

Influences of land use and ecological variables on trematode prevalence and intensity at the salt marsh-upland ecotone

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Abstract. Human-altered shorelines make up approximately 14% of the coastline in the United States, with consequences for marsh ecosystems ranging from altered physical and biological variables, to direct loss of intertidal marsh habitat, to diminished land–sea connectivity. Trophically transmitted parasites that require connectivity between upland host species and marsh host species to complete their complex lifecycles could be particularly sensitive to the effects of shoreline alterations. They can additionally respond to gradients in natural physical and biological factors, including the host communities, that are often sharp at the land–sea ecotone. Across 27 salt marshes over 45 km, we evaluated the effects of environmental variables and three types of land use (undeveloped; single-house adjacent to the marsh with small-scale shoreline armoring; and single-house adjacent to the marsh without shoreline armoring), on infection prevalence and intensity of the trematode *Microphallus basodactylophallus* in its second intermediate crab host, *Minuca pugnax*. The first intermediate hosts of *M. basodactylophallus* are Hydrobiid snail species that are obligate marsh residents, while the definitive hosts are terrestrial rodents and raccoons. Thus, trematode transmission must depend on cross-boundary movement by the definitive hosts. We found that although there was a trend of lower infection prevalence at undeveloped forested sites, there was no significant effect of adjacent land development on infection prevalence or intensity. Instead host, biotic and abiotic factors were correlated with infection; namely, larger *M. pugnax* had higher prevalence and intensity of *M. basodactylophallus*, and higher soil moisture and lower density of the ribbed mussel (*Geukensia demissa*) were associated with increased *M. basodactylophallus* prevalence. The small, indirect influence of upland development suggests that movement of definite hosts across the ecotone may be largely unaffected. Further, the robust trematode levels signify the ecosystem and the species interactions, upon which its complex lifecycle depends, are largely intact.

Key words: bulkheads; environmental impact; Georgia Coastal Ecosystem LTER; residential development; shoreline armoring; *Spartina alterniflora*; Special Feature: Honoring Charles H. Peterson; Ecologist.

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INTRODUCTION

Parasites can be helpful indicators of environmental stress and biological change because they are themselves often sensitive to change (Gehman

et al. 2018) and because they respond sensitively to changes in the abundance and distribution of their hosts. In fact, it has been posited that a healthy ecosystem is one rich in parasites, because for trophically transferred parasites to

thrive, the food web must function (Huspeni and Lafferty 2004, Hudson et al. 2006, Byers 2009). Because of their complex multi-host life cycles, trophically transferred parasites in particular can be sensitive indicators of their hosts' distributions and feeding behaviors (Byers et al. 2011). Several human land-use factors can disrupt parasite life-cycles and thus alter parasite prevalence; for example, road density around saltmarshes is a strong negative predictor of trematode infection prevalence (Altman and Byers 2014).

In fact, land use is a factor that is changing rapidly in coastal environments. Human populations are concentrated on the coasts of continents and have been increasingly altering and armor-ing the shorelines (Doody 2004, Popkin 2015). Currently, approximately 14% of the total US coastline has hard armoring (Gittman et al. 2015). Such structural armoring may influence boundary-crossing organisms that normally move across habitats, such as from terrestrial to marine, often to feed on resources found in the adjacent habitat (Polis and Hurd 1996). These transitional ecotones can have high biodiversity (Odum 1953, Kark and van Rensberg 2006) and high parasite richness (Weinstein et al. 2019) because they are composed of species from the two distinct adjacent habitats. However, some of these ecotones, such as the maritime forest-salt marsh ecotone, are subject to high levels of human encroachment and impact. Consequently, human land use (e.g., development and armor-ing) on this habitat transition area could alter the behavior or abundance of habitat-crossing animals and subsequently alter the strength of ecological interactions, like parasitism, of which they are a part.

The effects of human land use at the forest-marsh boundary on marsh ecology vary depend-ing on the type of development (Dugan et al. 2017). Across the coastal southeastern United States, there are many small bulkhead structures protecting single-family homes (Alexander 2010). Marshes adjacent to these small bulkheads in Georgia have lower tidal elevations and thus have marsh characteristics typical of a lower ele-vation marsh; for example, high coverage of *Spartina alterniflora* in place of the traditional high marsh plant community (Bozek and Burdick 2005, Gehman et al. 2017b). Even unarmored development, such as a lawn and house, can

increase soil water content and decrease salinity in the adjacent marsh, which suggests increased freshwater input (Gehman et al. 2017b). How-ever, for habitat-crossing species, both armored and unarmored development can alter their dis-tribution; for example, the high marsh crab *Armases cinerum* utilized the upland terrestrial system less in marshes adjacent to development (Gehman et al. 2017b). Thus, even small-scale armoring can alter the marsh abiotic and biotic factors, and we might expect these changes to alter marsh use by hosts and subsequently the distribution and prevalence of their parasites too.

Variation in abiotic and biotic factors, whether induced by human land use or naturally occur-ring, can alter parasite distribution and preva-lence across sites. If a host and parasite have differential physiological tolerances to environ-mental factors, physical gradients in these factors can decouple their distributions. For example, if the host can survive an environmental stressor such as salinity that its parasite cannot, low-salinity sites could have hosts with low to no infection (Dunn and Young 2015). Furthermore, species interactions can alter where parasites can survive and proliferate. If a predator preferen-tially feeds on infected prey, areas of high pred-ator abundance can be areas of low infection prevalence (Fong and Kuris 2017, Gehman and Byers 2017, Gehman et al. 2017a). For parasites with multiple hosts, the distribution of up-stream hosts can influence the parasite distribution. For example, in a trematode system where the parasite transmits from definitive bird hosts to first intermediate host snails, infection prevalence within the snail is dependent on bird abundance in snail habitats (Byers et al. 2008, 2015, Keogh et al. 2015).

Parasites with complex, multi-host life cycles have more opportunities to be affected by envi-ronmental influence. Digenetic trematode infec-tions often have a sexual stage in the definitive host, an asexual stage in the first intermediate host, a non-reproductive metacercarial stage in a second intermediate host, and often there are free-living cercarial stages that transmit between the first and second intermediate host. Thus, alter-ations to the first or second intermediate host, the definitive host, or the free-living cercariae could affect the parasite's abundance (Byers et al. 2011). Infection prevalence accumulates variably within

each host stage (Nikolaev et al. 2020), and since infection is often non-lethal in the second intermediate host, it can act as a collector of infective stages within the system (Leung et al. 2009). Here, we ask whether land-use development alters parasite prevalence in *Minuca pugnax*, a second intermediary host crab that resides in the salt-marsh. We chose to focus on a non-reproductive cyst stage in the second intermediate host, because the crab is the easier host to sample, and should accumulate infective metacercarial cysts for a relatively long time and thus be a good integrator of host presence and disease dynamics.

Although *M. pugnax* can be infected with a range of trematode parasites, we focused on *Microphallus basodactylophallus* (Fig. 1; Heard 1976, WoRMS 2021)). *M. basodactylophallus* is found in the digestive gland of several crab species including *Uca* species, as well as *Calinectes sapidus* and *Rhithropanopeus herbstii* from Massachusetts to

Louisiana (Heard 1976, Heard and Overstreet 1983, Johnson et al. 2020). It has also been found in the shrimp *Palaemonetes pugio*, and it remains unknown whether all the different hosts are hosting the same species (Heard, *personal communication*). The definitive hosts are the raccoon *Procyon lotor* and the marsh rat *Oryzomys palustris* (Heard, *personal communication*). The first intermediate host is a hydrobiid snail. Although *M. pugnax* is the second intermediate host for *M. basodactylophallus*, late-stage metacercariae can look remarkably similar to the adult worm stage (Fig. 1C, D) (Bridgman 1969, Heard 1976, Heard and Overstreet 1983, Shields and Overstreet 2003). We refer to this late developmental stage hereafter as “progenetic metacercariae.” In at least one of the second intermediate hosts of *M. basodactylophallus*, *C. sapidus*, heavy infection with metacercariae can be lethal (Heard and Overstreet 1983).

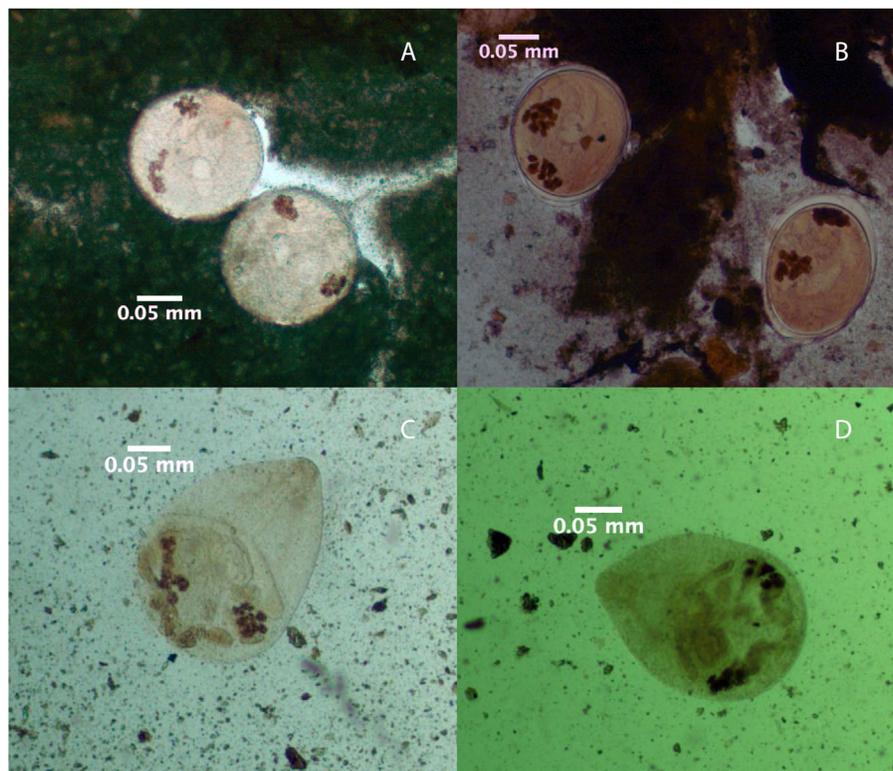


Fig. 1. Life stages of *Microphallus basodactylophallus* found in the digestive gland and gonad of *Minuca pugnax* collected from upland marsh in coastal Georgia. (A, B) The metacercarial stage and (C, D) progenetic metacercariae. (A, B) were dissected from a *M. pugnax* collected adjacent to armored land-use development, and (C, D) were dissected from *M. pugnax* collected adjacent to undeveloped land.

METHODS

To evaluate the *M. basodactylophallus* infection intensity and prevalence in *M. pugnax*, we collected *M. pugnax* at blocked stations along the Georgia coastline, in Chatham, Bryan, and Liberty counties, covering a range of ~45 km, in June and July of 2013. To examine the influence of development on parasites, we selected sites to sample *M. pugnax* with three types of land-use development. We used GIS data to locate armored shorelines with a single house (armored development) and then selected nearby unarmored sites with a house (unarmored development) and forested sites with no armoring or house (undeveloped). Collectively, one of each of these three site types comprised a single blocked station (seven stations with three sites per station for a total of 21 sites). Within a station, sites were separated by at least 20 m of shoreline. We collected *M. pugnax* at four additional armored sites and one additional undeveloped marsh site adjacent to armored sites (26 total sites). At each site, we collected 10 crabs haphazardly by hand in the saltmarsh within 8 m of the marsh–upland transition, with the exception of one site where we collected 13 crabs (Gehman et al. 2017b). We attempted to collect five males and five females for each site; however, this was not always possible. After collection, we brought crabs back to laboratory and froze them for later processing. There was a logistical trade-off between site-level replication and the number of individuals sampled within each site. Because our focus was to quantify the variance associated with adjacent land use, we chose to have higher site-level replication and fewer individuals sampled within site. This necessarily puts a higher level of influence for each detected infection within each site, and if there was a high level of variance in infection intensity within sites, it could diminish statistical power to discern site-level differences in that metric.

Crabs were dissected to determine trematode infection from November 2013 through May 2014. For each dissection, we removed one crab from the freezer, cleaned with tap water, and placed it in a glass dish. Sex and carapace width were recorded before each dissection. We dissected out all of the gonad and digestive tissue and screened the tissue for evidence of trematode

infection on a compound microscope at 100× magnification. We considered a crab infected if it contained any life stage of the parasite, including metacercariae and progenetic metacercariae (Fig. 1). We counted the number of metacercariae and progenetic metacercariae per host, and the sum of these two numbers was considered the worm intensity per host (Bush et al. 1997). In addition, we noted any parasitic bopyrid isopods present in the tissue.

At each site, we measured a wide range of environmental and biological variables, including aspects of soil water and nutrient content, soil grain composition, vegetation type and cover, and abundance of invertebrate species using standard techniques. Further details about variables measured can be found in (Gehman et al. 2017b). All metadata is available at (Gehman and G.C.E. LTER Project 2017). This work was completed as part of an NSF-funded long-term ecological research project, and as a result, we were able to measure many more field variables than could be included as predictor variables in our analysis. To select a subset of the available variables, we developed a set of a priori hypotheses on how upland development could alter definitive host abundance and use of the marsh (Appendix S1: Table S1). We included variables that we hypothesized would interact with *M. pugnax* infection prevalence, and in addition, we included environmental and biological variables that have been previously identified to be altered by armoring or development (Gehman et al. 2017b). The variables we used were as follows: land-use development practice immediately adjacent to the marsh, marsh elevation, the relative proportion of the marsh that was vegetated (vegetative cover estimated from an overhead photograph), proportion of wrack cover, soil water salinity and water content, marsh width (distance from upland to nearest creek), and density of the marsh snail *Littoraria irrorata*, the ribbed mussel *Geukensia demissa* and crab holes (a proxy for crab community abundance). We also used the size (carapace width, mm) of each dissected *Minuca pugnax* as an individual-level variable. It would have been ideal to also quantify hydrobiid snails, which are the first intermediate host of *M. basodactylophallus*; however, we could not achieve consistent reliability in our sampling methods for hydrobiids.

Statistical analysis was conducted in R (R Core Team 2020). We evaluated the multicollinearity of the selected environmental variables. All variables with a correlation coefficient greater than 0.60 were evaluated for biological relevance and only the most relevant variable was retained within the model evaluations. All models were fit using the package *glmmTMB* (Brooks et al. 2017). We ran analyses with two parasite metrics as response variables; (1) for prevalence, we used infection status (0 or 1) as the parasite response variable and fit a binomial mixed effects model, and (2) for intensity per host, we subset the data to include only the infected individuals and evaluated drivers of trematode abundance (combined metacercariae and progenetic metacercariae) per host, and fit a negative binomial generalized linear model. In the supplement, we further explored the within-host relationship between intensity of progenetic metacercariae and metacercariae (Appendix S1: Fig. S1, Table S2).

By selecting variables in our models that we knew were influenced by armoring, we were able to explore the indirect and direct effects of armoring in our analysis. First, we evaluated the direct effects of land-use development on infection and intensity, with land-use development category as a fixed variable (undeveloped, unarmored, and armored developed) and site as a random variable. Next, we evaluated the indirect effects of land-use development on infection and intensity by including the abiotic and biotic variables described above as predictor variables, and land-use development category as a crossed

random variable with site (Gehman et al. 2017b). Because there were multiple measurements of infection at each site we visited (~10 crabs per site), we included site as a random variable in all models. Our sites had some additional hierarchical structure, with unarmored and undeveloped sites selected to be adjacent to armored sites (Gehman et al. 2017b). Preliminary analysis included station in the hierarchical structuring as a random variable, but the analysis was singular, with no variance attributed to the station. Thus, to keep from overparameterizing our model, we removed station as a random variable and considered treatments to be randomly distributed within the region. We standardized each of the predictor variables using the *scale* function. Standardization allows for direct comparison of regression coefficients of each predictor variable. We evaluated an exhaustive suite of generalized linear models, calculated the relative variable importance (RVI) of each predictor variable, and then averaged the models using natural average method (i.e., non-shrinkage of estimates) across all models with a $\Delta AIC < 2$ (Grueber et al. 2011).

RESULTS

We dissected 263 crabs, with 201 males and 62 females. Overall, 80 (30.4%) of *Minuca pugnax* were infected by *M. basodactylphallus*, with 20 infected females (32.2%) and 60 infected males (29.9%). We found both metacercariae and progenetic metacercariae forms of *M. basodactylphallus* in the crabs (Table 1; Fig. 1). There were 36 crabs

Table 1. Summary statistics for *Minuca pugnax* for each of the three land-use categories describing the total number (Total) of each *Microphallus basodactylphallus* life stage (metacercariae and progenetic metacercariae), the per host intensity of infected hosts (mean and SD), the total number (N) of *M. pugnax* dissected, as well as the total number of uninfected and infected *M. pugnax* (with metacercariae and/or progenetic metacercariae of *M. basodactylphallus*), and the total number of bopyrid isopods detected (isopod).

Response	Statistic	Armored	Undeveloped	Unarmored
Metacercariae	Total	122	33	114
	Mean intensity	3.1	1.8	6.0
	SD	5.1	2.2	14.7
Progenetic metacercariae	Total	38	11	14
	Mean intensity	1.0	0.6	0.7
	SD	1.2	0.6	1.5
<i>N</i> (<i>M. pugnax</i>)	Total	113	80	70
	Infected	42	18	20
	Uninfected	71	62	50
Isopod	Total	4	4	4

with at least one progenetic metacercariae, 64 crabs had at least one metacercarial cyst, and 23 crabs had a combination of at least one metacercarial cyst and one progenetic metacercariae. The total count was 63 progenetic metacercariae and 269 metacercarial cysts, and infected *M. pugnax* had an average worm intensity of 4 (± 9 , SE). In addition to the trematodes, we found 12 parasitic bopyrid isopods in the branchial chamber spread across 10 hosts (Table 1).

We found that infection prevalence and intensity had high site-level variance (Fig. 2), and there was no direct effect of land use on either prevalence or intensity (Table 2). Infection prevalence was lowest at undeveloped sites (mean = 22.5%, SD = 19.1), intermediate at developed sites without armoring (mean = 28.6%, SD = 26.7), and highest at armored developed sites (mean = 37.3%, SD = 31.6; Fig. 2A, Table 2). Intensity was also lowest at undeveloped sites (mean = 2.4, SD = 2.3), but was intermediate at developed and armored sites (mean = 4.0, SD = 5.6), and highest at developed and unarmored site (mean = 6.7, SD = 16.0; Fig. 2B; Table 2). There were two individuals with high-intensity infections, one from an unarmored developed site (intensity = 72) and one from an armored developed site (intensity = 31), which are represented visually but were removed as outliers from all analyses.

We found significant relationships with abiotic and biotic variables that were associated with land-use development and second intermediate host characteristics. We found that *M. pugnax* size and soil water content were positively associated with infection prevalence, and *G. demissa* density was negatively associated with infection prevalence (Table 3; Figs. 3, 4). *G. demissa* and soil water content were each directly influenced by land-use development (Appendix S1: Table S3, Fig. 4). For trematode intensity, we found that only *M. pugnax* size was associated with intensity of infection, with higher intensity in larger individuals (Table 4; Figs. 5, 6). We did not find a significant effect of the variables we predicted would be correlated with definitive hosts (Appendix S1: Table S1; e.g., crab hole density, marsh width, vegetative, or wrack cover), affecting infection prevalence or intensity (Tables 3, 4; Figs. 3, 4). After removing two outliers with high intensity, there was no relationship between intensity of progenetic metacercariae and either metacercarial

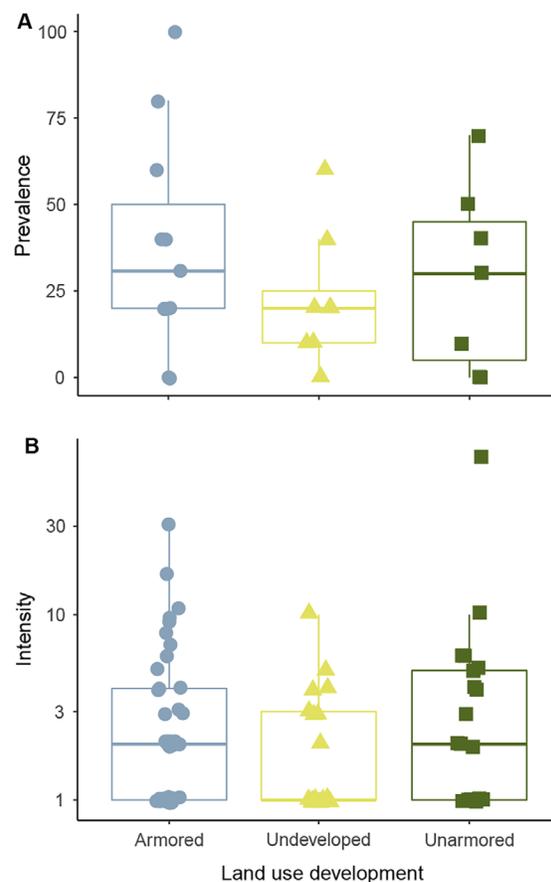


Fig. 2. Boxplots of site-level (A) prevalence and (B) intensity of *Microphallus basodactylphallus* by land-use practices directly adjacent to the marsh where we collected *M. pugnax*. The intensity *y*-axis is plotted on a log-scale to visualize the spread of the data, but the statistical analysis was done on the raw data (Table 2). Land-use development is indicated by color with armored development in blue, undeveloped in yellow, and unarmored development in dark green.

intensity alone or in concert with upland development (Appendix S1: Table S2, Fig. S1).

DISCUSSION

Although infection prevalence in *M. pugnax* trended lower in marshes adjacent to undeveloped (forested) sites, we did not find a strong direct effect of land-use development. This suggests either that land-use development does not directly influence raccoon and marsh rat use of

Table 2. Direct effect of land-use development on infection prevalence and intensity of *Microphallus basodactylphallus* in *Minuca pugnax*.

Predictors	Infection			Intensity		
	Odds ratios	CI	<i>P</i>	Incidence rate ratios	CI	<i>P</i>
(Intercept)	0.43	0.16–1.18	0.101	3.03	2.20–4.17	<0.001
Undeveloped	0.49	0.10–2.26	0.358	0.83	0.50–1.38	0.475
Unarmored Developed	0.59	0.12–2.95	0.522	1.03	0.63–1.68	0.91
	Random effects					
σ^2	3.29			0.54		
τ_{00}	2.04 _{site}			0.03 _{site}		
ICC	0.38			0.06		
<i>N</i>	26 _{site}			20 _{site}		
Observations	260			75		
Marginal <i>R</i> ² /Conditional <i>R</i> ²	0.019/0.395			0.012 / 0.070		

Notes: The intensity analysis was run with infected crabs only, and since not all sites had infections, there are fewer sites included in that analysis.

Table 3. Model average evaluating the drivers of *Microphallus basodactylphallus* prevalence (0,1) in *Minuca pugnax* in coastal Georgia.

Driver	Estimate	SE	2.50%	97.50%	<i>P</i>	RVI
(Intercept)	−1.25	0.27	−1.79	−0.71	<0.001	
<i>M. pugnax</i> size	0.77	0.22	0.34	1.2	<0.001	1.00
Soil water content	0.68	0.28	0.12	1.24	0.02	0.85
<i>G. demissa</i>	−0.82	0.4	−1.61	−0.03	0.04	0.83
<i>L. irrorata</i>	−0.6	0.41	−1.41	0.22	0.15	0.56
Wrack	−0.29	0.26	−0.81	0.23	0.28	0.39
Marsh width	0.3	0.34	−0.36	0.96	0.37	0.37
Vegetative fraction	−0.27	0.34	−0.94	0.4	0.43	0.34
Soil water salinity	−0.2	0.32	−0.84	0.44	0.54	0.32
Elevation	−0.12	0.45	−1	0.75	0.78	0.32
Crab hole density	−0.14	0.32	−0.77	0.5	0.67	0.30

Notes: Model averaged β estimate (Estimate), the standard error around that estimate (SE), the confidence intervals (2.50%, 97.50%), the *P* value (*P*) and the relative variable importance (RVI). Site and land-use development were included as crossed random variables.

the marsh or that metacercarial prevalence and intensity in the second intermediate host were not sufficiently linked to definitive host abundance or distribution to illuminate that response. We instead found that second intermediate host traits, community composition, and environmental characteristics were correlated with infection prevalence.

Larger hosts often have higher parasite burdens, and we found both higher infection prevalence and intensity within larger hosts. There are several possible explanations of this relationship. It could be that larger hosts have more resources for their parasites and so can support more

parasites (Alvarez et al. 1995, Gehman et al. 2017a). However, in this case, the metacercarial stage does not typically use resources from the host. Instead, because larger hosts are older crabs, it is likely this pattern is driven by the accumulation of metacercariae over a host's lifetime (Thieltges 2008). The existence of progenetic metacercariae within the crab host supports the idea that the infections in the second intermediate host could be long-lived. Development of progenetic metacercariae has been found to occur in other trematode second intermediate hosts (Poulin 2003). In one system where this has been evaluated, lack of predator cues was

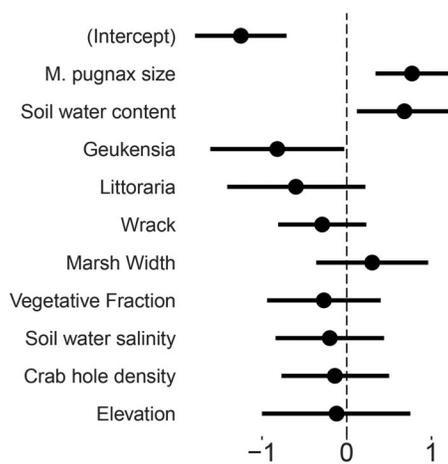


Fig. 3. Estimates and 95% confidence intervals for the effect of each predictor variable on the probability of finding a *Minuca pugnax* infected with trematode larvae, from a model average of all variables. Site and land-use development were included as crossed random variables.

associated with increased worm growth rate within the second intermediate host (Poulin 2003). Indeed, in that system, progenetic metacercariae within the second intermediate host can produce eggs that are infective to the first intermediate host, bypassing the definitive host (Holton 1984, Poulin 2003). The relationship between progenetic metacercariae and definitive host density has not been demonstrated for *M. basodactylphallus*, and we found no effect of land-use development or metacercariae intensity on intensity of progenetic metacercariae (Appendix S1: Table S2).

An unmeasured but potentially rate-limiting stage in the parasite's life cycle is the free-living cercarial stage between hydrobiid snails and the *M. pugnax* second intermediate host. Our result that *M. pugnax* at sites with higher densities of *G. demissa* had a lower proportion of trematode infection was not expected. It is possible that this correlation is not causal and instead represents that *G. demissa* and *M. basodactylphallus* are, for example, each responding in opposition to an environmental variable not measured in this study. If we instead assume some level of causation in the correlation, *G. demissa*, as a filter feeder, could affect parasite infection levels directly by consuming cercaria larvae. Filter feeders can

reduce the free-living infective stages of parasites as they move from one host to the next (Thieltges et al. 2008, Welsh et al. 2014, 2019, Fong and Kuris 2017). For example, barnacle hosts living near anemones have been known to have reduced infection burdens by their castrating isopod parasite, *Hemioniscus balani* (Fong and Kuris 2017). It is also possible that *G. demissa* shells simply provide an alternative settlement substrate for the cercariae, passively removing them from completing their life cycle (Welsh et al. 2014). Further work evaluating this relationship is necessary, for example experiments testing whether *G. demissa* is able to feed on trematode larvae.

Land-use development alters the water content and salinity in adjacent marshes, suggesting that marshes adjacent to unarmored development had higher freshwater input (Gehman et al. 2017b). We found that soil moisture was positively related to infection prevalence levels and had a positive (but not significant) relationship with infection intensity. This could potentially be the result of a relationship between soil moisture and Hydrobiid snails, the first intermediate host. While no data exist on the relationship between soil moisture and Hydrobiid snails, surveys in this same region as this study suggest that Hydrobiid snails prefer high and mid-level *Spartina* marshes with higher sand content (Pung et al. 2008).

We sought to use second intermediate host infection prevalence as an indicator for definitive host use of the marsh. This approach can be successful and save substantial effort in detecting movement of more elusive hosts (Byers et al. 2011). The other hosts of *M. basodactylphallus*—raccoons, marsh rats, and hydrobiid snails—are all far more difficult to quantify, so our intent was to infer their effects through measurement of a more readily available host, *M. pugnax*. While it remains true that the existence of *M. basodactylphallus* in *M. pugnax* necessitates the presence in the marsh of the other hosts in this lifecycle, we did not find support for any direct or indirect relationship between land-use development and the other hosts by using parasites in second intermediate host crabs as a proxy. This lack of effect could mean at least three things: Our proxies do not reflect well the abundance of the definitive hosts, there was no effect of land-use development on definitive host use of the marsh, or

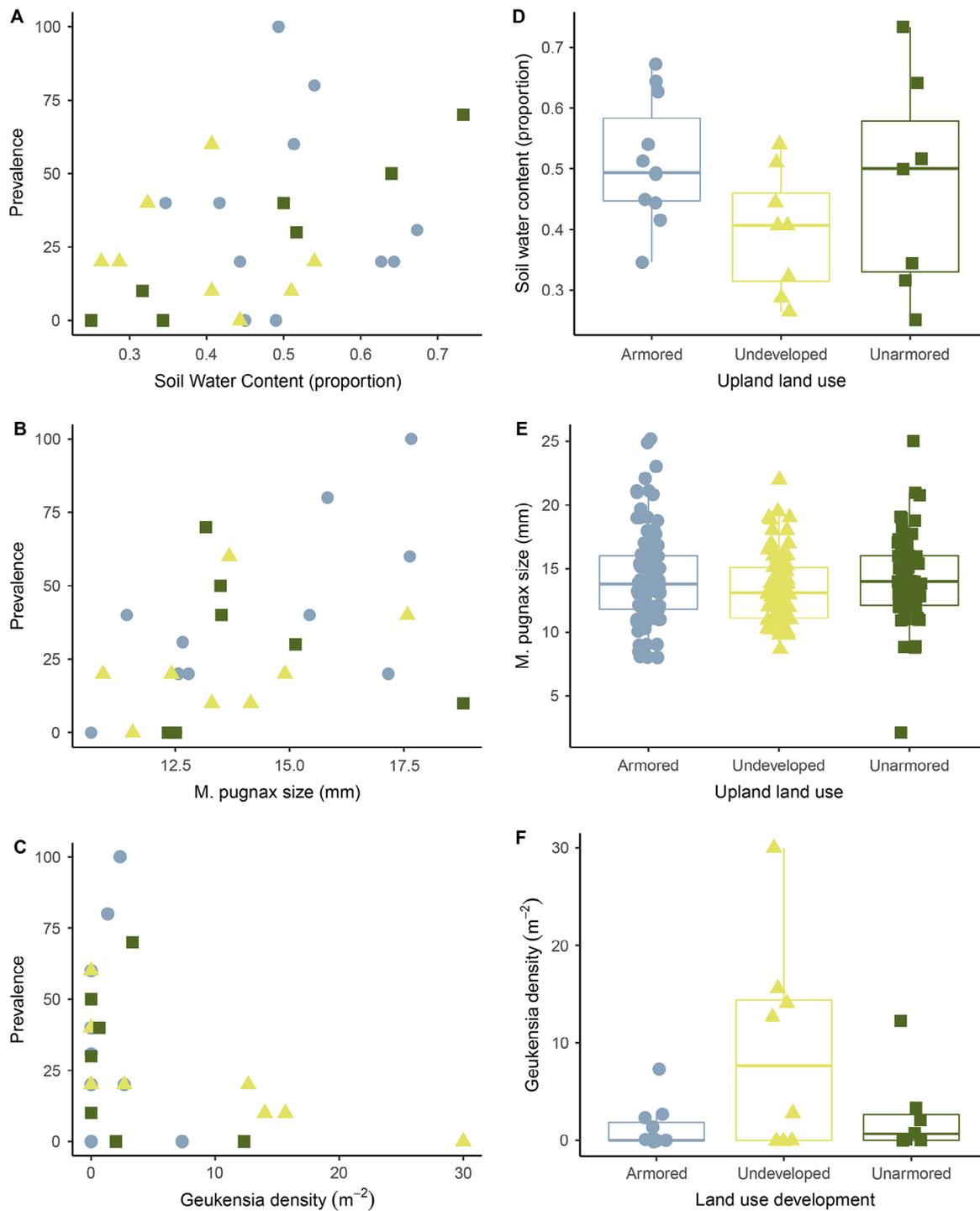


Fig. 4. (A–C) Infection prevalence of *Microphallus basodactylphallus* in *Minuca pugnax* within each site plotted against the three top predictor variables of infection probability (Table 3); the average soil water content (A), average crab size (B), and average *Geukensia demissa* density (C). Statistical analyses were run with a binomial response for infection in each individual crab host. (D–F) The relationship between the three top predictor

(Fig. 4. Continued)

variables of *M. basodactylphallus* infection prevalence in *M. pugnax* (Table 3) and land-use development including, soil water content (D), *M. pugnax* size (E), and the ribbed mussel *G. demissa* density (F), statistical analysis in Appendix S1: Table S3. Symbol shapes and colors indicate the land-use development for each site. Averages of each of these variables and the site-level prevalence are illustrated here for ease of visualization.

Table 4. Model average evaluating the drivers of infection by *Microphallus basodactylphallus* intensity (number of adults and metacercariae per host) in *Minuca pugnax* in coastal Georgia.

Driver	Estimate	SE	2.50%	97.50%	P	RVI
Intercept	0.87	0.16	0.55	1.2	<0.001	
<i>M. pugnax</i> size	0.27	0.09	0.09	0.45	<0.001	0.95
<i>L. irrorata</i>	-0.27	0.24	-0.75	0.21	0.27	0.35
Soil water salinity	-0.14	0.13	-0.4	0.13	0.31	0.34
Crab hole density	0.14	0.14	-0.14	0.42	0.32	0.32
Vegetative fraction	0.08	0.17	-0.25	0.41	0.62	0.27
<i>G. demissa</i>	0.15	0.24	-0.34	0.63	0.55	0.26
Soil water content	0.06	0.12	-0.18	0.3	0.63	0.25
Elevation	0.05	0.14	-0.23	0.34	0.71	0.24
Marsh width	-0.05	0.14	-0.33	0.23	0.71	0.24
Wrack	0.02	0.13	-0.25	0.28	0.91	0.23

Notes: Two outliers were removed from the statistical analysis (Fig. 6). Model averaged β estimate (Estimate), the standard error around that estimate (SE), the confidence intervals (2.50%, 97.5%), the *P* value (*P*), and the relative variable importance (RVI). Site and land-use development were included as crossed random variables.

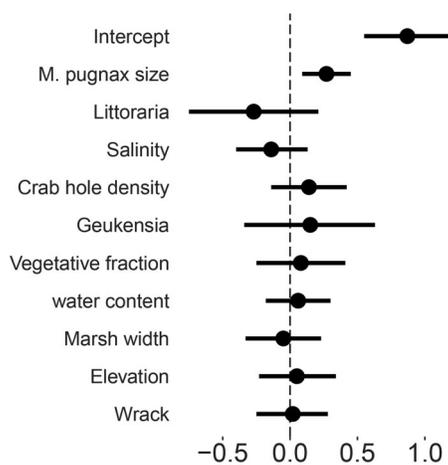


Fig. 5. Estimates and 95% confidence intervals for the effect of each predictor variable on the intensity of trematodes (metacercariae + progenetic metacercariae) in a *Minuca pugnax* from a model average of all variables.

cercariae supply from the first intermediate hydrobiid host is not limiting. Further, our results do not suggest that there is no direct effect of land-use development on other marsh

parasites, and it is possible that a parasite with a more sensitive response to human activity would have different results. Although logistically difficult, to understand the effects of land-use development on the raccoons, marsh rats and hydrobiid snails hosts in the life cycle may require direct further work on their distributions, habitat usage, and behavior.

Instead of being an indicator of other hosts, we found that *M. basodactylphallus* infection itself was sensitive to several abiotic and biotic factors that are associated with land-use development. With varying dependencies and rate-limiting steps throughout their life cycle, parasites with complex life cycles can be highly sensitive to alterations in ecosystem function. Metacercarial stages in the second intermediate hosts can often accumulate over years (within the limits of their hosts' lifespan), allowing them to reflect longer term influences of human driven disturbance. Our work shows that variables altered by shoreline armoring are correlated at a broad-scale with prevalence and intensity of *M. basodactylphallus*. Experiments evaluating the mechanistic links between the correlations we found in the field will likely be a fruitful direction for future work.

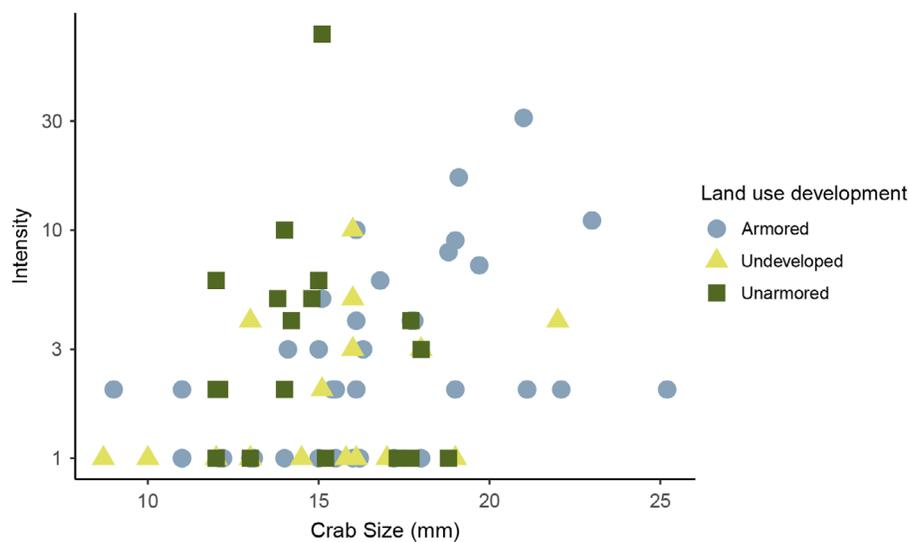


Fig. 6. Average infection intensity of *Minuca pugnax* infected with *Microphallus basodactylphallus* at each site in coastal Georgia. Two outliers were removed from the statistical analysis (metacercariae counts >25). The *y*-axis is on a log-scale for visualization, but the statistical analysis (Table 4) was run on the raw data. Colors and shapes indicate the land-use development adjacent to each site.

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DATA AVAILABILITY

Data and code are available in the Georgia Coastal Ecosystem Data Portal and Environmental Data Initiative: <https://doi.org/10.6073/pasta/c70d736e0750ef09566bff40c3730fd9>.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3723/full>