, $p = 0.0724$). *Spartina* biomass in uninfected plots was more than twice that of medium infection plots (Figure 11, Tukey HSD, $p < 0.0001$) and nearly four times greater than highly infected plots (Figure 11, Tukey HSD, $p < 0.0001$).

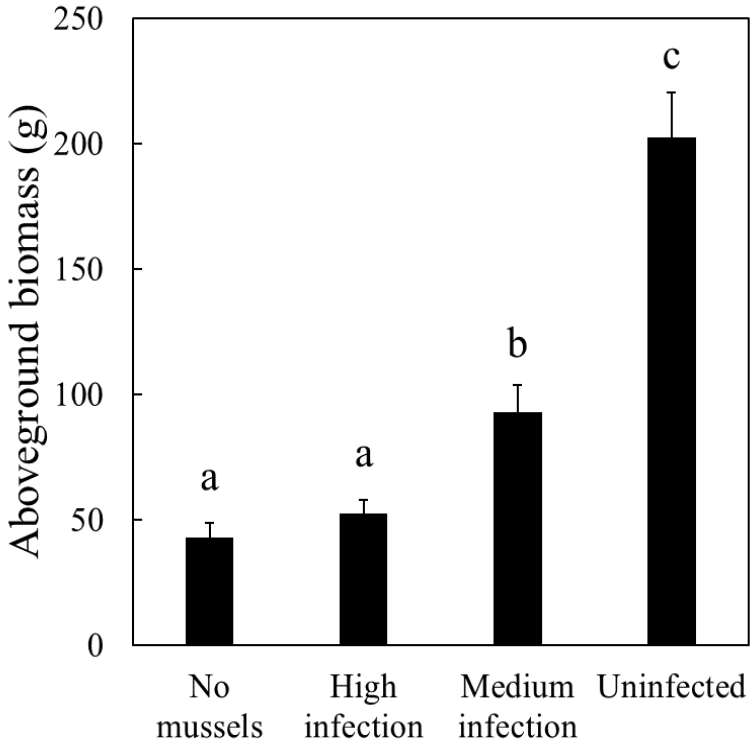

Figure 11: Spartina alterniflora aboveground biomass by treatment.Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

4.5.1.3 Spartina belowground biomass

Both *Spartina* root and rhizome biomass did not differ between high infection mounds and control plots with no mussels (Figure 12A-B, Tukey HSD, $p > 0.52$, both cases). Root and rhizome biomass was significantly higher in control mounds and mounds with middling infection intensity (Figure 12A-B, Tukey HSD, $p < 0.033$, both cases). There was no significant difference in belowground biomass metrics between highly infected mounds and mounds with middling infection intensity (Figure 12A-B, Tukey HSD, $p > 0.2$, both cases). Rhizome biomass was significantly lower in high infection intensity mounds compared to uninfected mounds (Figure 12A-B, Tukey HSD,

$p < 0.035$, both cases). There was no significant difference in either root or rhizome biomass between middling intensity mounds and uninfected mounds (Figure 12A-B, Tukey HSD, $p > 0.12$, both cases).

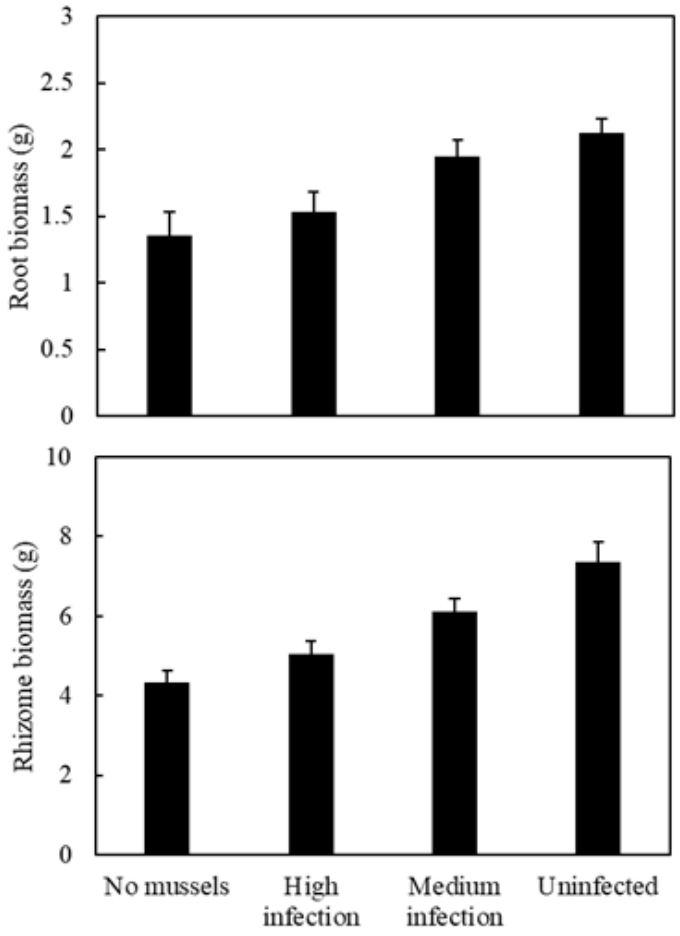

Figure 12: Spartina alterniflora root (A) and rhizome (B) biomass by treatment group.Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

4.5.1.4 Marsh elevation

Sediment accretion was significantly higher in plots with uninfected mussels relative to uninfected plots and plots with no mussels (Figure 13, Tukey HSD, $p < 0.11$ both cases). Sediment accretion in plots with a medium level of infection significantly greater than plots with no mussels (Figure 13, Tukey HSD, $p = 0.027$ both cases). There was no significant difference in sediment accretion between medium infection plots and uninfected plots (Figure 13, Tukey HSD, $p = 0.625$) and high infection plots and medium plots (Figure 13, Tukey HSD, $p = 0.204$). High infection plots were not significantly different than plots without mussels (Figure 13, Tukey HSD, $p = 0.822$).

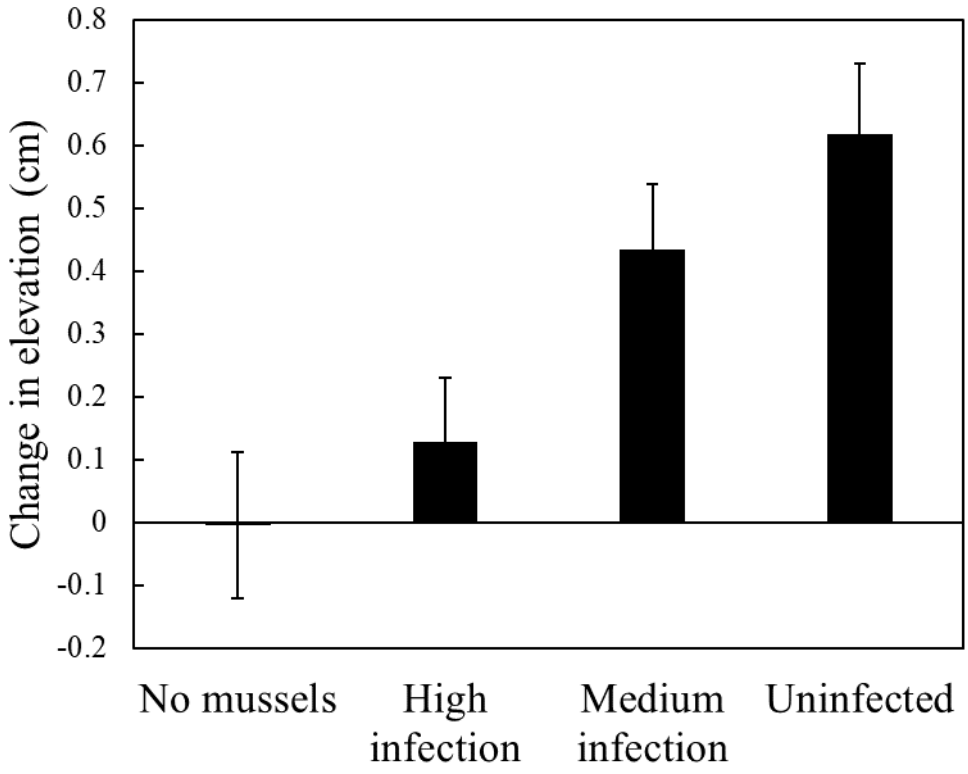

Figure 13: Change in sediment surface elevation by treatment. Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

4.5.1.5 Macrofauna abundance

Mud crab burrows were significantly more numerous in plots with uninfected mussels compared to plots without mussels and highly infected plots (Figure 14, Dunn's test, $p < 0.032$, both cases). Average mud crab abundance in medium infected plots was intermediate between highly infection and uninfected treatments, but was not significantly different from either (Figure 14, Dunn's test, $p > 0.23$, both cases). There was no difference in crab abundance between high infection plots and plots with no mussels (Figure 14, Dunn's test, $p > 0.999$).

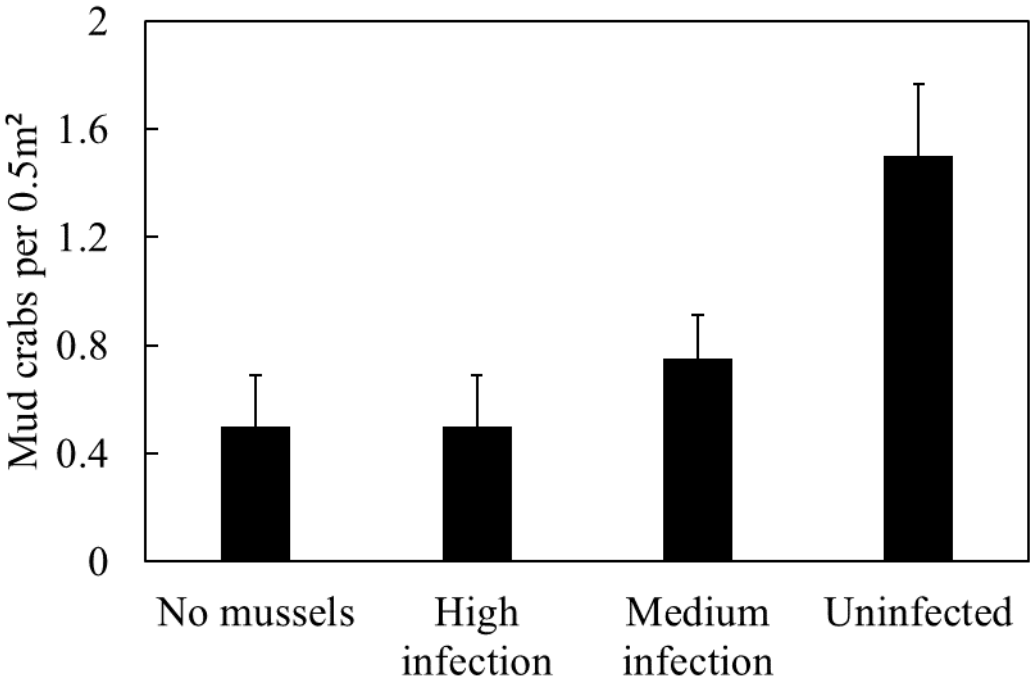

Figure 14: Mud crab burrow density by treatment group. Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

Juvenile fiddler crab burrow density in plots with uninfected mussels was significantly higher compared to plots without mussels (>1100% more burrows),

medium infected plots (>50% more burrows), and highly infected plots (>400% more burrows) (Figure 15A, Tukey HSD, $p < 0.0046$, all cases). Increasing level of parasite infection was associated with marked decreases in juvenile fiddler crab occupancy. At medium infection intensity, juvenile fiddler crab burrow density was significantly higher than both highly infected plots (Figure 15A, Tukey HSD, $p = 0.0084$) and plots without mussels (Figure 15A, Tukey HSD, $p = 0.00082$). There was no significant difference between highly infected plots and plots with no mussels (Figure 15A, Tukey HSD, $p = 0.805$). Adult fiddler crab burrows were significantly more numerous in uninfected plots compared to highly infected plots and plots without mussels (figure 15B, Tukey HSD, $p < 0.0007$, both cases). There was no significant difference in adult fiddler crab abundance between highly infected plots and plots without mussels (Figure 15B, Tukey HSD, $p = 0.998$).

Juvenile *Littoraria* recruitment was significantly higher in uninfected plots compared to all other treatments (Figure 16A, Tukey HSD, $p < 0.002$, all cases). There was no significant difference in juvenile *Littoraria* abundance between high infection plots, medium infection plots, and plots without mussels (Figure 16A, Tukey HSD, $p < 0.725$, all cases). There was no significant effect of treatment on the abundance of adult *Littoraria* (Figure 16B, ANOVA, $p = 0.837$).

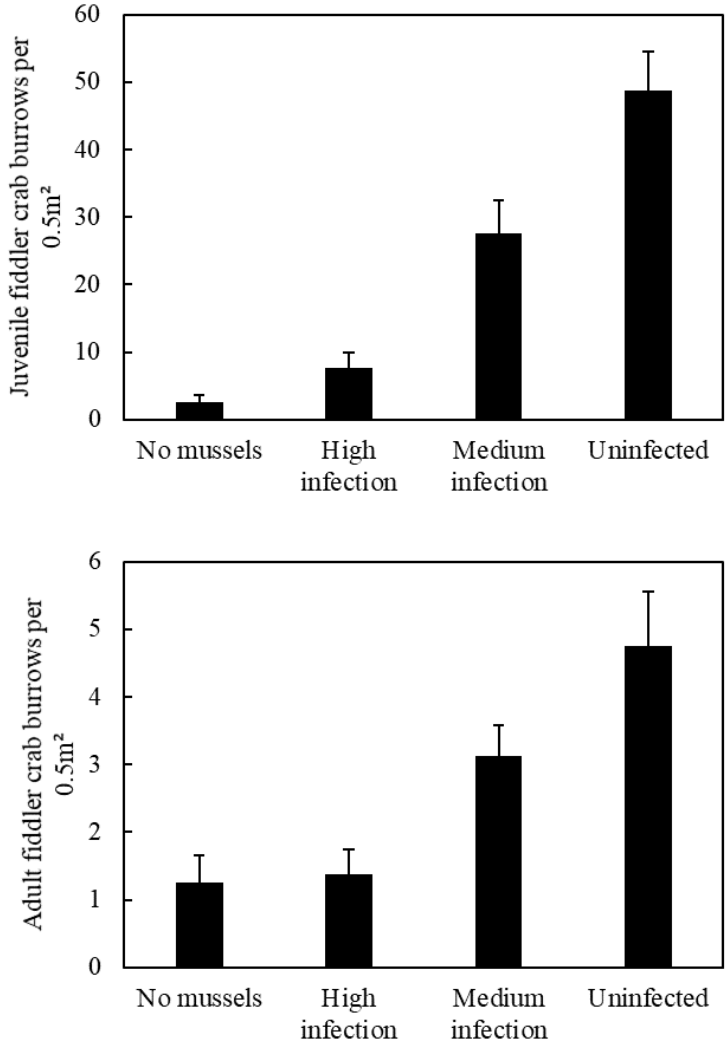

Figure 15: Burrow density of (A) juvenile and (B) adult fiddler crabs by treatment group. Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

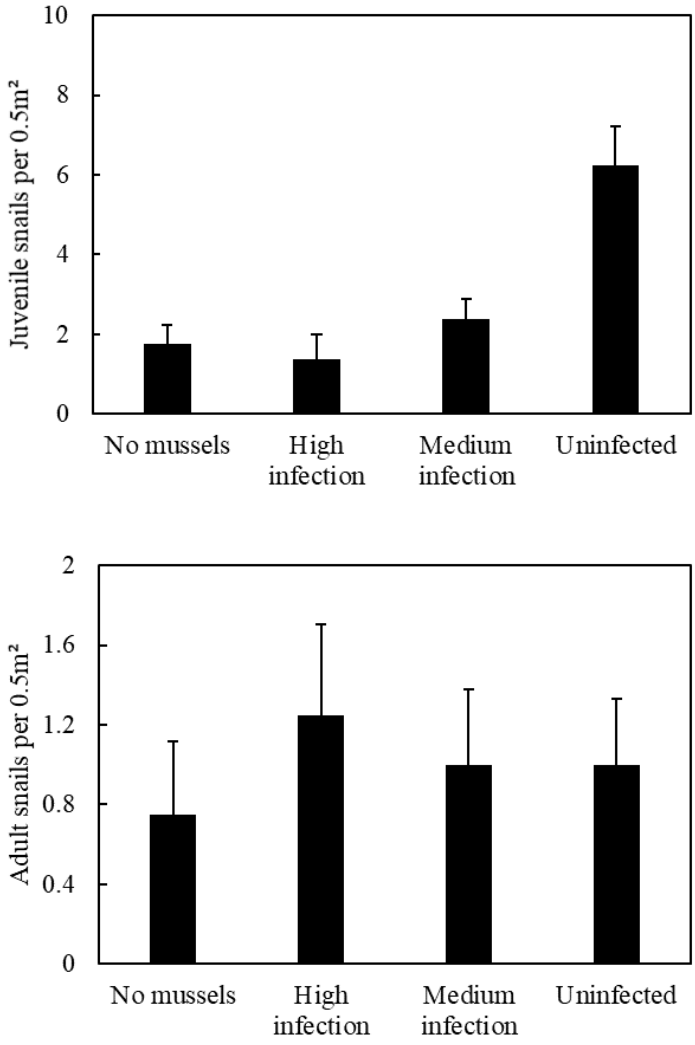

Figure 16: Density of (A) juvenile and (B) adult *Littoraria irrorata* by treatment group. Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

Sesarma reticulatum burrows were significantly more abundant in plots with uninfected mussels compared to highly infected plots and plots without mussels. There was no significant difference between high infection plots and plots with no mussels. Plots with middling levels of infection were not significantly different from uninfected

plots, highly infected plots, or plots with no mussels (Figure 17, Tukey HSD, $p > 0.314$, all cases).

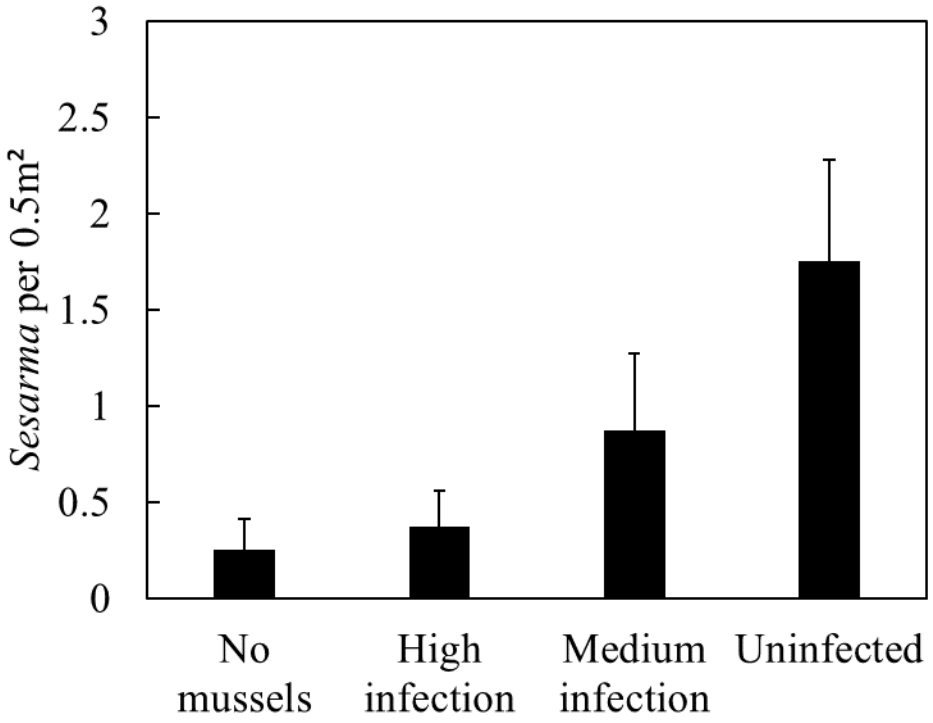

Figure 17: *Sesarma reticulatum* burrow density by treatment group. Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

4.5.1.6 Benthic Chl-a

Benthic chlorophyll-a concentration was significantly higher in the uninfected and intermediate infection mussel treatments compared to plots with no mussels (Figure 18, Tukey HSD, $p < 0.0016$, both cases). There was no significant difference in benthic chl-a between highly infected plots and plots with no mussels (Figure 18, Tukey HSD, $p = 0.584$). There was no significant difference in benthic chl-a between uninfected plots and intermediate infection plots (Figure 18, Tukey HSD, $p = 0.365$). Benthic chl-a was

more than 43% higher in uninfected plots than in high infection plots (Figure 18, Tukey HSD, $p = 0.0006$). Benthic chl-a was significantly greater in intermediate plots compared to high infection plots (Figure 18, Tukey HSD, $p = 0.039$).

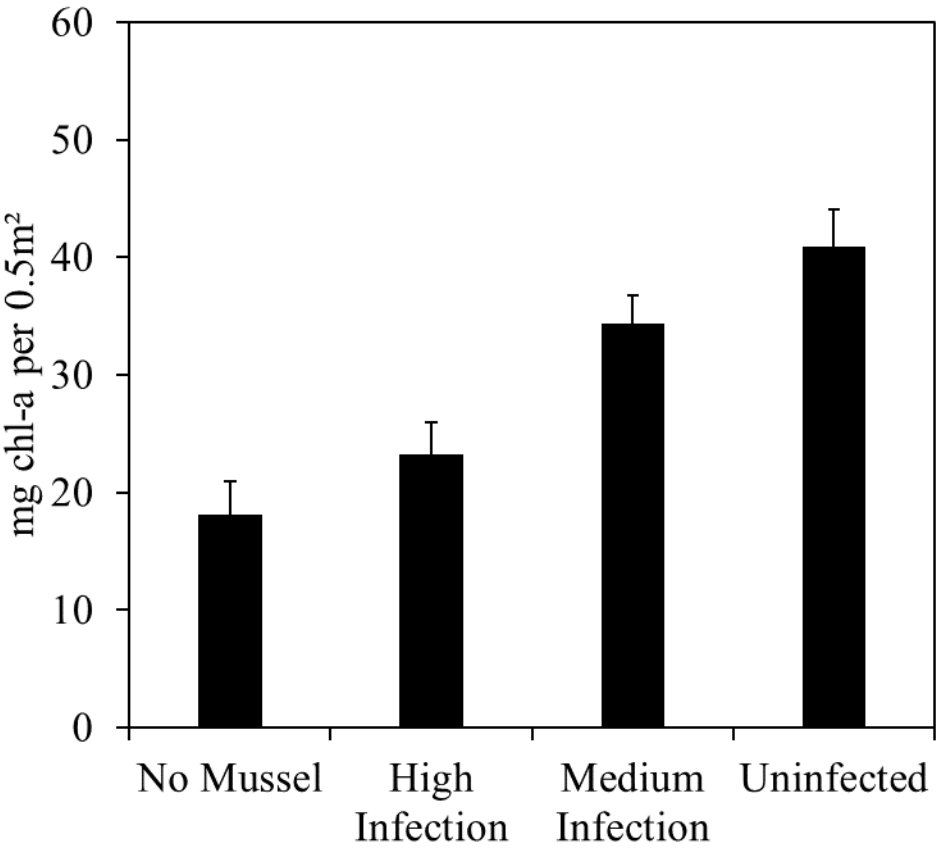

Figure 18: Benthic chlorophyl-a by treatment group.Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

4.5.1.7 Sediment redox potential

Sediment redox potential increased significantly with the addition of uninfected mussels relative to plots with no mussels (Figure 19, Tukey HSD, $p < 0.0001$) and all other treatments (Figure 19, Tukey HSD, $p < 0.021$ all cases). Redox potential of mounds

with a medium level of infection was significantly greater than highly infected mounds (Figure 19, Tukey HSD, p = 0.00034) or mounds without mussels (Figure 19, Tukey HSD, p = 0.00015). There was no significant difference between highly infected mounds and plots without mussels (Figure 19, Tukey HSD, p = 0.9908).

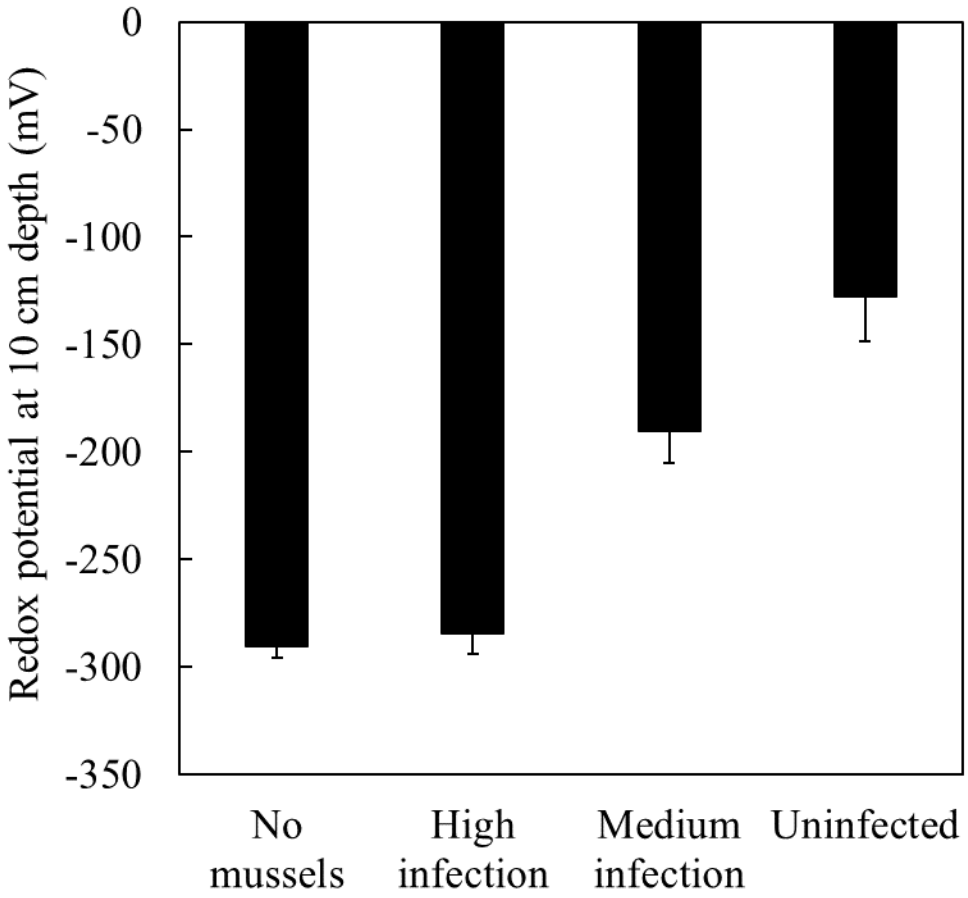

Figure 19: Sediment redox potential at 10 cm depth in each treatment group. Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

4.5.1.8 Flowering

Uninfected and medium infected mussel mounds yielded significant increases in flowering shoots of 114% and 93% (respectively) relative to plots without mussels (Figure. 20, Tukey HSD, $p < 0.02$ both cases). Uninfected plots had a significantly higher proportion of stems compared to uninfected plots (Figure 20, Tukey, $p = 0.016$). There was no significant difference in the proportion of flowering stems between uninfected and medium infection plots (Figure 20, Tukey, $p = 0.895$) or between high infection plots and medium infection plots (Figure. 20, Tukey, $p = 0.079$). High infection plots did not produce a significantly higher proportion of flowering shoots than plots without mussels (Figure 20, Tukey HSD, $p = 0.922$).

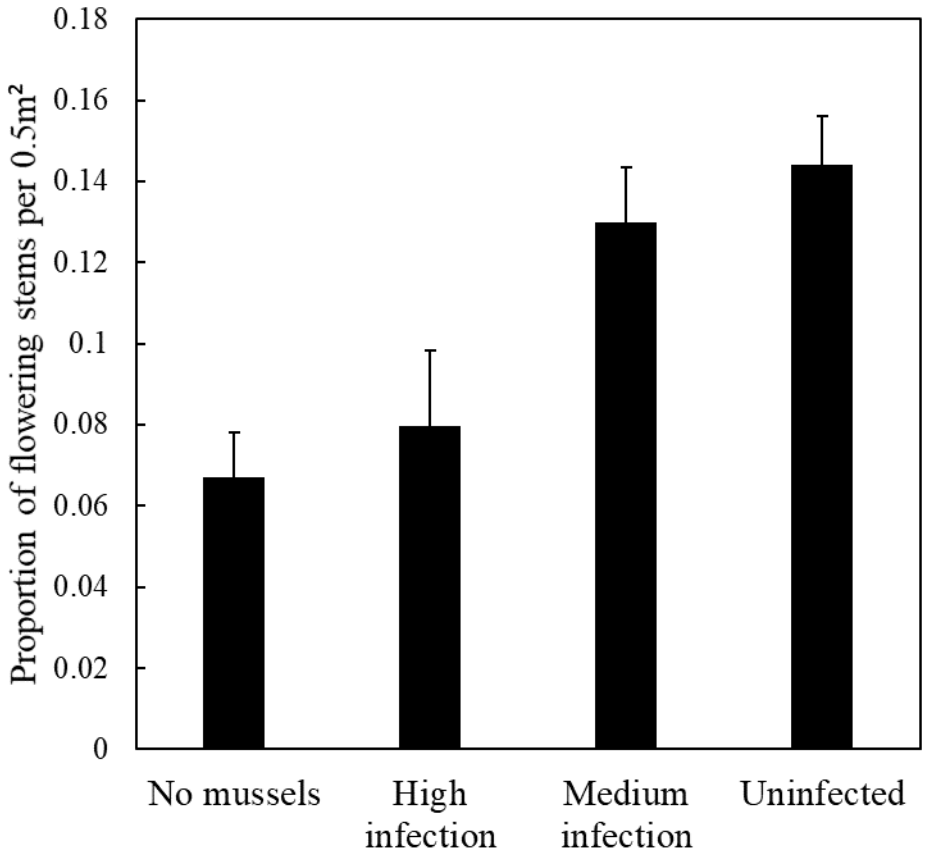

Figure 20: Proportion of *Spartina alterniflora* stems with flowers by treatment group. Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

4.5.2 Mechanisms underlying parasite disruption of the *Geukensia-Spartina* facultative mutualism

4.5.2.1 Effects on mussel mortality

Mortality of mussels occurred in all plots to which they had been added, although the mortality rate varied considerably (13-87%). Intermediately infected mussels did not experience mortality at a greater rate than uninfected conspecifics (Figure 21, Tukey HSD, p = 0.565). Mortality rate was significantly higher in highly

infected mussel plots compared to uninfected and intermediately infected treatments (Figure 21, Tukey HSD, $p < 0.006$). Shells of deceased mussels typically remained *in situ*, allowing us to assess the putative cause of death for each mussel during monitoring. There were significantly more mussel shells bearing obvious signs of crab predation in high infection treatment plots than in intermediately infected and uninfected treatments (Figure 21, Tukey HSD, $p < 0.017$) while there was no difference in the occurrence of crab-marred shells between uninfected and intermediately infected treatments (Figure 21, Tukey HSD, $p = 0.4$). There was a weakly significant effect of treatment on the proportion of dead mussel shells showing signs of crab predation (Figure 21, one-way ANOVA, $p = 0.044$) driven by a marginally significant difference between uninfected and highly infected treatments (Figure 21, Tukey HSD, $p = 0.056$).

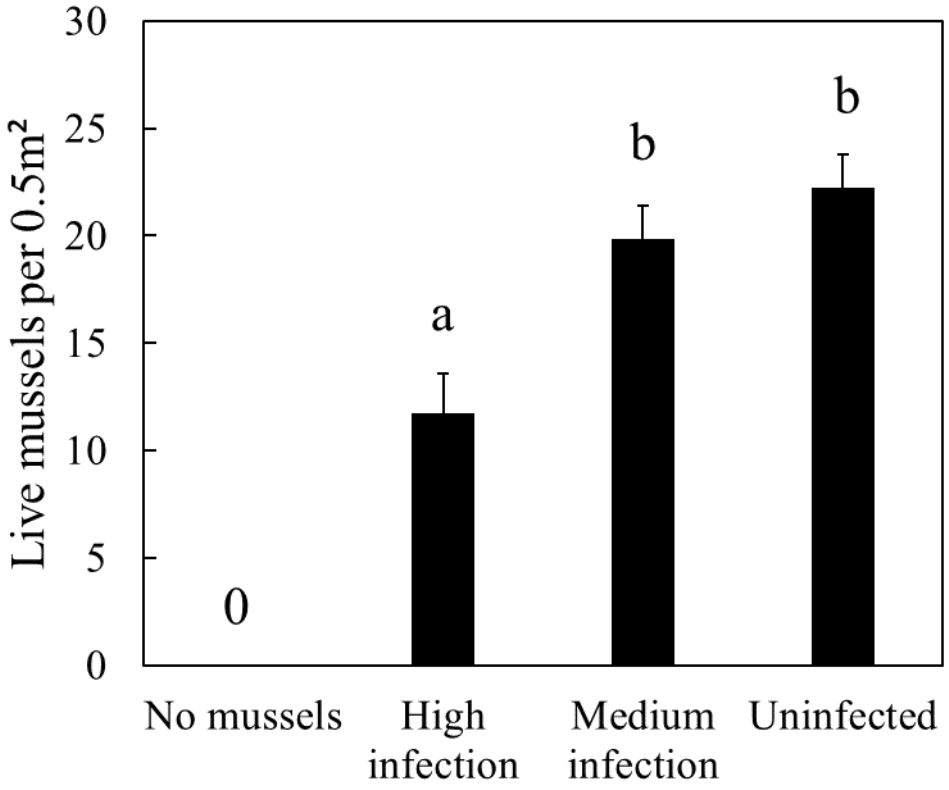

Figure 21: Live mussel density by treatment at the end of our field manipulation of parasite infection intensity. Data are treatment averages (n = 8 replicates per treatment) with standard error bars.

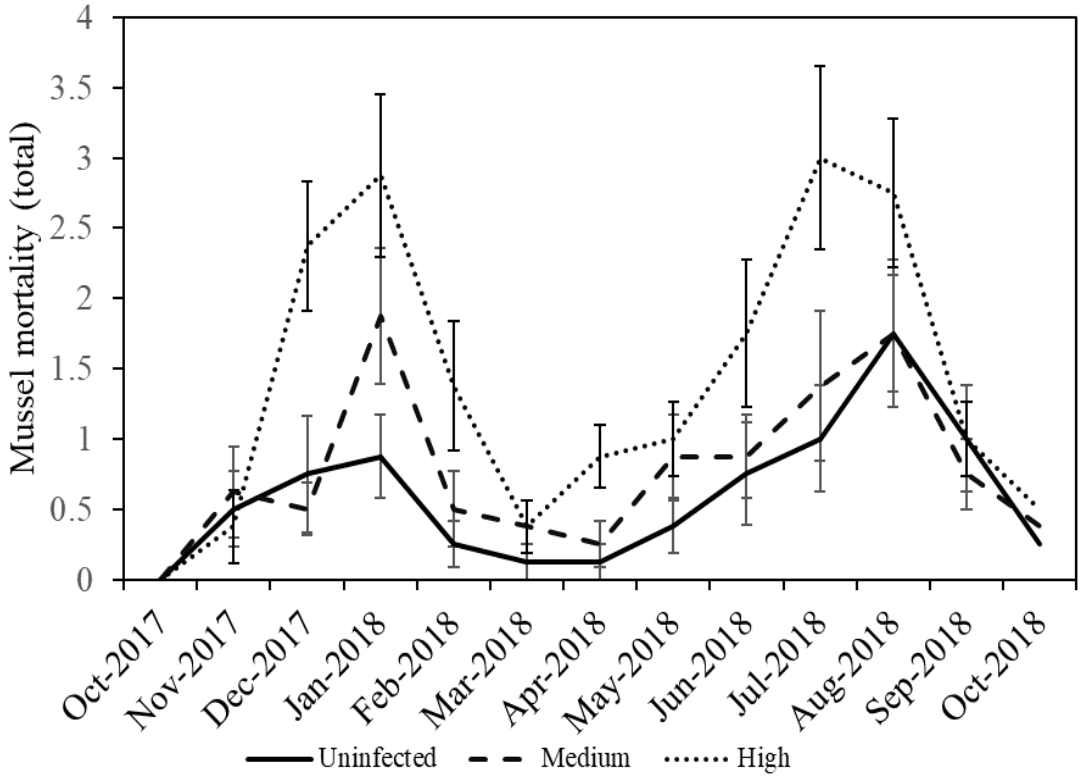

Figure 22: Total number of mussel deaths by treatment in each month of our experimental manipulation of infection intensity (October 2017 – October 2018). Data points are treatment averages (n = 8 replicates per treatment) with standard error bars.

There were distinct seasonal patterns in mussel mortality over the course of the experiment. Large spikes in mortality occurred in January-December, and July-August, corresponding to the coldest and hottest temperatures, respectively, experienced at the site (Figures 22-24). Predation accounted for roughly half of all mussel mortality during the spring and summer months, while mussels who died during the winter showed no signs of predation (Figure 23).

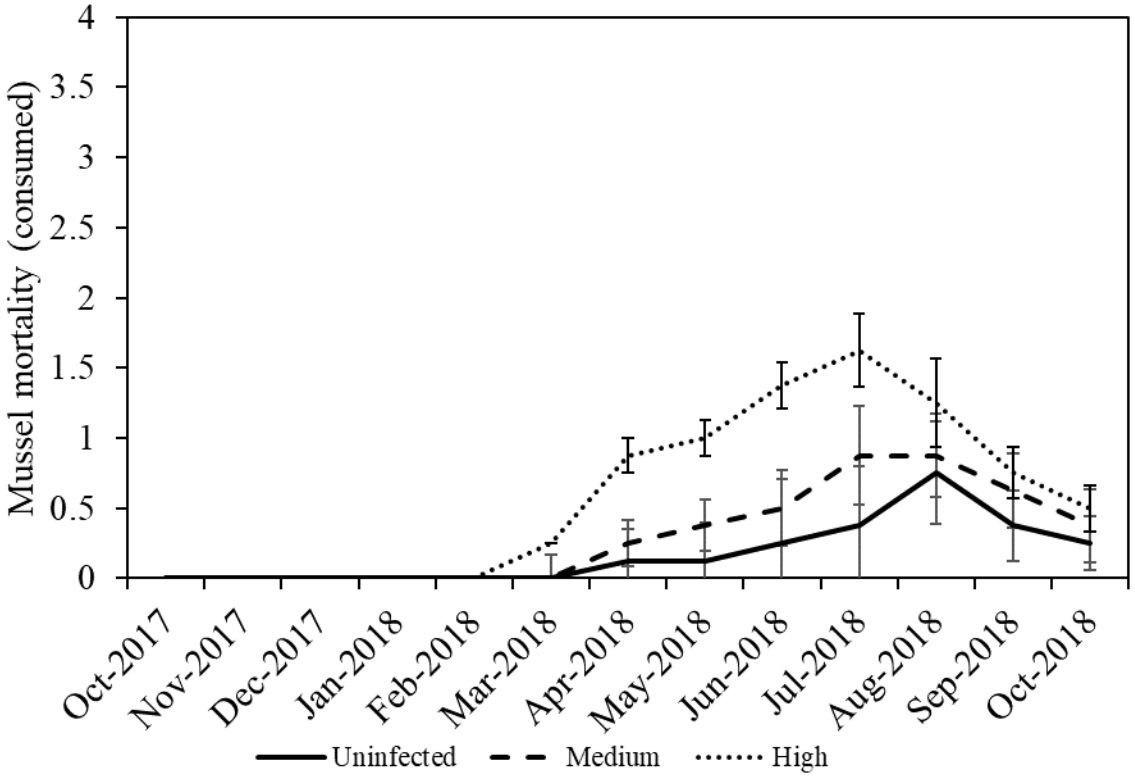

Figure 23: Total number of mussel deaths attributed to predation in each month of our experimental manipulation of infection intensity(October 2017 – October 2018). Data points are treatment averages (n = 8 replicates per treatment) with standard error bars.

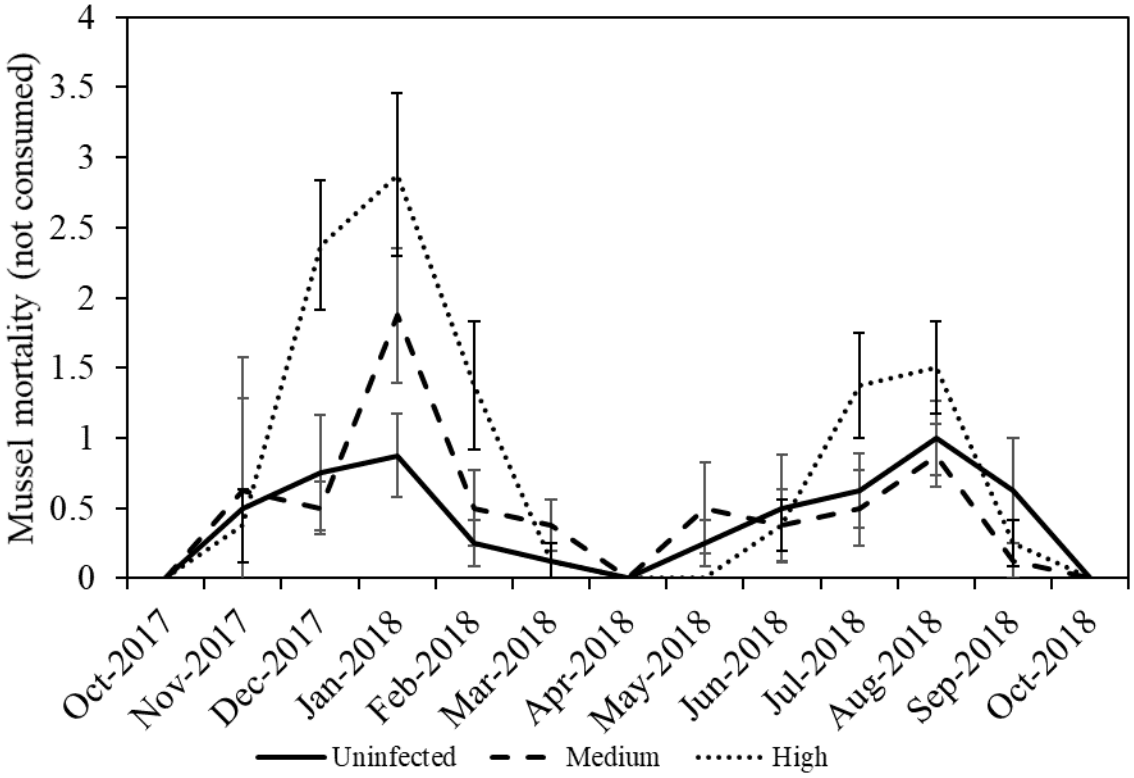

Figure 24: Total number of mussel deaths not attributed to predation in each month of our experimental manipulation of infection intensity (October 2017 – October 2018). Data points are treatment averages (n = 8 replicates per treatment) with standard error bars.

4.5.2.2 Effects on mussel biodeposit production (field assay)

The average infection intensity of mussels from a given plot did not differ significantly within treatments (Figure 25, one-way ANOVA, $p = 0.54$ at least, all treatments). The rate of biodeposit production decreased 42.5% in intermediately infected mussels and 67.3% in highly infected mussels, relative to uninfected conspecifics. Highly infected mussels produced biodeposits at a rate that was only 43% that of intermediately infected mussels (Figure 25, Tukey HSD, $p = 0.0016$). Biodeposit

production rate was strongly negatively correlated with infection intensity (Figure 25, Student's t-test, $p < 0.0001$).

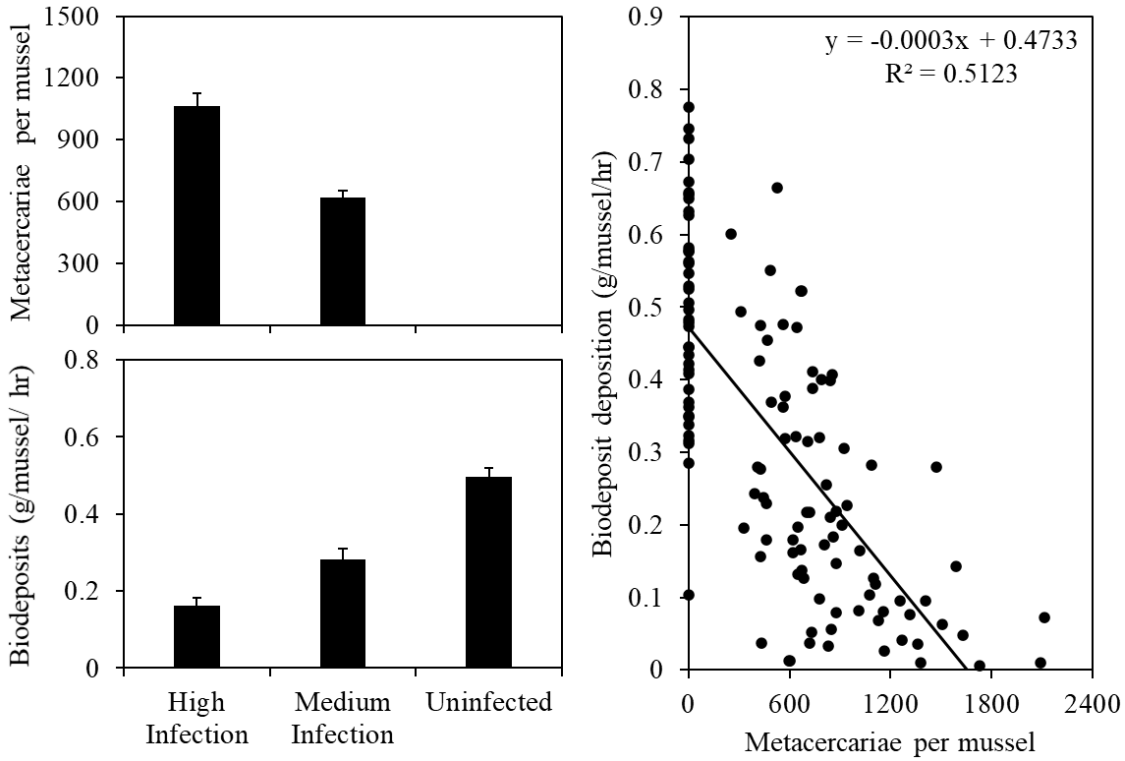

Figure 25: Results from our field mussel biodeposit production assay regarding (A) infection intensity of mussels from each treatment (B) biodeposit production of mussels from each treatment group (n = 40 individuals per treatment) and (C) the relationship between infection intensity and mussel biodeposit production.

4.5.2.3 Effects on mussel filtration and biodeposit production (lab assays)

The infection intensity of mussels used in our filtration assay ranged from 0 to 2020 metacercariae per individual (mean ± SE = 640 ± 92). Mussel filtration rate decreased significantly with increasing infection intensity (Figure 26, Student's t-test, p =

0.00018). Mussel shell length did not significantly impact filtration rate (Figure 18, Student's t-test, p = 0.14).

The infection intensity of mussels used in our biodeposit production assay ranged from 0 to 1745 metacercariae per individual (mean ± SE = 544 ± 86). Biodeposit production decreased significantly with increasing infection intensity (Figure 26, Student's t-test, p < 0.0001).

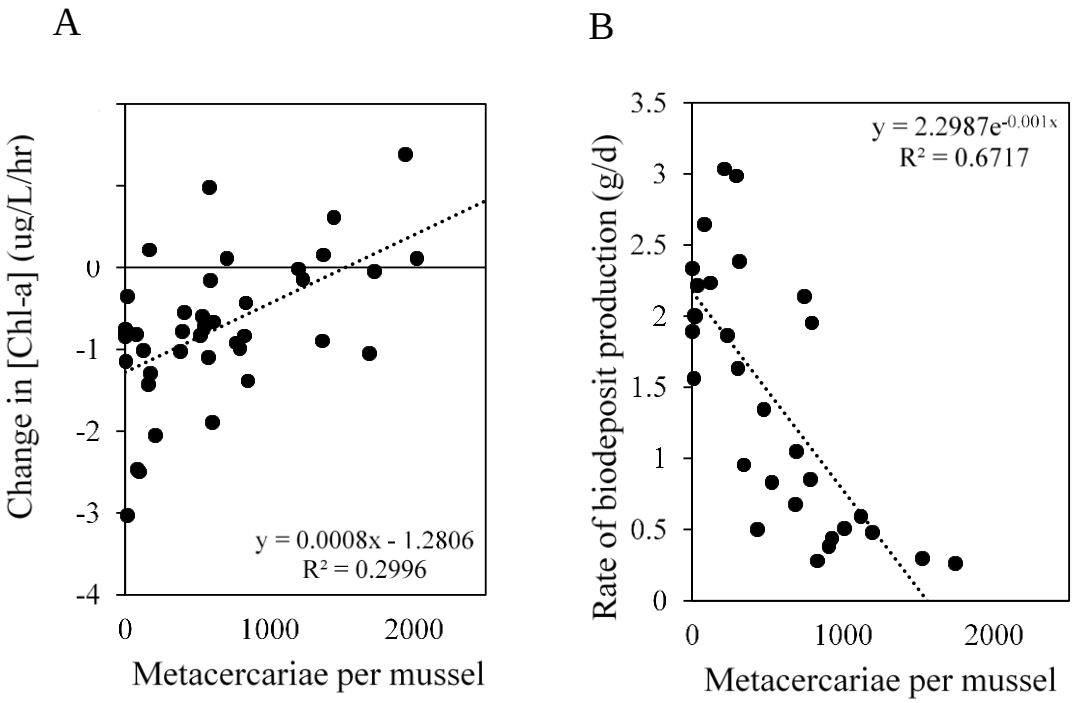

Figure 26: The relationship between (A) filtration rate and (B) biodeposit production rate of *Geukensia demissa* and infection intensity of *Cercaria opaca* from controlled laboratory experiments.

4.5.2.4 Effects on mussel shell thickness and strength

Infection intensity of mussels used in our shell strength assay ranged from 113-1476 metacercariae per individual (mean ± SE = 586 ± 49.79). The force required to yield

structural failure of mussel shells increased significantly with mussel size and decreased with increasing infection intensity (Figure 27, Student's t-test, $p < 0.00014$, both cases).

The average thickness of mussel shells used in our lab biodeposit production assay decreased significantly with increasing parasite intensity (Figure 28. Student's t-test, $p < 0.0001$). There was no detectable effect of mussel size (Student's t-test, $p = 0.83$), likely owing to the relatively uniformity in size of selected mussels (mean length ± SE = 7.87 cm ± 0.06).

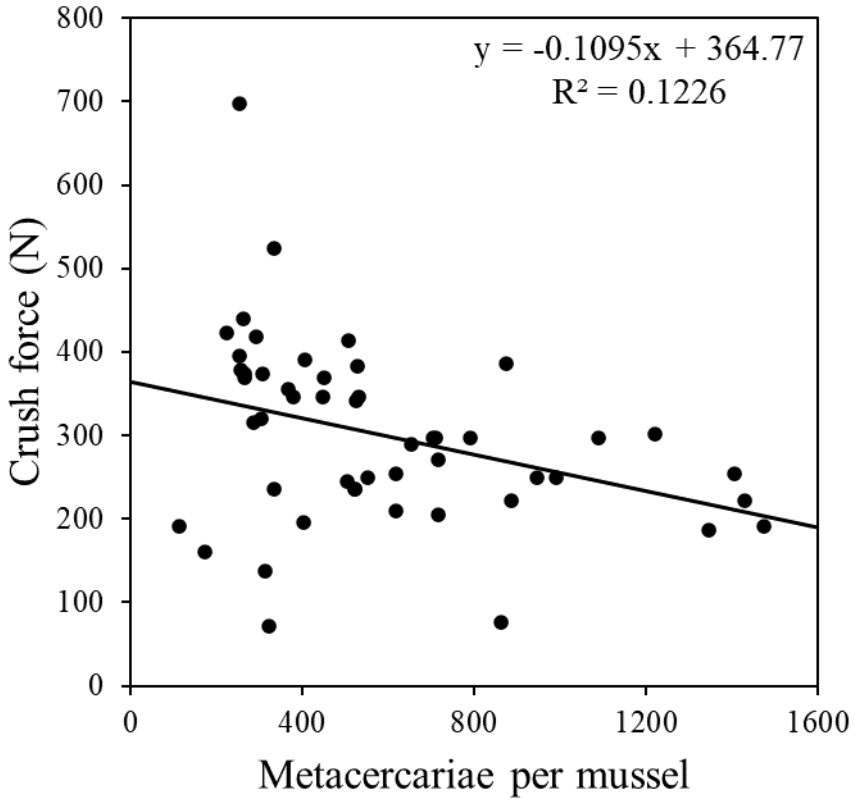

Figure 27: Least squares relationship between mussel infection intensity the amount of force required to break mussel shells.

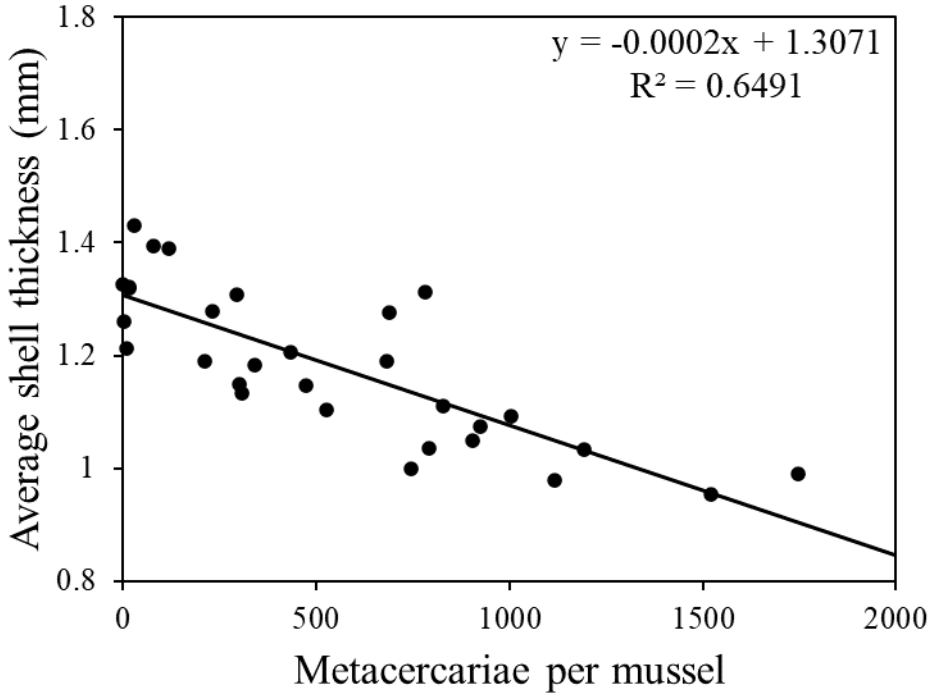

Figure 28: Least squares relationship between *C. opaca* infection intensity and average mussel shell thickness (mm).

4.5.2.5 Effects on byssal formation and attachment strength

Mussel infection intensity ranged from 201-3028 metacercariae per individual (mean ± SE = 1043 ± 82.69). Byssal thread number decreased significantly with increasing infection intensity (Figure 21, Student's t-test, $p = 0.021$) and increased with increasing mussel mass (Figure 29, Student's t-test, $p = 0.00049$). The force required to dislodge mussels from their holdfast was significantly negatively correlated with byssal thread number (Figure 29, Student's t-test, $p < 0.0001$). There was a marginally significant negative relationship between infection intensity and dislodgment force (Figure 29, Student's t-test, $p = 0.057$).

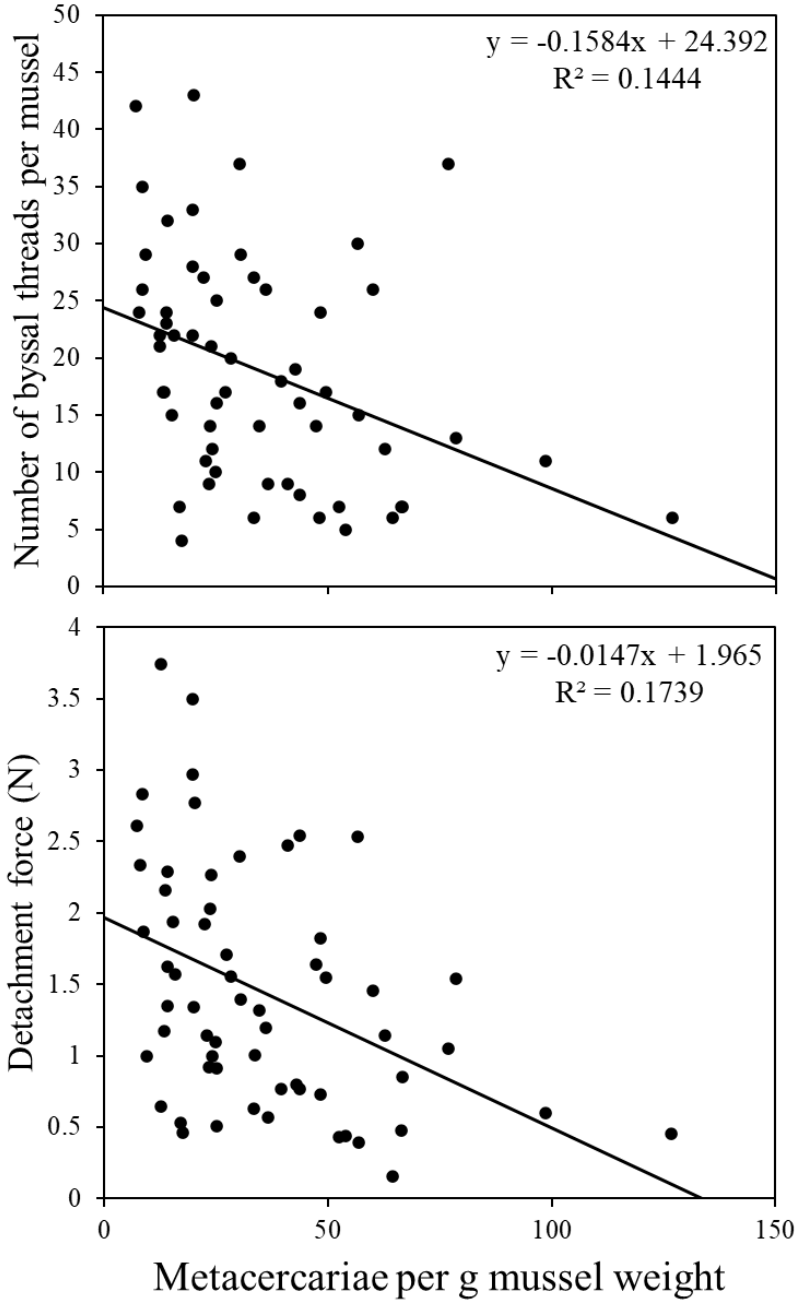

Figure 29: The least squares relationship between mass-standardized mussel infection intensity and (A) byssal thread production and (B) force required to detach holdfasts.

4.5.3 Survey of parasite infection within and outside of marsh die-off areas

Littoraria irrorata infected with *C. opaca* were present at all survey sites, though prevalence varied significantly by site and proximity to die-off areas. At all sites, parasite prevalence in snails was highest within snails on die-off borders and decreased sharply only 25 m away (Type II Wald χ^2 test, $p < 0.0001$). No snails infected with *C. opaca* were found 50 m away from any of the die-offs surveyed. Infection prevalence of *C. opaca* in snails increased significantly with increasing die-off size (Type II Wald χ^2 test, $p < 0.0001$).

Concomitant with the omnipresence of *C. opaca* infections in *Littoraria*, the metacercariae of *C. opaca* were found in mussels at each survey site. Infection intensity was consistently highest in mussels from within die-off interiors and decreased with increasing distance from the die-off edge (Figure 30, Type II Wald χ^2 test, $p < 0.0001$). Infection intensity in mussels increased significantly with increasing die-off size (Figure 30, Type II Wald χ^2 test, $p = 0.0021$).

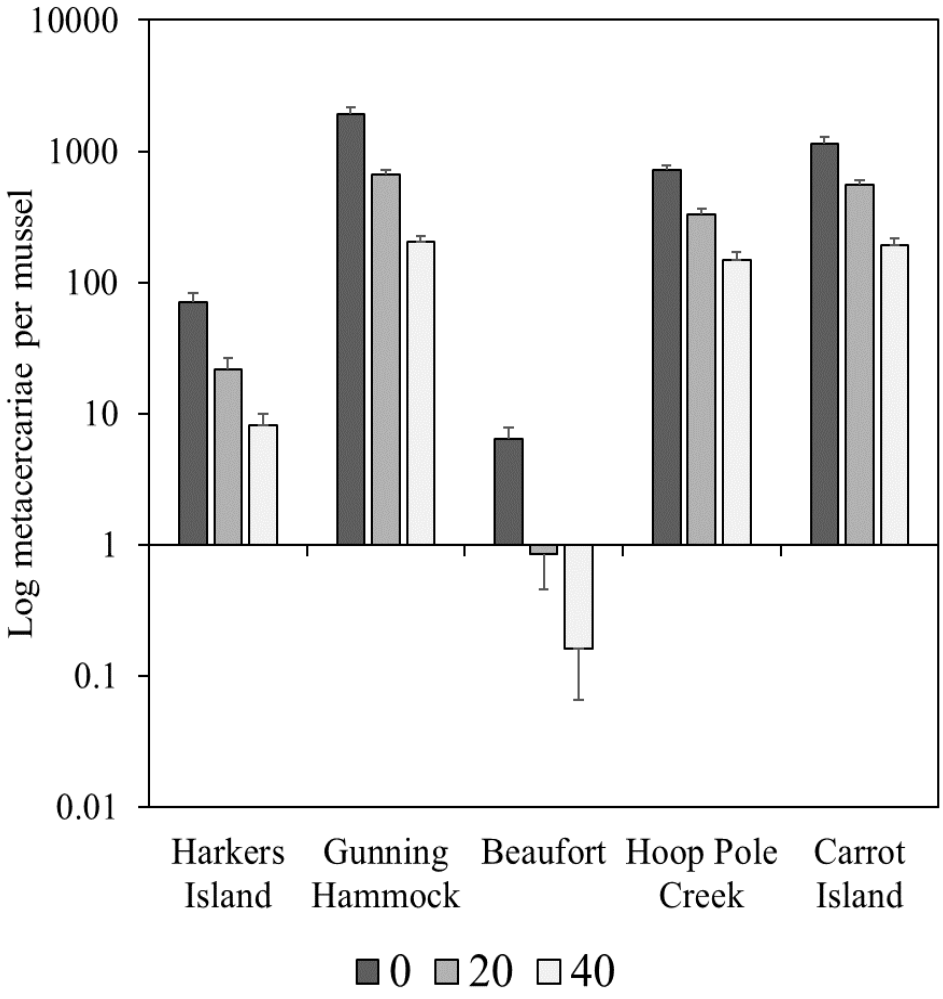

Figure 30: Infection intensity of mussels at 0, 20, and 40 meters distance from marsh die-off areas at five sites. Data points are averages with standard error bars.

4.6 Discussion

Our manipulative field experiment revealed that increasing intensity of *C. opaca* infection in *Guekensia* resulted in deceased facilitation of *Spartina* growth, affecting above- and belowground growth as well as flowering. (Figures 11, 12, 20). At intermediate levels of infection, *C. opaca* reduced mutualistic benefits to *Spartina* aboveground growth, and at high levels of infection, positive effects of mussels on any

metric of *Spartina* growth were eliminated. The primary mechanism by which mussels are known to facilitate *Spartina* on marsh flat habitats is through enhancing sediment nutrient availability through the production of biodeposits (Bertness 1984, Angelini et al. 2015). Both our laboratory filtration and biodeposit production assays using naturally infected mussels and our field biodeposit production assay using experimentally infected mussels demonstrate that increasing infection with *C. opaca* inhibited mussel feeding and biodeposit production. However, rather than being attributable to a singular proximate cause, it appears that the influence of *C. opaca* on the facultative mutualism between *Spartina* and *Geukensia* stems from a combination of effects on both mussel biodeposition and survivorship.

Mussels in the high infection intensity treatment of our field experiment experienced more than twice the level of mortality over the course of the experiment than did uninfected mussels. The loss of mussels in these treatments likely contributed to at least some of the observed differences in various response variables as the facultative effects of mussels on *Spartina* are density-dependent (Bertness 1984, Angelini et al. 2015, 2016). However, the final density of mussels in uninfected and intermediate intensity mounds did not differ significantly from one another, suggesting that the large differences in plant growth, macroinvertebrate abundances, and biogeochemical variables observed between these two treatments are more likely the product of parasite-induced reductions of per-capita biodeposit production.

Increased mortality of highly infected mussels in the field is partially attributable to relatively higher levels of predation on these mussels. Mortality due to predation peaked during the summer when known mussel predators (i.e., crabs, birds, and racoons) were active (Figure 23). Increased predation on infected mussels may be due in part to parasite-induced changes to host antipredator defenses. In laboratory assays, increasing numbers of *C. opaca* metacercariae in the viscera of mussels corresponded to thinner shells that were more easily crushed (Figures 27-28). The energetic costs of trematode infection can be substantial and preferential encystment in the tissues of the digestive gland may interrupt metabolic processes underlying shell growth and repair. Reduced growth rate and condition index of hosts with metacercarial infections has been observed in a number of other bivalve-trematode systems (Calvo-Ugarteburu & Mcquaid 1998, Wegeberg & Jensen 2003, Stier et al. 2015, Bakhmet et al. 2017). Additionally, our lab assays testing the effects of parasite infection intensity on byssal thread attachment strength showed that mussels with higher parasite loads produced fewer byssal threads and were more easily dislodged (Figure 29). Both of these parasite-induced changes are likely to make infected mussels more vulnerable to predation and may be responsible for the higher frequency of predation observed in the field. Such modifications to host defenses might increase the likelihood of successful trophic transmission to avian definitive hosts, although whether these changes to host phenotype constitute adaptive manipulations by *C. opaca* remains to be tested.

The majority of dead mussel shells recovered from our field experiment showed no obvious signs of predation and were often still planted in the sediment upon recovery, indicating that they had not been handled by predators (Figure 24). Ambient mortality increased with infection intensity treatment and was especially pronounced in highly-infected plots. This finding is consistent with a number of other studies that have found increased mortality of bivalves with heavy metacercarial infections (Desclaux et al. 2004, Thieltges 2006, Gam et al. 2009, Montaudouin et al. 2014). While the particular mechanisms by which *C. opaca* increased ambient mussel mortality remains to be tested, there were striking seasonal patterns. Most ambient mortality of infected mussels occurred in January and February when the experimental site experienced sub-zero temperatures. Another spike in mortality not associated with predation occurred in July and August when temperatures peaked on the marsh platform. Increasing parasite load may therefore adversely affect the ability of *Geukensia* to endure temperature extremes. The ability of trematode infections to alter the physiological tolerances of intertidal mollusks, including thermal tolerance, is well-documented (Lauckner 1983, Sousa & Gleason 1989, Wegeberg & Jensen 2003, Seuront et al. 2019, Selbach & Mouritsen 2020). Additionally, heavy infection by *C. opaca* may increase mussel susceptibility to heat stress through its indirect effects on *Spartina* aboveground growth. One of the principle mutualistic benefits conferred to mussels by association with *Spartina* is shade, which reduces thermal stress on mussels (Jost & Helmuth 2007, Angelini et al. 2015). Reduced

mutualistic benefits to *Spartina* growth from infected mussels may result in insufficient shade, leading to increased heat stress and enhanced mortality of mussels. It is also possible that infection with *C. opaca* may have contributed to increased ambient mussel mortality through other means not related to host physiological tolerances. For instance, reduced byssal attachment strength due to heavy infection may have increased mortality by making mussels more vulnerable to being buried and smothered by sediment. Regardless, we demonstrate that increased infection by *C. opaca* is associated with increased mortality of mussels.

Coincident with the negative effects of parasitism on the *Geukensia-Spartina* mutualism, increased infection of mussels by *C. opaca* negatively impacted the abundance of resident marsh macroinvertebrates, as well as important ecosystem functions that are typically enhanced by the presence of mussel mounds (Angelini et al. 2015b). *C. opaca* likely decreases benthic productivity indirectly through its negative effects on mussel nutrient-rich biodeposit production and survivorship. This reduction in the availability of benthic algae and bacteria is likely responsible for the concurrent reduction in the local abundance of fiddler crabs. Additionally, because of the strong association between fiddler crab abundance and habitat structure provided by *Spartina* (Teal 1958, Powers & Cole 1976, Ringold 1979, Bertness & Miller 1984, Angelini et al. 2015), *C. opaca* may have negatively impacted the abundance of these organisms through its negative indirect effects on *Spartina* aboveground growth. Similarly, *C. opaca* most

likely reduced the recruitment of juvenile *Littoraria* through its indirect effects on *Spartina* aboveground growth which altered the abundance of suitable microhabitats (senescent leaf furls) to which juvenile snails recruit.

In our field experiment, the addition of uninfected mussels significantly increased subsurface sediment oxygenation at a depth of 10 cm, while increasing infection with *C. opaca* was associated with diminished sediment oxygenation at this depth (Figure 19). Previous studies showed that mussels can aerate sediments by increasing infiltration of oxygen-rich surface water by excreting pseudofeces (Angelini et al. 2015a, Derksen-hooijberg et al. 2018). Increased aeration of sediments can increase *Spartina* growth by increasing oxidization of phytotoxic hydrogen sulfide to less harmful species (Koch & Mendelssohn 1989, Howes & Teal 1994, Lee 1999, Derksen-hooijberg et al. 2018). *C. opaca* may negatively impact sediment oxygenation by decreasing mussel feeding or by decreasing mussel abundance. Additionally, *C. opaca* may negatively impact sediment redox through its indirect effects on the abundance of fiddler crabs which aerate sediments through burrowing (Bertness 1985, Holdredge et al. 2010, Gittman & Keller 2013). *Spartina* itself is adapted to the anoxic sedimentary environment of salt marshes, able to shunt oxygen from the atmosphere to its roots, thereby reducing sulfide toxicity (Teal & Kanwisher 1966, Koch & Mendelssohn 1989, Lee 1999). By decreasing mutualistic benefits to *Spartina* growth, *C. opaca* may also decrease the ability

of *Spartina* to transport oxygen from the atmosphere to its root tissues (Teal & Kanwisher 1966, Hwang & Morris 1991, Arenovski & Howes 1992, Howes & Teal 1994).

The results of our survey of five marshes are consistent with the findings of previous studies that parasite prevalence in snail first intermediate hosts is locally elevated around marsh die-off areas, due to increased usage of these disturbed areas by birds, which act as definitive hosts for *C. opaca* and other trematodes that use *Littoraria* as first intermediate hosts (Morton & Silliman 2020, Morton & Silliman, in prep). The high observed prevalence of *C. opaca* infections in snails within these areas was associated with heightened infection intensity in mussel second intermediate hosts. Furthermore, the prevalence of *C. opaca* infection in snails and average parasite load in mussels increased with increasing die-off size, likely reflecting the known positive relationship between die-off size and bird usage (Morton & Silliman 2020, Morton et al. *in prep*). Additionally, since the post-disturbance recovery time of die-off areas is largely dependent on the size of the die-off area, larger die-offs that persist for longer may provide more time for mussels to accumulate infections (Angelini et al. 2016). Importantly, we found that the cercariae produced by infected snails along die-off borders appeared to find their way into downstream hosts located a considerable distance away. Mussels that were as far as 40 meters away from the borders of larger die-off areas still harbored considerable parasite loads. The findings of these surveys, coupled with the results of our field manipulation, indicate that the infection dynamics

generated as the result of vegetative die-off can have an ecological reach that extends well beyond the disturbed areas themselves. Therefore, even in the surrounding healthy marsh, parasite-mediated effects on the *Geukensia-Spartina* mutualism can have critical impacts on marsh functioning and ecosystem services.

While we found *C. opaca* to be a common member of the parasite component community within *Littoraria* and *Geukensia* associated with die-off areas, there is still very little known about this renicolid, including the identity of the bird(s) it uses as a definitive host. Previous surveys of Southeastern and Gulf Coast marshes suggest that *C. opaca* is widely distributed (Holliman 1961, Epstein 1972, Wardle 1974) and its impacts on the mutualism are unlikely to be limited to coastal North Carolina. Determining where *C. opaca* is most common and where it is likely to have the greatest impacts on marsh ecosystem structure and functioning requires clarification of its lifecycle, as well as more extensive latitudinal surveys examining its distribution. Although *C. opaca* was the dominant parasite infecting *Geukensia* in our surveys, it is unlikely to be the only parasite that may have deleterious effects on this important mutualism The echinostome trematode, *Himasthla quissitensis*, uses a suite of bivalves as a second intermediate host, including *Guekensia* (Cheng et al. 1966, Curtis 2007, Rossiter & Sukhdeo 2012). Likewise, an undescribed gymnophallid trematode uses *Guekensia* as a first and second intermediate host (Richard Heard, pers. comm.). While gymnophallids are known to have deleterious effects on other bivalve hosts at sufficiently high intensities (Lauckner

1983, Cremonte 2003, Thieltges 2006), gymnophallid metacercariae were very rarely found on the gills of mussels from our surveys of marsh die-off areas. Parasitic copepods were occasionally discovered in the viscera of mussels in small numbers (ranging from 1-6 individuals per mussel). These parasites are known to alter the condition and feeding behavior of other bivalve hosts in areas where they are common (Bayne et al. 1977, Gee & Davey 1986, Goedknegt et al. 2018). Whether or not these parasites affect *Geukensia* in ecologically meaningful ways is not yet known.

Our findings illustrate the complexity of understanding the impacts of parasites on ecosystems. Previous studies in this system found that trematodes, including *C. opaca*, can increase marsh ecosystem resistance to cascading vegetation loss by suppressing runaway grazing in *Littoraria* first intermediate hosts (Morton 2018, Morton and Silliman 2020, Morton et al. *In prep*). However, these same trematodes may ultimately yield *negative* impacts for ecosystem resilience by affecting their mussel second intermediate hosts. The facultative mutualism between *Geukensia demissa* and *Spartina alterniflora* enhances salt marsh ecosystem resistance to and recovery from drought (Angelini et al. 2016). Decreased evaporation and increased water storage within mussel mounds allows for increased survival of mound-associated patches during drought-induced die-off of foundational grasses. Surviving patches of *Spartina* associated with mussel aggregations then act as the nuclei from which drought-impacted marshes can regrow (Angelini et al. 2016). By altering mussel biodeposit

production and increasing their susceptibility to predation and environmental stress, *C. opaca* may diminish or negate the positive species interactions that support resistance to and recovery from disturbance in this ecosystem. We anticipate that there is some degree of lag time between an initial die-off event and the point at which mussels within relict patches within the marsh die-off acquire sufficient infections to yield detectable impacts on mussel behavior and survivorship. Mussels within larger die-offs may be especially vulnerable to infection, because these areas persist for longer and attract more avian final hosts that act as the dispersive vectors of *C. opaca* (Morton 2018, Morton and Silliman 2020, Morton et al. *In prep*). Our findings emphasize that examining the *net* effects of multi-host parasites on ecosystem stability requires investigating the impacts of such parasites on multiple hosts.

While we found that *C. opaca* had indirect negative effects on the strength of mutualistic interactions by altering the behavior of mussels, the impacts of behavior-altering parasites on mutualisms may not always be negative. Indeed, while the bulk of existing literature focuses on the negative impacts of parasites on host functional traits, parasites may enhance host performance if doing so increases the likelihood that they complete their lifecycle (Daniels 1985, Burns et al. 2005, Ziuganov 2005, Me´doc & Beisel 2008, Devevey et al. 2010, McElroy et al. 2015). If parasites alter the phenotypes of mutualistic partners in ways that lower mutualistic costs or increase mutualistic benefits, they may enhance rather than diminish the strength of mutualistic interactions.

For instance, the growth and persistence of many marine foundation species such as seagrasses and corals are increased by herbivorous mutualists that consume algal ephipytes (Stachowicz & Hay 1999, Reynolds et al. 2014). If such herbivores harbor parasites that increase host energetic demand, they may increase grazing rates, enhancing the facultative impacts of the host (Gérard & Théron 1997, Bernot & Lamberti 2008).

4.7 Conclusions

Considering the ubiquity of both positive species interactions and parasitism in ecosystems, it is likely that parasites commonly influence the strength of positive species interactions through indirect effects that alter the costs and benefits of the mutualistic interactions. If parasites alter the behavior or density of one or more mutualistic partners, they may impact the strength of mutualistic interactions that underpin processes related to ecosystem structure, functioning, and stability. Our study represents the first direct manipulation of parasitism in mutualistic hosts in the field. We were therefore able to experimentally demonstrate that increased trematode parasitism significantly impacts a keystone mutualism, with numerous effects on community structure and functioning. Studies in which parasites are experimentally added or removed should be employed when possible, to disentangle the role of parasites in mediating mutualistic interactions and their effect on ecological communities.

5. Conclusions

When considering the potential roles played by parasites in food webs, it has often been a useful heuristic to compare and contrast them with predators. Like predators, parasites can influence ecosystems by affecting competition, and exerting top-down control through trait- and density-mediated means. Predators can also increase ecosystem resistance and resilience to disturbances (Sala 2006, Wilmers et al. 2006b, Morissette et al. 2010). If parasites regulate top predators or mutualists that buffer stress and mediate ecosystem responses to perturbations, they might decrease ecosystem resistance and resilience. However, parasites might buffer disturbance when top predators are missing or by regulating free-living consumer populations. For instance, many organisms that form 'consumer fronts' in response to anthropogenic stressors and generate widespread, cascading loss to ecosystems (Silliman et al. 2013) are known to harbor parasites that can alter their behavior or survivorship and might mitigate their impacts (Wood et al. 2007, Clausen et al. 2008, Hoover 2011, Shi et al. 2014).

Despite the ubiquity of parasitism, there is strikingly little known about how parasites modify the phenotypes of many ecologically influential species. For instance, nearly all digenean trematodes infect a molluscan first intermediate host as part of their complex lifecycle, often resulting in profound changes to host physiology, including castration. While mollusk-trematode associations are common features of many aquatic ecosystems, little is known about the effects of trematode infection on

many molluscan hosts' behaviors, even for ecologically influential host species. However, given the few strong demonstrations that currently exist and the ubiquity of parasitic castrators in many consumer species, trait-mediated trophic cascades induced by these organisms may be common. Our results suggest that a more robust understanding of how ecosystems are organized and respond to disturbance will necessitate identifying and quantifying the effects of parasitism on host functional traits.

The work presented here also underscores the importance of using manipulative experiments to tease out the ecosystem-level consequences of parasitism. Manipulative in situ experiments with parasites can be technically challenging, which has contributed to the relative lack of examples of parasites' trait-mediated influences on ecosystems. While manipulating the prevalence or intensity of parasitism may not be possible in all systems, such experiments can be powerful tools for understanding the roles of parasites in food webs. If feasible, parasites can be excluded through the treatment with pharmaceuticals (e.g. antihelmithic drugs) or by manipulating host contact with disease vectors or dispersive agents (Hudson et al. 1998, Marvier 1998, Pedersen 2008). Alternatively, experimentally or naturally infected organisms can be added to a system (Wood et al. 2007).

While most free-living species act as hosts for numerous parasite species, most work has focused on single host/single parasite interactions to inform our current understanding of the effects of parasites on ecosystems. Indeed, much of the research

presented in this dissertation focuses on the impacts of single parasite species on salt marsh ecosystems. However, studies on the ecosystem-level effects of predators have revealed that different predator species exert differential effects on prey, and that the combined top-down effects of predators can be difficult to predict from their independent effects. Thus, understanding how parasites shape ecosystems will require examining the roles played by multiple parasites individually and simultaneously.

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