

Parasites enhance resistance to drought in a coastal ecosystem

JOSEPH P. MORTON¹ AND BRIAN R. SILLIMAN

Duke University Marine Lab, 135 Duke Marine Lab Road, Beaufort, North Carolina 28516 USA

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Abstract. Parasites are more diverse and numerous than their hosts and commonly control population dynamics. Whether parasites also regulate key ecosystem processes, such as resistance to climate stress, is unclear. In southern U.S. salt marshes, drought interacts synergistically with keystone grazing to generate extensive ecosystem die-off. Field manipulations of parasite prevalence and salt stress in sediments in healthy marshes demonstrated that trematode parasites, by suppressing feeding activity of grazers that overgraze on drought-stressed plants, have the potential to slow the rate of ecosystem loss. Surveys along 1,000 km of coastline during an intense drought event revealed parasitism is common in grazers on die-off borders and that increasing infection prevalence along marsh die-off borders is negatively correlated with per capita grazing. Combined, results from this field experiment and survey suggested, but did not show, that parasites could affect rates of drought-driven salt marsh die-off. To test whether parasites can indeed protect marshes under real drought conditions, we experimentally manipulated parasite prevalence in grazers over a month-long period on active die-off borders in three North Carolina marshes. Experimentally reducing parasite prevalence markedly increased the rate of plant ecosystem decline, an effect that scaled positively with prevalence. Thus parasites, by generating a trophic cascade, indirectly enhanced ecosystem resistance to overgrazing under intense drought in these North Carolina marshes. The generality of these results across the entire range of this keystone grazer in the southeastern United States needs to be tested, employing both experiments and extensive surveys that examine how the rate of ecosystem decline is mediated by parasitism. Given the ubiquity of parasites in ecosystems, our results suggest that more research effort should be invested in examining the possible roles for parasitism in regulating ecosystem function and stability.

Key words: behavior modification; drought; ecosystem resistance; grazing; *Littoraria irrorata*; parasites; *Parorchis acanthus*; salt marsh; trematode.

INTRODUCTION

Given their role in human and animal health, parasites have long been the focus of biological research, but only in recent decades have ecologists begun to thoroughly investigate the role of parasites in ecosystems. Beyond their individual-level effects, a significant body of research has demonstrated the ability of parasites to powerfully control host populations, both directly by reducing host densities or reproductive capacity or indirectly via modification of host phenotype (Lafferty 1993, Behrens and Lafferty 2004, Fredensborg et al. 2005, Hudson et al. 2007). More recently, ecologists have demonstrated the ability of parasites to control community structure in natural habitats (Mouritsen and Poulin 2005, Wood et al. 2007, Bernot and Lamberti 2008, Mouritsen and Haun 2008, Holdo et al. 2009,

Sato et al. 2012, Swope and Satterthwaite 2012, Callaway 2016, Sánchez et al. 2016). If a host species is ecologically influential and parasite prevalence is sufficiently high, parasite regulation of host density or behavior can have large-scale, cascading effects (Wood and Johnson 2015). For instance a study by Holdo et al. (2009) found that the rinderpest virus exerted strong top-down control over the East African Serengeti ecosystem through its regulation of influential grazer densities. Following the eradication of this microparasite in the 1960s, wildebeest populations underwent a dramatic irruption that resulted in a reduction in wildfires and subsequent increases in tree density (Holdo et al. 2009). Because hosts that are keystone grazers can not only modify the density of foundational plant species, but also their ability to resist and recover from disturbances (Silliman et al. 2005, 2013, He et al. 2017) parasites, like predators, may be able to exert strong control over the ability to resist and/or recover from large-scale stressors.

In southern U.S. salt marshes, intense and recurring drought interacts synergistically with grazing by the keystone grazing snail, *Littoraria irrorata*, to produce small-

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¹E-mail: joseph.morton@duke.edu

to large-scale marsh plant die-off events totaling well over 250,000 acres (McKee et al. 2004, Silliman et al. 2005, Angelini and Silliman 2012). During drought events, high- and intermediate-elevation areas of the marsh platform can become hypersaline, weakening defenses of the foundational marsh cordgrass, *Spartina alterniflora* (McKee et al. 2004, Angelini and Silliman 2012, Angelini et al. 2016). Weakened plant defenses due to sublethal edaphic stress, can lead to intensified grazing by *Littoraria*, which may then form high-density consumer fronts that yield cascading loss of foundational plants (Silliman et al. 2005). Die-off, triggered by regular, episodic drought events, can induce major shifts in the structure, function, and services provided by this valuable coastal ecosystem (Hensel and Silliman 2013, Silliman et al. 2013). Identifying indirect, positive species interactions that can enhance plant ecosystem resistance to climate stress will be critical for its long term survival and service provisioning.

While it is well documented that predators can facilitate marsh plants by controlling snail numbers (Silliman and Bertness 2002, Silliman et al. 2004, Griffin and Silliman 2011), no attention has yet been given to the similar, functional role animal parasites could play in modulating plant-grazer interactions. In these wetlands, the keystone grazer, *L. irrorata*, serves as the first intermediate host to at least four species of digenean trematodes. One common species, the philophthalmid *Parorchis acanthus*, yields marked changes to snail behavior, dramatically reducing snail feeding on live *Spartina* tissue (Morton 2018). Laboratory observations also suggest that infected snails may have a diminished propensity for circatidal climbing up *Spartina* stems, an effect that could reduce their interaction time with plants. Moreover, infection prevalence of this and other trematodes is high enough in some marsh areas (>30%), that parasites could have a positive localized effect on salt marsh community structure (Morton 2018).

Based on our observations in both lab and field, we hypothesized that increasing infection prevalence of *Parorchis* would result in cascading ecosystem effects, decreasing the impacts of snail grazing on salt marsh plant structure and increasing marsh ecosystem resistance to drought-driven die-off. To test for the potential of this tri-trophic cascade hypothesis, we employed a fully-factorial field experiment in a healthy marsh in which we modified soil salinity as a proxy for drought conditions and parasite infection prevalence and monitored impacts on marsh plant growth. We then surveyed multiple die-off areas across the east coast to see if parasites were common in die-off areas and if their presence was negatively correlated with per capita grazing by snails on die-off borders. Finally, to test if parasites could affect ecosystem resistance to drought-associated overgrazing, we experimentally manipulated parasite prevalence in grazers on actively expanding die-off borders during a drought event.

METHODS

Experiment I: field manipulation of parasite prevalence and drought stress

To test the hypothesis that widespread trematode infection can diminish the capacity of snails to overgraze marsh under simulated sublethal drought conditions, we established 0.7-m² caged plots in an area of ungrazed, healthy, intermediate-form *Spartina* marsh within the Hoop Pole Creek Clean Water Reserve in Atlantic Beach, North Carolina, USA (34.7082° N, 76.75° W) in May 2014. We chose this site because it was nearly identical in elevation, plant composition, and initial subsurface soil salinity (35 ppt) to nearby areas but had very low adult snail densities (>1 snail/m²), likely due to spatial differences in predation (J. P. Morton, *personal observation*). While a combination of drought-associated edaphic factors can contribute to stressed plants (soil drying, increased soil acidity, metal toxicity), we chose to simulate drought by elevating subsurface salinity because this a common manifestation of drought stress in marshes and has been experimentally shown to stimulate top-down control by snails (Silliman et al. 2005). Within caged plots we experimentally elevated marsh soil salinity to sublethal levels consistent with drought-stressed soils (45–55 ppt) by adding ocean salt and manipulated the prevalence of trematode infections in cage-enclosed snails (Silliman et al. 2005; see Appendix S1).

Infection status of field-collected *Littoraria* used in the experiment was determined via a proven cercariae shedding method that produced no false negatives (Morton 2018). Plots were assigned to one of the following treatments ($n = 8$ replicates): (1) salt addition and 30% infected, (2) salt addition and 10% infected, (3) salt addition and 0% infected, (4) no salt and 30% infected, (5) no salt and 10% infected, (6) no salt and 0% infected, (7) caged with no snails, (8) caged with salt added and no snails, and (9) uncaged with no snails. Caged treatments without snails tested for the effect of soil salt levels on marsh structure alone and uncaged plots acted as cage controls. The 10% infection prevalence treatment represented the mid-summer average from snails along marsh die-off borders within the Hoop Pole Creek study area, whereas the 30% treatment represented a naturally occurring high prevalence from the same site (Morton 2018). The 0% prevalence treatment constituted a removal of parasites from the marsh food web. We added 100 snails with shell length (mean \pm SE) 18.71 ± 0.45 mm and wet mass of 1.62 ± 0.11 g each to each grazer addition plot. The grazer density used was representative of naturally occurring densities around die-off areas of Hoop Pole Creek determined in May 2014 by haphazardly tossing a 0.7-m² quadrat 20 times in these areas and counting the snails therein.

Salt was delivered monthly to sediments in appropriate treatments for the duration of the experiment (May–

August) by inserting 16 50-mL plastic centrifuge tubes with 16 holes (hole diameter 0.16 cm) drilled in the sides into the sediments within all plots. Each tube held 150 g of salt (Silliman et al. 2005). We established a lysimeter in the center of each plot so we could periodically monitor pore water salinity of the *Spartina* rooting zone (Angelini and Silliman 2012). Pore water salinity samples taken from lysimeters were measured with a hand-held refractometer.

We marked each snail with a distinguishing dot (white for healthy, red for infected) using water-resistant paint pens, whose mark persists for long periods on the shells of aquatic gastropods without any discernable effect on behavior or longevity (Henry and Jarne 2007). Infected and uninfected snails within each plot were enumerated twice weekly and replaced when necessary to maintain constant density and assigned infection prevalence for the duration of the experiment. During weekly monitoring, any predatory mud crabs found within plots were removed and their burrows plugged with marsh sediment to discourage re-occupation.

Changes to salt marsh structure

We took measurements of several marsh characteristics in all plots initially and in each month following installation for the duration of the experiment. Initial plot biomass estimates were calculated from plant height measurements using a regression of stem height vs. stem biomass determined from stems taken from around the experimental area. 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 (Silliman et al. 2004), we enumerated the total length of radulations on the topmost unfurled leaf of five randomly selected stems within each plot. Additionally, we determined stem densities and measured the heights of 10 randomly selected stems in each plot. Random selection of stems was accomplished by tossing a plastic dowel into plots and then taking measurements on all stems touching the dowel. If the number of adjoining stems was insufficient, measurements were taken on stems closest to the dowel. At the end of the experimental period, all aboveground biomass was harvested and dried at 60°C for 2 weeks.

Snail climbing

Because trematode infection may affect snail circatidal climbing and such changes could affect grazing, we assessed differences in snail movement and position relative to the substrate at low and high tide. Within the first 3 weeks of the experiments' installation, we counted infected and uninfected snails in each plot above the water line at high tide. At low tide, the five infected and five uninfected snails closest to the center lysimeter were selected, their height above the marsh substrate was measured, and their position (on stems or the marsh substrate) was recorded.

Survey of parasite prevalence in die-off areas and healthy marsh

To determine if parasites commonly occurred in grazers on active die-off borders and whether parasite prevalence on die-off borders correlated with per capita grazing intensity, we surveyed nine Mid- and South Atlantic marshes in July 2016 when much of southeast was affected by moderate to severe drought (NOAA-NCEI 2016). Survey sites included Gaming Island, North Carolina (NC; 34.672689° N, 76.500426° W), Hoop Pole Creek, NC (34.705798° N, 76.75° W), Pine Knoll Shores, NC (34.6989° N, 76.8336° W), Harkers Island, NC (34.6914° N, 76.5265° W), Carrot Island, NC (34.705° N, 76.6214° W), Charleston, South Carolina (SC; 32.78° N, 79.965° W), Port Royal, SC (32.394° N, 80.771° W), Sapelo Island, Georgia (31.415° N, 81.2939° W), and Amelia Island, Florida (30.6294° N, 81.4752° W). At each site, eight 0.5-m² quadrats were haphazardly thrown both along the die-off border and in adjacent healthy intermediate *Spartina* 5 m from the die-off border. Within each quadrat, all visible snails were collected, transported back to the lab, measured, and dissected to determine infection status. Additionally, radulation and stem height data were collected in each quadrat as for the cages in the previous experiment.

Experiment II: parasite prevalence effects on die-off border movement

To experimentally test the hypothesis that trematode infection slows the rate of marsh loss after a die-off has been initiated and thereby increase ecosystem resistance to drought-driven die-off, we established 1.0-m² caged plots along actively expanding die-off borders at three North Carolina marshes in which we manipulated trematode infection prevalence in the same manner described for the previous field experiment. In July 2016, caged plots were installed within Hoop Pole Creek (34.70529° N, 76.74965° W), Pine Knoll Shores (34.6994° N, 76.83324° W), and Beaufort (34.72323° N, 76.6558° W). All sites contained large (~800–3,500-m²) die-off areas, and abundant snails. Caged plots were installed such that the die-off border edge ran along the plot's middle, with one-half of the plot consisting of denuded mudflat and the other live *Spartina*. We removed all snails from plots and estimated initial plant biomass using the method described previously. We placed two PVC flags to mark the initial die-off border position within each plot. Plots were assigned to one of the following treatments ($n = 6$ replicates): (1) high infection prevalence (30% infected), (2) control infection prevalence (10% infected), and (3) full parasite removal (0% infected). Snails were stocked and maintained at densities representing the naturally occurring mean at die-off sites in this area (100 snails/m²). Snails had shell lengths (mean \pm SE) 18.68 ± 0.4 mm and wet masses of 1.61 ± 0.12 g and were marked using methods described previously. Infected and uninfected snails within each plot were enumerated weekly and replaced when necessary to maintain constant snail

density and assigned prevalence. Any intrusive mud crabs discovered during monitoring were removed, though crabs were rare at all sites (<1 crab removed per site per month).

After a month, we evaluated die-off border movement in each plot. Border movement was determined by stretching taut a length of string between the flags that marked the initial die-off border in each plot and measuring the distance of the five *Spartina* stems closest to the string. Total border movement was calculated as the difference in average distance of these stems from the centerline. Because initial standing biomass can be a strong determinant of the rate of retreat, average rates of border retreat were normalized using estimates of initial biomass ($\frac{m}{\text{month}} \cdot \frac{g}{m^2} = \frac{g}{\text{month} \cdot m}$). All snails were collected from their respective plots and dissected to ensure that prevalence treatment remained constant. At all sites, final infection prevalence within all plots remained constant over the course of the experiment.

Statistical analysis

All data were analyzed using R (R Core Team 2014). Treatment differences in response variables measured at the end of the drought \times parasite field experiment were assessed using two-way ANOVA followed by Tukey's honestly significant difference (HSD) test for post hoc analysis. Differences in heights climbed by infected and uninfected snails within these plots at low tide and differences in the proportion of infected and uninfected snails above the mean high water line at high tide were evaluated with a one-way ANOVA.

To evaluate treatment differences in biomass-normalized border movement at the end of our experimental manipulation of parasite prevalence on active die-off borders, we used the lmerTest package in R (Kuznetsova et al. 2017) to perform a linear mixed effects analysis with the main effect being treatment group, blocked by marsh site. Tukey's HSD tests, calculated with the glht function from the multcomp library, were used for post hoc analysis (Hothorn et al. 2008). Data analyzed in this way exhibited both homogeneity of variance (confirmed through Bartlett's test) and were normally distributed (confirmed through Shapiro-Wilk test).

For regional survey data, differences in parasite prevalence in die-off border and healthy marsh areas were determined with a nested one-way ANOVA. Linear modeling was used to determine the relationship between radulation scar length, snail density, snail size, and parasite prevalence in die-off border survey plots.

RESULTS

Experiment I: field manipulation of parasite prevalence and drought stress

Initial plot conditions, infection prevalence, and snail densities.—Initial plot vegetation conditions (mean height, stem density, and stem biomass) were not

significantly different among treatments ($P > 0.35$ at least, two-way ANOVA, for each response variable). There were no significant differences in *Spartina*, NPP, stem density, or stem height means between caged controls and uncaged plots. Additionally, there were no significant differences in means of potentially confounding factors (i.e., fiddler crab abundance, elevation, pore water salinity) among treatments ($P > 0.6$, two-way ANOVA, for all response variables). The final mean infection prevalence for each treatment did not differ significantly from initial assignments ($F_{5,84} = 0.8494$, $P > 0.99$, two-way ANOVA, all cases). The mean weekly deviation in snail density in snail addition treatments never exceeded 10%.

Grazing intensity.—Elevated soil salinity acted synergistically with grazers, significantly increasing grazing effects (radulation scar length) in plots where salt was added (Fig. 1A). Mean radulation length decreased significantly with increasing parasite prevalence ($F_{2,42} = 60.14$, $P < 0.001$, two-way ANOVA). In salt-addition plots, parasites reduced average length of grazing scars by 38% at a 10% infection prevalence and 69% at a 30% infection prevalence compared to plots with only uninfected snails. In unsalted plots, the average length of grazing scars decreased by 33% at a 10% infection prevalence and 75% at a 30% infection prevalence. There was a significant interaction between salt addition and parasite prevalence ($F_{2,42} = 4.6$, $P = 0.037$, two-way ANOVA).

Changes to salt marsh structural characteristics.—Salt addition significantly decreased mean aboveground biomass (Fig. 1B, $F_{1,42} = 172.02$, $P < 0.0001$, two-way ANOVA), live stem density (Appendix S3: Fig. S1, $P < 0.03$, two-way ANOVA), and stem height (Appendix S3: Fig. S2, $F_{1,42} = 40.06$, $P < 0.0001$, two-way ANOVA). There was no significant interaction between salt addition and parasite prevalence for any of these factors ($P > 0.42$, at least, two-way ANOVA). Parasite-driven differences in the magnitude of grazer-induced wounds on live *Spartina* translated into marked changes in metrics of marsh structure (Fig. 2). Plots with only uninfected snails lost the greatest average amount of biomass compared to snail-free controls (70% in unsalted plots, 93% in salted plots). Mean aboveground biomass increased significantly with increasing parasite prevalence (Fig. 1B, $F_{2,42} = 118.37$, $P < 0.0001$, two-way ANOVA). Increases in biomass were roughly proportional to the level of infection prevalence. A 10% increase in infection prevalence decreased the amount of biomass lost by 13% in salted plots and 11% in unsalted plots (Tukey HSD, $P < 0.0001$, both cases). Likewise, a 30% increase in infection prevalence decreased the amount of biomass lost by 34% in salted plots and 24% in unsalted plots (Tukey HSD, $P < 0.0001$, both cases).

Parasite prevalence and salt addition affected not only the amount of available marsh structure but also

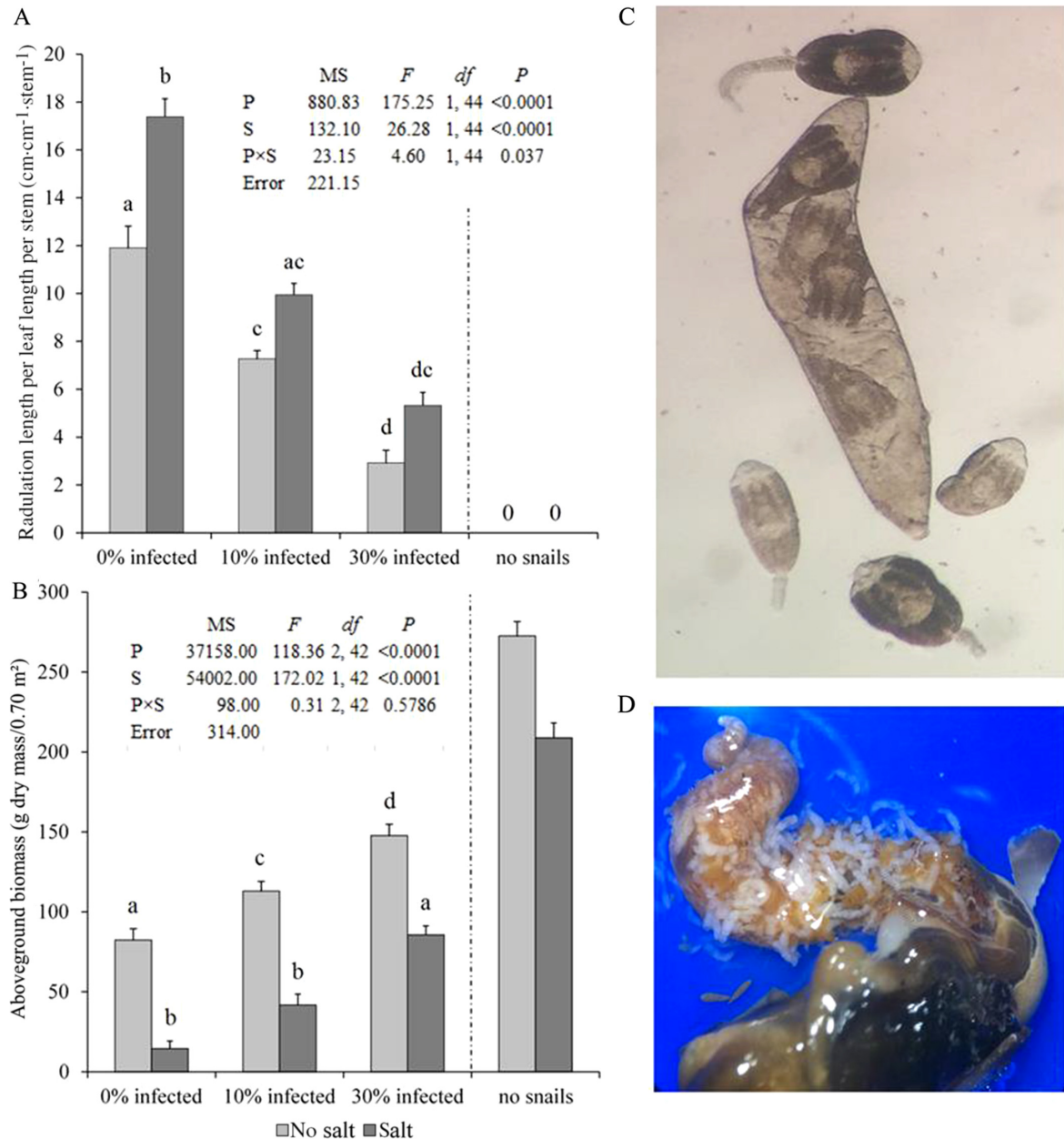


FIG. 1. (A) The effect of parasite prevalence on *Littoraria irrorata* radulations on *Spartina alterniflora*. Probability values are given for a two-way ANOVA testing for main and interactive effects. (B) The effect of parasite prevalence on *S. alterniflora* aboveground biomass. Probability values are given for a two-way ANOVA testing for main and interactive effects. For A and B, P stands for the main effect of parasite prevalence and S is the main effect of salt addition. Data are means and SE; $n = 8$ treatment replicates. Bars with different lowercase letters represent significant differences between treatments. (C) Redia and cercariae of *Parorchis acanthus*. (D) Rediae of *Parorchis acanthus* in the visceral hump of *Littoraria irrorata*.

structural qualities. Salt addition significantly decreased the difference in mean stem height over the course of the experiment ($F_{1,42} = 40.06$, $P < 0.0001$, two-way ANOVA), while increased parasite prevalence was associated with a significant increase in mean stem height (see Appendix S3: Fig. S2, $F_{2,42} = 357.90$, $P < 0.0001$, two-way ANOVA). Likewise, live stem density decreased significantly with both salt addition ($F_{1,42} = 37.20$,

$P < 0.0001$, two-way ANOVA) and increasing parasite prevalence (see Appendix S3: Fig. S1, $F_{2,42} = 59.57$, $P < 0.0001$, two-way ANOVA).

Snail climbing behavior.—Trematode infection significantly decreased average height climbed above the marsh substrate at low tide by more than 66% ($F_{1,158} = 172.66$, $P < 0.0001$, one-way ANOVA). Infected snails were six

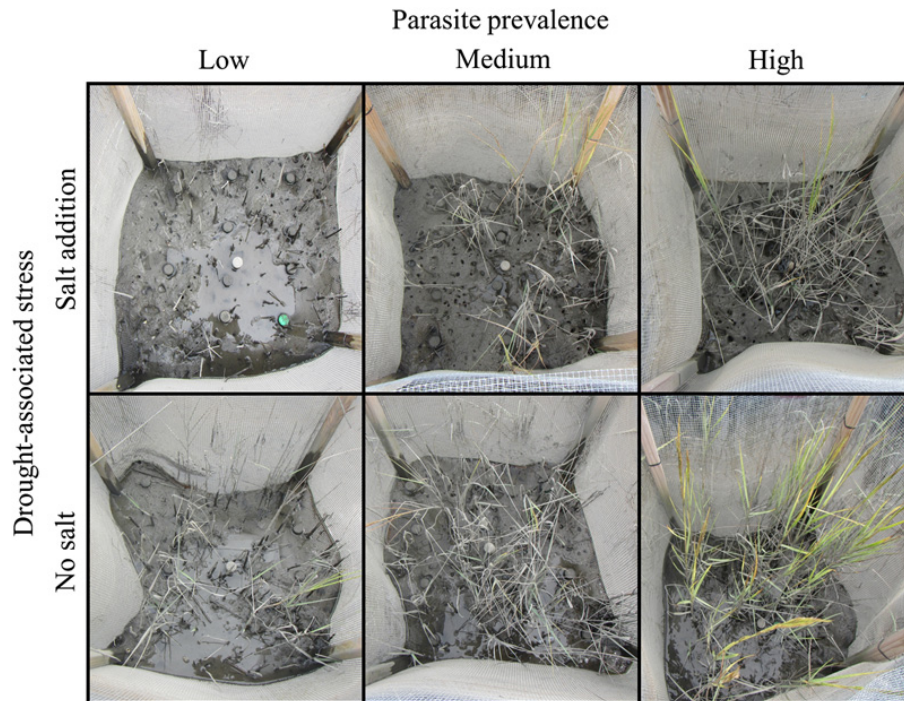


FIG. 2. Representative plots for each treatment at the end of our field manipulation of drought-associated soil salinity stress and parasite prevalence in *Littoraria*.

times more likely to be found on the marsh substrate at the base of stems than uninfected conspecifics at low tide. At high tide, a significantly smaller mean proportion of infected snails remained above the mean high water line (MHWL; 12.8%) compared to uninfected conspecifics (80.7%; see Appendix S3: Fig. S2, $F_{1,30} = 792.95$, $P < 0.0001$, one-way ANOVA).

Marsh surveys

Parasite prevalence and radulation data from the nine survey sites were pooled for analysis. Average snail density along die-off areas was more than 2.7 times higher on average than in adjacent healthy marsh areas (Fig. 3, $F_{1,16} = 131.1$, $P < 0.0001$, one-way ANOVA). We found that parasites occurred in grazers along all die-off borders at all sites and that the average parasite prevalence was significantly higher along die-off borders (11.2%) compared to healthy marsh areas (0.44%; Fig. 3, $F_{2,62} = 121.57$, $P < 0.0001$, one-way ANOVA). Lengths of radulations scars on stems sampled along die-off borders increased significantly with snail density and decreased with increased prevalence of infection (Fig. 4, $P < 0.04$, linear model, both cases).

Experiment II

At each of the three study sites, initial plot conditions (mean stem height, pore water salinity, snail density,

stem density, ribbed mussel density, adult fiddler crab burrow abundance, and estimated stem biomass) were not significantly different among treatments and the interaction of treatments ($P = 0.08$ at least, two-way ANOVA, for each response variable). The final mean infection prevalence for each treatment at each site did not significantly differ from initial conditions ($F_{1,52} = P > 0.92$ at least, two-way ANOVA). Weekly snail loss was low at all sites, never exceeding 5% for any plot.

At all study sites, the rate of border retreat decreased significantly with increasing of infection prevalence (Fig. 5, $P < 0.0005$, GLMM). Compared to uninfected parasite removal plots, border retreat was significantly slower (33%) in the control (10%) infection prevalence treatments, ($P = 0.0005$, Tukey HSD). In high prevalence plots, border retreat was 79% slower compared to parasite removal plots ($P < 0.00034$, Tukey HSD). In control prevalence plots, borders retreated at a little more than half the rate of high infection plots ($P = 0.044$, Tukey HSD).

DISCUSSION

Results from our experimental manipulation of parasite prevalence in healthy marsh suggested that increasing parasite prevalence in keystone grazers could slow rates of ecosystem die-off under simulated drought conditions by reducing per capita grazing rates. Our experimental manipulation of parasite prevalence on actively

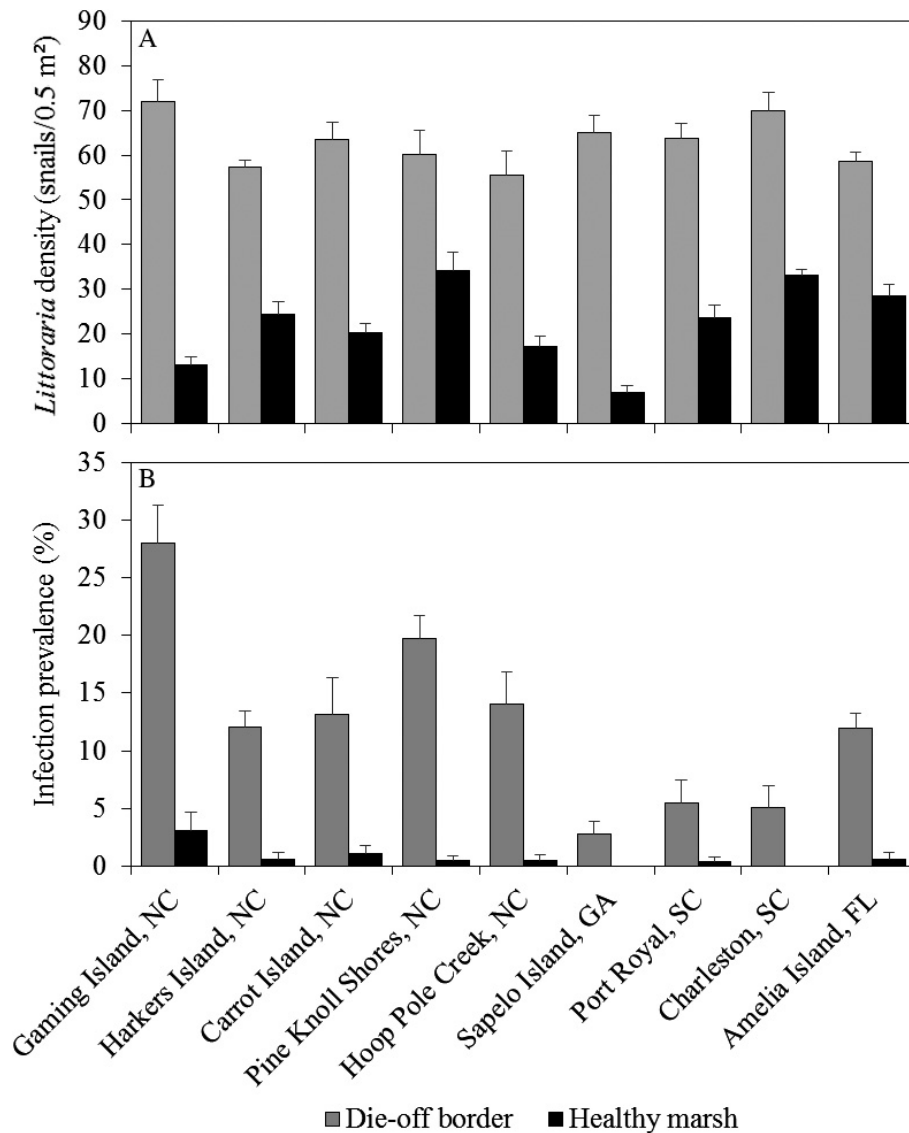


FIG. 3. (A) Average snail density and (B) trematode infection prevalence in *Littoraria* within die-off border areas and healthy marsh areas of nine east coast marshes. Data are means with SE; $n = 8$ samples per area per site. NC, North Carolina; GA, Georgia; SC, South Carolina; FL, Florida.

expanding die-off borders in three North Carolina marshes demonstrated that parasites can regulate plant ecosystem resistance to die-off from drought-associated overgrazing by generating a tritrophic cascade that protects against elevated herbivory on salt-stressed plants. Our latitudinal surveys of parasite prevalence and grazing intensity show that parasitism in *Littoraria* is common within southern marsh die-off areas and that increased parasitism is correlated with decreased per capita grazing by snails, suggesting that the relationships elucidated in our experiments could be more spatially general. Further manipulative experiments across the range of *Littoraria* will be necessary to determine the spatial generality of our experimental

results in North Carolina showing that parasites increase ecosystem resistance to drought-driven die-off. In light of these results, the well-documented ubiquity of parasites and parasite-induced behavioral modification of their hosts (Lafferty and Morris 1996, Clausen et al. 2008, Hernandez and Sukhdeo 2008, Libersat et al. 2009, Shi et al. 2014) and the common occurrence of top-down control of ecosystem processes and resilience, it is possible that parasites may indirectly regulate ecosystem resistance in many other natural communities.

The results of our simulated drought \times parasite experiment show that (1) sublethal drought stress conditions act additively with grazing snails to produce die-off

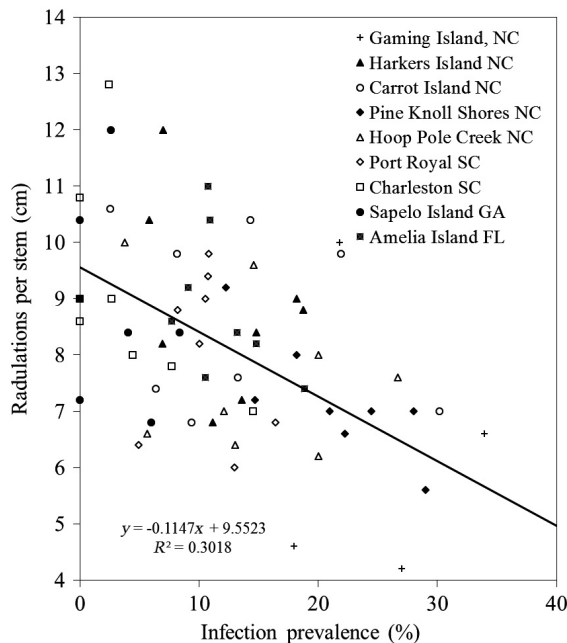


FIG. 4. Least squares linear relationship between trematode infection prevalence and snail grazing intensity (radulations per stem) along die-off borders for nine marshes along the eastern U.S. coast.

conditions, consistent with the findings of previous research (Silliman et al. 2005), and (2) parasites can reduce the strength of this additive effect between grazers and physical stress at sufficiently high prevalence. In the absence of parasites, sublethal drought stress and grazers yielded die-off of foundational vegetation (Fig. 2). The presence of parasites at intermediate and high prevalence rescued plants from overgrazing and led to relative increases in marsh plant biomass (Fig. 1B). These experimental results demonstrate that, at moderate snail densities (~ 100 snails/ 0.7 m^2), parasites have the potential to increase marsh resistance to drought and prevent die-off from occurring.

The mostly likely mechanism by which parasites prevent die-off of marsh plants is by reducing the top-down effect of infected snails on plants to near zero (Morton 2018). Past experiments have shown that infection with *Parorchis acanthus* greatly reduces snail grazing on live *Spartina alterniflora* (Morton 2018). In our field manipulation of drought stress and parasite prevalence, we saw a similar pattern, as grazing intensity per plot significantly decreased with increasing prevalence of infection (Fig. 1A). We found a significant interaction between grazing intensity and salt addition in this experiment, but not aboveground biomass. This disparity may be the result of our sampling method, which did not account for trematode-induced changes to snail climbing. At least some of the differences observed in the intensity of snail grazing likely reflect our method of measuring radulations on the topmost unfurled leaves of individual

plants where infected snails were less likely to be found feeding (Appendix S3: Fig. S3).

Concomitant with decreased grazing rates in plots with parasites, there were similar reductions in the top-down impacts of grazers on plant biomass that were roughly proportionate to the level of infection prevalence. Specifically, regardless of salt level, snails in uninfected treatments reduced plant biomass by $\sim 80\%$ relative to plots with no grazers present while in plots with 10% and 30% infection prevalence the magnitude of the top-down effect of snails decreased and reduced biomass by 67% and 51%, respectively. Thus, increasing parasite prevalence to 10% and 30% yielded relative decreases in top-down impacts on plant biomass of 12% and 28%, respectively (Fig. 1B).

At high tide, infected snails did not climb as high as uninfected conspecifics and were more likely to be found below the mean high water line. At low tide, the feeding activities of infected snails were confined mainly to plant bases or the marsh substrate (Appendix S3: Fig. S3). This behavioral modification may be adaptive for the parasites, increasing the likelihood that the snail will be submerged with the rising tide so that transmission to the next host may be facilitated through cercarial shedding (Belgrad and Smith 2014). Reduced circatidal migration and an increased tendency to remain closer to the marsh substrate could also work in concert with known parasite-induced reductions in grazing to affect field grazing rates by limiting the amount of time infected snails have access to their grazed resource. Additionally, reduction of snail reproduction via parasitic castration could potentially have compounding negative effects on snail grazing, but the temporal limitations of our study did not allow us to examine this.

Our survey of southeastern U.S. salt marshes showed that parasites occurred in grazers at all die-off borders and that infection prevalence was higher in die-off areas than in healthy marsh areas. This survey also found that increased infection prevalence along die-off borders in surveyed marshes was significantly related to a decrease in the average total length of radulations, consistent with the results of our field experiments. These survey results suggest, but do not show, that die-off borders may be infection hotspots, where increased prevalence reduces *Littoraria* grazing intensity. This pattern is likely driven by increased usage of these areas by foraging marsh birds. Bird droppings, which potentially harbor trematode eggs, were frequently noted inside sample plots along die-off borders in all surveyed marshes, while droppings were rarely found within healthy marsh plots (Appendix S3: Fig. S4). Moreover, direct observations in the field indicated that birds, including known final hosts of *Parorchis*, were found in greater abundance around die-off borders than in healthy marsh areas. This is consistent with previous observations where trematode infections were much more abundant in close proximity to clapper rail nests and roosting areas (Heard 1970). The existence of high infection rates around die-off

borders where snail densities are elevated would represent a potential negative feedback that could suppress the expansion of such borders, even in the absence of predators. While bird population declines are a recognized global trend, data on many coastal wetland-associated birds are particularly sparse (Conway et al. 2011, Spooner et al. 2018). However, there is some evidence to indicate that some marsh birds, including some known to harbor *Parorchis*, might indeed be experiencing population declines (Correll et al. 2012, Wiest 2018). The loss these hosts and the parasites they harbor could have ramifications for marsh ecosystem resistance. It has yet to be experimentally determined, however, whether high prevalence of infection along die-off borders is the result of elevated infection rates due to an abundance of bird final hosts or a diminished abundance of predators in these areas that may preferentially consume infected snails at higher rates due to differential climbing behavior.

Our manipulation of parasite prevalence along three marsh die-off borders in North Carolina provides direct evidence that parasites can slow the rate of ecosystem die-off by modifying host phenotype. Removal of parasites from active die-off borders resulted in accelerated rates of ecosystem loss, while increasing parasite prevalence significantly increased marsh resistance. At all sites, a 10% control prevalence was able to significantly slow marsh loss compared to uninfected parasite removal plots (Fig. 5). When parasite prevalence was raised to 30%, the decrease in mean die-off border expansion rate was even more pronounced and almost ceased. Thus, at moderately high snail densities, characteristic of many die-off border areas, the 10% infection prevalence, representing the midsummer average, appears sufficient to significantly slow ecosystem retreat due to overgrazing of drought-stressed plants. Previous experiments paired with latitudinal surveys found that snail grazing was an important top-down force throughout the southeastern United States and that there can be substantial biogeographical variation in consumer impacts (He and Silliman 2015, Pennings and Silliman 2005). More experiments are needed to determine how parasitism alters consumer impacts across their range.

Previous surveys have suggested that Mid-Atlantic Coast populations of *L. irrorata* do not harbor trematode parasites (Rossiter 2013) and that infection prevalence rarely exceeds 1% in Southern Atlantic and Gulf Coast marshes (Holliman 1961, Hamilton 1978). The findings of a previous survey (Morton 2018) and our survey in the current study demonstrate that summertime prevalence of *Parorchis* is low in healthy *Spartina* marsh, but can exceed 10% and be as high as 30% in marsh die-off areas. Our treatments used in field manipulations encompassed this range in infection prevalence and, therefore, tested the maximum potential of *Parorchis* to indirectly affect salt marsh structure that we could expect to observe under current natural conditions. Additionally, while we have demonstrated that

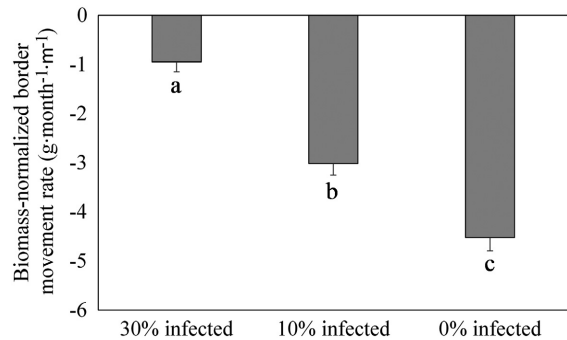


FIG. 5. The effect of parasite prevalence on the biomass-normalized die-off border movement. Data are means and SE; $n = 18$ treatment replicates.

parasitism can have ameliorating effects at moderately high host densities (100 snails/0.7 m²), snail densities associated with some consumer fronts (where snails are smaller) can be much higher (>600 snails/m²; Silliman and Zieman 2001, Silliman and Bertness 2002). It is likely that, over a certain threshold of grazer density, any ameliorating effects of parasitism on marsh structure would be entirely overshadowed by the strong top-down effects of grazing, but we did not encounter this in our surveys or experiments with naturally occurring densities. Determination of critical grazer host density thresholds where parasitism can have the highest impact will be necessary to better understand and contextualize the trait-mediated impacts of parasitism on marsh ecosystem resistance.

These results add to a growing body of evidence demonstrating parasites can modify ecosystem properties. Many parasites have powerful effects on host behavior and can profoundly alter or initiate trophic cascades (Wood et al. 2007, Hernandez and Sukhdeo 2008, Libersat et al. 2009, Sato et al. 2012, Buck and Ripple 2017). While parasites are common community components, the effects of many parasites on the behavior of their hosts, and how such changes to host phenotype affect ecosystem properties, are poorly understood. Parasites may influence aspects of resilience in many ecosystems by modifying the behavior of influential hosts, dampening consumer effects, or facilitating processes that contribute to diversity (such as predation). Because of the limitations that often exist in acquiring or identifying infected individuals, this investigation is one of few studies to manipulate parasite prevalence in the field and quantify the indirect ecosystem-level consequences of parasite-induced behavioral modifications of their host species (Mouritsen and Poulin 2005, Wood et al. 2007). In order to better understand how natural systems will respond to global scale disturbances, it is important that the roles played by parasites in aspects of ecosystem resilience are better elucidated through manipulative field experiments. Additionally, the potential for parasites to have positive effects on ecosystem stability, as illustrated

by the current study, may provide greater impetus to conserve certain parasitic fauna and the habitat-forming or keystone hosts on which they depend.

A variety of consumers including locusts (Krall et al. 1997, Hunter 2004), gypsy moths (Elkinton and Leibold 1990), herbivorous crabs (Holdredge et al. 2009), urchins (Estes and Palmisano 1974, Lauzon-Guay and Scheibling 2007), and snails (Silliman et al. 2005, Lauzon-Guay and Scheibling 2009) form consumer fronts in response to anthropogenic stressors and can generate widespread, cascading ecosystem loss in disparate systems around the world (Silliman et al. 2013). These abundant and influential grazers are also host to a variety of parasites, many of which are known to or have the potential to alter host behavior (Hagen 1996, Wood et al. 2007, Clausen et al. 2008, Hoover et al. 2011, Shi et al. 2014). For instance, microsporidian parasites modify the behavior of their locust hosts, preventing them from forming gregarious migratory swarms that can cause transcontinental plagues (Shi et al. 2014). Based on the results of our study, we suggest that parasite control over ecosystem resistance via trait-mediated effects could be more common than previously thought. Parasites might buffer disturbance when top predators are missing or by regulating free-living consumer populations. Parasites that modify host density or behavior may influence ecosystem resistance to and recovery from disturbances, including buffering cascading effects associated with predator extirpation. Whether parasite modification of grazer host behavior has a positive, negative, or neutral effect on ecosystem resistance to consumer-driven die-off depends on both the strength and direction of modification and the prevalence of parasitism in consumer populations before or following the formation of fronts.

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