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Geographic and Environmental Patterns in Infection by Oyster Macroparasites Along the Georgia Coast

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Geographic and Environmental Patterns in Infection by Oyster Macroparasites Along the Georgia Coast

A Thesis Presented

by

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of

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ABSTRACT

The eastern oyster (*Crassostrea virginica*) is a keystone species that stabilizes sediments, cycles nutrients, filters water, and provides habitats and food for estuarine fauna. Oysters are prone to several macroparasites, including oyster pea crabs (*Zaops ostreum*), mud blister worms (*Polydora websteri*), and boring sponges (*Cliona sp*.). Prior research has shown that shellfish infected with blister worms and boring sponges exhibit weaker shells, are more vulnerable to damage and predation, and potentially experience reduced condition. Pea crabs may cause reduced condition, growth, and fertility in their hosts. However, the extent to which these parasites harm their hosts is still unclear. We collected oysters from 24 reefs across eight sites on Georgia's coastline and examined them for macroparasites, then quantified the relationships between macroparasite prevalence and intensity, oyster condition, site location, and reef characteristics. We found no correlation between location and infection prevalence or intensity for any macroparasites. However, increased blister worm prevalence was correlated with low salinity and low reef rugosity. Additionally, while oyster condition varied between sites, it was not a significant predictor of individual oyster condition, indicating that a non-blister worm factor is responsible for the differences in condition across the state. The geographic and environmental factors influencing oyster macroparasite infections and their effects on oyster health have been extensively explored, but have been subject to long-term study in only a few locations in Georgia, and are critical for maintaining and restoring high-quality reef habitats. The large tidal amplitude, high tidal intensity, and exclusively intertidal oyster reefs on Georgia's coast, which differ from the conditions at other highly researched oyster communities in the eastern United States, mean that it is crucial to conduct studies in Georgia to inform decisions about oyster populations within the state. The effects of climate change may also cause macroparasite infection patterns to shift in the future, and the ability to predict these changes accurately relies on a clear understanding of how environmental conditions affect macroparasite distribution.

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INTRODUCTION

i. Eastern Oysters

The eastern oyster (*Crassostrea virginica*) is a keystone species and ecosystem engineer in Georgia's coastal salt marshes, as well as in other salt marsh and estuarine ecosystems across the Atlantic coast of the United States. In their natural environment, *C. virginica* populations form large reefs in the intertidal zones of salt marshes and estuaries. These reefs contribute to the continued health and maintenance of the salt marsh ecosystem and carry out a wide variety of ecological functions, including cycling and sequestering of carbon, nitrogen, and phosphorus (Newell and Langdon 1986, Dame et al. 1989, Hurst et al. 2022), particulate organic matter filtration and water turbidity regulation (Newell 1988), protection of the marsh edge from erosion by waves and harsh weather (Smith et al. 2009, Wiberg et al. 2019), and habitat and food provisioning for other estuarine species (Meyer and Townsend 2000).

All oyster species, including *C. virginica,* are prone to a variety of macroparasites. In the northwestern Atlantic Ocean, three of the most common macroparasite species affecting oyster populations are mud blister worms (*Polydora websteri*), boring sponges (*Cliona* spp.), and oyster pea crabs (*Zaops ostreum*). Macroparasite infection is rarely directly fatal, unlike infection by disease-causing protozoan microparasites such as *Perkinsus marinus* (Dermo) and *Haplosporidum nelsoni* (MSX), which can wipe out entire oyster populations during outbreaks and cause widespread environmental and economic devastation. As a result, many oyster disease studies focus primarily on the detrimental effects of oyster microparasites. However, despite their lack of oyster-killing potential, all three of these common macroparasites may still have significant effects on both individual oyster health and whole-reef health.

ii. Mud Blister Worms

Mud blister worms are parasitic spionid polychaetes that use a variety of shellfish for shelter. After entering crevices in an oyster's shell, blister worms burrow into the interior of the shell, bringing external sediment into their burrows that irritates the oyster's internal soft tissues (Dorgan et al. 2021). Multiple worms can burrow into an oyster at once, and burrows may merge together into large open cavities in the shell. In response to a blister worm infection, an oyster will lay down fresh shell layers over open burrows (Wargo and Ford 1993, Dorgan et al. 2021), creating characteristic dark, raised, mud-filled blisters that are more fragile than the surrounding shell (Figure 1). Worm burrows often extend much further across the interior of the shell than the external blisters indicate, causing further structural damage (Dorgan et al. 2021).

Burrowing action from blister worms weakens oyster shells, making infected oysters more fragile. Prior research indicates that shell-covered blisters require much less force to break than undamaged shells do (Dorgan et al. 2021). Additionally, in experiments using scallop and snail shells, shells infected with blister worms demonstrated a smaller average crush force than shells that were worm-free (Bergman et al. 1982, Buschbaum et al. 2007). Predatory crabs consumed snails bored by the closely related mud worm species *Polydora ciliata,* which exhibits similar shell-boring behavior to *P. websteri*, at a faster rate than uninfected snails, and were also more likely to select the infected snails as prey (Buschbaum et al. 2007). This effect is especially apparent in smaller individuals that have high-intensity blister worm infections, which are the easiest for predators to consume and the most vulnerable to predation (Ambariyanto and Seed 1991).

The shell damage caused by a blister worm infection may force parasitized oysters to redirect energy into laying down new shell layers. Depositing fresh shell over damaged areas is

Figure 1*: Oyster shells infected with blister worms, demonstrating characteristic black blisters.*

energetically costly (Palmer 1992) and quickly depletes the oyster's energy reserves. Continuous damage caused by blister worms may cause a chronic energy shortage for other essential functions, such as soft tissue growth and maintenance, leading to reduced oyster condition. In practice, the specific effects of blister worms on oyster condition are varied. Some studies (Ambariyanto and Seed 1991, Wargo and Ford 1993) have implicated blister worm infection in reduced condition, but it is still unclear whether infection is always related to reduced condition. Cole et al. 2020 determined that *P. websteri* abundance in diploid (wild-type) oysters in Alabama oyster farms was weakly negatively correlated with oyster condition during some but not all of the study's deployments, indicating that blister worms' effects on condition may be situational. Additionally, not all studied sites exhibited oyster condition reduction during deployments when it was observed, meaning that another, unknown factor was potentially responsible for the observed decrease in condition. Other studies have failed to find a correlation between blister worm prevalence and oyster condition (Hanley et al. 2019, Diez et al. 2022).

Blister worms are unable to tolerate pure freshwater for extended periods, but can survive in a wide range of salinities, ranging from brackish 10 ppt water to ocean water at 30 ppt (Nel et al. 1996, Brown 2012). Cole et al. 2020 and Hanley et al. 2019 both determined that blister worm prevalence was consistently higher in estuarine areas with lower salinity resulting from increased freshwater outflow from the mainland. Hanley et al. 2019 also indicated that blister worms were most common on reefs below the tidal line with intermediate levels of oyster density. Increased siltation is also a correlate for high blister worm infection prevalence (Clements et al. 2017, Cole et al. 2020), potentially due to particulate matter in the water providing more plentiful food for filter-feeding worms (Cole et al. 2020). Water temperature is also known to influence both mud blister worm prevalence and intensity. Higher temperatures are positively correlated with increased infection prevalence; this effect occurs both seasonally, resulting in decreased blister worm prevalence in the winter, and across multiple years, where increased summer water temperatures predicted increased blister worm infection rates (Cole et al. 2020). High levels of blister worm infection intensity have been shown to be connected to increased water temperature (Hanley et al. 2019). Co-infection with other shell-boring organisms, including boring sponges, is also known to promote blister worm infection. In Patagonia, oysters that had been attacked by the shell-boring bivalve *Lithophagia patagonia* were more likely to also be infected with blister worms (Diez et al. 2014), and shell-boring polychaetes, including *P. websteri,* have been observed inhabiting the holes in shellfish and other calcified organisms created by boring sponges (Bower et al. 1994, Martin 1996).

iii. Boring Sponges

Boring sponges are parasitic sponges that colonize the calcified structures of a variety of organisms, including oyster shells. Infected shells can be identified by characteristic pitting around the hinge and dark spots and streaks on the interior shell cavity (Figure 2), increased fragility, and leakage of yellow and brown fluid from broken shells (Carver et al. 2010, Carroll et al. 2015). In some cases of advanced infection, visible sponges can be seen growing on the outside of the shell; this is most common on oysters that are already dead (Carver et al. 2010). While boring sponges may play a beneficial role in maintaining oyster reef density at healthy levels, at high levels of abundance they have been theorized to be a major contributor to both reduced oyster condition and decreased oyster population density in the lower intertidal zone (Carroll et al. 2021).

Like with blister worms, repairing the damage caused by growing sponges is energetically costly (Palmer 1992) and redirects energy away from other biological processes toward shell repair. Boring sponge infection has demonstrated negative impacts on oyster growth rate and condition (Carroll et al. 2015, 2021, Watts et al. 2018, Dieudonne and Carroll 2022). The shell fragility caused by a boring sponge infection may also make oysters more vulnerable to predation or other forms of environmental damage. Experiments conducted with sponge-infected snails have demonstrated that predatory crabs can crush infected snails with much less force, and that infected snails were more likely to be eaten due to being less able to withstand predators' attempts to crush their shells (Stefaniak et al. 2005). Although similar experiments with oysters did not indicate that predatory crabs had a specific preference for sponge-infected oysters, infected oysters also exhibited greater prey value in terms of food gained per unit force exerted to break apart their shells (Coleman 2014), indicating that predators without powerful

Figure 2*: Interior (left) and exterior (right) of oyster shells infected with boring sponges, demonstrating pitting around the hinge on the exterior shell and yellow spotting on the interior.*

shell-crushing abilities may elect to prey on infected oysters that require less effort to open. Boring sponge infection may also cause increased vulnerability to other macroparasites; pea crab prevalence increases in oysters that are also infected with boring sponges, potentially due to the energy drain from a boring sponge infection decreasing an oyster's ability to fight off pea crabs (Watts et al. 2018, Carroll et al. 2021), and as mentioned previously, blister worms and other shell-boring polychaetes will readily inhabit boring sponge tunnels (Bower et al. 1994, Martin 1996).

Because boring sponges are unable to tolerate open air for extended periods, there is a strong established correlation between boring sponge prevalence and tidal inundation of oyster reefs, with boring sponge infections being more common in oysters that are primarily or entirely subtidal and rarer in intertidal oysters. In Georgia, where high tidal amplitudes mean that most oysters are intertidal, boring sponge infections are more common in the lower intertidal zone where oysters spend more time underwater, and decrease in prevalence in the upper intertidal zone (Carroll et al. 2021, Dieudonne and Carroll 2022). Salinity is also a key predictor of boring sponge prevalence. *Cliona celata*, the most abundant boring sponge species in the southeastern United States, is adapted to higher salinities and its prevalence on estuarine oyster reefs decreases in fresher water, especially at salinities of below 15 ppt (Hopkins 1962). Increasing water temperatures, especially temperatures in excess of 15°C during the summer months, have also been implicated in increased boring sponge prevalence (Carver et al. 2010). Water temperature in Georgia exceeds 15°C for most of the year, with temperatures typically rising above this threshold beginning in mid-March and remaining high until early December ("Physical Oceanography - NOAA Tides & Currents"), and is therefore generally favorable to boring sponges.

iv. Oyster Pea Crabs

Oyster pea crabs are soft-bodied endoparasitic crabs that live inside the mantle and gill tissue of oysters (Figure 3). Established estimates of pea crab prevalence in the southeastern United States vary widely, even for studies conducted in the same place: prevalence measurements near Skidaway Island at the northern end of the Georgia Coast range from 1-8% in the late 1990s (O'Beirn and Walker 1999) to as high as 73% several years later (Byers et al. 2014). Planktonic juvenile pea crabs are carried to oyster reefs by currents, are filtered out of the water by growing oysters, and embed themselves in the oysters' mantle and gill tissue. Female pea crabs remain inside the oyster for their entire lifespan, growing up to 10 mm across, and use their oyster hosts as a source of food and protection.

Figure 3*: Oyster pea crab buried in an oyster's mantle tissue (left) and outside of the oyster host (right).*

Once inside a host, pea crabs facilitate their growth by stealing filtered food from their host's gills. This decreases the amount of energy available to the oyster for growth and other essential biological processes, and has been implicated in reduced oyster body condition. Besides taking food from parasitized oysters, pea crab feeding can also cause gill damage that decreases its host's feeding ability and negatively impact its condition (O'Beirn and Walker 1999, Mercado-Silva 2005). Pea crabs have also been linked to diminished host gonad development, which may be detrimental to oyster fertility in areas with high infection prevalence (O'Beirn and Walker 1999).

Along with boring sponge infection, several other key environmental factors influence pea crab prevalence. Because pea crab larvae are planktonic, pea crab prevalence is most strongly correlated with oyster recruitment, which is primarily driven by high water flow resulting from currents and tides (Mercado-Silva 2005, Byers et al. 2014). Oysters in estuaries located on rivers and sounds, which move larger volumes of water, are more prone to pea crab infection than those located in estuaries on smaller creeks (Mercado-Silva 2005). Pea crabs are more prevalent in regions with larger tidal amplitudes, since the increased movement of water deposits more oyster and pea crab larvae (Byers et al. 2014). Pea crab infection is also more common in smaller, younger oysters; crabs may have a higher chance of successfully invading spat or juvenile oysters, which have less developed antiparasitic defenses, than more mature oysters, which can block pea crab infection by coating crab larvae in mucus (Christensen and McDermott 1958). Water temperature has also been shown to influence pea crab infection rates; like blister worms and boring sponges, pea crabs are also more prevalent when water temperatures are higher (Byers et al. 2014).

v. Study Importance and Aims

Due to the demonstrated effects that all three macroparasites have on oyster health and reef predation dynamics, macroparasite dynamics have a significant impact on oyster reef health and, by extension, on the functioning of the environmental systems that rely on the services the reefs provide. However, there is little research about the wide-scale geographic distribution of any of these macroparasites outside of an aquaculture context, which deals almost exclusively with parasite prevalence in high-density farmed oysters. The majority of existing long-term macroparasite studies about wild oysters in Georgia have been conducted in only a few locations, such as the areas surrounding the established marine stations on Skidaway and Sapelo islands, excluding the rest of the state’s 110 miles of coastline and limiting available data from the rest of the state. To our knowledge, only one other study (Byers et al. 2014) has examined macroparasite distribution in Georgia on this large of a geographic scale, but it only examined drivers of variation in pea crab prevalence and did not investigate parasites' effects on oyster condition. Because little information is available about macroparasite distributions in other areas of the state, it is still unclear whether the information from these studies is generalizable to the rest of Georgia's coastline.

A large portion of research on estuarine oyster reefs in the southeastern United States has been conducted in the Chesapeake Bay and the Gulf of Mexico. Parasite prevalence and distribution patterns in Georgia may differ from observations from these regions due to the major differences in tidal flow and reef submergence, and predictions about parasite dynamics created using data from those areas may not be accurate in Georgia. Tidal amplitude is the main oceanographic factor that differentiates Georgia from these other heavily researched oyster habitats. The Georgia-South Carolina coastline has a very large tidal amplitude; the mouth of the Savannah River regularly sees a difference of 6 to 8 feet between high and low tide, while the regions in the Chesapeake Bay with the highest amplitudes only experience differences of 2 to 3 feet, and most of the Gulf of Mexico only sees differences of 1 to 2 feet ("Physical Oceanography - NOAA Tides & Currents"). Due to the drastic difference between tidal extremes, oysters in Georgia experience much longer periods exposed to open air and sunlight than oysters in the other two regions, which are primarily subtidal and experience permanent or near-permanent submergence. Most studies from the Chesapeake Bay and the Gulf of Mexico are conducted on subtidal oysters, which are able to feed for longer periods and experience less exposure to stressful open-air conditions but are also more prone to macroparasites. Conversely, most studies in Georgia are conducted on intertidal oysters, which are the only occurring type of wild oysters in the state (Carroll et al. 2021). Decreased oyster submergence time has an established correlation with decreased prevalence of all three macroparasites (Hanley et al. 2019, Carroll et al. 2021), indicating that intertidal oysters in Georgia may have lower prevalences of both of these parasites than subtidal oysters from regions with less extreme tidal cycles. However, regions with increased tidal amplitude also exhibit increased pea crab recruitment (Byers et al. 2014) due to increased water flow into and out of oyster reefs, and the stress caused by longer exposure times may also have detrimental effects on oysters' ability to cope with parasite infections (Carroll et al. 2021).

This study aimed to investigate variations in macroparasite prevalence and intensity on Georgia's coastline. Key goals were to determine regions where any of the studied parasites were especially prevalent or infections were especially severe, what environmental factors drive parasite prevalence, and whether oyster condition is significantly affected by parasite prevalence. This research is part of one of the largest-scale comprehensive field studies conducted on oyster disease in the state of Georgia to date, and is crucial for both oyster reef restoration and preservation efforts as well as designating healthy commercial farming and harvest areas for Georgia's burgeoning aquaculture industry. Environmentally informed restoration, conservation, and harvest policies are especially important in the wake of wide-scale devastation of oyster populations caused by unsustainable harvesting. Georgia has experienced oyster reef declines that may be as severe as 90% (Beck et al. 2011), although the exact extent of these declines and their specific cause is disputed due to the inaccuracy and limitations of historical measurements (Power et al. 2010). In addition, a clear understanding of which environmental factors affect oyster disease will be critically important as climate change continues to alter conditions in the northeastern Atlantic Ocean, which may have drastic effects on community composition and ecosystem health.

METHODS

i. Field Methods

Oysters were sampled at three replicate reefs at each of eight sites along the Georgia coast during the first three weeks of June 2022 (Figure 1a, Table A1). Six of the eight sampling sites were located within state-designated wild oyster harvest areas. Reefs chosen for sampling all had a seaward edge within one meter of the marsh and a slope of approximately 30%. Selected reefs were also generally representative of the character of oyster communities within the site, and did not appear to be significantly more or less dense or degraded than surrounding reefs. All reefs within a site were located at least 1 kilometer away from the other sampled reefs (Table A1).

At each reef, a 0.25-square meter quadrat was deployed in a random position representative of general reef composition within a 1-meter distance of the marsh edge (Figure 1b). Within the bounds of the quadrat, all live oysters, associated dead shells, and other organisms were collected. Any live animals that were seen in the quadrat but escaped collection or could not be collected were recorded. Following oyster collection, the depth of the excavated quadrat was also recorded. At each reef, data about water temperature, salinity, and dissolved oxygen were taken using a YSI water quality meter. Reef rugosity was measured via the chain method, where a chain was conformed to the reef topography in a straight line and the distance covered by the conformed chain was measured. The rugosity index for each reef, R_q, was calculated as $R_q = d / l$, where d was the horizontal distance covered by the conformed chain and l was the length of the chain when fully extended (Aronson and Precht 1995). The heights of the three tallest oyster clusters along the rugosity chain were also measured.

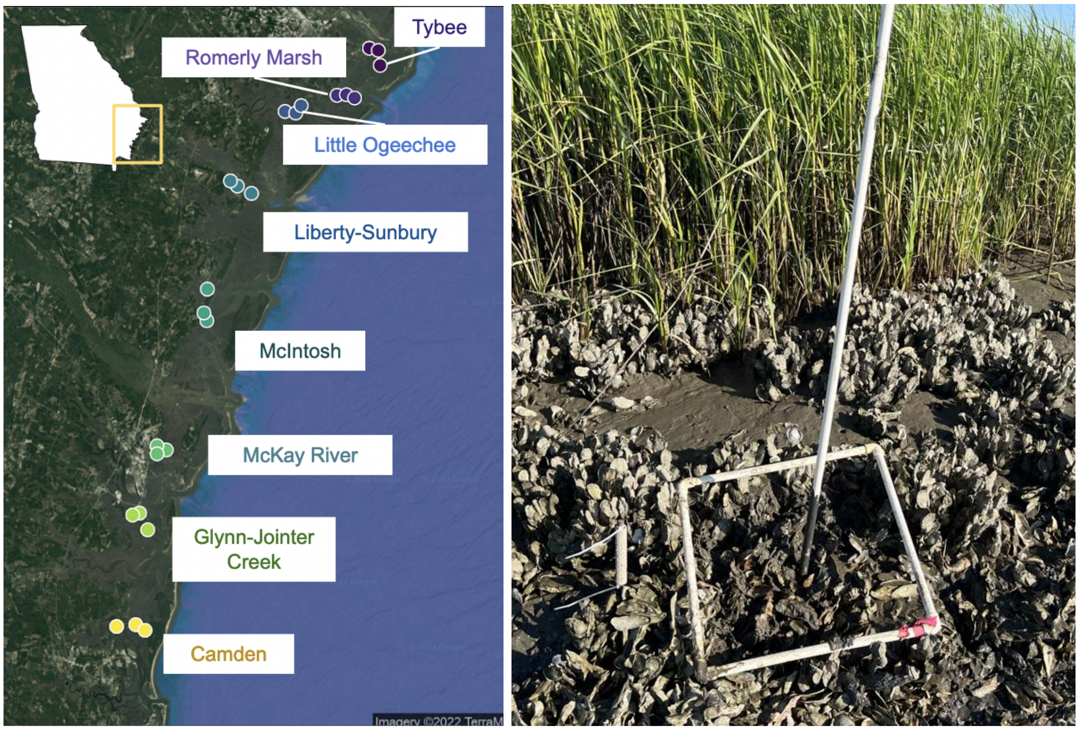

Figure 4: *Map of sampling sites (left) and excavated quadrat deployed on an oyster reef (right).*

Within 24 hours of collection, oysters from each quadrat were divided into live oyster clusters and dead shells. As much dead shell as possible was removed from the live clusters to ensure they consisted primarily or entirely of living oysters. Attached clams, mussels, bryozoan, barnacles, and other extraneous material were also removed and discarded from both the live clusters and dead shell. The numbers of large live oysters (>10mm long) and spat (0-10 mm) from the quadrat were counted and recorded. The total weights of the clean live clusters and dead shells were then taken using a spring scale.

Live oyster clusters were disassembled into single oysters, which were sorted into large (>60 mm) and small (30-60 mm) size classes. Single oysters were cleaned with a shucking knife to remove any attached spat and smaller oysters. Up to 25 unbroken single oysters of each size class were retained and refrigerated for macroparasite processing and condition analysis.

ii. Lab Methods

Each oyster designated for macroparasite processing and condition analysis was cleaned in the lab with the help of a shucking knife to remove any attached organisms missed during initial processing. After cleaning, the shell height (umbo to farthest point on tip) and shell length (across the widest portion of the shell) of each oyster were taken.

Each oyster was investigated for external boring sponge cover, which included both live sponge on the shell and the presence of pitting on the shell left by sponge infection. We then used the shucking knife to open each oyster and examine the internal tissue and shell for boring sponge, blister worms and pea crabs. We measured the carapace width of any pea crabs found inside of the oysters; pea crabs were discarded after measurement. To quantify infection intensity, total boring sponge and blister worm cover on each oyster's shell were both categorized as none, 0-5%, 5-10%, 10-25%, 25-50%, 50-75%, or 75-100%

With the aid of the shucking knife, we removed all of the oyster's internal tissue and fluids, including any tissue stuck to the inside of the shell, and deposited them into a pre-weighed foil weigh boat. Empty shells were retained for drying and condition analysis. The boat containing the tissue and fluid was weighed to obtain the wet tissue mass before being dried for 48 hours in a drying oven at 60°C to evaporate out all the water in the sample. Following the 48-hour drying period, we weighed the tissue samples again to obtain dry tissue mass. After opening and tissue removal, the empty shells were partially wrapped in aluminum foil and dried them in the drying oven at 60°C for 48 hours. The dried shells were also weighed following the 48-hour drying period to obtain dry shell mass. Condition index was calculated for each oyster using the formula *CI* = (*100* x *dry tissue weight) / dry shell weight* (Hanley et al. 2019)

iii. Statistical Methods

We determined individual and combined parasite prevalence, defined as the proportion of oysters infected with each parasite (individual prevalence), as well as the proportion of oysters infected with any of the three parasites (combined prevalence), for each of the 24 sampled reefs. We used skewness, kurtosis, and the Shapiro-Wilk test to confirm the presence or absence of a normal distribution for each of the individual parasite prevalence datasets and the combined prevalence dataset. To visually examine for normality, we also created a density plot for each dataset. Because none of the datasets were normally distributed, we transformed each of them using an arcsine transformation and checked them again for normality using the Shapiro-Wilk test and heteroscedasticity using the Breusch-Pagan test. Following transformation, the boring sponge and pea crab datasets were still not normally distributed, so we performed a Kruskal-Wallis non-parametric ANOVA on each of the transformed datasets to determine the relationship between parasite prevalence and site. Although the blister worm and total parasite datasets were normally distributed following transformation, we used the Kruskal-Wallis ANOVA for all four datasets to maintain consistency.

Due to the low prevalence of boring sponge and pea crabs, only blister worm prevalence was used for the other three statistical analyses. Blister worm intensity measurements were converted from percent cover to a 0-6 intensity scale. In order to account for the majority of the oysters at all of the sites having no blister worm infection, we removed the measurements for oysters in the “0”/no infection category from the dataset. We then used pairwise Kolmogorov-Smirnov tests, comparing only the infected oysters from each of the eight sites, to determine if any of the sites had significantly different parasite intensity distributions from one another.

To examine the relationship between macroparasite prevalence at individual reefs and eight reef-level environmental variables, eight separate linear regressions were performed to compare reef-level blister worm prevalence to each of the eight variables (rugosity, salinity, dissolved oxygen, water temperature, number of large live oysters in quadrat, live shell mass, dead shell mass, and live-to-dead-shell ratio).

We compared average oyster condition at each site using a one-way ANOVA to test whether condition index varied between sites, followed by a posthoc Tukey test to determine which of the sites significantly differed from one another. In addition, we performed a binomial regression comparing individual oyster condition to blister worm infection status, accounting for the fixed effect from different sampling sites, and conducted a Wald chi-squared test on the binomial regression model to determine whether blister worm infection was a significant predictor of decreased individual oyster condition. All statistical analyses were performed in the R statistical software (v4.1.0; R Core Team 2021).

RESULTS

i. Geographic Macroparasite Prevalence

Overall, there was no significant difference in combined macroparasite prevalence between sites ($H_7 = 9.69$, $p = 0.21$). The MacKay River had the highest combined macroparasite prevalence, with an average of 53.8% of oysters infected with at least one macroparasite across three sampled reefs. Camden had the lowest combined macroparasite prevalence, with an average of 10.5% oysters infected across two sampled reefs (Figure 5D).

There was no significant difference in blister worm prevalence between sites ($H_7 = 9.35$, $p = 0.23$). The MacKay River had the highest average blister worm prevalence, with 53.0% of oysters showing signs of infection across three sampled reefs. Camden had the lowest average blister worm prevalence, with 10.5% of oysters showing signs of infection across two sampled reefs (Figure 5A).

There was no significant difference in combined boring sponge prevalence between sites ($H_7 = 0.98$, $p = 1.00$). McIntosh had the highest average boring sponge prevalence, with an average of 5.4% of oysters showing signs of infection across three sampled reefs. Camden had the lowest average boring sponge prevalence, with an average of 1.2% of oysters showing signs of infection across two sampled reefs (Figure 5B).

There was no significant difference in pea crab prevalence between sites ($H_7 = 7.31$, $p = 0.40$). Romerly Marsh had the highest average pea crab prevalence, with 5.3% of oysters containing crabs across three sampled reefs. There were no pea crabs found at any of the three reefs on Glynn-Jointer Creek, and the site with the lowest nonzero pea crab prevalence was McIntosh, where 0.7% of oysters across three sampled reefs contained crabs (Figure 5C).

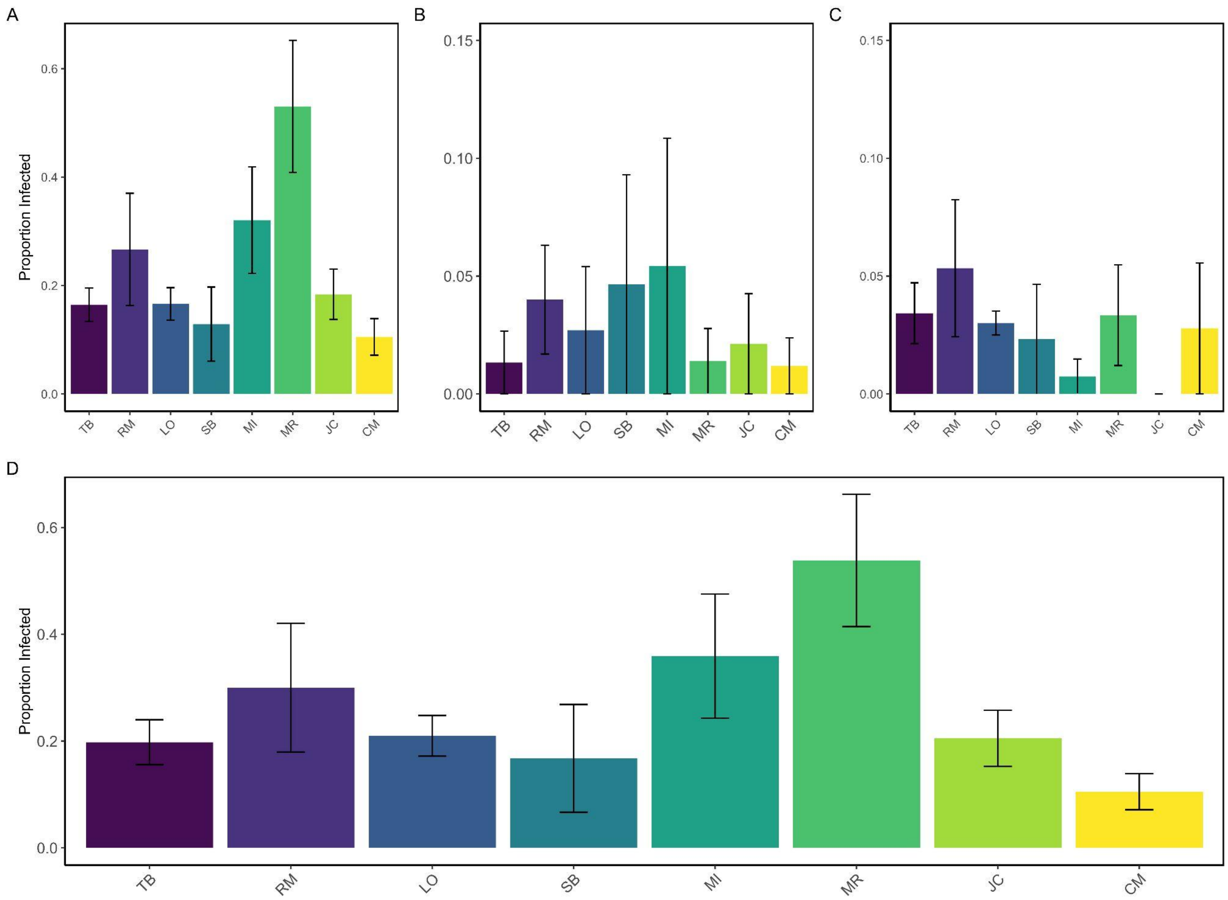

Figure 5: *Prevalence of (a) blister worms, (b) boring sponges, (c) pea crabs, and (d) all macroparasites combined at each of the eight sampling sites (mean proportion infected ± SE).*

ii. Geographic Mud Blister Worm Intensity

None of the eight sampling sites had significantly different distributions of blister worm infection intensity from one another (Tables A2 and A3). The Romerly Marsh, Liberty-Sunbury, and Glynn-Jointer Creek sites had the highest-intensity infections, with 2.5% of infected oysters at Romerly Marsh, 8.3% of infected oysters at Liberty-Sunbury, and 3.7% of infected oysters at Glynn-Jointer Creek having 75-100% blister cover (Figure 6). The MacKay River had the largest proportion of infected oysters with high-intensity (>50% cover) infections, at 26.9%. However,

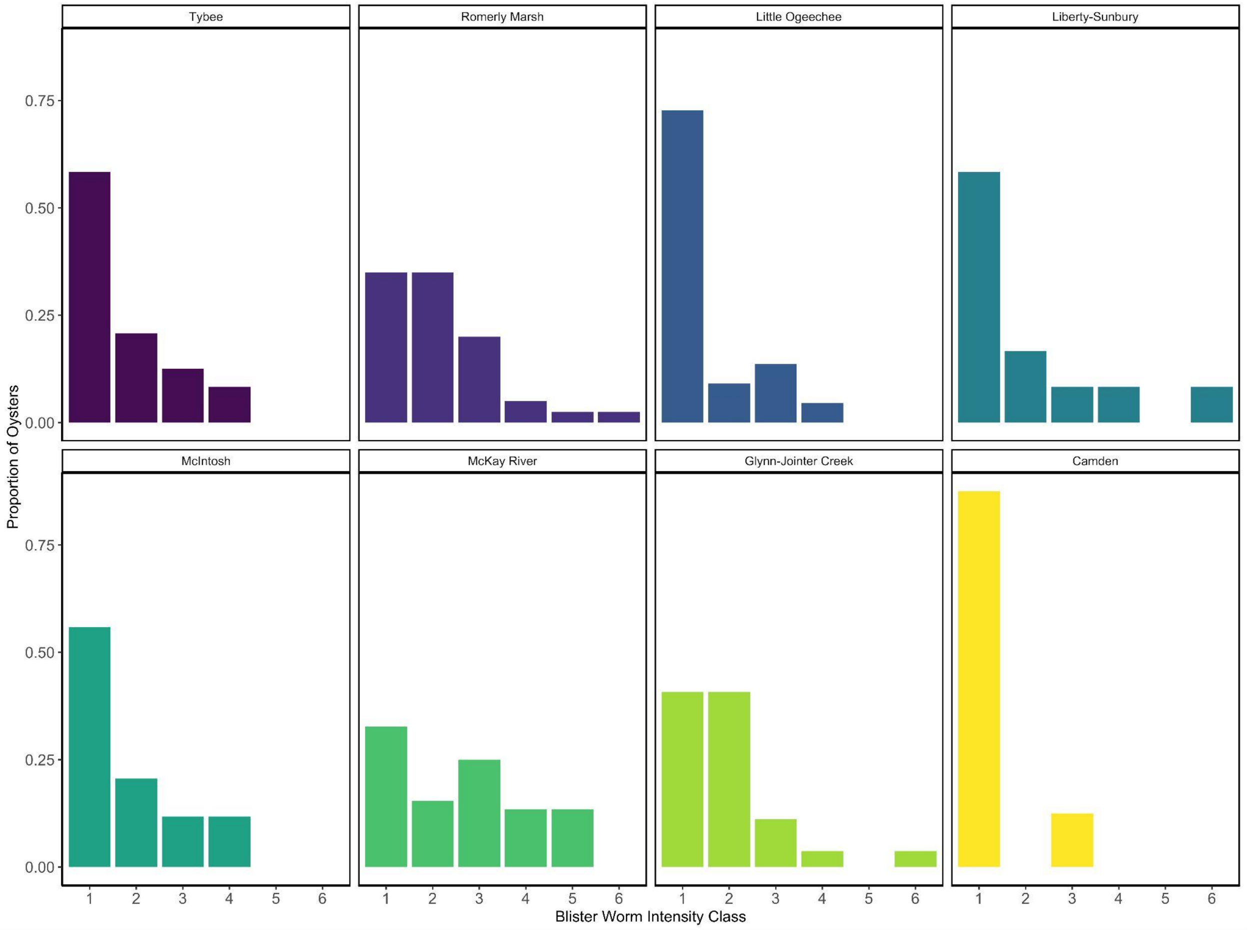

Figure 6: *Mud blister worm infection intensity among the blister worm-infected oyster populations at each of the eight sampling sites (mean proportion of infected oysters in each intensity class ± SE), where 1 = 0-5% blister cover across the oyster's whole shell, 2 = 5-10% blister cover, 3 = 10-25% blister cover, 4 = 25-50% blister cover, 5 = 50-75% blister cover, and 6 = 75-100% blister cover. Oysters with no blister worm infections have been removed from the dataset.*

at all eight sites, the majority of infected oysters had lower-intensity (<50% cover) infections (Figure 6). At the Tybee, Little Ogeechee, McIntosh, and Camden sites, no oysters had blister worm infections resulting in over 50% blister cover. Camden in particular had a high proportion of very low-intensity infections, with 87.5% of infected oysters at the site being in the least severe infection intensity category.

iii. Mud Blister Worm Prevalence and Environmental Conditions

There was a negative correlation between salinity and blister worm prevalence at individual reefs ($R^2 = 0.34$, $F_{1,21} = 10.89$, $p = 0.003$) (Figure 7A-7B). All three of the lowest-salinity reefs were at the MacKay River site, with salinities of 16.4, 17.8, and 18.0 ppt. Two of these three reefs also had the highest observed blister worm prevalences (58.5 and 70.8% infection at the reefs at 16.4 and 18.0 ppt, respectively). The third highest blister worm prevalence (46.5%) was observed at a reef at the McIntosh site with a salinity of 26.3 ppt. There was also a positive correlation between reef rugosity and blister worm prevalence at individual reefs ($R^2 = 0.22$, $F_{1,21} = 6.04$, $p = 0.02$). Since a smaller rugosity value indicates a higher level of reef complexity, there is a negative correlation between reef complexity and blister worm prevalence. Two of the three least rugose reefs were also from the MacKay River, with rugosity values of 0.875 and 0.90. These two reefs were also the reefs with the two highest blister worm prevalences. The third least rugose reef was from Camden, with a rugosity value of 0.850 and a blister worm prevalence of 13.9%.

There was no correlation between water temperature ($R^2 = 0.05$, $F_{1,21} = 1.27$, $p = 0.27$), dissolved oxygen ($R^2 = 0.06$, $F_{1,21} = 1.48$, $p = 0.24$), number of large live oysters ($R^2 = 0$, $F_{1,21} = 0.01$, $p = 0.92$), live oyster mass ($R^2 = 0.01$, $F_{1,21} = 0.24$, $p = 0.63$), dead shell mass ($R^2 = 0.15$, $F_{1,21} = 3.75$, $p = 0.06$), or live oyster mass to total shell mass ratio ($R^2 = 0.14$, $F_{1,21} = 3.29$, $p = 0.08$) and blister worm prevalence at individual reefs (Figure 7 C-7G).

iv. Oyster Condition

Average site condition index significantly varied across sites ($H_{7,911} = 14.8$, $p < 0.001$) (Figure 8A). However, blister worm infection status was not a significant predictor of individual

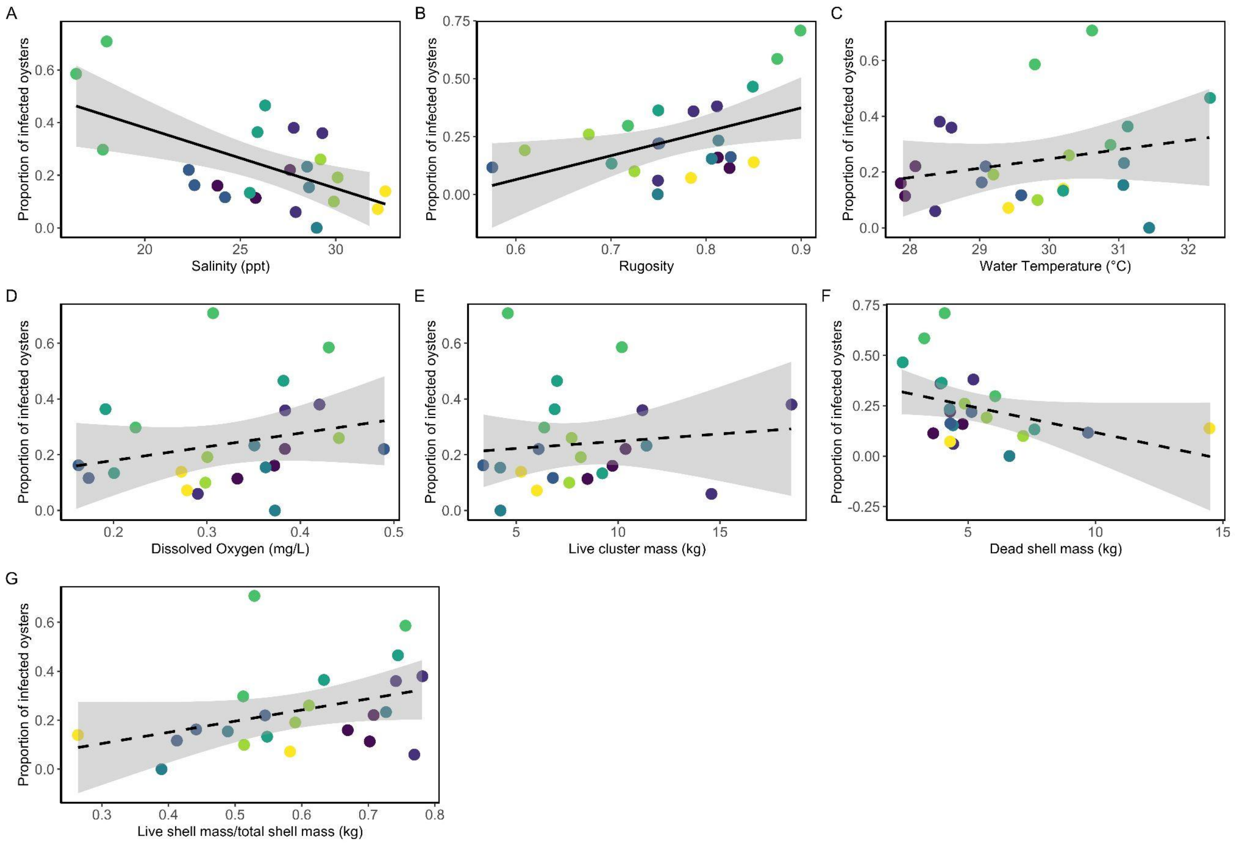

Figure 7: *Correlation between (a) water temperature (°C), (b) dissolved oxygen (mg O_2/L water), (c) salinity (ppt), (d) live oyster mass (kg), (e) dead shell mass (kg), (f) live oyster mass to total shell mass ratio, and (g) rugosity at individual reefs within the 7 sampled sites, and blister worm infection prevalence at each reef. Dashed lines indicate non-significant relationships.*

oyster condition ($X^2(1) = 0.64$, $p = 0.43$) (Figure 8C). The site with the highest average oyster condition was the MacKay River, with an average condition index of 3.72, and the site with the lowest average oyster condition was Romerly Marsh, with an average condition index of 2.44. The MacKay River had significantly higher average condition than all seven other sites: Tybee ($p< 0.001$), Romerly Marsh ($p < 0.001$), Little Ogeechee ($p < 0.001$), Liberty-Sunbury ($p < 0.001$), McIntosh ($p < 0.001$), Glynn-Jointer Creek ($p < 0.001$), and Camden ($p = 0.01$). Romerly Marsh had significantly lower average condition than Tybee ($p = 0.001$), Little Ogeechee ($p < 0.001$), McIntosh ($p < 0.001$), MacKay River ($p < 0.001$), Glynn-Jointer Creek ($p < 0.001$),

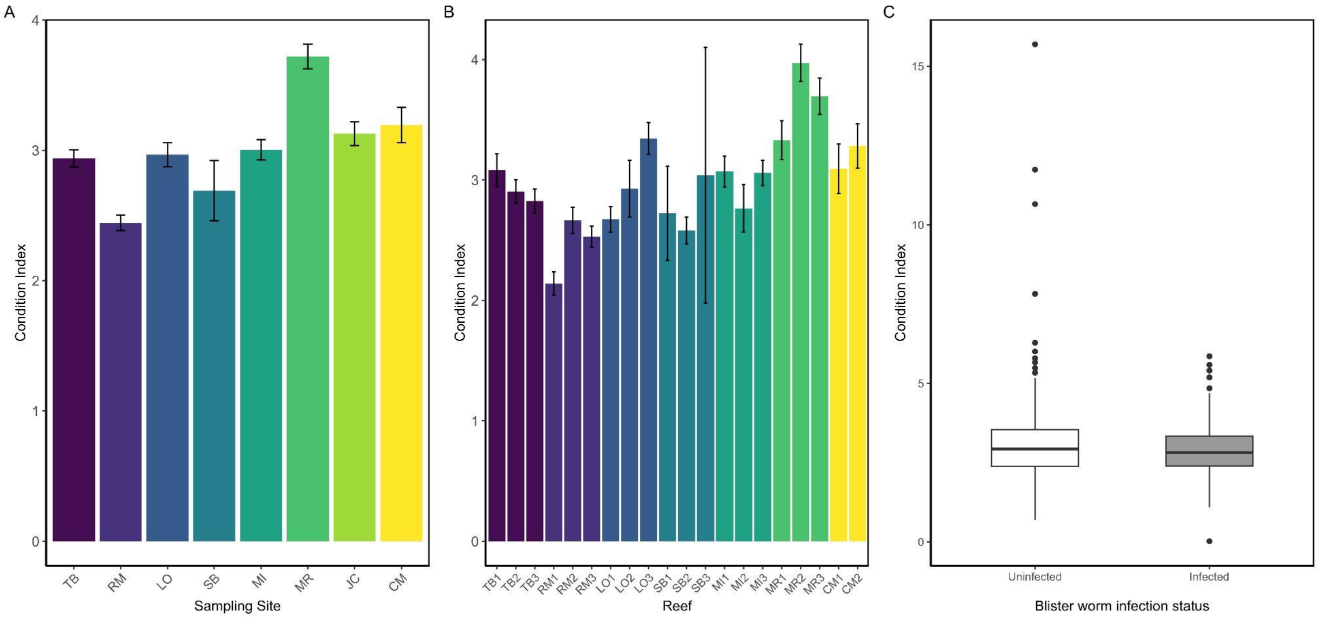

Figure 8: *(a) Site-level and (b) reef-level average oyster condition indexes across the eight sampling sites (mean condition ± SE). (c) Effects of blister worm infection status on condition index.*

and Camden ($p < 0.001$), but not Liberty-Sunbury ($p = 0.759$).

Within the sampling sites, two of the three reefs with the highest average per-reef condition were from the MacKay River, and the third was from Little Ogeechee (Figure 8B). The highest-condition reef, which had an average condition of 3.97, was also the reef with the second-highest prevalence (58.5%) of blister worm infections. In addition, the reef with the highest prevalence of blister worm infections of any reef sampled (70.8%) also had the fourth-highest average condition (3.33). Two of the three reefs with the lowest condition were from Romerly Marsh, and the third was from Liberty-Sunbury. None of these three reefs were also reefs in the top three proportions of blister worm infections.

DISCUSSION

We found that there was no significant difference in combined macroparasite distribution between sampling sites, as well in as the distributions of any of the individual parasites. The lack of wide-scale geographic variation within any of the parasite categories partially aligns with results from another parasite study on a similar scale, Byers et al. 2014, where pea crab prevalence was shown to vary between sites but did not exhibit a latitudinal gradient. Unlike in that study, though, we also did not find significant variation in the prevalence of any of the parasites between sites. Due to the very low observed prevalences of pea crabs and boring sponges, we were only able to examine the environmental factors that drive blister worm prevalence. However, the fact that blister worm prevalence did not vary significantly between sites but did vary between individual reefs due to salinity and reef rugosity indicates that environmental factors that vary on a smaller scale, especially between reefs in the same estuary, are more important drivers of blister worm prevalence than geographic location. Additional sampling at a lower tidal elevation to achieve sufficient numbers of infected oysters would be necessary to determine conclusively whether pea crabs and boring sponges are also primarily driven by reef-level factors instead of site-level factors, as well as to find more robust estimates for the prevalences of these two parasites at the sampling sites.

Due to sampling relatively high in the intertidal zone, pea crab and boring sponge infection prevalence was much lower than blister worm prevalence, leading to worm prevalence having an outsized influence on the combined data. As a result, the per-site prevalence for all parasites combined was primarily driven by the prevalence of blister worms. Pea crabs and boring sponges are generally more common farther down in the intertidal zone and exhibit lower prevalence in areas exposed to open air more frequently (Carroll et al. 2021), so lower levels of these two macroparasites were expected, albeit inconvenient for this arm of the study. Air exposure in the upper intertidal zone causes a major decrease in the prevalence of both boring sponges and pea crabs, which cannot tolerate prolonged periods out of the water, at higher tidal elevations (Hanley et al. 2019, Carroll et al. 2021). Unlike boring sponges, tidal elevation has only minor effects on blister worm prevalence (Ambariyanto and Seed 1991, Hanley et al. 2019), so they would be expected to be the most prevalent parasite higher in the intertidal zone. While the 5.4% maximum pea crab prevalence found in our survey aligns with some previous estimates (O'Beirn and Walker 1999) of pea crab prevalence on the Georgia coast, more recent studies have indicated that pea crab prevalence may be as high as 73% (Byers et al. 2014). This may have been because all of the oysters we examined for parasites in the lab were mature oysters that were over 30 mm long. Because pea crab prevalence is highest in spat and smaller, younger oysters (Christensen and McDermott 1958, Byers et al. 2014), which we did not use for parasite analysis, we would expect to see pea crab prevalence levels comparable to those reported in studies like O'Beirn and Walker 1999 that also only sampled mature oysters.

Patterns of blister worm infection intensity did not vary significantly between sites. At all of the sites, a majority of oysters were uninfected. Even when only the oysters with blister worm infections were taken into account, a majority of the infections were relatively low-intensity -- over half of the infected oysters at all sites had less than 50% blister worm cover, even at sites like the MacKay River that exhibited very high blister worm prevalence. In the Gulf of Mexico, blister worm infection intensity has also been shown to exhibit little to no geographic variation (Hanley et al. 2019). However, the same study also indicated that high blister worm infection intensity was correlated with decreased salinity, likely as a result of the increased density of worms in low-salinity areas. Increasing temperature, which had no effect on blister worm prevalence in our study, was also shown to be correlated with higher-intensity infection across the temperature range from 28 to 31°C (Hanley et al. 2019), a similar range to our study.

Blister worm prevalence was positively correlated with decreased reef rugosity. To our knowledge, few studies have examined the effects of reef rugosity on blister worm prevalence. One possible explanation for this relationship is that the decreased rugosity at MacKay River reefs increases the amount of food available to blister worms by encouraging increased water flow rates over the reef, making suspension-feeding action easier for blister worms and increasing their abundance and growth. Like most polydorids, blister worms are suspension feeders that filter particulate matter from moving water (David 2021). Several studies have demonstrated that suspension-feeding polydorid worms grow faster in habitats with higher flow rates due to increased food capture rates (Hentschel 2004, Hentschel and Herrick 2005). Rougher surfaces experience significantly decreased flow velocity directly adjacent to the surface (Chakroun et al. 1997, Styles 2015), so a less rugose oyster reef would be expected to have a higher flow rate close to the reef, where blister worms living on the reef are engaging in feeding action. Food availability for filter feeders is correlated with increased flow rates (Wilson-Ormond et al. 1997, Styles 2015). At flatter reefs, such as the ones located on the MacKay River, blister worms may therefore have more plentiful access to food that allows increased worm abundance and growth. Additionally, increased blister worm prevalence may have a mitigating effect on oyster growth that keeps overall reef rugosity low when blister worms are more abundant. High blister worm prevalence makes oyster communities, especially smaller oysters, more vulnerable to predation (Stefaniak et al. 2005, Ambariyanto and Seed 1991). At reefs with increased blister worm prevalence, smaller, younger oysters are therefore more likely to be infected and picked off by predators before they can grow significantly, preventing reefs from growing upwards and becoming more complex. Oysters that do survive, however, benefit from increased food provided by high flow rates over the flatter reef and decreased spatial competition from other large oysters (Marshall and Dunham 2013, Hanke et al. 2017), allowing them to grow larger and potentially accounting for the relatively large average oyster size at reefs on the MacKay River (Table A2) despite the overall low rugosity on the same reefs. Since oysters are also filter feeders, they benefit from increased flow around reefs increasing their food capture rate in a similar manner to blister worms (Wilson-Ormond et al. 1997). Shorter reef height does cause filtering to be less efficient, but only becomes problematic for food acquisition at densely packed reefs where oysters are competing for food access (Wilson-Ormond et al. 1997), which is unlikely to be an issue at the sparser reefs on the MacKay River. Oysters exhibit increased weights and growth rates in high-flow areas (Bishop and Hooper 2005, Lee et al. 2017). The combined effects of low reef rugosity benefiting blister worms and blister worms maintaining low reef rugosity could potentially reinforce each other, keeping rugosity low and blister worm prevalence high at sites like the MacKay River.

Blister worm prevalence was also high in lower-salinity areas. Several previous studies that have noted that blister worms are more prevalent in brackish, inshore waters than marine waters support this observation. While they cannot survive in pure freshwater, blister worms are able to survive salinities as low as 10 ppt (Brown 2012), compared to marine environments with salinities of over 30 ppt. Patterns of increasing worm prevalence in fresher water have been noted by several other studies (Hanley et al. 2019, Cole et al. 2020). Cole et al. 2020 speculated that increased freshwater outflow could drive increased blister worm prevalence in the Gulf of Mexico through increased siltation and turbidity from particulate matter washed into the ocean by streams and rivers. This would provide worms located closer to areas of increased outflow with a more plentiful source of food, since their diet primarily consists of particulate organic matter. Although we did not take direct turbidity measurements, turbidity is consistently high in estuarine areas along the Georgia coastline (Johnston et al. 1974, Alber 2000), meaning that particulate matter in the water from freshwater runoff may not account for the variation in blister worm prevalence observed between reefs in this region. However, since blister worms thrive in areas with increased water flow rates due to increased food capture rates (Hentschel 2004, Hentschel and Herrick 2005), if decreased salinity at the MacKay River is related to increased freshwater outflow from the river itself during the time period that we were collecting oysters, the increased rate of flow from the river could possibly also increase blister worm food capture rates by moving existing organic particulate matter to reefs at a faster rate than at sites with decreased freshwater outflow (Wilson-Ormond et al. 1997). To investigate whether the decreased salinity at the MacKay River is a result of increased outflow from the river at the time of our study, we would need to take additional salinity measurements, in conjunction with river outflow measurements, at each reef over a longer time period. We would also need to take long-term outflow and salinity measurements near our other sampled reefs to determine whether salinity at other sites was related to freshwater outflow from their respective creeks and rivers, and whether the MacKay River experienced outflow that was significantly higher than at the other sites.

Average site condition index differed significantly between sampling sites, but blister worm infection status was not a significant predictor of whether individual oysters exhibited reduced condition. Additionally, despite the high blister worm prevalence we observed at the MacKay River, that site also had the highest average condition overall; the oysters at the MacKay River appeared to be relatively unaffected, at least in terms of condition, by the high blister worm prevalence. This suggests that blister worm prevalence is unlikely to be the primary factor driving differences in condition, both in individual oysters and across the state as a whole. Several studies (Hanley et al. 2019, Diez et al. 2022) that have failed to find significantly reduced condition in blister-worm-infected oysters support this conclusion. As discussed previously, most sampled oysters infected with blister worms had relatively low-intensity infections, regardless of blister worm prevalence at the site -- even at the MacKay River, only 26% of oysters had blisters covering more than 50% of their shells. This may indicate that most of the sampled oysters did not have severe enough blister worm infections to affect their condition. Our results aligned with previous studies (Hanley et al. 2019) that did not find a correlation between high blister worm infection intensity and decreased condition. Oysters from the MacKay River, while not the largest overall, were also of fairly typical average size for this study, indicating that they are probably not experiencing stunted shell growth due to blister worms. MacKay River oysters had the fourth-largest average shell height out of the eight sampling sites, and were only 4 mm shorter on average than oysters from Camden, the site with the lowest blister worm prevalence (Table A2).

Increased flow velocity around reefs and decreased crowding due to low reef rugosity at the MacKay River could also benefit oyster condition, as well as growth rate. Decreased oyster density on less rugose reefs could reduce overcrowding-related stress on oysters, resulting in improved oyster condition. In simulated oyster farming operations, oysters stocked at high densities exhibit significantly decreased condition indexes compared to oysters stocked at low densities, although the oyster densities used in these studies are often far higher than those seen on natural oyster reefs (Marshall and Dunham 2013). In wild oysters, average oyster size has been shown to decrease at denser reefs, and oyster condition index increases towards the edge of reefs as opposed to in the more crowded centers of reefs (Hanke et al. 2017). In addition to directly promoting good condition, increased energy availability from improved access to food could help offset the drain on an oyster's energy reserves caused by a blister worm infection (Wargo and Ford 1993), helping to prevent condition reduction.

Condition index at the MacKay River may have possibly be artificially inflated by the condition index calculation that we used, but this is unlikely. The calculation we used, which was derived from Hanley et al. 2019, divides dry tissue weight by shell weight. While this method for calculating condition is relatively straightforward, the high prevalence of blister worm infections at the MacKay River could potentially mean that most of the oysters collected there had lost shell mass from worm burrows, which would lead to decreased overall shell weight and seemingly increased condition compared to a similarly-sized uninfected oyster. However, mud blister worm infection presence does not appear to have a major effect on dry shell weight, so any gains in perceived condition would likely be small. Blister worm burrowing has generally been observed to either have no effect on shell weight (Dorgan et al. 2021), cause only a minimal decrease in shell weight (Handley and Bergquist 1997), or only cause significant decreases in shell weight at high infection intensities (Royer et al. 2006). Because a majority of oysters at the MacKay River had relatively low-intensity infections, it is unlikely that a large proportion of oysters at the site would have lost enough shell mass from worm boring to significantly artificially increase the site's overall condition index.

Condition is also not the only possible measurement of individual oyster health, and while blister worms may not affect oyster condition, they may have health effects on oysters through other pathways. Other studies have examined metrics such as oyster growth rate (Chambon et al. 2007), oyster mortality (Bishop and Hooper 2005), immune response (Malek and Byers 2016), and oxidative stress (Chambon et al. 2007) as proxies for oyster health rather then condition. In particular, Chambon et al. 2007 connected infection by a closely related *Polydora* species to increases in oxidative stress indicators in infected oysters, such as increased opening frequency, decreased opening time, and hyperoxia-associated gene upregulation in circulatory tissue. The same study proposed that polydorid infection may primarily weaken oysters by causing them to hyperventilate, increasing oxidative stress on the oyster's circulatory system, instead of by directly affecting their condition by forcing them to waste energy constantly repairing their shells. Alternative effects of blister worm infection such as circulatory stress may have effects on oyster survival or reproductive success rates that are not apparent from using only reduced condition as an indicator of poor health.

Understanding the patterns of infection and health impacts of macroparasites on Georgia's oyster population is critical for the ongoing success of reef restoration efforts. Oyster habitat restoration on the Atlantic coast is an important piece of re-establishing biodiversity (Manley et al. 2010), ensuring the maintenance of proper nutrient cycling (Hurst et al. 2022), and protecting shorelines from erosion (Ridge et al. 2017). This study is crucial for helping to develop focused knowledge about where restoration efforts are more likely to succeed or fail due to the effects of macroparasite infections, which will be invaluable in developing future reef protection and restoration plans along Georgia's coast.

In addition, understanding how parasites respond to different environmental factors will be incredibly important as climate change accelerates, altering many physical characteristics of marine habitats. While studies on the specific effects of climate change on blister worms are rare, existing research has indicated that they may exhibit decreased hatching time but also increasing larval mortality (David 2021) as a result of increasing ocean temperatures. Although we failed to find a correlation between temperature and blister worm prevalence during this specific summer, Cole et al. 2020 reported that over a period of several years, increased summer water temperature was positively correlated with higher blister worm prevalence. Salinity's effects on blister worm prevalence are also especially pertinent to climate change. Even minor sea level rise from climate change has been shown to cause increasing saltwater intrusion from the ocean into coastal estuaries worldwide, causing higher-salinity zones to extend further inland (Barendregt and Swarth 2013). In Georgia, there has been a pattern of slow but steady sea level rise since the 1940s, with modern-day sea levels being on average 0.33 meters higher than 1940s levels ("Sea Level Trends - NOAA Tides & Currents"). Estuarine ecosystems in some areas of the Georgia coast are already experiencing saltwater intrusion due to sea level rise (Craft 2012). Combined with additional predicted increases in ocean salinity on the coastline of the southeastern United States due to changes in rainfall patterns (Alexander et al. 2020), which decreases freshwater outflow from rivers and raises the salinity of ocean water intruding into estuaries, climate change is likely to cause estuary salinity in Georgia to continue to increase. This may have positive effects on oysters, at least for some time, by decreasing blister worm pressure on oyster populations; conversely, it may increase the level of other parasites, such as boring sponges, which thrive in higher-salinity conditions (Hopkins 1962). It may also have more detrimental effects on the surrounding estuarine community as a whole, since increasing the salinity of the estuary may irreversibly change its aquatic species composition. Information about the potential drivers and outcomes of these environmental dynamics is highly beneficial for determining which ecosystems will require increased monitoring and protection in the face of climate change, and which ecosystems will potentially function more normally or even thrive.

APPENDIX

Table A1: Site location, latitude, and longitude of sampled reefs.

Site	Reef	Latitude	Longitude
Tybee	TB1	32.02171	-80.94308
	TB2	32.02171	-80.92203
	TB3	31.98741	-80.91755
Romerly Marsh	RM1	31.92759	-81.01943
	RM2	31.92879	-80.99755
	RM3	31.92217	-80.97818
Little Ogeechee	LO1	31.89519	-81.14262
	LO2	31.89197	-81.11729
	LO3	31.90686	-81.10466
Liberty-Sunbury	SB1	31.73083	-81.22192
	SB2	31.74574	-81.25545
	SB3	31.75607	-81.27174
McIntosh	MI1	31.47537	-81.32270
	MI2	31.49091	-81.33453
	MI3	31.53877	-81.32639
MacKay River	MR1	31.21492	-81.42349
	MR2	31.22319	-81.44549
	MR3	31.20721	-81.44522
Glynn-Jointer Creek	GJ1	31.08752	-81.48679
	GJ2	31.08360	-81.50299
	GJ3	31.05379	-81.46747
Camden	CM1	30.86197	-81.49571
	CM2	30.85055	-81.47381
	CM3	30.85826	-81.54015

Table A2: Mean, minimum, and maximum oyster heights (mm) by site. Oyster heights were measured from the umbo (base of the hinge) to the tip of the shell.

Site	Mean Oyster Height (mm)	Minimum Oyster Height (mm)	Maximum Oyster Height (mm)
Tybee	63	29	127
Romerly Marsh	68	32	117
Little Ogeechee	61	30	124
Liberty-Sunbury	52	30	91
McIntosh	60	32	115
MacKay River	64	26	105
Glynn-Jointer Creek	66	32	138
Camden	68	32	124

Table A3: P-values from pairwise Kolmogorov-Smirnov testing between sites.

	Tybee	Romerly Marsh	Little Ogeechee	Liberty-Sunbury	McIntosh	MacKay River	Glynn-Jointer Creek	Camden
Tybee								
Romerly Marsh	0.95							
Little Ogeechee	0.99	0.99						
Liberty-Sunbury	0.40	0.50	0.94					
McIntosh	0.69	0.58	0.21	0.12				
MacKay River	0.46	0.50	0.16	0.08	0.64			
Glynn-Jointer Creek	0.86	0.92	0.99	0.81	0.40	0.33		
Camden	0.93	0.84	1.00	0.99	0.89	0.68	0.98	

Table A4: T-values from pairwise Kolmogorov-Smirnov testing between sites.

	Tybee	Romerly Marsh	Little Ogeechee	Liberty-Sunbury	McIntosh	MacKay River	Glynn-Jointer Creek	Camden
Tybee								
Romerly Marsh	0.33							
Little Ogeechee	0.25	0.25						
Liberty-Sunbury	0.60	0.50	0.35					
McIntosh	0.50	0.50	0.75	0.80				
MacKay River	0.75	0.50	0.75	0.80	0.50			
Glynn-Jointer Creek	0.40	0.33	0.25	0.40	0.60	0.60		
Camden	0.50	0.50	0.25	0.30	0.50	0.60	0.40	

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