

Coexistence in the intertidal: Understanding relationships between mud snails (*Ilyanassa obsoleta*), parasitism, and the structure of soft-sediment ecosystems

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ABSTRACT

Parasite diversity and host-parasite relationships are often indicative of community diversity and ecosystem structure, especially when an organism like the eastern mud snail (*Ilyanassa obsoleta*) hosts nine distinct species of parasitic trematodes and is an ecologically influential macroconsumer. Individuals in the same parasite community can cause varying behavioral and physiological changes in snail hosts, and it is these modifications that could play a role in driving cascading direct and indirect effects onto ecosystems like seagrass beds and mudflats. This abundant gastropod host and its suite of trematode parasites represent a valuable study group to investigate potential indirect relationships between parasite diversity and the functioning and structure of these vital habitats. Two approaches assessing the direct and indirect impacts of this parasite component community were conducted from 2024 to 2025 in Bogue Sound and the Straits of North Carolina: (1) observational surveys of seagrass system inhabitants (trematode diversity in *I. obsoleta*, invertebrate community diversity, and shoalgrass (*Halodule wrightii*), and (2) a treatment-based field experiment in productive mudflats designed to assess how species-specific infection within *I. obsoleta* indirectly affects productivity and biodiversity properties. My results show how trematode infection correlates with abiotic and biotic variables, specifically water temperature, water depth, shell length, and salinity. These show unique connections from parasitic infection onto the structure of soft-sediment systems through a host.

For seagrass communities, I sampled *I. obsoleta*, *H. wrightii*, epibionts growing on *H. wrightii* blades, and seagrass-associated invertebrate taxa across seasonally replicated surveys

from eight sites across Bogue Sound and the Straits. I used this data to understand how patterns of trematode and invertebrate diversity fluctuated, what factors correlated with probability of infection by all trematodes, if those factors continued when assessing host-specific infection (i.e. birds versus fish), and what factors correlated with epibiont biomass. Trematode species richness decreased with rising water temperatures, reflecting negative correlations between temperature and infection probability from all species and fish-using trematodes. Infection probability from bird-using trematodes positively correlated with salinity and water depth, while probability from birds versus fish only negatively correlated with water depth. Epibiont biomass positively correlated with seagrass-associated taxa abundance, yet negatively correlated with shoot density, depth, snail density, and salinity. Such associations linked highly variable environmental conditions to patterns of parasite and seagrass communities within a complex environment. Further, these results demonstrate that multiple forms of biodiversity, especially parasites, are important to consider in investigations of seagrass communities and structure.

Within mudflat communities, I monitored changes in nutrient concentrations (chlorophyll-*a* and organic carbon) and meiofaunal diversity using experimental cages containing uninfected and infected *I. obsoleta* with treatments differentiated by trematode species. As part of future analysis and research, I am currently analyzing whether species-specific infection is behind nutrient variation and meiofaunal community composition.

Altogether, this study points to the importance of parasite diversity in complex ecosystems, especially where multiple parasites from the same component community infect a single host and thus individually and collectively impact and are affected by and abiotic and biotic variables. The identified cascading effects from trematode infection in *I. obsoleta* provide opportunity for future investigation on parasite-induced indirect effects.

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LIST OF ABBREVIATIONS

1. **HQ:** *Himasthla quissetensis*
2. **AV:** *Austrotilharzia variglandis*
3. **GA:** *Gynaecotyla adunca*
4. **DN:** *Diplostomum nassa*
5. **LS:** *Lepocreadium setiferoides*
6. **ZL:** *Zoogonus lasius*
7. **SD:** *Stephanostomum dentatum*
8. **ST:** *Stephanostomum tenue*
9. **PM:** *Pleurogonius malaclemys*
10. **JL:** Jones Landing
11. **MP:** Manatee Street Park
12. **ML:** Mary Lane
13. **MM:** Miramar
14. **OH:** Oak Hammock
15. **PKS:** Pine Knoll Shores
16. **SP:** Sunny Point
17. **IMS:** University of North Carolina: Institute of Marine Science
18. **nMDS:** Non-metric multidimensional scaling plot
19. **PERMANOVA:** Permutational Analysis of Variance
20. **ANOSIM:** Analysis of Similarities
21. **SIMPER:** Similarity Percentage
22. **Avg.:** average

- 23. **PCoA:** Principal Coordinate Analysis
- 24. **GLMM:** Generalized linear mixed model
- 25. **AICc:** Corrected Akaike Information Criterion
- 26. **DOY:** Day of year
- 27. **THRO:** Theodore Roosevelt State Natural Area
- 28. **DUML:** Duke University Marine Lab
- 29. **ERL:** East Carolina University Environmental Research Laboratory
- 30. **SOP:** Standard operating procedure
- 31. **LOI%:** Loss of Ignition (%)
- 32. **U:** Uninfected snail treatment
- 33. **CC:** Caged-control treatment
- 34. **UC:** Uncaged-control treatment

PREFACE

A fundamental goal in community ecology is to understand the manners at which parasites shape communities and the coexistence between them and their hosts. Being ubiquitous organisms, parasites have been increasingly recognized as important high-level consumers that can alter abundance, size structure, or behavior of their hosts (Dobson 1988; Curtis 2007; Wood et al. 2007; Clausen et al. 2008; Poulin 2010; Soghigian et al. 2017; Buck 2019). In the study of biodiversity and ecosystem functioning, parasites and their impact on these vital community components have been considered minimally, especially when addressing multiple species under a single host versus a single parasite species. For instance, Thomas et al. (1998) considered how a parasitic trematode manipulated behavior of cockles, leading to habitat creation for associated invertebrate species. In another case, nematomorph parasites induce cricket suicide in streams, providing food sources for trout while achieving reproduction (Sato et al. 2012). These examples indicate how one species of parasite can have ecosystem-level effects when infecting key hosts in food webs, as well as the value of considering the connection between parasitism and biodiversity (Frainer et al. 2018). This also addresses the general importance of including parasites in food webs, as they are commonly left out of. Despite their known importance, parasites are often neglected in community ecology investigations due to the difficulty in quantifying them using standard ecological methods and the task of disentangling complicated host-parasite networks (Christoffersen et al. 2011). However, inclusion of parasites should become a standard protocol due to their prominent roles in ecological networks through augmentation of energy flow and changing productivity in hosts (Christoffersen et al. 2011), as well as causing an increase in species richness (Lafferty et al. 2006).

Incorporating parasites in food webs can become even more complicated when parasites themselves are diverse, specifically when a host is affected by multiple species. Parasite diversity can be particularly indicative of host diversity and food web structure by association (Lafferty et al. 2006). For example, a strong connection stands between multiple bird species that serve as definitive hosts to 19 known trematode species in the Carpinteria salt marsh food web with a gastropod *Cerithidea californica* as the first intermediate host (Hechinger and Lafferty 2005). In addition, *Littorina littorea*, a highly abundant gastropod on the northern Atlantic coast, acts as a host to a large diversity of digenean trematode species that each alter *L. littorea* feeding behavior to different degrees, likely causing consumption-related indirect effects onto ecosystem structure (Wood et al. 2007; Byers et al. 2008; Clausen et al. 2008). When hosts are ecologically influential macroconsumers and are influenced by a parasite component community (i.e., all parasite species exploiting a host population), the parasite community can have cascading effects on ecosystem structure and functioning.

The eastern mud snail (*Ilyanassa obsoleta*) is an ecologically influential macroconsumer that is an abundant member of mudflat and saltmarsh communities along the eastern seaboard (Gulf of St. Lawrence, Canada to Florida), forming dense aggregations (up to 1000-1500 m⁻²), and foraging on microorganisms (e.g. bacteria, blue-green algae and diatoms), carrion, and macroalgae (Pace et al. 1979; Curtis 2002, 2005; Kelaher et al. 2003; Blakeslee et al. 2012). The snail is also a first intermediate host to nine known trophically transmitted, digenean trematode parasites (Lauckner 1987; Curtis 1997, 2002; Altman and Byers 2014). Trophic transmission simply refers to organisms in a prey host transmitted to the next host in its life cycle via predation all without killing such host (Buck 2019). Parasitic infection is common in *I. obsoleta*, with prevalence ranging from 15.5-37.8% (Altman 2010) to 8.7-78.9% (Curtis 1997),

respectively, along the eastern coast of the United States. Each *I. obsoleta* trematode has a different complement of downstream second intermediate and definitive hosts. Mud snail infections consist of asexually produced sporocysts, rediae, or both, and their asexually produced cercariae (Curtis 2009). Cercariae represent a free-swimming stage that ranges in morphological characteristics per trematode species, and these characters can be used to assist with species identification upon mud snail dissection (Blakeslee et al. 2012). Trematodes *Himasthla quissetensis* (HQ), *Austrobilharzia variglandis* (AV), *Gynaecotyla adunca* (GA), and *Diplostomum nassa* (DN) use birds as definitive hosts, while *Lepocreadium setiferoides* (LS), *Zoogonus lasius* (ZL), *Stephanostomum dentatum* (SD), and *Stephanostomum tenue* (ST) use fish as definitive hosts, and lastly, *Pleurogonius malaclemys* (PM) uses terrapins as definitive hosts (Curtis 2007, 2009; Altman 2010). Infection strategies involving diverse host cycles of individual trematode species may differ dramatically, leading mud snail hosts to experience highly specific modifications of consumption, movement (e.g. carrion response, locomotion), and distribution (e.g. site fidelity) behaviors (Clausen et al. 2008; Curtis 1985, 1987, 1990, 1995, 2002, 2005, 2007; Nadzam et al. *in prep*; Rossiter and Sukhdeo 2012). It is these modifications in mud snail behavior that could be driving direct and indirect effects within mudflat and seagrass habitats.

I. obsoleta resides in soft sediment intertidal systems along the Atlantic coast of the United States, including mudflats and seagrass beds rich in nutrient levels. These habitats create coastal structure, nutrient cycling, and enhanced living space and food for hundreds of species including myriad infaunal and epifaunal organisms (Best and Stachowicz 2012; Hovel et al. 2002). In seagrass ecosystems, *I. obsoleta* are known to facilitate shoal grass (*Halodule wrightii*) and eelgrass (*Zostera marina*) growth through the consumption of epiphytic microalgae,

bacteria, microfauna, and detritus (otherwise known as “epibionts”) that foul individual seagrass blades, outcompeting grasses for light and limiting nutrient uptake from the water column (Kinlan et al. 1997; Lewis and Boyer 2014). Multiple studies have addressed the direct influence of epibiont and epiphyte biomass upon seagrass productivity through grazer activity (e.g. Penhale 1977; Kinlan et al. 1997; Mattila et al. 1999; Voigt and Hovel 2019). Fauna other than *I. obsoleta* also graze on grasses and epibionts within such beds, and still others use seagrass for habitat and protection. Thus, to understand the influence of this fauna within seagrass communities, it is important to also survey for the abundance and diversity of seagrass-associated taxa, particularly those serving as epibiont grazers (Hovel et al. 2002). This also includes sampling and analyzing different species within a parasite component community within one host and the direct effects happening between them. However, little effort has gone towards parasite-induced indirect effects differentiated by species.

These indirect effects stemming from parasitism are not frequently considered when evaluating community structure. Even though parasitism comprises the most common lifestyle among animals, we know little about the indirect consequences of parasite abundance and diversity on community dynamics (Christofferson et al. 2011). Assessing trophically transmitted parasites is especially difficult due to some parasites requiring one or multiple hosts to complete life cycles (Buck 2019). Indirect effects stemming from trophically transmitted parasites are formally referred to as trait-mediated indirect effects (TMIE): indirect interactions where one species (i.e. the parasite) alters the phenotype (i.e. trait) of another species (i.e. the host), thereby changing its effect onto another species (i.e. receiver) (Buck 2019). TMIEs caused by parasites are known to influence the consumptive and nonconsumptive traits or behaviors of a host, and such changes can cause fluctuations in host populations and promote biodiversity in a

surrounding system (Wetzel et al. 2016; Buck 2019). The logical and ordered sequencing of effects on how a system responds to interventions, actions, or perturbations is referred to as a ‘chain of causation’ with each event being linked to the next with a direct or indirect relationship (Qui et al. 2018). Most work has focused on single host-single parasite interactions to inform our current understanding of the chains of causation occurring in ecosystems (Wetzel et al. 2016, Richard et al. 2021). Here, I will focus on the parasite component community of *I. obsoleta* and its nine trematodes within intertidal mudflats and seagrass beds.

My work in this study system is divided into two chapters. In Chapter One, I examine the component community of trematode parasites in *I. obsoleta* to address multiple knowledge gaps in our understanding of host-parasite influences on seagrass communities. To this end, this chapter investigated four research questions, including: (1) what factors coordinate with patterns of trematode species diversity in *I. obsoleta* populations in Bogue Sound and the Straits?; (2a) what abiotic and biotic factors correlate with the probability of trematode infection (all trematode species) in *I. obsoleta* in Bogue Sound and the Straits; (2b) what abiotic and biotic factors correlate with probability of infection from bird-using trematode species versus fish-using species in *I. obsoleta* in Bogue Sound and the Straits?; (3) what abiotic and biotic factors correlate with epibiont biomass on *H. wrightii* blades in Bogue Sound and the Straits?; and (4) what factors correlate with patterns of diversity of seagrass-associated invertebrate taxa in beds of *H. wrightii* in Bogue Sound and the Straits? For this chapter, I present a complete examination of my questions posed above, including introduction, methods, data analyses, results, discussion, and conclusions.

In Chapter Two, I address the influence of trematode infection on mudflat system functioning through experimental treatments, with the following research question: How does

species-specific infection within *I. obsoleta* affect productivity and biodiversity properties within mudflat ecosystems? This chapter represents research in progress, and here, I present my introduction, methods, and future directions.

CHAPTER ONE

INTRODUCTION

North Carolina ecosystems are enduring ongoing impacts from climate change and human activity, requiring investigations into species persistence and biodiversity in seagrass and mudflat habitats. Seagrass beds of North Carolina (NC) are particularly vital habitats, as they reside in the biogeographic transition zone between temperate and tropical bioregions (Stevenson et al. 2025). Seagrass systems are under threat due to multiple human impacts, including habitat destruction and degradation, pollution, and invasive species (Mallin et al. 2000; Flowers et al. 2024). Further, as global climate change is known to increase water temperatures as well as sea levels, allowing existing seagrass habitats to be redistributed or cause die-off, anthropogenic activities such as trawling, boating, and nutrient over-enrichment of coastal waters also intensely impact seagrass habitats (Short and Coles 2001). In NC, seagrass areal extent was shown to decline at a rate of 1.5% per year between 2007 and 2013 and become patchier due to temperature driven losses of *Z. marina* biomass (Field et al. 2020; Bartenfelder et al. 2022). While playing pivotal roles in sediment accumulation and stabilization (Short and Coles 2001; Short et al. 2007; Lewis and Boyer 2014; Masselink et al. 2024) and habitat for diverse fauna (Webster et al. 1998; Mattila et al. 1999; Short et al. 2007; Lewis and Boyer 2014; Best and Stachowicz 2012), *Z. marina* is dominant in winter to spring seasons while *H. wrightii* is more prominent from summer to early fall. Temperatures >28-30 °C cause heat stress on *Z. marina* while similar temperatures, specifically 25-35 °C, promote increased biomass for *H. wrightii* (Bartenfelder et al. 2022; Stevenson et al. 2025). As coastal waters of NC oscillate between temperate and tropical characteristics, these conditions still permit growth of *Z. marina*, even on the edge of seasonal stressful water temperatures >28-30 °C with positive influence of water clarity and meadow area stability (Bartenfelder et al. 2022). For example, Bartenfelder et al.

(2022) observed meadow biomass and area of *Z. marina* versus *H. wrightii*, finding the former to be largely absent from NC seagrass meadows from August to September 2019, months where water temperatures are still optimal for the latter. While *Z. marina* becomes heat-stressed in warmer months, water clarity and meadow area stability are due in part even during winter months to *H. wrightii* density not thinning (i.e. decreased shoot density) (Bartenfelder et al. 2022; Stevenson et al. 2025). With that, the thermal tolerance of *H. wrightii* is predicted to eventually result in species replacement because of rising coastal water temperatures in NC (Wilson and Lotze 2019), likely allowing seagrass habitat sustainability to persist.

The abundance and distribution of organisms in seagrass beds across time and space has been linked to change in habitat structure and functioning in multiple studies (e.g. Fonseca and Bell 1998; Beck et al. 2001; Hovel et al. 2002; Short et al. 2007; Best and Stachowicz 2012). The diverse range of organisms using seagrass beds as habitats and nurseries can be connected to a variety of services, such as fish that consume smaller vertebrates, crustaceans, and mesograzers (i.e. small herbivorous invertebrates ≤ 2.5 cm) (Beerman et al. 2018), sediment stabilization (Newell and Koch 2004), and productivity (Penhale 1977). This can be connected to parasite distribution and successful transmission from free-living host to free-living host, as Marcogliese (2002) found that helminth parasite species composition changes in heavily exploited areas to reflect modifications in fish and invertebrate communities. Further, major contributors to diversity (i.e. clams, snails, worms, crabs, amphipods, seagrass species) in soft sediment systems are hosts for micro- and macroparasites (May and Anderson 1979; Dobson and Hudson 1986; Sousa 1991). Therefore, parasitic species within the same component community are likely heavily influenced by the diversity of hosts needed to complete their life cycles within seagrass ecosystems. Relationships between species in seagrass beds could influence the presence of

parasite diversity infecting *I. obsoleta* living in beds of *H. wrightii* and the indirect influences parasites have on the habitat their host is intertwined with. Therefore, it is important to study how and to what extent parasites can indirectly influence these vital ecosystems, as climate change is expected to strongly affect parasite abundance (Harvell et al. 2002; Richard et al. 2021).

Regarding my study species, *I. obsoleta*, the gastropod densely aggregates amongst saltmarshes and seagrass beds, using the system as habitat and source of food including microalgae, detritus, and the seagrasses themselves (Curtis 2005; Best and Stachowicz 2012). Meanwhile, the larval stages of the parasite component community infecting *I. obsoleta* can be found suspended in seagrass beds by baffling action, leading to higher abundances of infauna and greater potential for invertebrates inhabiting grasses to become infected by trematodes (Sousa 1991). Such abundances of diversity in parasitic trematodes can add to biodiversity and production of an ecosystem as previously mentioned, further contributing to the understanding of how parasitism overall augments the flow of energy and causes trophic cascades in food webs (Christoffersen et al. 2012). Designing hypotheses around *H. wrightii* and its surrounding intertidal community are beneficial, as this species is becoming the more prominent species in my study system during the months I sampled, due to the higher water temperatures and overall biomass of *H. wrightii* remaining steady in NC coastal waters from 1979-2019 (Bartenfelder et al. 2022).

As little research addresses the chains of causation set off by parasite diversity or even consequences of parasite diversity, my 2021 field experiment (Nadzam et al. *in prep*) provides preliminary evidence for the influence certain species of trematodes can have on grazing behaviors of gastropod hosts. In comparison to previous work focusing on individual host-

parasite interactions, this work involved examination of roles played by multiple parasites individually and simultaneously. Trematode infection prevalence of HQ, LS, and ZL were manipulated in mud snail hosts within multiple treatments deployed in seagrass beds of *H. wrightii*. The study found HQ and LS, within mud snails, indirectly promoted seagrass growth by changing the behavior of the snails which increased their epiphyte consumption from seagrass blades (Nadzam et al. *in prep*). These findings support previous work on how parasitism can increase consumption behavior in first-intermediate gastropod hosts, with higher amounts of epiphytes consumed leading to higher rates of seagrass productivity and shoot extension (Kinlan et al. 1997; Jaschinski and Sommer 2008). While this work begins to help us understand how parasites can indirectly affect ecosystem structure through one chain of causation, it did not explore the ramifications of the natural prevalence percentages of parasitism on the observed seagrass embayment in North Carolina (Nadzam et al. *in prep*). As a result, it is important to determine whether the impacts on snail consumption behavior observed in this field experiment at one site would be broadly observed during field surveys across multiple sites and with season. In other words, with parasite prevalence and diversity likely fluctuating interannually per season and site, the chains of causation and any quantified indirect effects will inevitably be different across time and space.

The relationship between *I. obsoleta* and its parasite-component community within the *H. wrightii* seagrass habitat is important to examine, as indirect effects resulting from this relationship could mitigate growth of epibionts depending on the trematode species infecting *I. obsoleta* at a single site (Nadzam et al. *in prep*). Snail consumption of epibionts could be occurring in additional seagrass beds other than the one bed analyzed in Nadzam et al. (*in prep*). Assessing variation of parasite diversity in multiple beds of *H. wrightii* could provide more

evidence of how a macroconsumer such as *I. obsoleta* is heavily influential on seagrass ecosystem functioning and structure.

Research Questions and Hypotheses

Here, I investigated the diversity of parasitic trematodes within populations of their first intermediate host, *Ilyanassa obsoleta*, and the direct and indirect connections this parasite-host relationship has with the abiotic and biotic factors driving seagrass (*Halodule wrightii*) ecosystem functioning and structure. My study addressed the following research questions:

Research Question 1 (Q1): What factors coordinate with patterns of trematode species diversity in *I. obsoleta* populations in Bogue Sound and the Straits?

Research Question 2A (Q2A): What abiotic and biotic factors correlate with the probability of trematode infection (all trematode species) in *I. obsoleta* in Bogue Sound and the Straits?

Q2B: What abiotic and biotic factors correlate with probability of infection from bird-using trematode species versus fish-using species in *I. obsoleta* in Bogue Sound and the Straits?

Regarding Q1, in coordination with trends of rising temperatures from seasons spring to fall (Mallin et al. 2000; Bartenfelder et al. 2022; Stevenson et al. 2025), I hypothesized (**H1.1**) that the diversity of parasites infecting *I. obsoleta* occupying seagrass beds of *H. wrightii* would increase with temperature, specifically that warmer temperatures would correlate with greater trematode species richness. As this study was observational and only took place over two years, we can only make inferences about the patterns across replicated, seasonal sampling of parasite diversity. Therefore, if patterns of diversity are consistent over time, there is likely a seasonal effect on parasite diversity, as in temperate systems, changing temperatures over the course of a year tend to correlate with seasonal patterns of diversity. Climate change and global warming are expected to significantly alter parasite transmission dynamics from season to season, increasing

or decreasing infection levels and/or richness of trematodes in most ecosystems depending on the severity of factors such as temperature (Poulin 2006). Features of trematodes such as size, morphology, or numbers of transmission stages produced result from trade-offs among several factors, ranging from resource availability (i.e. snail size) and second intermediate and definitive host identity (abundance, mobility, etc.) to environmental factors such as temperature (Selbach and Poulin 2020). Temperature-mediated changes in the output of the cercarial life stage can vary widely among trematodes, from small reductions to 200-fold increases in response to a 10 °C rise in temperature, with this output likely occurring in temperature-regulated pulses (Poulin 2006). In addition, *I. obsoleta* gain sexual maturity once reaching ~12-14 mm in shell length, typically during their third summer (Curtis 1995; Blakeslee et al. 2012). Larger (older) snails (≥ 19 mm) are also seen to be more infected than younger (smaller) snails (Curtis 1995), most likely for older snails to have been exposed longer to an environment inhabited by parasites (Lafferty 1993; Curtis 2002). With that, regarding question 2A, I hypothesize (**H2.1**) that greater quantities of larger snails (> 14.00 mm) to be infected in comparison to smaller snails (< 14.00 mm). I also expect more infected snails to be found in time periods with higher water temperatures.

Similarly with question 2B (**H2.2**), the probability of infection from specific trematode species will also likely be influenced by snail size and temperature, and then specifically whether those species use birds or fish as their definitive hosts. Trematode species that are more abundant in sampled snail populations are representing that habitat's population of *I. obsoleta* in that season and year, leading to assumptions about what second intermediate and definitive hosts are present for successful parasite reproduction and infection (i.e. fish versus birds). Generally, parasites need to be in the environment of where its host is most likely to be, leaving a better chance for the parasite to infect its host in a favored location (Combes 2005). How the larval

stage of digenean trematodes, miracidium, enter the environment can greatly increase or decrease the probability of infecting a snail host, specifically if the egg and larval stages are being deposited by a bird, fish, or terrapin (Combes 2005). While dispersal of trematode eggs from definitive hosts is unpredictable and can be from site to site (Curtis 2002), reflecting on parasite life cycles can help evaluate differences between trematodes once they have infected a snail to understand which species has a higher infection prevalence in a sampled snail population (Combes 2005). For instance, LS, a fish-using trematode, is one of the most numerically dominant species comprising the “core” members of the parasite component community of *I. obsoleta* (Curtis 1997). Thus, more prevalent species like LS could lead to a higher probability of infection in *I. obsoleta*.

Research Question 3: What abiotic and biotic factors correlate with epibiont biomass on *H. wrightii* blades in Bogue Sound and the Straits?

With primary productivity of seagrasses and microalgae increasing with increasing water temperatures from spring through summer to early fall (Mallin et al. 2000), I hypothesized (**H3**) that high parasite prevalence in *I. obsoleta* would correlate with low epibiont biomass. This hypothesis is supported by preliminary results by Nadzam et al. (in prep) suggesting that LS infection of *I. obsoleta* increased consumption of epiphytes and indirectly regulated epiphyte biomass on *H. wrightii* blades (Nadzam et al. *in prep*). I also expect greater quantities of average blade length and average shoot density of *H. wrightii* to correlate with high epibiont biomass. This would also be in coordination with the higher primary production granting increased shoot densities and epibiont biomass found growing on seagrass blades seen previously in warmer months (Mallin et al. 2000).

Research Question 4: What factors correlate with patterns of diversity of seagrass-associated invertebrate taxa in beds of *H. wrightii* in Bogue Sound and the Straits?

In addition to *I. obsoleta*, other grazers and non-grazers will be found associated with seagrass habitat. I hypothesized (**H4**) that the majority of identified seagrass-associated invertebrate taxa would be made up of crustaceans and molluscs, particularly amphipods, shrimps, gastropods, and copepods, due to their high mobility, reproduction rates, and overall influence on macrophyte composition in benthic communities (Brawley and Adey 1981; Coull 1999; Montagna 1995; Duffy and Hay 2000; Jaschinski and Sommer 2008; Beerman et al. 2018; Voigt and Hovel 2019). I expected change in taxa diversity to positively correlate with changes in abiotic and biotic factors, as seen in previous studies (e.g. Beerman et al. 2018; Brawley and Adey 1981; Coull 1999; Duffy et al. 2015). Greater abundance of mesograzers and/or non-grazers could also correlate with growth of food sources (i.e. epibiont biomass) on the seagrass, thus directly affecting presence and abundance of specific taxa.

MATERIALS AND METHODS

Study System

The Albemarle-Pamlico Sound ecosystem supports two million acres of estuarine waters containing mudflats and submerged aquatic vegetation (Albemarle-Pamlico National Estuary Partnership 2012). This large estuary is composed of multiple interconnecting sounds, one of which being Bogue Sound where much of this work is based (Albemarle-Pamlico National Estuary Partnership 2012). This setting provided a variety of habitat types (i.e. natural shorelines, artificial shorelines, boat ramps) inhabited by our study species, *Ilyanassa obsoleta*, within its native geographical distribution along the Atlantic coast of North America (Cranford 1986; Blakeslee et al. 2019). Initial observational surveys of habitat and biodiversity in the Bogue,

Back, and Pamlico Sounds of the Albemarle-Pamlico estuary began in March 2024. Out of 17 sites visited, site locations were narrowed down to a total of eight for a representation of biodiversity in seagrass beds in Bogue Sound and the Straits of NC. an overall representation of the area. These sites were chosen based on the following criteria: presence of *H. wrightii*, populations of *I. obsoleta*, seagrass-associated taxa, and accessibility by foot. Five of the eight sites abut homeowner properties with artificial oyster restoration reefs or boat ramps (JL, MP, ML, OH, SP) and three alongside university-owned, private properties designated for field research (MM, PKS, IMS) (Table 1, Fig. 1).

Table 1. Site locations. This table lists each site's name and abbreviation that corresponds with latitude and longitude coordinates in the Albemarle-Pamlico estuary.

Site	Coordinates
Jones Landing (JL)	34°43'11"N 76°55'21"W
Manatee Street (MP)	34°40'48"N 77°03'54"W
Mary Lane (ML)	34°43'38"N 76°52'08"W
Miramar (MM)	34°43'26"N 76°32'57"W
Oak Hammock (OH)	34°42'39"N 76°33'52"W
Pine Knoll Shores Aquarium (PKS)	34°42'12"N 76°49'29"W
Sunny Point (SP)	34°43'01"N 76°55'50"W
University of North Carolina: Institute of Marine Science (IMS)	34°43'19"N 76°45'38"W

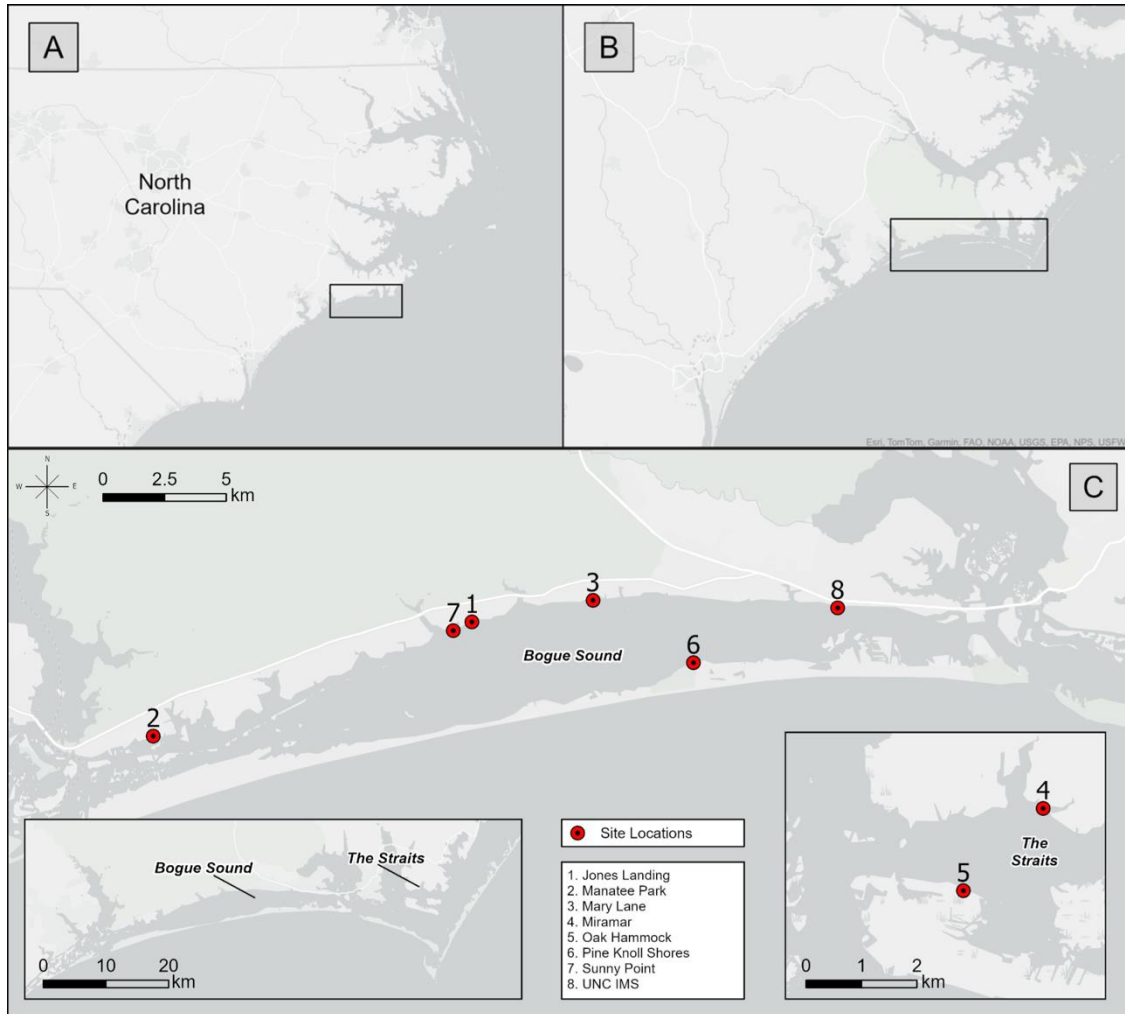


Figure 1. Map of study sites. (A) The general location of Bogue Sound and the Straits in North Carolina. (B) The extent of Bogue Sound and the Straits in relation to the surrounding Albemarle-Pamlico estuary and North Carolinian coastline. (C) Specific study sites are depicted with red markers and numbers based on their location and name ($n = 8$).

Experimental Design

This was a two-year study (2024-2025) designed to capture community diversity at multiple sites throughout the areas of Bogue Sound and the Straits, NC. Sampling was structured to encompass entire years and seasons during which *I. obsoleta* were active in the intertidal. It is well known that *I. obsoleta* migrate from the intertidal into deeper waters for the late fall to winter months (November – February in a temperate region) (Batchelder 1915, 43-46; Stambaugh and McDermott 1969), so sampling did not occur in these time periods. Sampling

began in April of 2024 and concluded in October of 2025 with each site sampled at least once per year. This was done to best capture the range of environmental conditions and variation that was present each year. Two sites, Manatee Park (MP, no. 2) and UNC IMS (IMS, no. 8) were added after May 2024 to expand the geographical range of the study, resulting in fewer replicates for these two sites across both years (Fig. 1). Preliminary results from the 2024 seasons were also used as a baseline for data analysis modifications, as well as overall cumulative comparison between 2024 and 2025 seasons. All sampling took place during low tide (< 0.2 m) for best access to study species.

Field Sampling: Seagrass and Habitat Characteristics

As I was interested in how communities affiliated with *H. wrightii* would change over seasonal-temporal factors, I used previously established protocols (Moorman et al. 2017; US Geological Survey 2012) as a baseline to derive my own methodology to characterize habitat characteristics in terms of: (1) seagrass shoot abundance and density, (2) *I. obsoleta* population abundance and density, and (3) benthic seagrass-associated invertebrate diversity sieving with a Rose Bengal solution.

Initial observations at each site identified stretches of soft sediment intertidal containing patches of seagrass and snail aggregations that extend offshore. One stretch was randomly selected along the shoreline, extending 24-m from the high tide line into the water. *H. wrightii* shoot abundance was counted within a 0.5 m^2 quadrat ($n = 5$ replicates) with quadrat placement randomized along the transect. Post-sampling, average seagrass shoot density per survey was then calculated to fit a 1-m quadrat. Randomization of quadrat placement along the 24-m transect allows an unbiased estimate of seagrass shoot abundance at a particular site (i.e. if no shoots were found within a placed quadrat, shoot abundance was recorded as zero). Shoot abundance

was hand-counted by the number of individual shoots intact in the sediment within each quadrat. Any dead seagrass blades were carefully removed from each quadrat prior to counting shoot abundance to avoid false data. Within each quadrat, at least five blades of *H. wrightii* present were snipped with shears just above the sediment line and placed into sample bags labeled with the corresponding quadrat number, site name, season, and date. Distance between each quadrat (m) was also recorded via transect, as well as water depth at each quadrat location (cm). Water temperature (°C) and salinity (ppt) measurements were collected using a handheld YSI probe at the first quadrat's location. The YSI probe was fully submerged in water before recording the measurements.

In different 0.5-m² quadrats positioned every 6-m along a 24-m transect set at the highest point in elevation where *I. obsoleta* were found, I quantified *I. obsoleta* population abundance with the first recording at 0 m (n = 5 quadrats). Post-sampling, average snail density per survey was calculated as the number of snails per m². The starting point of each transect was randomized, extending down shore elevation near or within seagrass beds. I aimed to collect >100 snails per habitat survey using active sampling (hand-collecting) to gain a population's natural range of shell size and infection prevalence. Snails were collected haphazardly in and around the seagrass beds to gain a wide representation of the snail population. *I. obsoleta* snails smaller than 10-mm were not collected due to infection status being more likely in larger (older) snails with longer exposure time to parasites in their habitats, and because snail gonad development needs to occur for infection to be successful (Lafferty 1993; Blakeslee et al. 2012). Snails were transported to the lab and dissected (see parasite dissection protocol below).

Seagrass-associated invertebrate taxa samples were collected in each seagrass sampling quadrat (n = 5 per site). Before recording water depth and while the quadrat was submerged, I

used a small mesh aquarium net (1-mm, 14.5-cm width) to collect taxa on, in, around the seagrasses in the quadrat. The mesh net was swept in a zig-zag pattern across the quadrat three times without digging down into the substrate. The sample was then deposited into a plastic container with 25 mL of 70% isopropyl ethanol and seawater, each labeled with quadrat number, site name, season, and sample date. Mesh nets were rinsed thoroughly in between each quadrat. These samples were brought back to the lab for taxonomic sorting (see Lab Processing for Seagrass-associated taxa).

Lab Processing for Seagrasses

Seagrasses were transported on ice to East Carolina University (ECU) and frozen (-80 °C) until laboratory processing. Samples were thawed prior to processing by briefly soaking the sealed sample bags in warm tap water. To calculate seagrass and epibiont metrics, we adapted protocols used in Nadzam et al. (in prep). Five random blades were selected from a sample and scraped of epibionts gently using a sterilized metal razor onto pre-weighed (g) microfiber filters. Both the sides and edges of each seagrass blade were scraped to remove all possible epibionts present. *I. obsoleta* eggs were observed on seagrass blades upon scraping. *I. obsoleta* have shown preference for egg deposition on seagrasses (Harmon and Allen 2018), but since no research has yet investigated how the survival of seagrass is impeded by *I. obsoleta* eggs, all eggs were carefully removed and not scraped onto the filters to add weight to overall measurements. Filters were placed in labeled, pre-weighed aluminum weighing dishes (volume = 42 ml). Scraped blades were measured by length to the nearest mm and placed in labeled, pre-weighed aluminum foil envelopes (g). Both filters and blades were dried in a drying oven until a constant weight at 70 °C (~72 hrs.). Samples were transported to an analytical balance from the oven in a glass desiccator to prevent inaccurate weight measurements. Epibiont biomass (g) per seagrass sample

was then determined to the nearest 0.0001-g using an analytical balance. Average epibiont biomass (mg/mm) found on seagrass blades for a particular habitat survey was calculated as epibionts (mg) per blade length (mm).

Lab Processing for Parasites

Prior to dissection, I recorded the size (mm) of the snail measured as the distance from the bottom of the aperture to the apex of the snail's spire using digital calipers. Following this, each snail was dissected using a hammer, with forceps to remove the gonadal and intestinal region from within the snail's spire, which is where trematodes of *I. obsoleta* concentrate (Blakeslee et al. 2012). The gonads were pulled apart using forceps and studied under a dissection microscope (1-5X) for parasites. Positive cases were recorded along with the species identification of the parasite. Species identifications were made by analyzing the cercariae (larval stage) and sporocysts or rediae of the parasite and using morphological traits to arrive at an identification by referencing several sources: Altman (2010), Blakeslee et al. (2012), Phelan et al. (2016), Stunkard (1973), Stunkard (1983), and Curtis (2007).

Lab Processing for Seagrass-Associated Taxa

For the seagrass-associated taxa data, I sorted through each site's five quadrats worth of samples, identifying each organism at the lowest level possible using taxonomic guides and keys. Sample containers were condensed using a 63-micrometer sieve and tweezers into small glass vials labeled with site, quadrat number, date, season, and sampler initials. The vials were filled with 91% isopropyl ethanol and stored until processing. Upon processing and taxa identification, each sample was submerged in a 70% ethanol solution with Rose Bengal dye for 48-72 hrs. The Rose Bengal solution was made following the protocol outlined in US Geological Survey (2012) due to samples containing large amounts of organic matter. All samples were sorted and

observed under a Zeiss dissection scope (0.63-5.0X). Images were taken of organisms to assist with identification using a Jenoptik Graphax camera attached to the scope. All organisms were first identified as mesograzers or non-grazers, then organized and preserved into individual sample jars with 70% ethanol by taxonomic group, site, and season. Based on the quantitative diversity observed, all seagrass-associated taxa were sorted into the following 16 taxonomic groups: Amphipods, *Astyris lunata*, *Bittium* spp., bivalves, calanoid copepods, cumacea, *Palaemon* spp., foraminifera, *I. obsoleta* (< 10 mm), isopods, nematodes, oligochaetes, ostracods, polychaetes, Portunidae, and *Tozeuma carolinense*. As some organisms (i.e. amphipods, isopods) can be difficult to identify by morphology alone at species-specific levels without DNA analysis, those organisms were grouped together in larger taxonomic groups (Bousfield 1973). The following taxonomic groups were identified as mesograzers (i.e. small herbivorous invertebrates), as these have been previously recorded to graze on epiphytic material in seagrass habitats: amphipods, *Astyris lunata*, *Bittium* spp., calanoid copepods, *Palaemon* spp., *I. obsoleta*, isopods, polychaetes, Portunidae, *Tozeuma carolinense* (Heck and Orth 1980; van Montfrans 1982; Fulton III 1984; Ruesink 2000; Moore and Hovel 2010; Best and Stanchowicz 2012; Beerman et al. 2018; Voigt and Hovel 2018).

Statistical Analyses

Research Question 1 (Q1):

The snail trematode data included all parasites identified in snails brought back to the lab for dissection. The data was analyzed in terms of prevalence, in which the number of individual snails infected by a certain parasite species was divided by the total number of snails dissected in that sample survey (n = 100). Trematode abundance was first visualized through rank-abundance plots to understand patterns regarding water temperature (< 30 °C, > 30 °C) in R (*BiodiveristyR*

package version 2.17.4) (Moore et al. 2024). Bins of less than and greater than 30 °C are based on this temperature being the high threshold for temperature tolerance for *I. obsoleta* before becoming stressed (Fofonoff et al. 2018; Blakeslee et al. 2019). Snail–trematode data was arranged in decreasing order of abundance, and abundance (y-axis) was plotted against community rank (x-axis). Curves were fitted to the same domain (1–8 species) to facilitate comparison (Moore et al. 2024). To explore the patterns of similarities and differences between trematode species, Bray-Curtis similarities were calculated for square-root transformed data of species abundance (Moore et al. 2023).

A form of nonmetric multidimensional scaling (nMDS) called principal coordinate analyses (PCoAs), an unconstrained ordination model, was used to investigate trematode diversity analyses and visualizations to examine the patterns of trematode abundance by species and when factored by water temperature range ($< 30\text{ }^{\circ}\text{C}$ and $> 30\text{ }^{\circ}\text{C}$, which was treated as an independent, categorical variable (Chen et al. 2022; Jankowska and Wlodarska-Kowalczyk 2022). The trematode species dataset was first square-root transformed before using a Bray-Curtis resemblance matrix. This data contained a small proportion of zero's and therefore a dummy variable with a value of 0.0001 was added to the Bray-Curtis similarity matrix to accommodate for random similarities where no trematode species were recorded in compared samples (Baldanzi et al. 2013; Legendre and Anderson 1999). Models had overlaying vectors associated with trematode species, all based on Pearson correlations of individual trematode species selected from the list of eight identified trematodes found in sampled snails (Chen et al. 2022). To test the effects of the chosen factor, analyses were carried out using partial main and pairwise permutational analysis of variance (PERMANOVA) models, followed by one-way Analysis of Similarities (ANOSIM) models and one-way Similarity Percentage analyses

(SIMPERs) with 70% cut off for low contribution. Additional nMDS bubble plots were also created to focus on the change of infection prevalence between specific trematode species, with bubble size indicating the number of snails infected with a specific trematode sampled from one habitat survey. I performed all the multivariate analyses and permutation tests (999 permutations) presented above in Primer v7 with Anderson (2024).

Additionally, infection prevalence in sampled *I. obsoleta* was examined alongside all environmental variables with the same categorical variable (water temperature range) using PCoAs and Bray-Curtis similarity index in Primer v7 (Moore et al. 2023). Data was initially square-root transformed based on overall visuals in each plot. Running one-way environmental SIMPERs and ANOSIMs (999 permutations) identified high right skewness on the data's distribution from certain environmental variables (i.e. water temperature, salinity, avg. water depth), so the data was normalized to construct a Euclidean resemblance matrix, refining infection prevalence in relation to all variables through removal of outliers and environmental ranking of the variables (Chen et al. 2022). Each PCoA displayed overlaying vectors of environmental variables based on Pearson correlations and specified selection from all variables in the dataset (Chen et al. 2022). The original environmental dataset included 16 environmental variables calculated per habitat survey: water temperature, salinity, average water depth, average shoot density, average shoot abundance, average epibiont biomass, average snail density, average snail abundance, infection richness, infection prevalence, mesograzer abundance, average mesograzer abundance, non-grazer abundance, average non-grazer abundance, total taxa abundance, and average total taxa abundance. Averages (avg.) of the above variables were calculated from the five quadrats taken per site visit to provide site-level measurements for each.

Data on snail sex, as well as presence of eggs in the gonadal tissue, were collected during snail dissection, but were not analyzed here. Intensity data, specifically number of trematode cysts or adults, was also not collected, as intensity of larval trematode cercariae was not easily quantifiable in snail dissections. However, presence or absence of identifiable trematode life stages (cercariae, rediae, sporocysts) was noted upon dissection but not analyzed.

Research Questions 2A and 2B (Q2A and Q2B):

Following analyses in Primer, further refinement of correlated patterns between environmental variables and probability of any trematode infection were quantified using generalized linear mixed models (GLMM) in R (*lme4* package version 2.0-1). In all relevant analyses, the best model was selected using corrected Akaike Information Criterion (AICc) model performance tests (Moore et al. 2020). I first fit a correlation matrix with variables that could directly influence infection. This matrix was built from a dataset listing each snail (n = 4,600) and the abiotic and biotic variables taken during the habitat surveys and snail dissections. Infection was referred to binomially, with a “1” indicating an infected snail, and a “0” indicating an uninfected snail. The variables used in the correlation matrix were also rescaled to use the binomial family.

The selection for specific GLMMs regarding patterns of probability of parasitic infection were based on the correlation coefficients and the outcomes from all possible combinations of factors and their interactions probability of infection. All models used site as a random effect to account for non-independence of observations within sites. Due to the large number of model combinations, model selection was conducted using the *dredge ()* function in the *MuMIn* package (version 1.48.19). GLMMs were constructed with a logit-link function (i.e. logistic regression), with probability of infection (infected snail /uninfected snail) as the response (Potter

et al. 2026), followed by a fixed effect with a strong AICc set on the x-axis. Models within $\Delta\text{AICc} \leq 2.0$ were considered to have substantial support.

Similar models were used to assess the probability of infection from specific trematodes that use different definitive hosts. GLMMs for specific trematode infection in snails was based on overall trematode species abundance across all snail sample populations. Thus, for these calculations, I used the results from the nMDS and rank-abundance plot visualizations on trematode diversity. The observed trematode species in dissections provided a division between those that used fish as definitive hosts (i.e. LS, SD, ST, ZL) versus those that used birds (AV, DN, GA, HQ) (Altman 2010; Curtis 2007). Models on infection probability were then run in groups based on use of definitive host: bird-using trematodes versus fish-using trematodes. Model selection was again conducted using the *dredge ()* function. Models within $\Delta\text{AICc} \leq 2.0$ were considered to have substantial support. Here, GLMMs under the binary family were constructed with a logit-link function (i.e. logistic regression), with either bird-using or fish-using (infected snail = “1”, uninfected snail = “0”) as the response, followed by a single or multiple fixed effect(s) with a strong AICc set on the x-axis. Additional models were then conducted based on comparing only infected snails, this time with the binomials differentiating between bird-using trematodes (0) and fish-using trematodes (1) followed by single or multiple fixed effect(s) with a strong AICc set on the x-axis.

Research Question 3 (Q3):

For the relationship between epibiont biomass and the other environmental variables, data was reorganized so each habitat survey corresponded to one value of avg. epibiont biomass (mg/mm). When assessing the relationship between avg. epibiont biomass and environmental factors, I conducted similar generalized linear mixed models to Q2A and 2B using the *glmmTMB*

package with influence from AICcs in R but included additional predictors to prevent skewness and provide clarity in this complex dataset. These predictors included avg. shoot length, avg. shoot density, infection prevalence, and infection richness. Variables were first analyzed categorically (abiotic versus biotic) in forest plots to identify direction of relationships per predictor with epibiont biomass and the strength of those predictors. Each plot was constructed from exponentiated fixed-effect coefficients ($\exp(\beta)$) and their 95% confidence intervals. Coefficients represent the multiplicative effect of a one standard deviation increase in each predictor; 1 = no effect, < 1 = decrease in avg. epibiont biomass and a negative association, > 1 = increase in avg. epibiont biomass and a positive association. Predictor variables in each plot were ranked according to relative effect magnitude on a scale from 0 to 1. Effect size of each variable does not indicate significance.

Following the identification of the most strongly affiliated variables, models used a Gamma mixed-effects formula with logit-link to constrain the response variable (avg. epibiont biomass), as its values were always greater than zero and similar to previous work (e.g., Foster and Bravington 2013; Olson et al. 2022). Models used site as the only random effect to reduce the complexity of the model but also provide variation from the independent effects. Due to the large number of model combinations, model selection was conducted using the *dredge()* function. A global GLMM containing all candidate predictor variables was constructed, with predictors selected based on results from the forest plots: `glmmTMB(formula = avg_epibiont_biomass_mg_mm ~ doy_s + tempwater_s + salinity_s + avgwaterdepth_s + avgshootdensity_s + avgsnaildensity_s + avgngong_s + avgmeso_s + (1 | site_id), family = Gamma (link = "log"), data = clean_grassdata, control = ctrl_grass)`. The *dredge()* function generated all subsets of fixed effects while retaining the same crossed random effects. Models

within $\Delta AICc \leq 2.0$ were considered to have substantial support. Dredge outputs indicated that variables low tide height, DOY, avg. blade length were not present in any models with $\Delta AICc \leq 2.0$, so they were removed from the global model.

Research Question 4 (Q4):

In terms of biodiversity of seagrass-associated taxa, data included all organisms brought back to the lab for sorting and identified at the lowest taxonomic level into our 16 taxonomic groups. This dataset was analyzed in Primer v7 using the abundance of recorded taxonomic groups (numbers of individuals recorded per group) using the statistical methods and visualizations described for Q1. Changes in seagrass-associated taxa abundance and diversity visualized using shade plots, nMDS bubble plots, and PCoA models using the Bray-Curtis similarity index with a dummy variable of 1.00 similar to previous studies (Baldanzi et al. 2013; Legendre and Anderson 1999). Data was also square-root transformed and run with SIMPER, ANOSIM, and PERMANOVA tests (Moore et al. 2023).

RESULTS AND DISCUSSION

Q1: Trematode Diversity

Trematode species diversity changed markedly over the study period and was relative to temperature variation. Changes in diversity were first visualized through rank-abundance plots arranged by temperature range (Fig. 2), then temperature range and year (Fig. 3). With rank-abundance plots, a steeper slope between species reflects a less evenly diverse community, while a shallower slope indicates a more equal relative abundance across all species in the community. Eight of the nine known trematode species were observed over the study period. Trematodes LS, ZL, ST, and SD were the most abundant species with slight fluctuation between ranks 1, 2, 3, and 4 (Fig. 2). LS was the most abundant trematode over the study period and across both

temperature ranges. The number of snails infected with LS, as well as all other species, decreased as temperature rose over 30 °C (Fig. 2), leading to overall less infection prevalence in sampled snail populations as temperature increased. Species richness also decreased in the higher temperature range, for eight trematode species were identified in the < 30 °C range while only six were identified in the >30 °C range. This could be tied to how gastropod hosts become more stressed as temperature rises, and those that are infected have higher mortality rates than those uninfected in the same habitat (e.g., Lee et al. 2025). Further, Studer et al. (2010) indicated that successful transmission of trematodes utilizing multiple hosts in their life cycles may be negatively impacted by temperatures ≥ 30 °C due to the increased mortality of hosts. Even when more secondary and third definitive hosts of the trematode life cycle are typically present in warmer months (Curtis 2007), the trematode life cycle would not reach completion if snails and/or their parasites became too stressed and died off, resulting in lower trematode abundance.

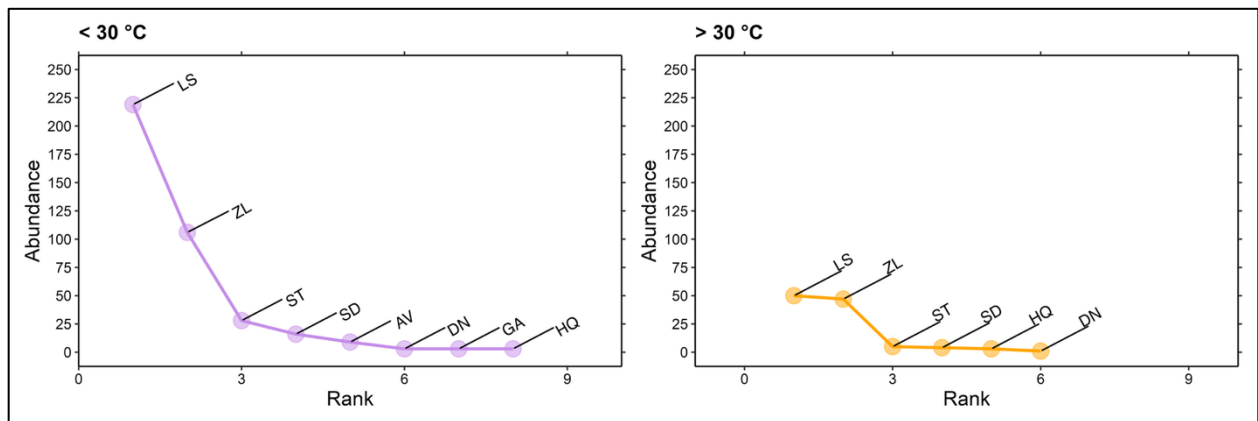


Figure 2. Rank-abundance curves depicting the raw trematode species abundance affiliated with sampled eastern mud snails (*I. obsoleta*) amongst two temperature ranges (< 30 °C ($n = 30$ habitat surveys) and >30 °C ($n = 16$ habitat surveys)). All trematode data was derived from all sites in Bogue Sound and the Straits over 2024 and 2025 ($n = 46$ total habitat surveys). Letters within the figure represent abbreviations of the scientific name of the trematode species represented at that point; AV = *Austroilharzia variglandis*, DN = *Diplostomum nassa*, GA = *Gynaecotyla adunca*, HQ = *Himasthla quissetensis*, LS = *Lepocreadium setiferoides*, SD = *Stephanostomum dentatum*, ST = *Stephanostomum tenue*, ZL = *Zoogonus lasius*.

The result of species richness decreasing as temperature increases does not support **H1.1**. However, there are patterns in trematode diversity between our two temperature ranges. Trematodes LS and ZL are consistently more abundant in both the higher and lower temperatures, holding ranks 1 and 2 in each (Fig. 2); however, they differ dramatically in abundance between the two temperature ranges, with LS being ~ 4x and ZL ~ 2x more abundant in the < 30 °C versus > 30 °C temperature ranges. ST and SD also show this pattern, with both ranking 3 and 4 consistently, but the difference in abundance between the two temperature ranges was not as striking. In addition, all four of these species are fish-using trematodes, meaning that these parasite life cycles are playing strong roles in infection prevalence, trematode community composition, and seagrass community diversity. It also alludes to possible seasonal effects in the abundance of these trematode species since the warmest temperatures would be associated with temperate seasons of late summer and early fall and the cooler temperatures would be associated with fall and spring.

Addressing how trematode diversity changed over time (year to year), species richness decreased from 2024 into 2025 (Fig. 3). While eight species were identified in temperatures < 30 °C and six in temperatures > 30 °C for 2024, only seven were seen in temperatures < 30 °C and five in temperatures > 30 °C for 2025. Regardless of the temperature ranges, ZL was the most abundant trematode in 2024, while LS was the most abundant in 2025. Overall, species richness decreased from 2024 to 2025, and infection prevalence and trematode diversity in *I. obsoleta* also decreased when water temperatures were > 30 °C. Avg. water temperature per year deviated only slightly (2024 mean = 27.745 °C, 2025 mean = 27.651 °C), but a larger standard deviation in 2025 ($x = 5.572$) versus 2024 ($x = 3.884$) indicates there was greater environmental variability in 2025 (Fig. 4). The few extremes of sampling in lower temperatures that took place in March

2025 likely caused this deviation. Overall, 2025 had slightly higher median ($\bar{x} = 29.15\text{ }^{\circ}\text{C}$) and maximum temperatures ($\bar{x} = 34.00\text{ }^{\circ}\text{C}$) compared to 2024 (median = $28.85\text{ }^{\circ}\text{C}$, max = 33.4) (Fig. 4). Analyzing trematode diversity over longer periods of time (i.e. more than two years) would be necessary to truly elucidate any annual patterns between temperature, infection prevalence, and species richness. We can only take note that temperatures greater than $30\text{ }^{\circ}\text{C}$ were recorded, as die-off of both parasites and gastropods due to heat stress are known to happen in shallow waters along the North Carolina coast reaching at least $31\text{-}33\text{ }^{\circ}\text{C}$ (Mallin et al. 2000). This is a potential explanation as to why lower species richness was observed, so building a long-term dataset is needed to confirm temperature-regulated patterns in trematode diversity, and how that may change with rising temperatures in the region impacting trematode prevalence and diversity, especially if the number of days with temperatures $>30\text{ }^{\circ}\text{C}$ increases over time.

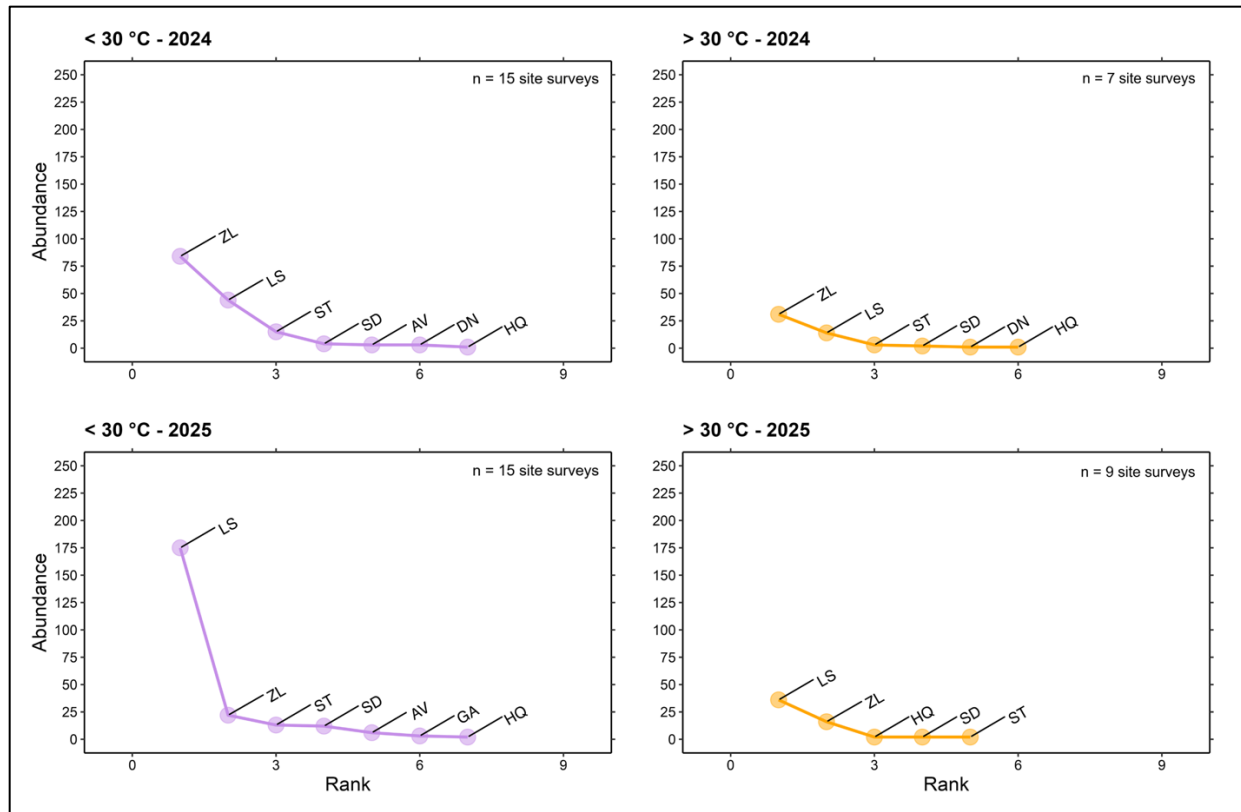


Figure 3. Rank-abundance curves depicting the yearly raw trematode species abundance affiliated with sampled eastern mud snails (*I. obsoleta*) amongst two temperature ranges (< 30 °C and >30 °C) and year (2024 and 2025). All trematode data was derived from all sites in Bogue Sound and the Straits over 2024 and 2025 (n = 46 total habitat surveys). Letters within the figure represent abbreviations of the scientific name of the trematode species represented at that point; AV = *Austrobilharzia variglandis*, DN = *Diplostomum nassa*, GA = *Gynaecotyla adunca*, HQ = *Himasthla quissetensis*, LS = *Lepocreadium setiferoides*, SD = *Stephanostomum dentatum*, ST = *Stephanostomum tenue*, ZL = *Zoogonus lasius*.

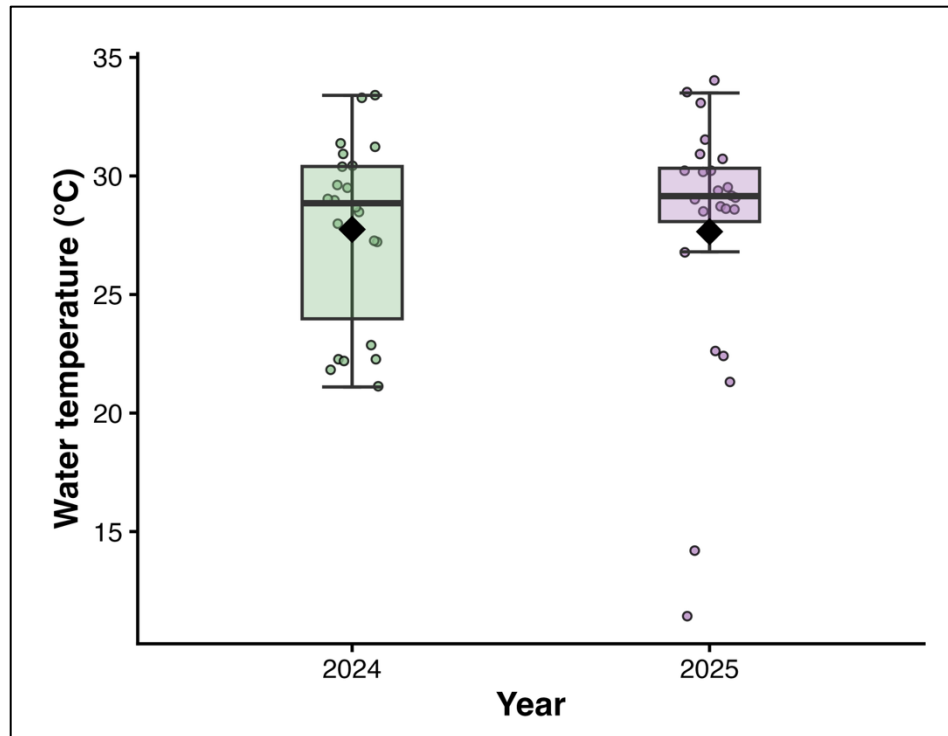


Figure 4. Box plot showing water temperature (°C) by year (2024 in green and 2025 in purple). Box plot showing water temperature (°C) by year (2024 in green and 2025 in purple). Boxes represent the interquartile range (IQR), with the horizontal line indicating the median, whiskers extending to 1.5 x IQR, and black diamonds indicating the average. Points represent individual surveys. Point color is associated with year.

Overall trematode diversity was also visualized in PCoA plots. A multivariate analysis supported the visual separation of the data by water temperature ranges versus a site-by-site comparison (Fig. 5), as site was not significant, as highlighted by a PERMANOVA ($p < 0.05$). However, with many points overlapping between our water temperature ranges of > 30 °C and < 30 °C, pair-wise tests indicated that the assemblages of trematode diversity between each range were not significantly different ($F = 2.4072$, $p = 0.079$). SIMPER analyses revealed that the average dissimilarity between < 30 °C and > 30 °C was 51.03%, with ZL contributing 44.49% in the < 30 °C range and 34.47% in the > 30 °C range, while LS contributed a similar 46.50% in the < 30 °C range, yet a higher percentage of 58.89% in the > 30 °C range. Greater abundances of ZL and LS in the lower temperature range likely caused this, as the abundance of both species

was drastically lower when temperatures rose over 30 °C (Fig. 2). This can be visualized in the slight clustering of the purple triangles on the higher end of the PCO2 axis in Figure 5, coordinating with the positive angle of vectors associated with LS, ZL, and ST, the three most abundant trematode species observed.

Additionally, a PERMANOVA revealed that year as an individual factor in relation to trematode diversity was significant ($F = 0.002$, $p = 0.002$). Pair-wise tests further indicated that trematode diversity in 2024 was significantly different from that in 2025 ($p = 0.001$). However, the ANOSIM indicated a significant difference in trematode diversity between years ($R = 0.154$, $p = 0.002$), the low R value indicates that yearly differences in trematode community composition were weak, meaning that this statistically significant difference likely stems from the lower abundance of infection prevalence from our more abundant species. SIMPER analysis showed that the average dissimilarity between 2024 and 2025 communities was 55.16%, with LS (36.98%), ZL (28.55%), and ST (12.87%) contributing most to this difference, together making up 76.40% of the total dissimilarity. Currently, this can only be considered as observational, as only two years of diversity data is not enough to confirm annual patterns of trematode diversity or that trematode diversity has a negative correlation with time. Each year also contained slightly different quantities of habitat surveys ($n = 22$ in 2024; $n = 24$ in 2025), and the months during which sampling occurred deviated between years, leading to factors of year, season and month being statistical artifacts.

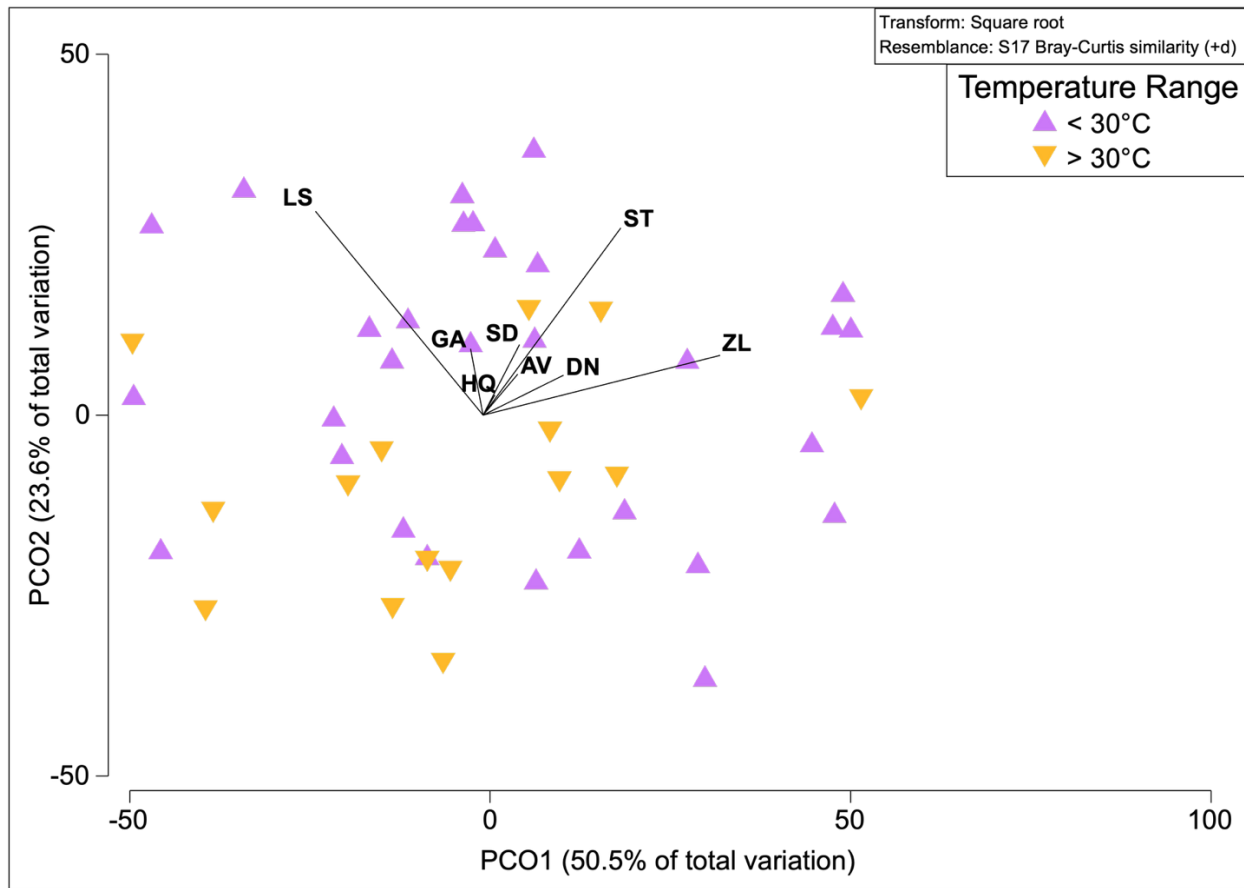


Figure 5. Principal coordinate analysis (PCoA) showing an unconstrained ordination plot in a 2-dimensional space using the Bray-Curtis similarity index (+ 0.0001 dummy variable) to assess the changes in trematode species diversity between water temperature ranges (< 30 °C and > 30 °C). Each individual symbol represents one habitat survey ($n = 46$) from Bogue Sound and the Straits. Temperature ranges are differentiated by color and shape: < 30 °C in light purple triangles and > 30 °C in yellow, inverted triangles. The overlay vectors are based on Pearson correlation. Letters within the figure represent abbreviations of the scientific name of the trematode species represented at that point; AV = *Austrobilharzia variglandis*, DN = *Diplostomum nassa*, GA = *Gynaecotyla adunca*, HQ = *Himasthla quissetensis*, LS = *Lepocreadium setiferoides*, SD = *Stephanostomum dentatum*, ST = *Stephanostomum tenue*, ZL = *Zoogonus lasius*.

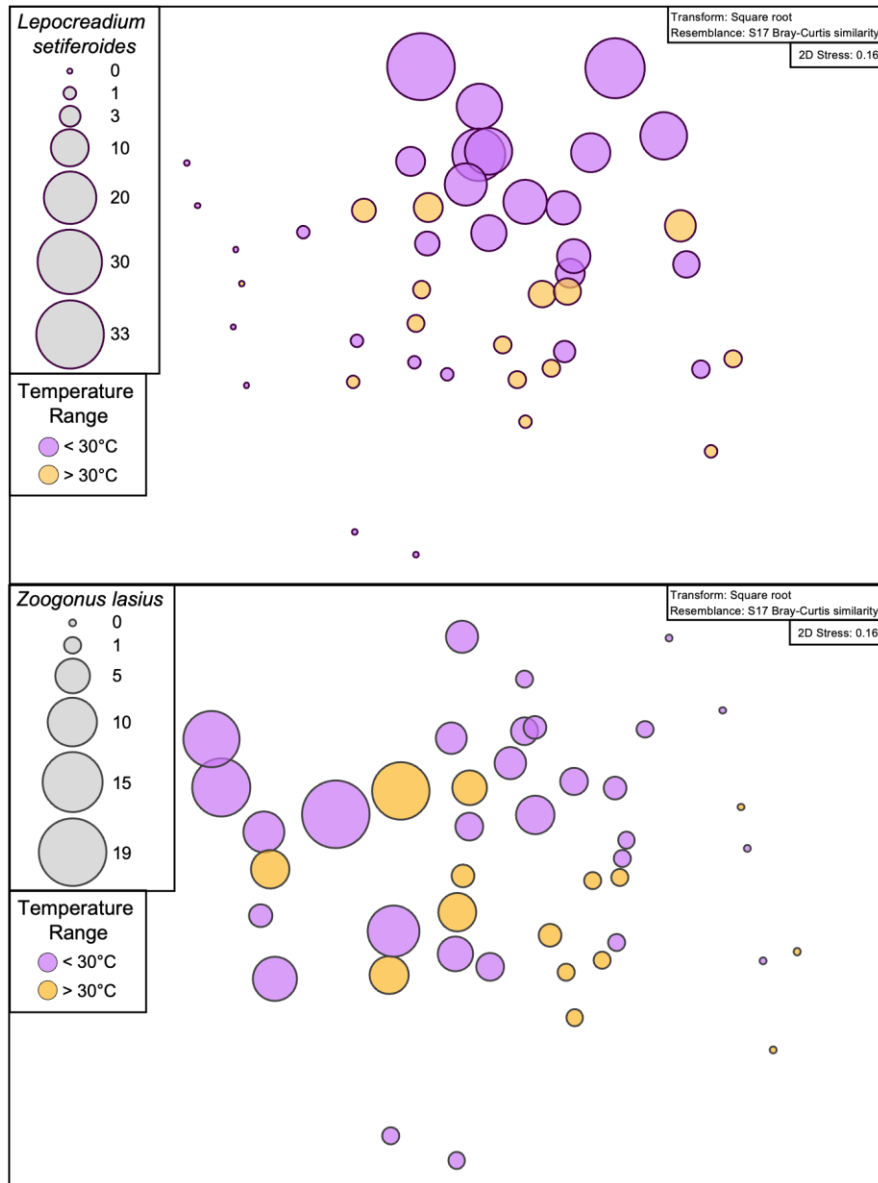


Figure 6. Two panels of non-metric multidimensional scaling bubble plots using the Bray-Curtis similarity to assess the changes between temperature ranges in infection prevalence in *I. obsoleta* from *Lepocreadium setiferoides* and *Zoogonus lasius* at all habitat surveys in Bogue Sound and the Straits. Each individual bubble represents one habitat survey ($n = 46$). The bubble size indicates the number of infections from a specific trematode species per 100 snails. Year and water temperature range are differentiated by color (< 30 °C in light purple and > 30 °C in yellow).

Fortunately, differences on a species-specific level exist when focusing visualization on our more abundant species: *L. setiferoides* (LS) and *Z. lasius* (ZL). This can be seen further in Figure 6, with larger bubble sizes in < 30 °C for ZL and in < 30 °C for LS, indicating that there

is change in which species of trematode is most abundant in seagrass beds. This visualization also reflects the higher abundances of ZL and LS in the lower temperature range over the course of sampling observed in Figure 3. The change in species-specific trematode abundance is possible due to change occurring on the community scale within each seagrass habitat, such as snail distribution from onshore to into the seagrass beds or snail population density, as well as distribution of second and definitive hosts across Bogue Sound and the Straits. Furthermore, change in trematode diversity could also be more intricate, such as the specific fecundity of a single trematode species. Both LS and ZL utilize complex life cycles that rely on trophic transmission from host to host, with each living in two “habitats”: *I. obsoleta* as the first, and the seagrass system as the second. Both species must be able to detect and endure changes in both “habitats” to acquire successful transmission from host to host and life cycle completion; it is this complexity that is difficult to measure and quantify when studying parasite component communities, especially as parasite diversity changes in tandem with the environment.

Moreover, while LS and ZL are similar in their use of fish as their definitive hosts, their morphologies, reproductive cycles, and physiological effects differ. With LS as a digenean fluke (Miller and Northup 1926; Stunkard 1972), it produces actively feeding rediae and cercariae that can alter the host’s protein metabolism when exposed to carrion (Curtis 1985), leading to varying degrees of tissue damage in the snail’s gonads (Cheng et al. 1983). These rediae possess a mouth, pharynx, and primitive gut, leading to LS at this life stage to directly consume host tissue via tearing and consumption (Esch et al. 2002; Kuris 1990). In comparison, ZL is a plagiorchiid digenean producing sporocysts which appear to be less capable of causing tissue damage (Rossiter and Sukhdeo 2012; Stambaugh and McDermott 1969; Stunkard 1938), as these sporocysts lack gut and mouth anatomy and instead absorb nutrients from the host’s body (Kuris

1990; Magalhães et al. 2020). These well-identified differences can assist in understanding how parasite diversity changes over time and space in seagrass ecosystems. For instance, changes in temperature can lead to wide ranges of cercarial output, from small reductions to 200-fold in response to a 10-degree rise (Poulin 2006). Addressing temperature in addition to the other measured environmental variables in relation to trematode infection prevalence and the patterns between them can help predict the true drivers of trematode diversity in seagrass ecosystems.

Patterns among environmental variables were visualized in PCoA plots (Fig. 7). Given the similar length of the vectors associated with each environmental variable, the patterns between each variable were less discernable than the previous PCoA. An ANOSIM showed that there was a weak, significant difference among environmental variables between temperature ranges of $< 30\text{ }^{\circ}\text{C}$ and $> 30\text{ }^{\circ}\text{C}$ ($R = 0.145$, $p = 0.008$). The low R value indicates that interannual variation was limited relative to within-range variability. This suggests that short-term dynamics (i.e. dramatic temperature fluctuation in a short time) may play a key role in structuring environmental conditions within the system. Patterns between specific environmental variables are present, specifically between the two water temperature ranges and the angle of specific vectors. SIMPER analysis showed that differences in environmental variables between $< 30\text{ }^{\circ}\text{C}$ and $> 30\text{ }^{\circ}\text{C}$ were primarily driven by water temperature (10.30 %), avg. water depth (cm) (6.74 %), avg. mesograzer abundance (6.19 %), salinity (6.19 %) and infection prevalence (6.17 %), followed by differences in avg. snail density (6.06 %), avg. epibiont biomass (mg/mm) (6.03 %), and avg. total taxa abundance (5.95 %). Together, these variables accounted for over 50% of the observed dissimilarity between the two ranges of water temperature. These shifts suggest a potential change in seagrass habitat structure and water column conditions, which may influence both host distribution and parasite transmission dynamics. More importantly, they also suggest

increased biodiversity and epibiont biomass in the system, supporting the need for future analyses on such variables. Patterns between specific environmental variables are present, specifically between variables such as temperature and epibiont biomass that can alter habitat complexity and resource availability for seagrasses (Voigt and Hovel 2018), while reduced salinity and increased water depth may affect the physiology and distribution of both hosts and intermediate hosts (Pochtar et al. 2025).

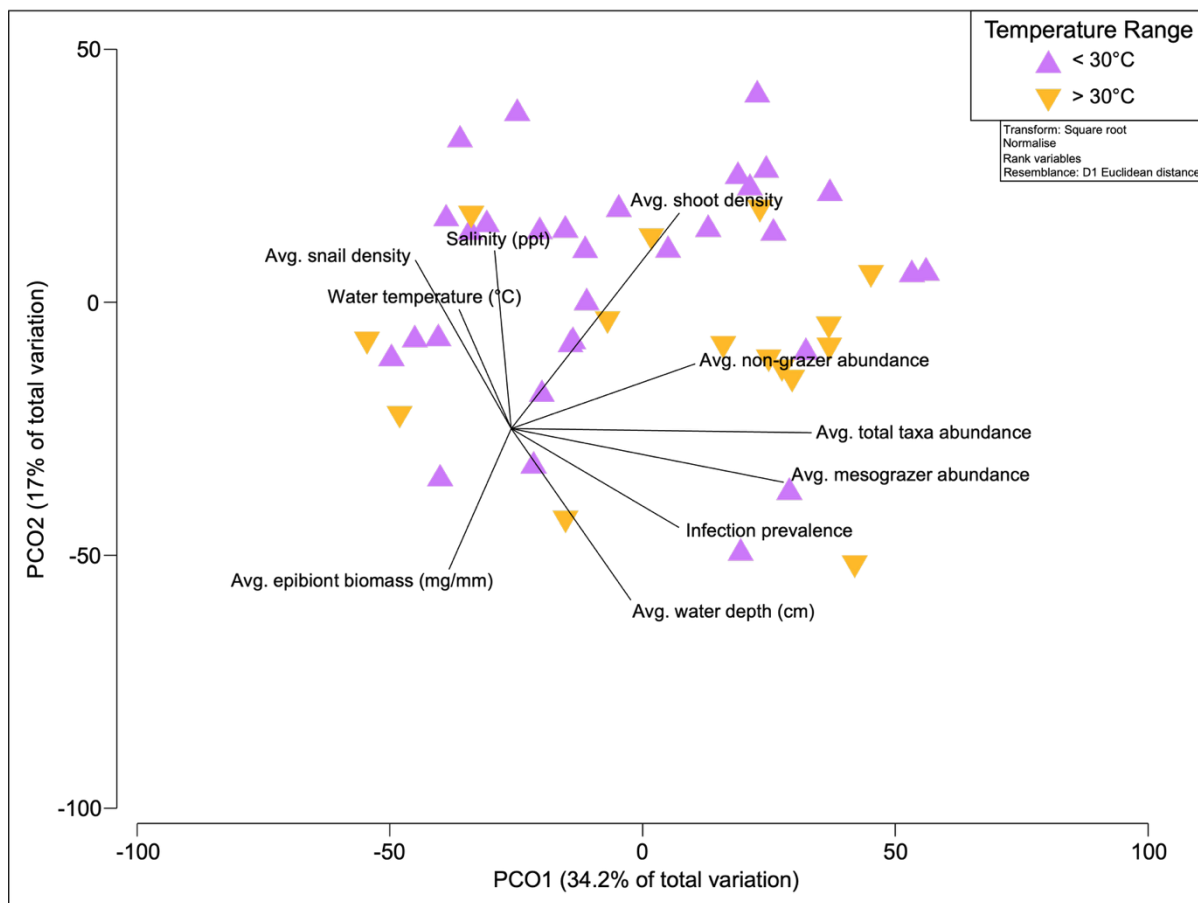


Figure 7. Principal coordinate analysis (PCoA) showing the unconstrained ordination plots in a 2-dimensional space using Euclidean distance to assess ranked and normalized environmental variables from habitat surveys within Bogue Sound and the Straits by range of water temperature (< 30 °C and > 30 °C). Each individual symbol represents one habitat survey (n = 46). Temperature ranges are differentiated by color and shape (< 30 °C in light purple triangles, and > 30 °C in yellow inverted triangles). The overlay vectors are based on specified selection with Pearson correlation.

Further, it is worth continuing to investigate the distribution of diversity in general in seagrass ecosystems. Invertebrate diversity alone can drive ecosystem functioning and biodiversity in seagrass beds, as seen in an experimental study with *Z. marina* conducted by Zhang and Silliman (2019) in North Carolina. A secondary foundation species, pen clams (*Atrina rigida*) drove local biodiversity in its marine system, specifically increasing abundance and species richness of epiphytic macroalgae and fouling invertebrate fauna that consume the epiphytes. With increased diversity of grazers and food sources for such grazers, results indicated the important role invertebrates have in trophic cascades in seagrass ecosystems. As this cascade alludes to the direct relationship between grazers and epiphytes with facilitation from a bivalve, investigating how parasite component communities add to biodiversity and production of an ecosystem can provide understanding of parasitism overall causing trophic cascades in food webs (Christoffersen et al. 2012) and indirect effects through their hosts.

Q2A: Probability of Infection – All Species

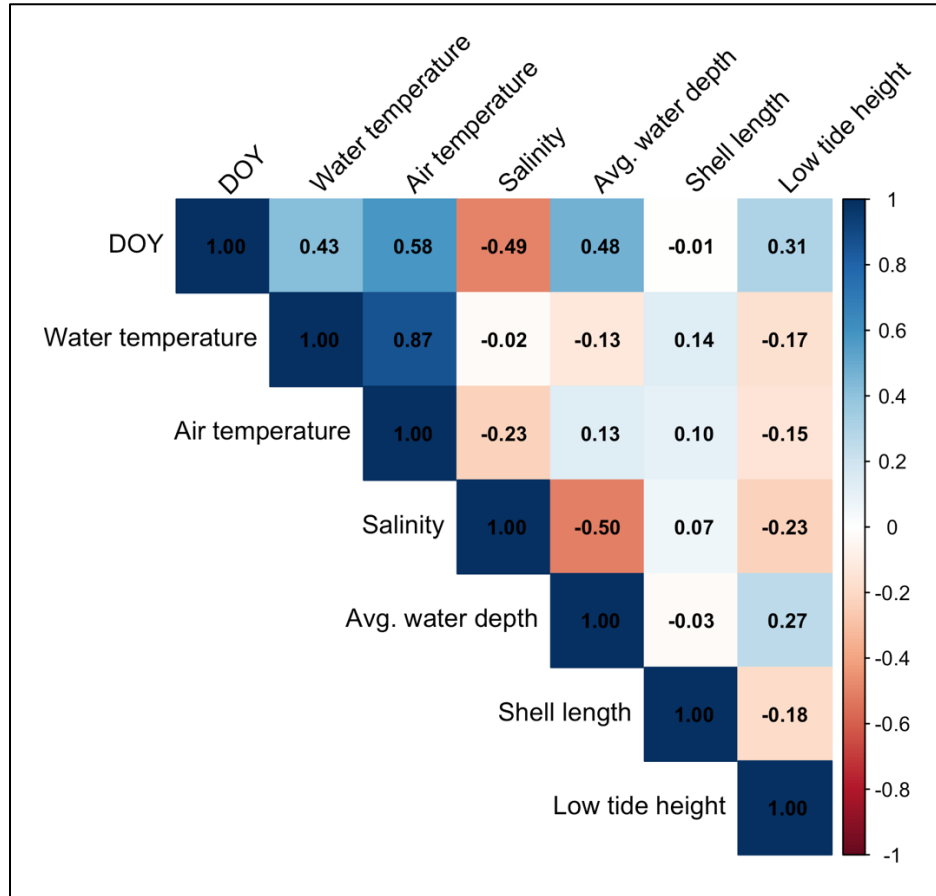


Figure 8. Correlation matrix showing pairwise Pearson correlation coefficients among abiotic and biotic variables relating to infection prevalence per individual snail. Cells display correlation coefficients. Color intensity and shading indicate the strength of each variable, with positive correlations shown in blue and negative correlations in red. Higher correlation coefficients represent stronger associations between variables.

Selection of variables for models stemmed from Pearson correlation coefficients (ρ) (Fig. 8). Due to year and DOY having a negative correlation ($\rho = -0.32$), the use of DOY as a predictor variable instead of a random effect proved beneficial, as DOY indicated a numerical form of seasonality and site would be then considered a random effect in models. Air temperature and water temperature displayed collinearity ($\rho = 0.87$), therefore models only included water temperature due to its lower correlation with DOY ($\rho = 0.42$). Water temperature was of interest because while the early life stages of these trematodes in this component community have

seldom been studied in the lab, let alone in the field, Stunkard (1938a, 1938b) observed the ciliated miracidia of HQ and ZL in water that actively infect *I. obsoleta*. With little known about the remaining species' miracidia and the parasitic egg deposition by definitive hosts, we can only assume, based on knowledge of most trematode life cycles, that their miracidia stages shed out of the eggs, are ciliated, and seek out a molluscan host (Curtis 2009; Esch et al. 2002; Hechinger and Lafferty 2005; Rossiter and Sukhdeo 2012). Predictor variables included were DOY, salinity, avg. water depth, water temperature, low tide height, and shell length, creating the following formula to run variations of GLMMs: $\text{infection_status} \sim \text{tempwater_s} + \text{doy_s} + \text{salinity_s} + \text{avgwaterdepth_s} + \text{shelllength_s} + \text{lheight_s} + (1 \mid \text{site_id})$. Shell length proved integral to the model, as infection is more likely in larger (i.e. older) snails that have been exposed longer to parasites in their habitats, and an older snail's fully developed gonad allows infection to be successful (Lafferty 1993; Blakeslee et al. 2012). A total of six models addressing different combinations of these variables were conducted alongside the null model provided that these models contained values of $\Delta\text{AICc} \leq 2.0$ (Table 2).

In the following paragraphs, I report only the top model and its estimates of probability of infection with fixed effects of shell height and water temperature, ($w = 0.203$, Table 2). I provided AICc values, differences between AICc values (Δ) and weight of each model in Table 2. The influence from each of the fixed and random effect variables in the top model is listed in Table 3.

Table 2. Infection prevalence model selection table where models are ranked by AICc from lowest to highest. This table lists the model number, predictors for each model, the number of parameters (df), the negative log-likelihood (-2LL), AICc score, the difference in AICc score between each model and the top-ranked model ($\Delta AICc$), and weight given to each model (w).

Model	Predictors	df	-2LL	AICc	$\Delta AICc$	w
1	shell length + water temp.	4	-1,445.570	2,899.1	0.00	0.203
2	avg. water depth + shell length + water temp.	5	-1,445.091	2,900.2	1.05	0.121
3	salinity + shell length + water temp.	5	-1,445.378	2,900.8	1.62	0.091
4	low tide height + shell length + water temp.	5	-1,445.489	2,901.0	1.84	0.081
5	avg. water depth + DOY + shell length + water temp.	6	-1,444.488	2,901.0	1.85	0.081
6	avg. water depth + low tide height + shell length + water temp.	5	-1,445.561	2,901.1	1.99	0.075

Table 3. Fixed and random effect variables of the top model for infection prevalence. This table lists each variable, if that variable is fixed or random, effect size estimate (β), standard error, test statistic (z value), p-value associated with the z value ($Pr(>|z|)$), and marginal R^2 (R^2) for the mixed model. Number of snails = 4,537; n = 46 habitat surveys. Asterisks indicate significance.

Variable	Effect type	β	Std. error	z value	$Pr(> z)$	R^2
site id	Random	-2.30619	0.28996	-7.954	1.81e-15	0.042
shell length*	Fixed	0.22997	0.06783	3.390	0.00069	
water temp. *	Fixed	-0.37487	0.04252	-8.816	< 2e-16	

The top model outlined that water temperature ($^{\circ}\text{C}$) and shell length (mm) were the strongest predictors for probability of infection of any trematode species in *I. obsoleta* (Table 2). This is supported by the change in trematode species richness and abundance between temperature ranges seen in Figs. 2 and 3, as well as the greater abundances of ZL and LS in different time periods. Based on the conditional and marginal R^2 values for the top model (conditional $R^2 = 0.199$, marginal $R^2 = 0.042$), our nested random effect of site ID explains the variance between variables and each model, showing improvement with the use of the single random effect and the *dredge* () function in R. This hints at the numerous other variables I did not assess in this study that could heavily influence infection prevalence in *I. obsoleta*.

populations inhabiting seagrass beds. For instance, previous work has found host fitness (Lochmiller and Deerenberg 2000), spatial distribution of host species (Byers et al. 2011; Ni and Doherty 2022; Rossiter and Sukhdeo 2012), light exposure (Kuntz 1947), mollusc bioturbation (Dairain et al. 2019), human-influenced variables (i.e. nitrogen in coastal waters, host proximity to human developments) (Altman and Byers 2014), and abundance of multiple parasite life stages per host (Magalhães et al. 2020) to influence parasite infection prevalence in a variety of hosts. Most of these supplemental variables, however, have yet to be analyzed in studies focused on an entire parasite component community within a single host, providing opportunity for future analysis of additional drivers of infection prevalence and, eventually, indirect effects from parasitism.

The logistic regression predicted water temperature to be inversely correlated with infection prevalence; as water temperature increased, infection prevalence decreased (Fig. 9, $\beta = -0.37487 \pm 0.04252$, $p = < 2e^{-16}$). As sampling of all *I. obsoleta* took place in all months except those temperately associated with winter (i.e. November, December, January, February), the warmer parts of the year showed decreased infection prevalence. Previous studies have indicated that hosts become more stressed as temperature rises, and those that are infected have higher mortality rates than those uninfected in the same habitat (e.g., Lee et al. 2025). In addition, other studies indicated that successful transmission of trematodes utilizing multiple hosts in their life cycles may be negatively impacted by temperatures $> 30^{\circ}\text{C}$ due to the increased mortality of hosts (Studer et al. 2010). It would be interesting to assess this question of parasite fecundity in a future study with a similar number of infected mud snails, as in this work, I did not record the loads of cercariae, sporocysts, or rediae within each snail. With that, ciliated miracidia of HQ and ZL are known to actively hatch and seek *I. obsoleta* in the environment (Stunkard 1938a;

1938b). It is possible that higher temperatures increase mortality of this life stage before they reach the mud snails, leading to more uninfected snails (0) than infected (1) in our dataset (Fig. 9). As previously stated, there is little record of the miracidia in this host-parasite network, let alone their temperature thresholds; this eludes to another knowledge gap in how probability of infection can fluctuate between species belonging to the same parasite component community.

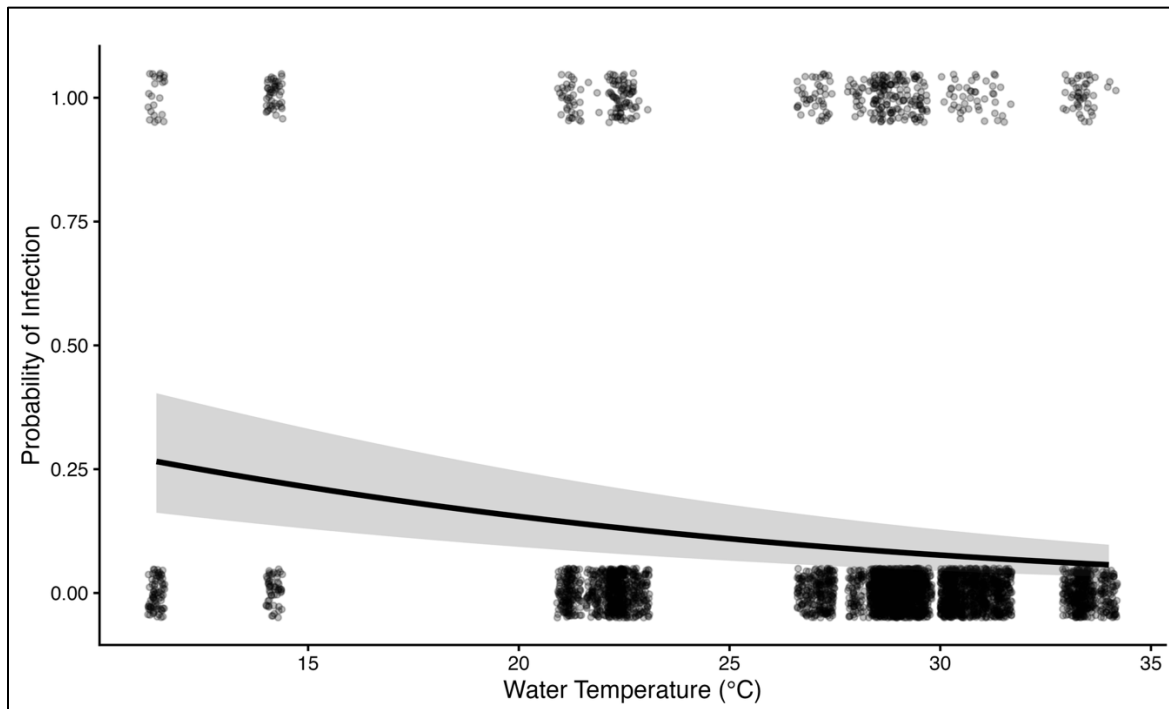


Figure 9. Logistic regression of the relationship between water temperature ($^{\circ}\text{C}$) and the probability of infection from all trematode species. Data is derived from the top generalized linear mixed effects model from Table 2 (Model 1). Points represent individual *I. obsoleta* ($n = 4,537$) and their infection status (0 = uninfected, 1 = infected). Points have been jittered vertically for visualization. The solid black line indicates Model 1's predicted probability of infection across the recorded water temperature ranges from all habitat surveys ($n = 46$). The gray band represents the 95% confidence interval. Note that water temperature was converted back to raw values from the standardized values used in Model 1 to highlight the relationship between temperature and infection.

It is clear that most snails, regardless of infection, were collected in natural water temperature ranges for *I. obsoleta*, as seen in the heavy clustering of points (i.e. individual snails) to the left of 30 $^{\circ}\text{C}$ compared to the smaller cluster to the right of 30 $^{\circ}\text{C}$ (Fig. 9). It is also worth

noting that in infected ($y = 1$) versus uninfected snails ($y = 0$), there are fewer infected snails found in water temperatures $> 30\text{ }^{\circ}\text{C}$; this follows the pattern of lower abundance of all trematode species in that temperature range seen in the rank curves (Figs. 2, 3). This negates our prediction of more infected snails being found in warmer temperatures. However, with sites only visited once per season and 100 snails collected per habitat survey ($n = 46$ habitat surveys; $n = 4,600$ snails), there is a possible sample size issue, particularly due to lack of sampling done in cooler months. This is a potential explanation for the inverse relationship between infection and water temperature seen in Fig. 9, despite some studies finding higher temperatures to correlate with increased trematode infection (e.g., Poulin 2006; Thieltges and Rick 2006) and cercarial emergence (e.g., Kuntz 1947). However, lower environmental temperatures have been seen to limit the growing season of *I. obsoleta* (i.e. tissue development and shell growth) to only five to six months out of the year (Cranford 1986). As successful infection from a trematode relies on a fully developed gonadal tissue (Blakeslee et al. 2012), it is possible that snails reach full development in warmer months, leading to increased infection in those periods. In future assessments, sampling from *I. obsoleta* populations during colder time periods to assess their infection statuses could compensate for the lack of data in the temperature ranges of $\leq 15 - 20\text{ }^{\circ}\text{C}$ and $> 30\text{ }^{\circ}\text{C}$, as well as aim to provide further insight to the relationship between temperature and the members of this parasite component community.

The next logistic regression predicted shell length to be positively correlated with infection prevalence; as shell length increased, infection prevalence increased (Fig. 10, $\beta = 0.22997 \pm 0.06783$, $p = 0.000698$). This supports previous findings where larger (i.e. older) snails that have been exposed longer to parasites in their habitats and those with more fully developed gonads are more likely to be infected (Curtis 1995; Lafferty 1993; Blakeslee et al.

2012). These correlations between mud snail shell size, water temperature, and infection prevalence support my hypothesis on how greater quantities of larger snails (> 14 mm) are to be infected versus smaller snails (< 14 mm).

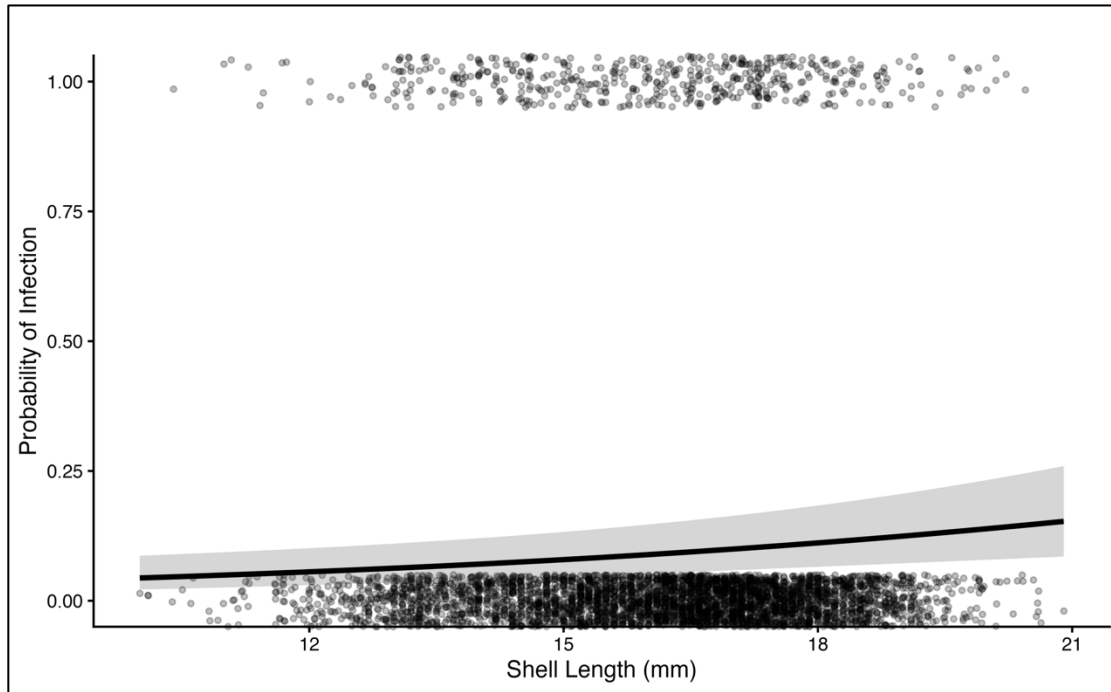


Figure 10. Logistic regression of the relationship between shell length (mm) and the probability of infection from all trematode species. Data is derived from a generalized linear mixed effects model (Model 1) from Table 2. Points represent individual *I. obsoleta* ($n = 4,537$) and their infection status (0 = uninfected, 1 = infected) along the y-axis. Points have been jittered vertically for visualization. The solid black line indicates Model 1's predicted probability of infection across the recorded shell length ranges from all habitat surveys ($n = 46$). The gray band represents the 95% confidence interval. The x-axis was scaled to show only where points appeared. Note that shell length was converted back to raw values from the standardized values used in Model 1 to highlight the relationship between shell length and infection.

Q2B: Probability of Infection – Host Specific

GLMM models built with one or more predictor variables using the *dredge()* function and either of the response variables, bird-using or fish-using, indicating correlations between variables. The best model for bird-using trematodes took the following form: $\text{bird_host} \sim \text{avgwaterdepth_s} + \text{salinity_s} + (1 \mid \text{site_id})$ ($w = 0.077$, $\Delta\text{AICc} = 0.00$, $\text{AICc} = 267.1$, $\text{df} = 4$, conditional $R^2 = 0.189$, marginal $R^2 = 0.099$). The best model for fish-using trematodes took the

following form: (1) $\text{fish_host} \sim \text{shelllength_s} + \text{tempwater_s} + (1 \mid \text{site_id})$ ($w = 0.140$, $\Delta\text{AICc} = 0.00$, $\text{AICc} = 2795.1$, $\text{df} = 4$, conditional $R^2 = 0.189$, marginal $R^2 = 0.040$). Both of these top models and their logistic regressions were derived from the following global models addressing host-specific infection probability: (1) $\text{glmer}(\text{formula} = \text{bird_host} \sim \text{tempwater_s} + \text{doy_s} + \text{salinity_s} + \text{avgwaterdepth_s} + \text{shellheight_s} + \text{ltheight_s} + (1 \mid \text{site_id}), \text{data} = \text{cleanbird_data}, \text{family} = \text{binomial}, \text{control} = \text{ctrlbird})$; (2) $\text{glmer}(\text{formula} = \text{fish_host} \sim \text{tempwater_s} + \text{doy_s} + \text{salinity_s} + \text{avgwaterdepth_s} + \text{shellheight_s} + \text{ltheight_s} + (1 \mid \text{site_id}), \text{data} = \text{cleanfish_data}, \text{family} = \text{binomial}, \text{control} = \text{ctrlfish})$. Like the previous models addressing infection probability from all trematode species, the use of the single random effect of site ID helped explain the variation between each model. Within the bird model, both avg. water depth ($p = 0.0330$) and salinity ($p = 0.0458$) were found to be significant. Within the fish model, both shell length ($p = 0.00511$) and water temperature ($p = < 2e-16$) were found to be significant.

The first final host-specific logistic regression regarding bird-using trematodes predicted avg. water depth to be positively correlated with infection probability from bird-using trematodes (Fig. 11, $\beta = 0.6441 \pm 0.3020$, $p = 0.0330$). Most snails, uninfected and infected with bird-using parasites, were found in shallower depths (< 40 cm, Fig. 11). In general, intertidal habitats, shorebirds aggregate at the unsubmerged areas for prey consumption, and it is here that passive or active transmission of digenean trematodes can occur (Rohde 2005). This has also been seen with a similar gastropod, *L. littorea*, and its parasite component community (), as trematode prevalence was seen to be quite high (50-90%) particularly where birds congregated to feed or brood (Byers et al. 2008; Sindermann and Farrin 1962; Wood et al. 2008). However, out of all snails dissected, only 22 snails were infected with bird-using trematodes; this is a potential explanation for a moderate significance and an almost flat line of fit in the regression. Further,

differences in habitat structure can affect the distribution of estuarine organisms that serve as explanation for a moderate significance and an almost flat line of fit in the regression. Further, differences in habitat structure can affect the distribution of estuarine organisms that serve as hosts for *I. obsoleta* trematodes in saltmarshes including birds, and distribution of the snails themselves can lead to spatial heterogeneity in infection (Altman 2010; Baltz et al. 1993). As previously mentioned, snails move to the upper intertidal and shores when migratory birds congregate, leading to infections from birds (Curtis 2002). As habitat surveys took on haphazard hand-collection methods for *I. obsoleta* all around our site and not just the higher inertial zone, it is possible that additional infections from bird-using trematodes were simply not collected. Increasing sample sizes to be greater than 100 snails per habitat survey would have increase the probability of finding infections from bird-using trematodes, as snails often remain in those elevated, shallower zones in summer where prevalence has been known to increase (Curtis 2002). While I did not collect specific bird data over time to interpret bird abundance in comparison to bird-using trematodes, it is worth investigating, as a diverse bird community can transmit several trematode species to snail populations (Huspeni and Lafferty 2004). However, larval trematodes in first intermediate hosts are known to engage in fierce interspecific competition (Kuris and Lafferty 1994), so those highly abundant trematodes (e.g. LS and ZL) are likely outcompeting subordinate trematode species due to being competitively dominant.

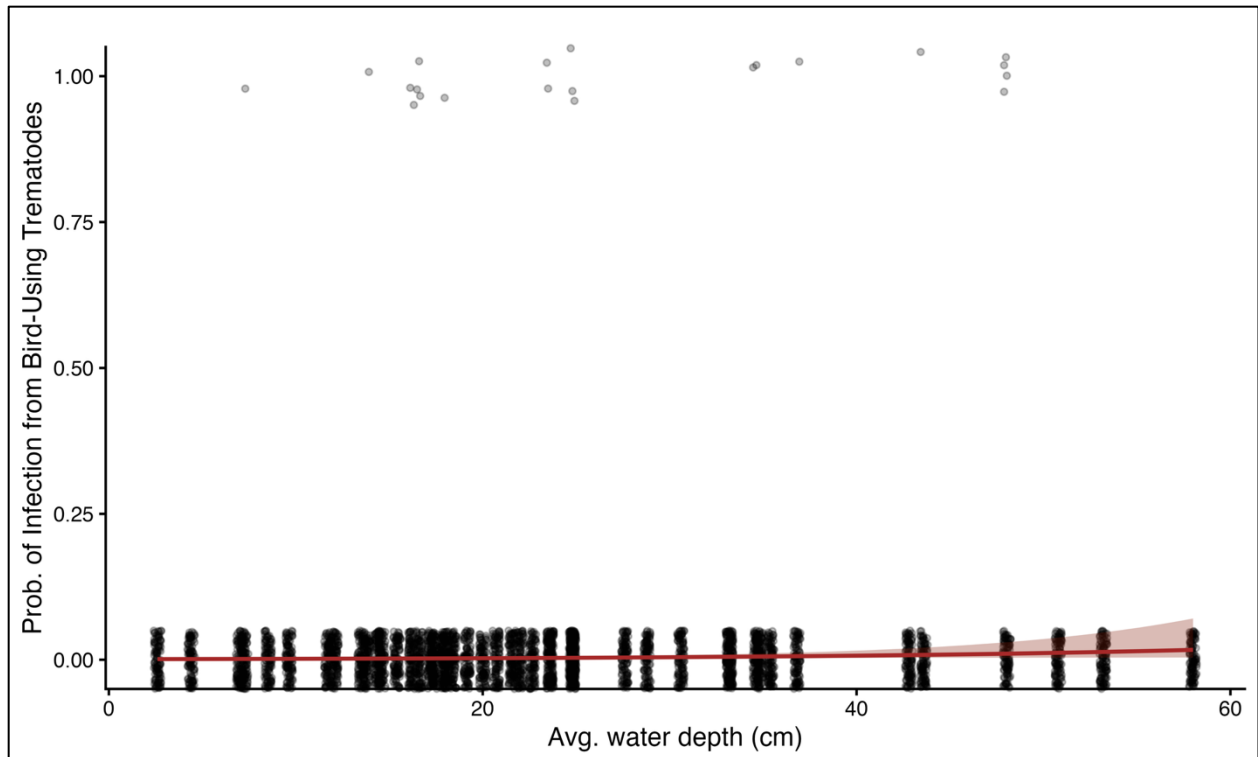


Figure 11. Logistic regression of the relationship between average water depth (cm) and the probability of infection from bird-using trematodes. Data is derived from a generalized linear mixed effects model with average water depth as a predictor ($p = 0.0330$). Points represent individual *I. obsoleta* ($n = 4,537$) and their infection status (0 = uninfected, 1 = infected) along the y-axis. Points have been jittered vertically for visualization. The solid red line indicates the model's predicted probability of infection across the recorded depth ranges from all habitat surveys ($n = 46$). The red band represents the 95% confidence interval. The x-axis was scaled to show only where points appeared. Note that water depth was converted back to raw values from the standardized values used in the model to highlight the relationship between water depth and infection from bird-using trematodes.

The second final host-specific logistic regression regarding bird-using trematodes predicted salinity to be positively correlated with infection probability from bird-using trematodes (Fig. 12, $\beta = 0.6378 \pm 0.3193$, $p = 0.0458$). Salinity can influence the physiologies of mud snails (e.g., Richmond and Woodin 1996) and trematodes (e.g., Koprivnikar and Poulin 2009). *I. obsoleta* has high salinity tolerances (10-35 ppt) (Fofonoff et al. 2018), and the salinity ranges observed did not exceed 36 ppt. However, as both snail and parasite must constantly adapt to every-changing environmental conditions, tolerances and physiology may vary depending on the stress

endured by the individual. In Vernberg (1969), cercariae of *H. quissetensis* (a bird-using trematode) have been able to better handle salinity extremes with respiratory rates from 10 to 25 ppt, while the rate of *I. obsoleta* in this study declined below 15 ppt. In comparison, some cercariae are unable to endure changes in salinity; *A. variglandis* cercarial output reduced due to salinity extremes in addition to low temperatures and reduce oxygen tension (Sindermann et al. 1957; Sindermann 1960). This parasite component community may be particularly sensitive to abiotic factors and dramatic environmental conditions when in its free-living stages. Therefore, our model could be improved by including snail dispersion, water pH, run-off, nutrient levels, and the like.

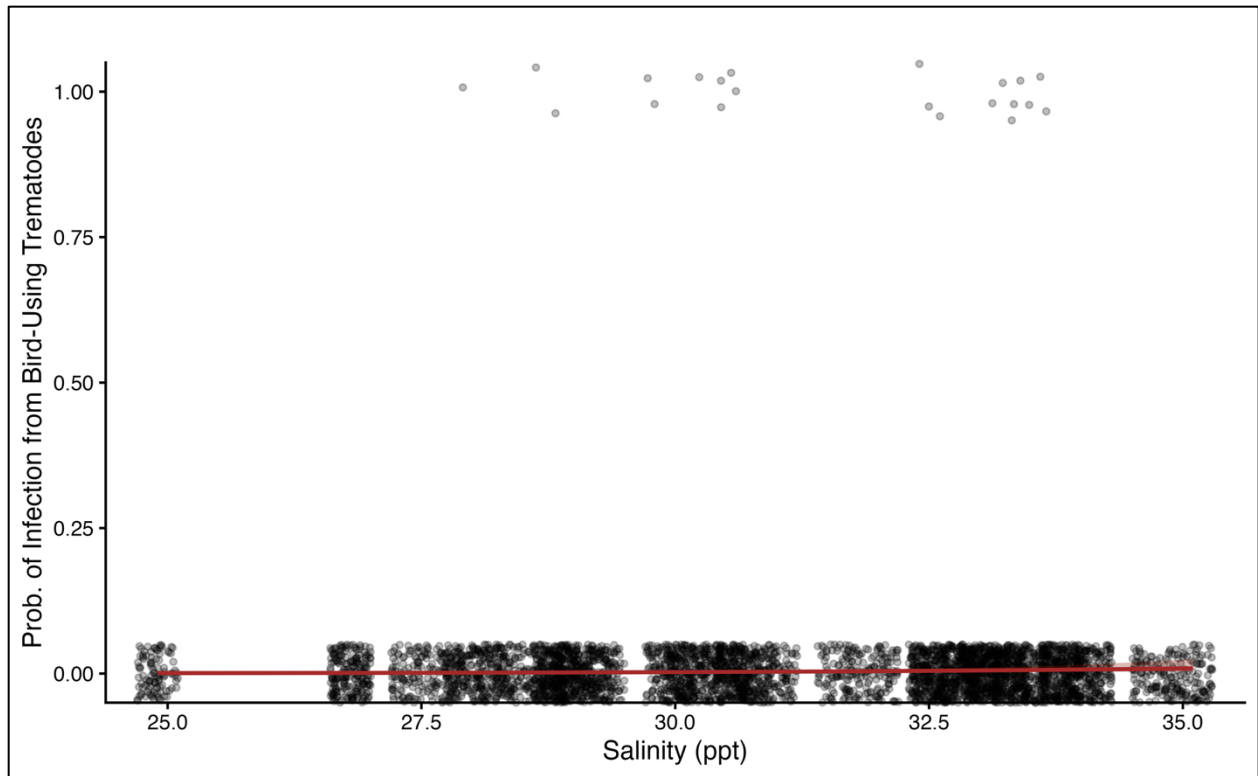


Figure 12. Logistic regression of the relationship between salinity (ppt) and the probability of infection from bird-using trematodes. Data is derived from a generalized linear mixed effects model with salinity as a predictor ($p = 0.0458$). Points represent individual *I. obsoleta* ($n = 4,537$) and their infection status (0 = uninfected, 1 = infected) along the y-axis. Points have been jittered vertically for visualization. The solid red line indicates the model's predicted probability of infection across the recorded salinity ranges from all habitat surveys ($n = 46$). The red band represents the 95% confidence interval. The x-axis was scaled to show only where points appeared. Note that salinity was converted back to raw values from the standardized values used in the model to highlight the relationship between salinity and infection from bird-using trematodes.

The first final host-specific logistic regression regarding fish-using trematodes predicted shell length to be positively correlated with infection probability from fish-using trematodes (Fig. 13, $\beta = 0.19413 \pm 0.06933$, $p = 0.00511$). Most points along 1.00 of the y-axis cluster over larger shell length sizes (≥ 15.00 mm), supporting, once again, the pattern of infection in larger (i.e. older) mud snails (Curtis 1995; Lafferty 1993; Blakeslee et al. 2012). Data on fish abundance was not recorded, let alone that on species identification for fish seen, during habitat surveys. Inclusion of final host abundance as a predictor can prove difficult, as it can come off as a

negative predictor for trematode response, meaning that the role of final hosts as predators may drive down second intermediate host abundance at sites (Altman and Byers 2014). Further research of how fish abundance affects trematode prevalence is needed to better understand the diversity of this parasite component community. This would provide further insight into which specific species of fish are used in each species' life cycle, as only a handful of studies has obtained trematodes from their definitive hosts (e.g., Martin 1939; Rankin 1940; Stunkard 1938a, 1938b, 1961, 1972, 1973, 1983; Stunkard and Hinchcliffe 1952).

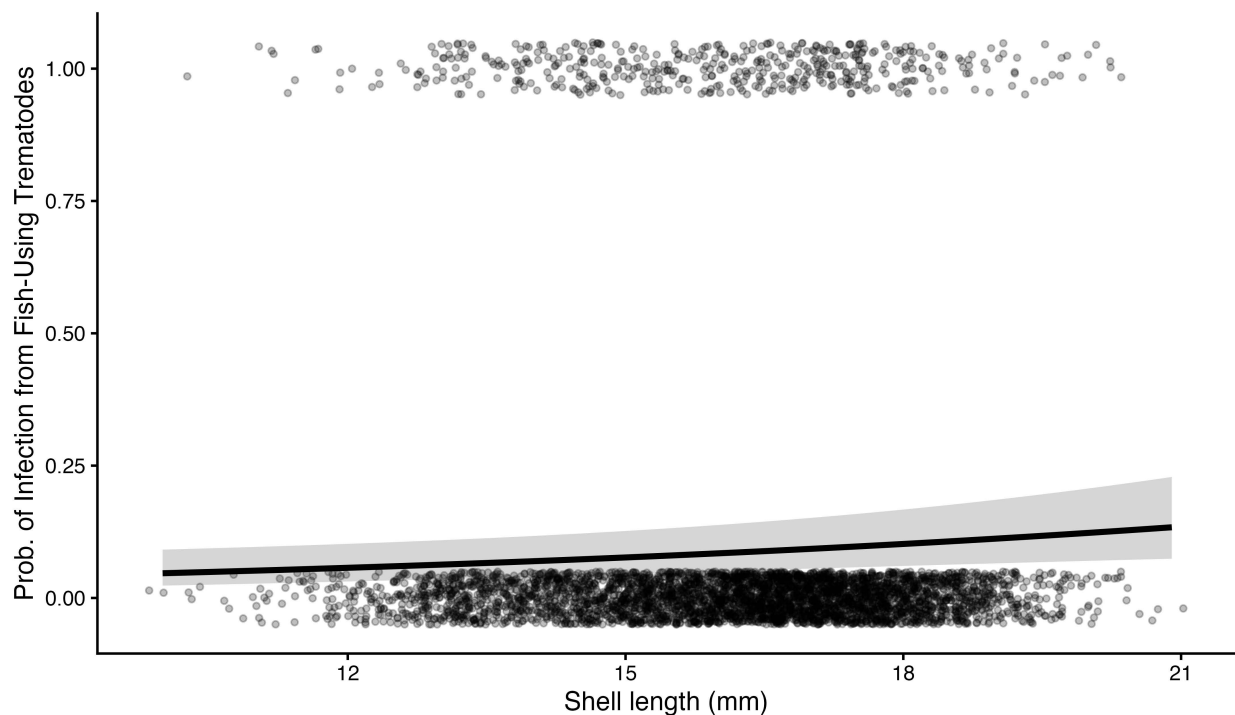


Figure 13. Logistic regression of the relationship between shell length (mm) and the probability of infection from fish-using trematodes. Data is derived from a generalized linear mixed effects model with salinity as a predictor ($p = 0.00511$). Points represent individual *I. obsoleta* ($n = 4,537$) and their infection status (0 = uninfected, 1 = infected) along the y-axis. Points have been jittered vertically for visualization. The solid black line indicates the model's predicted probability of infection across the recorded shell length ranges from all habitat surveys ($n = 46$). The gray band represents the 95% confidence interval. The x-axis was scaled to show only where points appeared. Note that shell length was converted back to raw values from the standardized values used in the model to highlight the relationship between shell length and infection from fish-using trematodes.

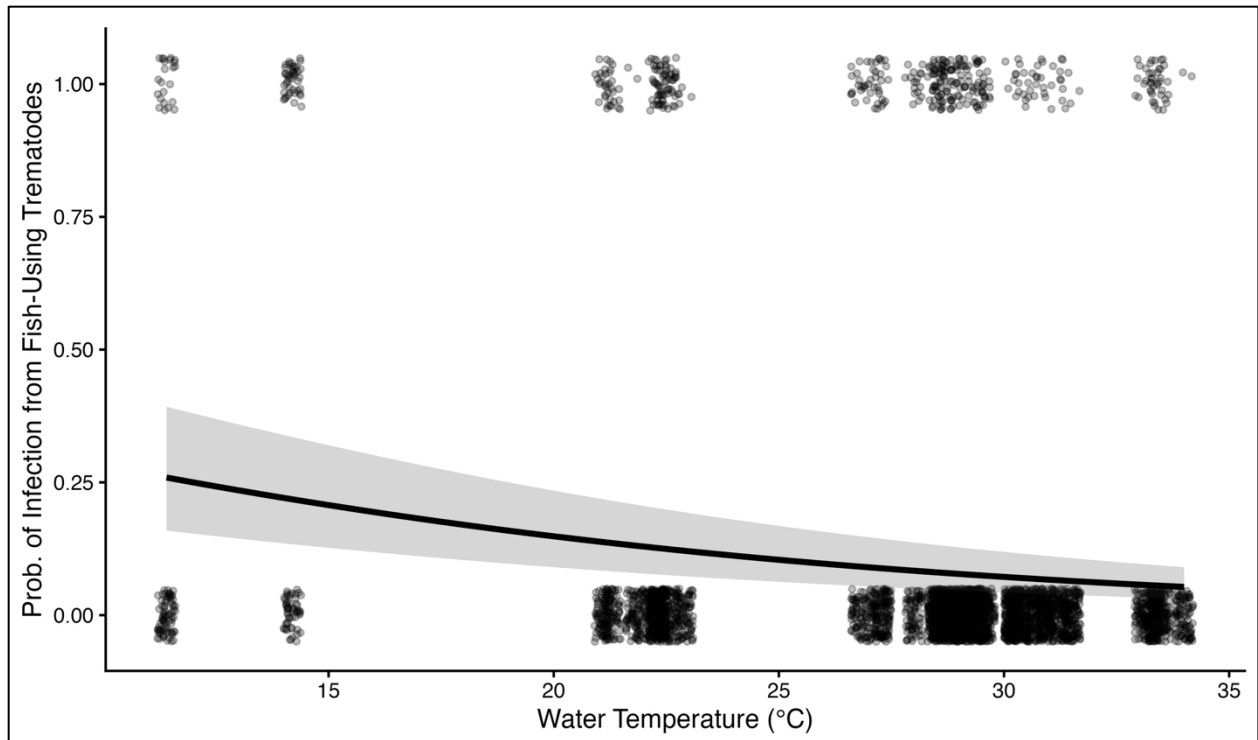


Figure 14. Logistic regression of the relationship between water temperature ($^{\circ}\text{C}$) and the probability of infection from fish-using trematodes. Data is derived from a generalized linear mixed effects model with water temperature as a predictor ($p = < 2e^{-16}$). Points represent individual *I. obsoleta* ($n = 4,537$) and their infection status (0 = uninfected, 1 = infected) along the y-axis. Points have been jittered vertically for visualization. The solid black line indicates the model's predicted probability of infection across the recorded water temperature ranges from all habitat surveys ($n = 46$). The gray band represents the 95% confidence interval. Note that water temperature was converted back to raw values from the standardized values used in the model to highlight the relationship between water temperature and infection by fish-using trematodes.

The second final host-specific logistic regression regarding fish-using trematodes predicted water temperature to be negatively correlated with infection probability from fish-using trematodes; as water temperature increased, probability of infection decreased (Fig. 14, $\beta = -0.38122 \pm 0.04342$, $p = < 2e^{-16}$). Temperature has previously been an important environmental driver for the spatial heterogeneity and habitat use of coastal fishes (Chen et al. 2022), but assessment on fish host diversity used by this parasite component community in seagrass beds of NC has yet to be conducted. As previously discussed, water temperature can have positive and negative influences on species distribution and prevalence; this includes fish populations. Rising

temperatures may negatively affect the physiological capabilities of fish to endure dramatic environmental changes (Lee et. al 2024). For instance, higher water temperatures and warming events like marine heat waves can negatively impact reproduction of coastal invertebrates (Shanks et a. 2021), or they can increase the occurrence of harmful algal blooms that lead to fish and invertebrate mortalities (Karlson et al. 2021). By association, the threats the diversity of fish face reflects onto the distribution and prevalence of their parasites, as to why these trematodes can be valuable indicators of fish populations beyond abiotic factors (Marcogliese 2002). Given that more fish-using parasites (e.g., LS, ST, SD, ZL) were observed in comparison to bird-using parasites (e.g., AV, DN, GA, HQ, Fig. 2), it is worth noting that the seasonal mobility of fish communities can lead to links in food webs and parasitic transmission from different areas (e.g., from Bogue Sound to the Straits of North Carolina) (Marcogliese 2002). The timing (i.e. month and day of year) of the habitat samples likely correlated with fish abundance, providing more support and need for additional sampling of fish populations that are associated with this trematode community. Monitoring such mobility can add to long-term datasets addressing the changes in parasite prevalence and abundance and / or their relationships with environmental conditions. As such, this work provides numerous avenues for more analysis between the fish-using parasites of this component community and their hosts. As previously stated, data on fish abundance and richness was not collected during habitat surveys, and conducting additional sampling would aid this effort, let alone helping determine the trophic levels of fishes in these NC seagrass beds. Identifying such metrics would assist in understanding food web models that included parasitic trematodes, providing further insight on the influence of parasites on that system's structure and energy augmentation (Christoffersen et al. 2011).

Lastly, a global model addressing the difference between infection from bird-using and fish-using trematodes took the following form: `glmer (formula = bird_fish ~ tempwater_s + doy_s + salinity_s + avgwaterdepth_s + shellheight_s + ltheight_s + (1 | site_id), data = cleanbf_data, family = binomial, control = ctrlbf)`. A total of nine models contained values of $\Delta AICc \leq 2.0$, but only the top model showed significant variables. Following the outcome from *dredge ()*, the best model took the following form to create a logistic regression: (1) `bird_fish ~ avgwaterdepth_s + salinity_s + shellheight_s + (1 | site_id)` ($w = 0.076$, $\Delta AICc = 0.00$, $AICc = 174.2$, $df = 5$, marginal $R^2 = 0.141$). Only avg. water depth was significant ($p = 0.03037$, Fig. 15), while salinity ($p = 0.08708$) and shell length ($p = 0.06328$) were nearly significant. The bird-using versus fish-using logistic regression regarding predicted avg. water depth to be negatively correlated with infection probability; as water depth increased, probability of infection decreased (Fig. 15, $\beta = -0.64110 \pm 0.29608$). Regardless of being bird-using or fish-using, more infected snails were generally found in shallower waters (< 40 cm). This coincides with Curtis (2002) identifying how *I. obsoleta* often remained in those elevated, shallower zones where infection prevalence was higher. Further, our habitat surveys took place in and around seagrass beds up and down elevated shorelines; locations of seagrass beds in soft-sediment habitats can affect the density of fish species (Beck et al. 2001), and then by association, could affect the community diversity of those trematodes and their abundances in comparison to bird-using trematodes. Despite there being a significantly higher abundance of fish-using trematodes in *I. obsoleta* in comparison to bird-using trematodes, these larger macrofauna like shorebirds and flounders, for instance, occasionally rely on mud snails for prey (Curtis 2007), linking soft-sediment intertidal productivity to avian and fish populations (Bhuiyan et al. 2025). By deepening the understanding of seagrass food webs, new trophic links can reinforce the importance of macrofauna (e.g., fish,

birds, snails) and their parasites in maintaining complex importance of macrofauna (e.g., fish, birds, snails) and their parasites in maintaining complex ecological balances.

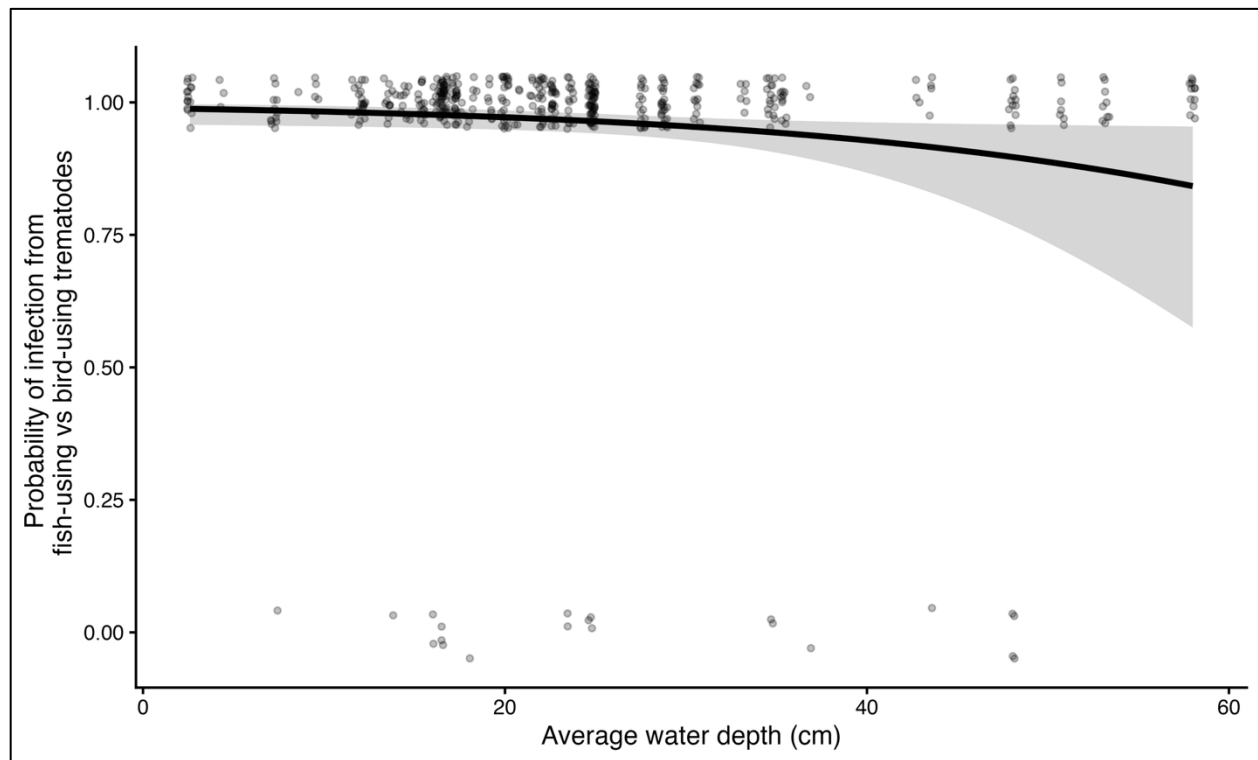


Figure 15. Logistic regression of the relationship between average water depth (cm) and the probability of infection from fish-using trematodes versus bird-using trematodes. Data is derived from a generalized linear mixed effects model with water depth as a predictor ($p = 0.03037$). Points represent individual *I. obsoleta* ($n = 4,537$) and their infection status (0 = bird-using trematode infection, 1 = fish-using trematode infection) along the y-axis. Points have been jittered vertically for visualization. The solid black line indicates the model's predicted probability of infection across the recorded water depth ranges from all habitat surveys ($n = 46$). The gray band represents the 95% confidence interval. Note that water depth was converted back to raw values from the standardized values used in the model to highlight the relationship between water depth and infection probability.

Across the host-specific regressions, patterns continued from those with probability of infection from all trematode species, specifically regarding shell length and water temperature. This supports our hypothesis that the significant patterns would carry over when addressing host specificity. These findings support the benefit of comparting parasite population structure between different final hosts, as it provided insight to the correlations between this diverse

component community and the surrounding abiotic and biotic factors, all of which are acting interdependently. With that, this study adds to the significant quantity of literature demonstrating how snail-trematode surveys can be indicators of community diversity and ecosystem structuring (see Huspeni and Lafferty 2004).

Q3: Epibiont Biomass and the Seagrass Ecosystem

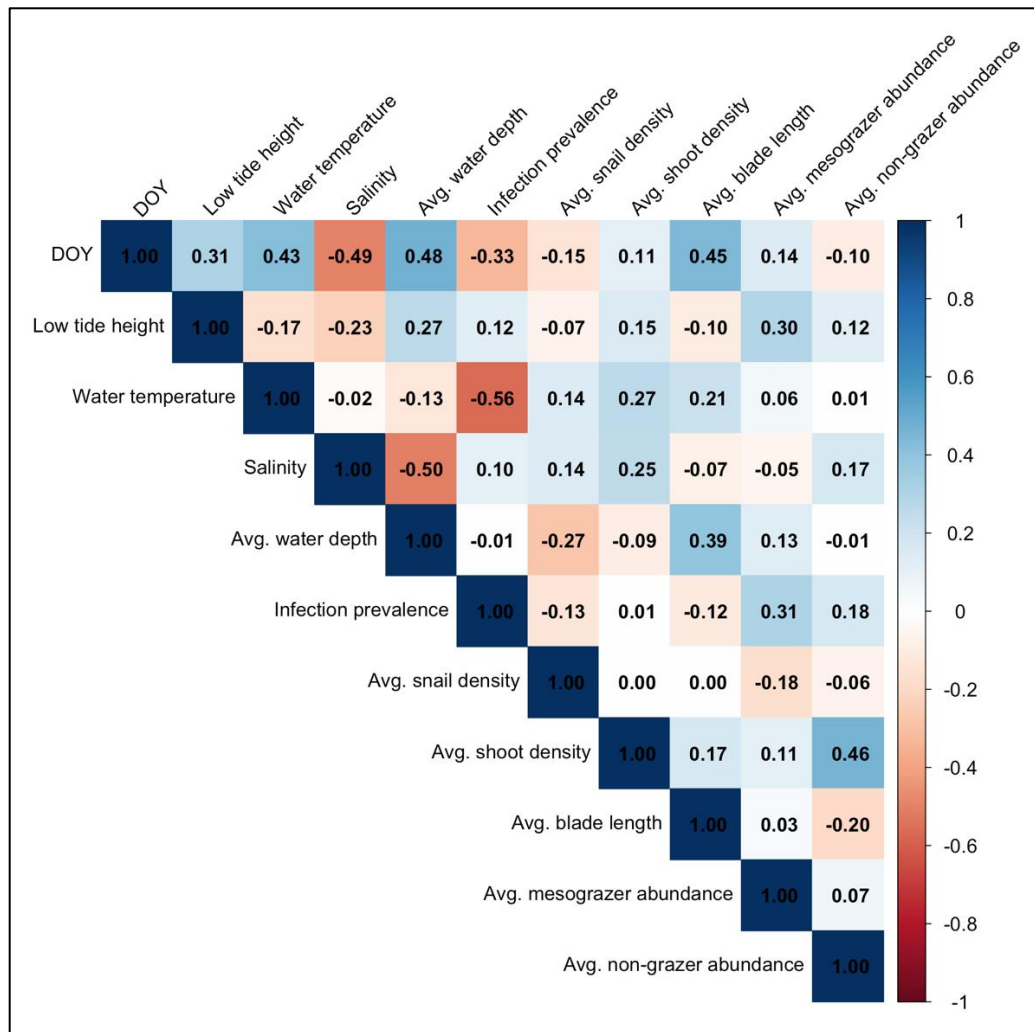


Figure 16. Correlation matrix showing pairwise Pearson correlation coefficients among abiotic and biotic variables relating to average epibiont biomass (mg/mm) per habitat survey. Cells display correlation coefficients. Color intensity and shading indicate the strength of each variable, with positive correlations shown in blue and negative correlations in red. Higher correlation coefficients represent stronger associations between variables.

Like Figure 7, selection of variables for models stemmed from Pearson correlation coefficients (ρ) (Fig. 16). Site was the only random effect. Air temperature was not included in these models, as previously explained. Given that high collinearity was present between avg. mesograzer abundance and avg. taxa abundance ($\rho > 0.6$), avg. taxa abundance was not included in the final correlation matrix or any model, as it contained averaged data between both mesograzers and non-grazers. Infection richness was also not present in the final matrix or any model, given its collinearity between infection prevalence; infection prevalence remained in the model, as it was the response variable in the previous matrix.

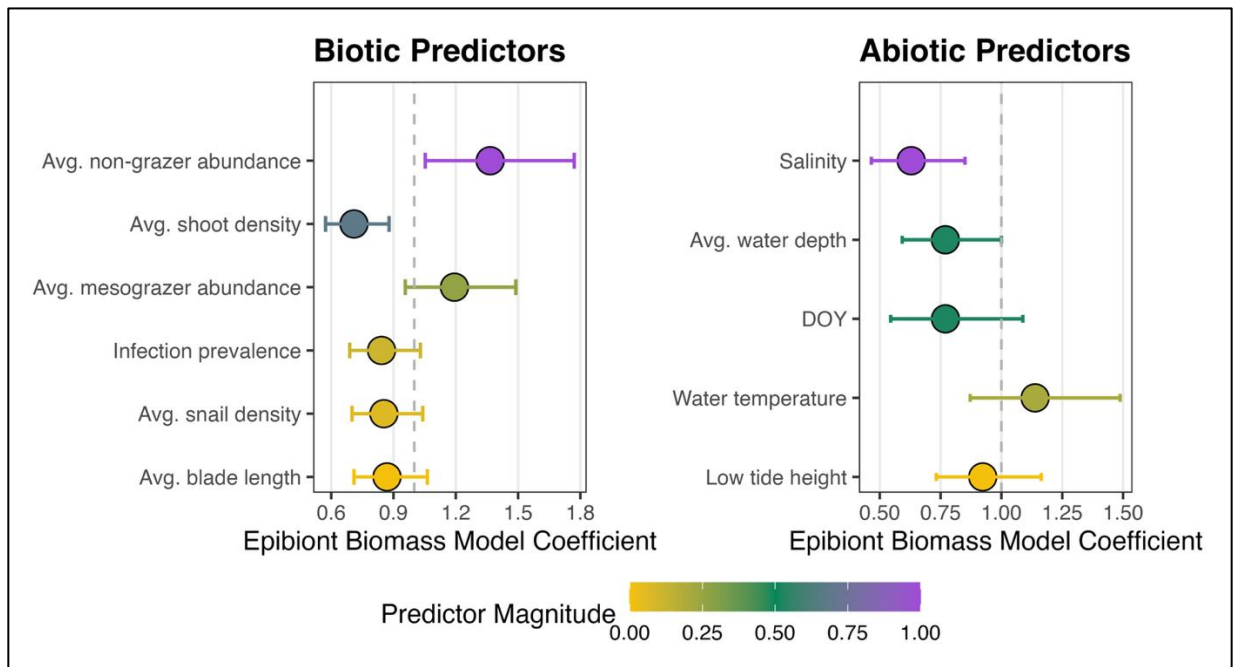


Figure 17. Effects of biotic and abiotic predictor variables on average epibiont biomass per habitat survey displayed as forest plots with Gamma generalized linear mixed models. Points represent estimated multiplicative effects ($\exp(\beta)$). Horizontal bars indicate 95% confidence intervals. The grey vertical dashed line at 1.00 denotes no effect; values > 1.00 indicate a positive association with epibiont biomass, while values < 1.00 indicate a negative association. Predictors are separated into biotic (left panel) and abiotic (right panel) variables and are ordered by relative importance within each panel. Point colour represents scaled predictor magnitude (0.00-1.00), with purple (1.00) denoting highest magnitude.

Forest plots of abiotic and biotic predictor variables indicated strong correlations with of avg. epibiont biomass across the dataset (Fig. 17). The strongest correlations, as discussed below,

were initially used in GLMMs before additional model variations were run. The biotic drivers with highest coefficients and magnitude toward avg. epibiont biomass were avg. non-grazer abundance ($\exp(\beta) = 1.3654$), avg. shoot density ($\exp(\beta) = 0.7095$), and avg. mesograzers abundance ($\exp(\beta) = 1.1934$). Both variables regarding invertebrate taxa show strong positive correlations with epibiont biomass due to coefficients > 1.00 and the confidence intervals are leaning positively. Therefore, community structure and productivity are key to the levels of epibiont biomass in beds of *H. wrightii*. The simple correlation between presence of mesograzers and epibiont biomass suggests that there is facilitation of epibionts by such grazers. For instance, lower density of grazers such as amphipods have been seen to correlate with higher percentages of epiphyte biomass, and that natural densities of amphipods can reduce epiphyte biomass (Cook et al. 2011). However, in contradiction, our data shows an unexpected trophic relationship, as there is a positive association between epibiont biomass and mesograzers. Beds of *H. wrightii* with more epibiont biomass support greater abundances of mesograzers and non-grazers, suggesting that other unmeasured environmental factors may be at play directly and indirectly, including nutrient availability, damage to seagrass blades from taxa, predators of mesograzers, and recruitment of new seagrass shoots per site (Heck and Valentine 2006). Abundance of these grazers can be characterized by high levels of secondary production by epibionts and then in turn can be sources of prey for non-grazers or consumers on a higher trophic level (Goecker et al. 2005; Heck and Valentine 2006). The different taxonomic groups of grazers (i.e. gastropods versus amphipods or shrimps) also can have different effects on epibiont biomass due to the diversity of feeding methods and preference for feeding on different epibiont material (Jernakoff et al. 1996; Duffy and Harvilicz 2001; Duffy et al. 2003; Heck and Valentine 2006). This indicates that different mesograzers can cause variation in the accumulation and composition of

epibiont biomass based on the nutrient content per epiphytic material. Therefore, there is increasing recognition for the importance of identifying and monitoring abundance of seagrass-associated taxa, regardless if they are grazers or non-grazers. It also leads to assumptions on what beyond epibiont biomass correlates with mesograzer and non-grazer abundances, such as factors that vary temporally and seasonally.

Further, avg. shoot density had moderate correlation with avg. epibiont biomass due to its coefficient being < 1.00 . This indicates that higher shoot density is associated with lower epibiont biomass, hinting at a potential dilution effect, whereas if there is more surface area for epibiont accumulation, less epibiont biomass per mg/mm will be present. This also suggests that seagrass landscape contiguity and seagrass metrics beyond those assessed in this study (e.g., canopy height, seagrass biomass, light intensity) may have implications for the changes in epibiont biomass over time (Fonseca and Bell 1998). Moreover, the high rate of decline of seagrasses (1.5 % per year) in Back and Bogue Sounds, NC (Field et al. 2020) likely leads to decrease of epibiont biomass by association. Given the small scope of this study and lack of regularly scheduled field monitoring of North Carolina's seagrasses to provide data needed to explain the connections between epibiont biomass, seagrass system health, and time (Field et al. 2020), there is an urgent need to build long-term datasets on these variables and their associates. With the largest documented polyhaline seagrass ecosystem on the Atlantic seaboard of North America occurring in the shallow coastal waters (< 2.0 m depth) of North Carolina (Bartenfelder et al. 2022), investigating further growth patterns of epibiont biomass on seagrasses, let alone their rates of productivity, is essential.

In comparison, the weak, negative association between infection prevalence ($\exp(\beta) = 0.8424$) and epibiont biomass eludes to the complex indirect relationship between parasitism and

epibionts growing on *H. wrightii* (Fig. 17). Despite the weak correlation and a lower magnitude, it suggests that higher infection prevalence is associated with lower epibiont biomass. As Sousa (1991) identified that *I. obsoleta* and their larvae find refuge in seagrass beds and constitutes an important component of the habitat's structure, it also leads to the presence of trematodes and free-swimming cercaria life stage being baffled by seagrass blades when seeking the next host in the life cycle. When not infected by trematodes, *I. obsoleta* are known to facilitate shoal grass (*Halodule wrightii*) and eelgrass (*Zostera marina*) growth through the consumption of epibionts (Kinlan et al. 1997; Lewis and Boyer 2014). Preliminary work focusing on infection prevalence in seagrass beds identified that LS infection of *I. obsoleta* increased consumption of epiphytes and indirectly regulated epiphyte biomass on *H. wrightii* blades (Nadzam et al. *in prep*). While this current work did not quantitatively measure indirect effects between infection prevalence and epibiont biomass, we can only assume that this weak, yet negative, correlation is associated with LS, as it was the most abundant trematode species in our sampled *I. obsoleta* populations as time progressed (Fig. 2).

The abiotic drivers with highest coefficients and magnitude toward avg. epibiont biomass were salinity ($\exp(\beta) = 0.6291$), avg. water depth ($\exp(\beta) = 0.7697$), and day of year (DOY; $\exp(\beta) = 0.7700$) (Fig. 17). As none of these variables had coefficients > 1.00 but had positively leaning confidence intervals, it suggests that as salinity and water depth increased, epibiont biomass decreased. Physical parameters that regulate the physiological activity of seagrasses (e.g. temperature, salinity, waves, depth, day length), natural parameters that limit photosynthetic ability (e.g. light, nutrients, epiphytes), and the various combinations of such parameters could permit, encourage, or eliminate seagrasses and epibionts from the study sites (Short and Coles 2001). DOY can not only suggest potential seasonal patterns, but also tidal range, number of low

tides versus high tides, and changing tidal channels, leading to fluctuations in water depth and salinity. This reinforces the need for data on additional variables like light intensity, as it is reduced in greater water depths and leads to inhibition of photosynthesis (Short and Coles 2001). Seagrasses are able to survive in the intertidal when sheltered from wave actions, associated turbidity, and low salinity inflow from freshwater creeks, but when salinity intrusion does occur, it can affect vegetation propagation, reproduction, and distribution. This is similar for epibionts, and these parameters can greatly affect their rapid growth rates and short generation times that leads to significant productivity (Duffy and Harvilicz 2001; Morgan and Kitting 1984). In the vulnerable habitat of the soft-sediment intertidal, the studied seagrass beds are experiencing these parameters to many degrees, likely leading to the correlations observed with epibiont biomass and possible reasoning as to why a weak correlation appeared between infection prevalence of trematodes in *I. obsoleta* and avg. epibiont biomass. Avg. snail density ($\exp(\beta) = 0.8536$), avg. blade length (0.8686), water temperature ($\exp(\beta) = 1.1384$), and low tide height ($\exp(\beta) = 0.9231$) were weak abiotic and biotic drivers of avg. epibiont biomass and ranked last in predictor magnitude.

Overall, these findings support our hypothesis (**H3**) of increased prevalence being correlated with lower epibiont biomass and alludes to the support from Nadzam et al *in prep* regarding LS increasing consumption of epibionts. In addition, the strong correlations between epibiont biomass and avg. shoot density continue the productivity-related patterns seen in previous work (e.g. Mallin et al. 2000). The results of this study provided additional data on the distribution of parasites belonging to the same component community, and especially the presence of trematodes that previously caused varying indirect effects between parasitism and seagrass health (i.e. LS, HQ, ZL) (Nadzam et al. *in prep*). It also provided evidence of additional

seagrass beds in Bogue Sound and the Straits, NC besides the single site used in the preliminary work that supported strong populations of *I. obsoleta* and its parasite community. However, further experiments at these additional locations will be necessary to determine if epibiont biomass is truly indirectly affected by parasitism in *I. obsoleta*, or if other environmental variables not assessed in this study drive epibiont biomass.

To confirm the specific abiotic and biotic drivers of epibiont biomass in *H. wrightii* beds of Bogue Sound and the Straits, multiple models were run with the *dredge ()* function using the previously outlined global Gamma mixed-effects formula. A total of five models addressing different combinations of these variables contained values of $\Delta AICc \leq 2.0$ and were considered to have substantial support for influence on epibiont biomass (Table 4). In the following paragraphs, the top five models outline that avg. water depth, avg. shoot density, avg. snail density, avg. non-grazer abundance, and salinity were significant and strongly correlated with avg. epibiont biomass (mg/mm) per site in seagrass beds of *H. wrightii* (Table 4).

Table 4. Candidate generalized linear mixed models derived from the dredge output assessing abiotic and biotic predictors of average epibiont biomass with models ranked by $\Delta AICc$ from lowest to highest (≤ 2.0). This table lists the epibiont model number (EB), predictors for each model, AICc score, the difference in AICc score between each model and the top-ranked model ($\Delta AICc$), weight given to each model (w), and the p -value associated with each variable per model (p). Gray cells indicate that the associated variable was not included in that aligned model. Water temp. = water temperature. Meso. = mesograzers.

EB	AICc	Δ AICc	w	p							
				Water temp.	Avg. water depth	Avg. shoot density	Avg. snail density	Avg. meso. abundance	Avg. non- grazer abundance	Salinity	Infection prevalence
1	-191.2	0.00	0.138		0.0004	0.0007	0.0051		0.0072	0.0232	
2	-189.8	1.40	0.068		9.45 e-05	0.0001	0.0056	0.0686	0.0017	0.0236	0.0526
3	-189.8	1.41	0.068		0.0002	0.0002	0.0123	0.2086	0.0042	0.0259	
4	-189.6	1.61	0.062	0.2061	0.0004	0.0002	0.0031		0.0048	0.0178	
5	-189.4	1.76	0.057		0.0003	0.0006	0.0025		0.0054	0.0200	0.2275

Based on the marginal R^2 values for all five models (R^2 in order of EB = 0.460, 0.515, 0.478, 0.484, 0.477), our single random effect of site ID helped explain the correlation between

variables and each model. However, in similarity with analyses on trematode diversity, data collection on a small selection of abiotic and biotic variables highlights numerous other variables not assessed in this study that could heavily influence epibiont biomass in *H. wrightii* beds. For instance, while the variable avg. mesograzer abundance did not appear to be significant in any of our top models, it is well known that mesograzers have markedly strong impacts on the fouling assemblages on seagrass blades (Kinlan et al. 1997; Duffy and Hay 2000; Duffy and Harvilicz 2001; Jaschinski and Sommer 2008; Voigt and Hovel 2018). However, diversity of all seagrass fauna present at the study sites was not assessed, for complete assessment on biodiversity on any scale is difficult to achieve. These additions would include fishes and larger decapods that would identify the studied mesograzers as prey, thus affecting the community composition and range of specific mesograzer taxa present. The complexity of each model and divulging which statistical models best fit the data only supports that more assessment is needed on seagrass community structure and how certain abiotic and biotic factors govern the presence of epibiont biomass.

All models in Table 4 indicated that both variables of year and avg. blade length were significant. Therefore, the top model was used for all logistic regressions. The model took the following form: $\text{avg_epibiont_biomass_mg_mm} \sim \text{avgnon_g} + \text{avgshootdensity_s} + \text{avgsnaildensity_s} + \text{avgwaterdepth_s} + \text{salinity} + (1 \mid \text{site_id})$ ($w = 0.138$, $\Delta\text{AICc} = 0.00$, $\text{AICc} = -191.2$, $\text{df} = 8$, $\text{marginal } R^2 = 0.460$). A logistic regression predicted avg. epibiont biomass to be inversely correlated with avg. water depth; as water depth increased, biomass decreased (Fig. 18; $\beta = -0.4117 \pm 0.1171$, $p = 0.0004$). The second regression predicted avg. epibiont biomass to be inversely correlated with salinity; as salinity increased, biomass decreased (Fig. 19; $\beta = -0.2643 \pm 0.1164$, $p = 0.0232$). The slopes of the lines of fit in each abiotic regression, as well as the

affiliated negative β values confirm the correlations between the significant variables with higher magnitude and epibiont biomass seen in Fig. 17 and described above.

Regarding the biotic variables, a logistic regression predicted avg. epibiont biomass to be inversely correlated with avg. shoot density; as shoot density increased, biomass decreased (Fig. 20; $\beta = -0.3472 \pm 0.1025$, $p = 0.0007$). A second regression predicted avg. epibiont biomass to be positively correlated with avg. non-grazer abundance; as non-grazer abundance increased, biomass increased (Fig. 21; $\beta = 0.3118 \pm 0.1162$, $p = 0.0072$). A third regression predicted avg. epibiont biomass to be negatively correlated with avg. snail density; as snail density increased, biomass decreased (Fig. 22; $\beta = -0.2733 \pm 0.0977$, $p = 0.0051$). Like the abiotic regressions, the slopes of the lines of fit in each biotic regression, as well as the affiliated β values confirm the correlations between the significant variables with higher magnitude and epibiont biomass seen in Fig. 17.

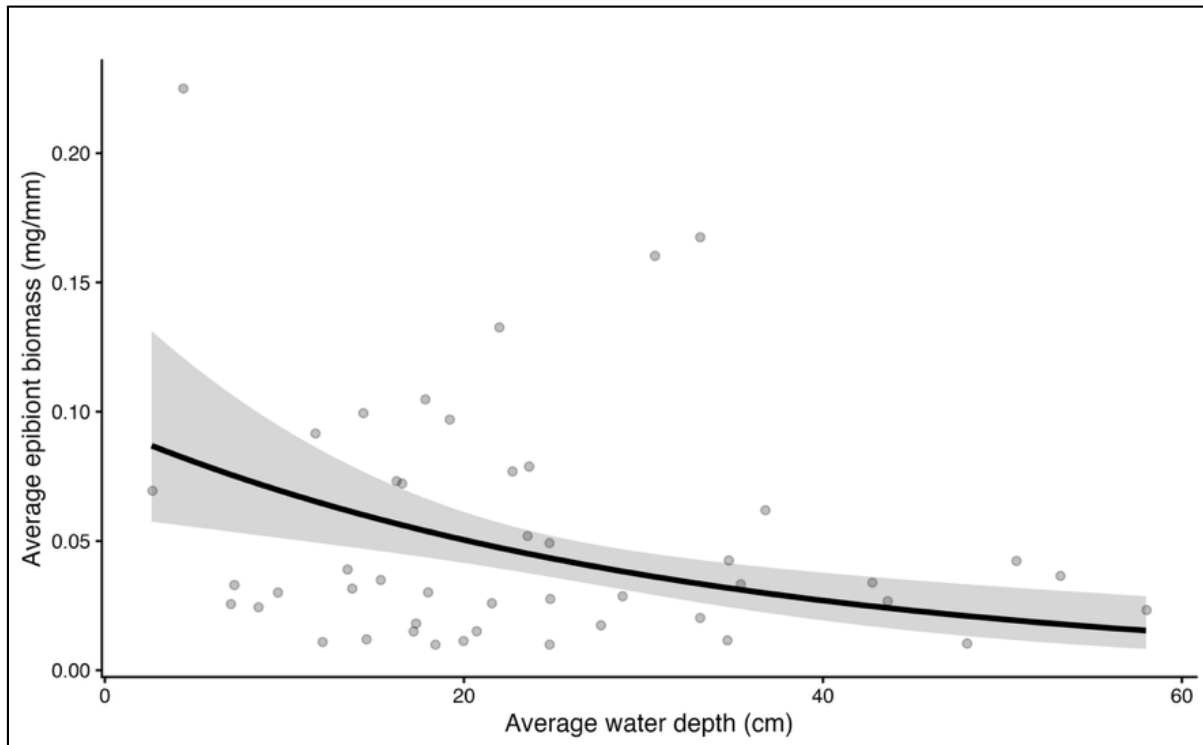


Figure 18. Logistic regression of the relationship between average water depth (cm) and average epibiont biomass (mg/mm). Data is derived from a Gamma generalized linear mixed effects model with average water depth as a predictor (0.0004). Points represent individual habitat surveys ($n=46$). Points have been jittered along both axes for visualization. The solid black line indicates the model's predicted correlation between average water depth and average epibiont biomass across all habitat surveys. The gray band represents the 95% confidence interval. Note that both variables were converted back to raw values from the standardized values used in the model to highlight their relationship.

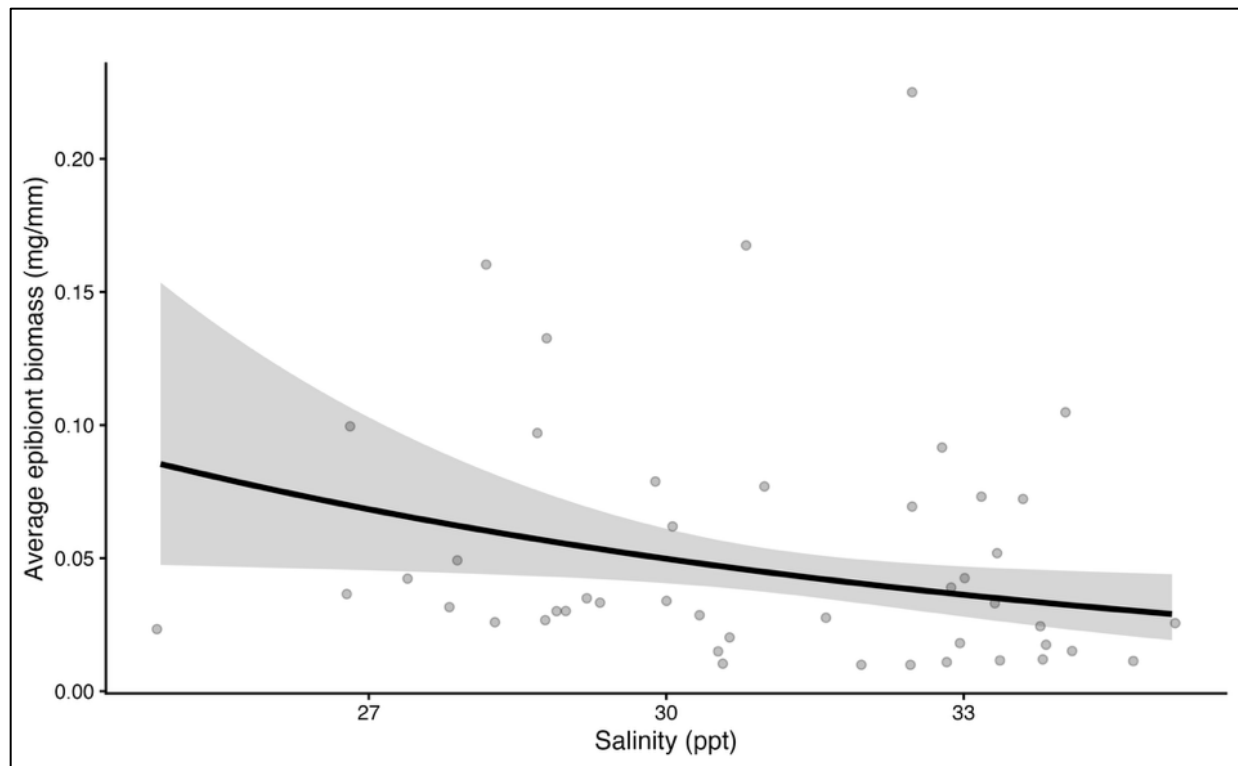


Figure 19. Logistic regression of the relationship between salinity (ppt) and average epibiont biomass (mg/mm). Data is derived from a Gamma generalized linear mixed effects model with salinity as a predictor ($p = 0.0232$). Points represent individual habitat surveys ($n=46$). Points have been jittered along both axes for visualization. The solid black line indicates the model's predicted correlation between salinity and average epibiont biomass across all habitat surveys. The gray band represents the 95% confidence interval. Note that both variables were converted back to raw values from the standardized values used in the model to highlight their relationship.

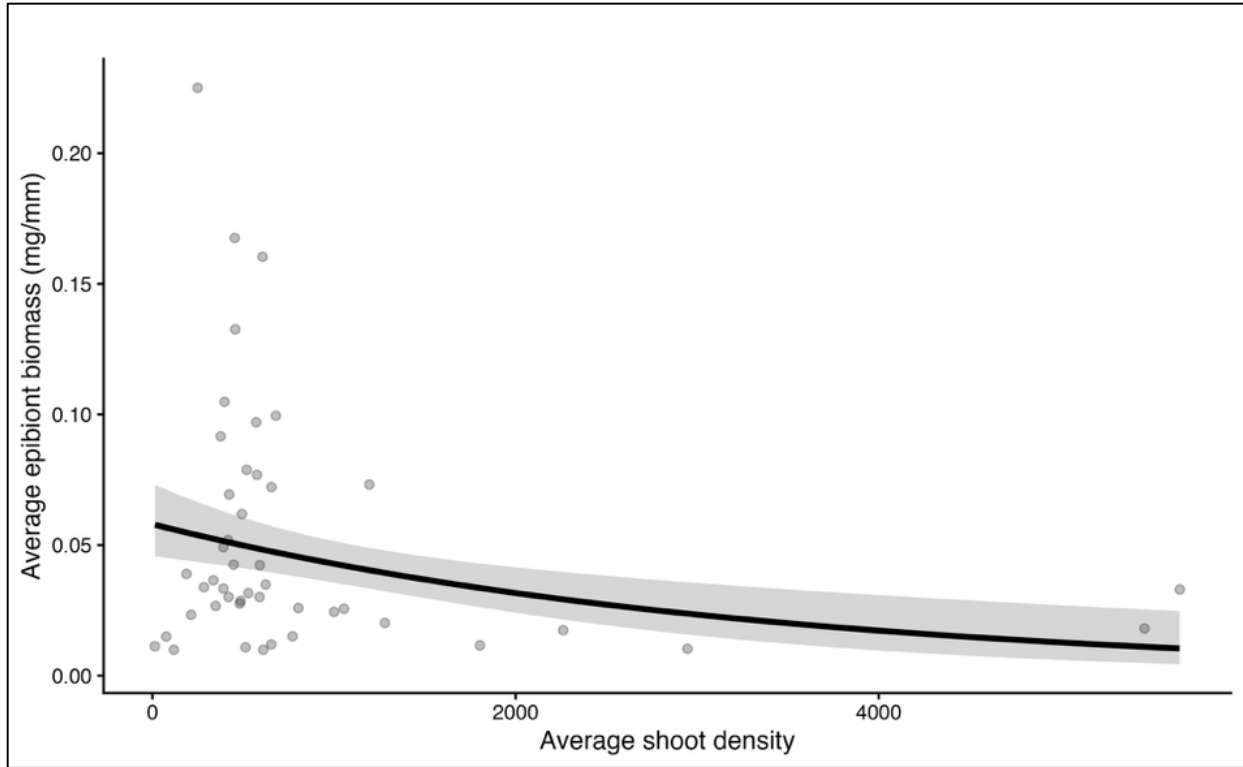


Figure 20. Logistic regression of the relationship between average shoot density and average epibiont biomass (mg/mm). Data is derived from a Gamma generalized linear mixed effects model with average shoot density as a predictor ($p = 0.0007$). Points represent individual habitat surveys ($n=46$). Points have been jittered along both axes for visualization. The solid black line indicates the model's predicted correlation between average shoot density and average epibiont biomass across all habitat surveys. The gray band represents the 95% confidence interval. Note that both variables were converted back to raw values from the standardized values used in the model to highlight their relationship.

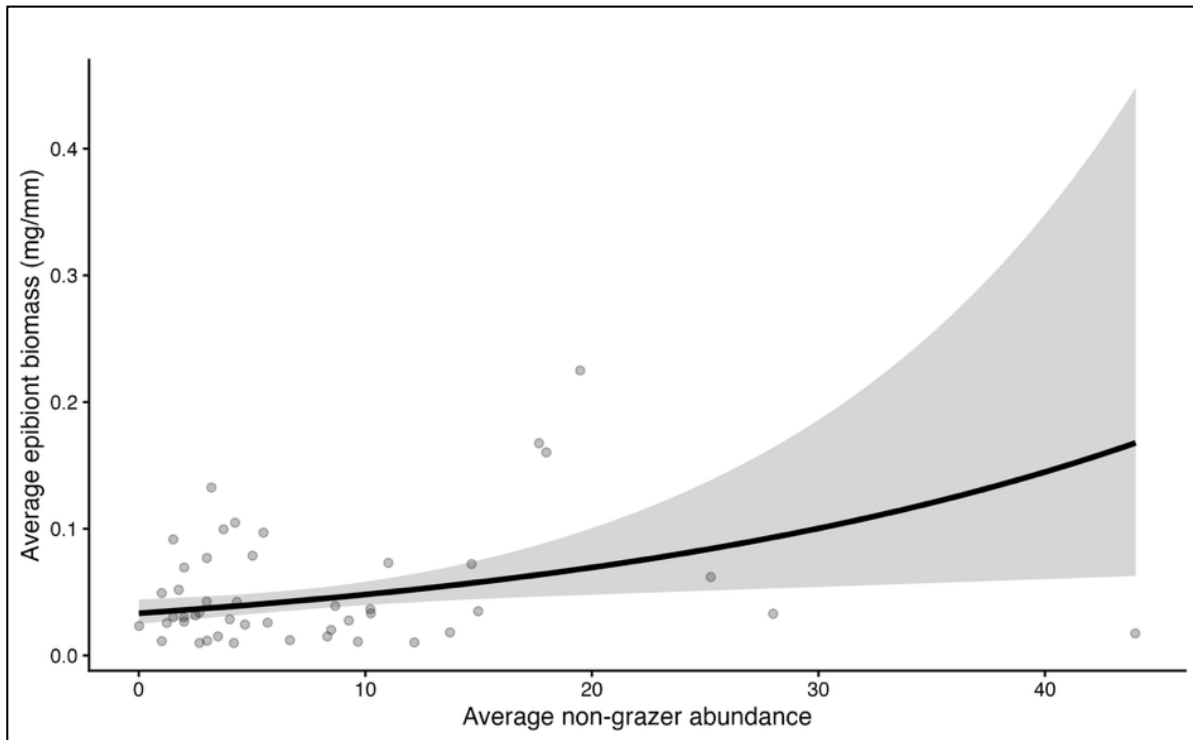


Figure 21. Logistic regression of the relationship between average non-grazer abundance and average epibiont biomass (mg/mm). Data is derived from a Gamma generalized linear mixed effects model with abundance as a predictor ($p = 0.0072$). Points represent individual habitat surveys ($n=46$). Points have been jittered along both axes for visualization. The solid black line indicates the model's predicted correlation between abundance and average epibiont biomass across all habitat surveys. The gray band represents the 95% confidence interval. Note that both variables were converted back to raw values from the standardized values used in the model to highlight their relationship.

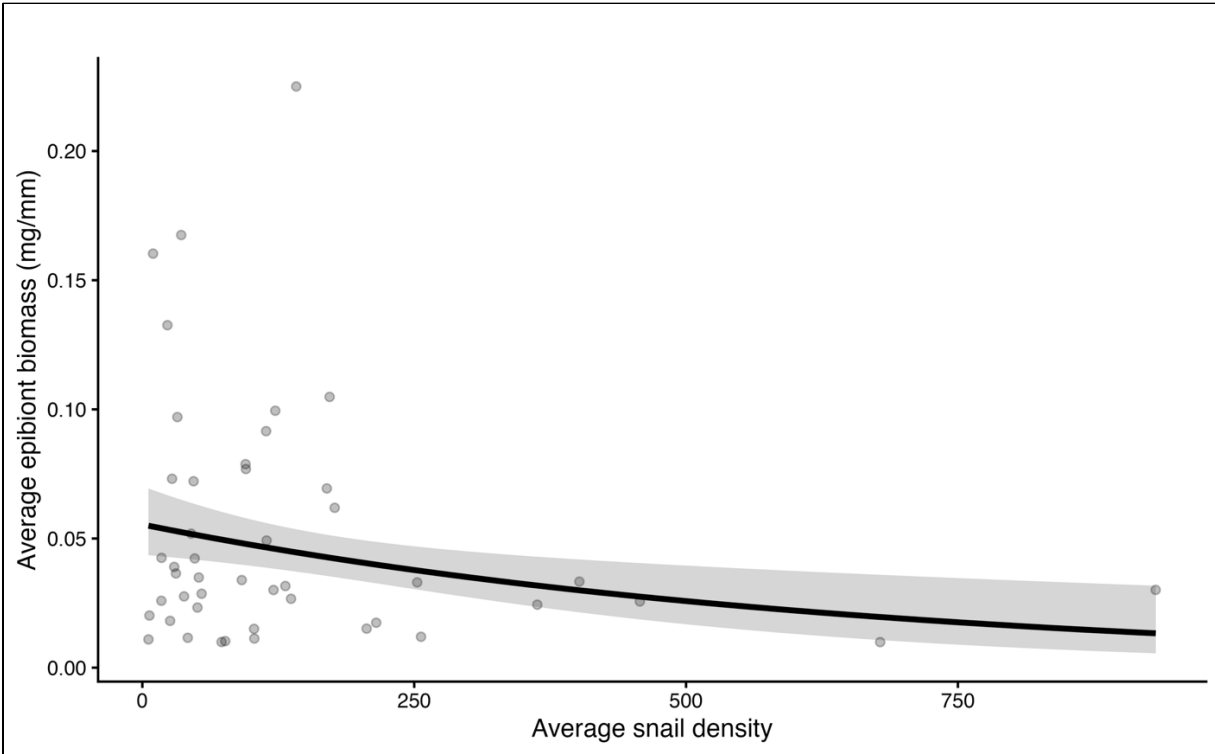


Figure 22. Logistic regression of the relationship between average snail density and average epibiont biomass (mg/mm). Data is derived from a Gamma generalized linear mixed effects model with average snail density as a predictor ($p = 0.0051$). Points represent individual habitat surveys ($n=46$). Points have been jittered along both axes for visualization. The solid black line indicates the model's predicted correlation between average snail density and average epibiont biomass across all habitat surveys. The gray band represents the 95% confidence interval. Note that both variables were converted back to raw values from the standardized values used in the model to highlight their relationship.

Considering non-grazers, the majority of seagrass-associated taxa identified were mesograzers instead of non-grazers (see Q4: Biodiversity of Seagrass-Associated Taxa), and habitat and community complexity in general is likely due broad overlap in species composition in *H. wrightii* beds compared to *Z. marina* that are also sparsely present in the area (Jernakoff et al. 1996; Field et al. 2020; Bartenfelder et al. 2022). For instance, competition between grazers and non-grazers for shelter and from predation is possible, as there could be trade-offs occurring between shelter and food (i.e. epibiont) acquisition (Beerman et al. 2018). In addition, seagrasses in warm temperate regions such as North Carolina could serve as better nurseries than cool

temperate regions, especially for fishes or seagrass beds near areas where prey larvae enter estuaries and therefore have higher fish densities (Orth and van Montfrans 1990; Beck et al. 2001). While *I. obsoleta* is a grazer, these gastropods are known to treat seagrass beds as nurseries by coating seagrass blades with large quantities of eggs, affecting light viability for epibionts and the grasses themselves (Lewis and Boyer 2014), but also contributing to the overall community diversity of the system. Due to seagrasses being such a highly productive ecosystem undergoing constant biotic and abiotic changes, production from epibionts and the grasses themselves allows the community composition to follow many pathways. This suggests that temporal or seasonal shifts in abundance of *H. wrightii* likely affect faunal recruitment and utilization of these critical habitats (Micheli et al. 2008), regardless of the fauna being mesograzers or non-grazers.

As small spatial and temporal scales were assessed in this study, the methods used in analyzing epibiont abundance and community composition are relevant when simply attempting to understand the small changes in seagrass biodiversity and community ecology. This is especially the case for parasite diversity, even if infection prevalence was not significant in any top model despite it being a moderate biotic driver of epibiont biomass (Fig. 17; Table 4). That association between epibionts and parasitism, while weak, only warrants more investigation beyond what was assessed experimentally in Nadzam et al. (in prep) and observationally in this study using *I. obsoleta*.

Q4: Biodiversity of Seagrass-Associated Taxa

Ten out of the 16 taxonomic groups were identified as mesograzers (amphipods, *Astyris lunata*, *Bittium* spp., calanoid copepods, *Palaemon* spp., *I. obsoleta* (< 10 mm), isopods, polychaetes, Portunidae, and *Tozeuma carolinense*), while the remaining 6 were identified as

non-grazers (bivalves, cumacean, foraminifera, nematodes, oligochaetes, and ostracods). A total of 12,598 individual organisms were counted, with 90.09% being mesograzers (n = 11,350) and 9.91% being non-grazers (n = 1,248). Raw abundance data indicated that *Bittium spp.* (gastropod, n = 6,158 total), *Palaemon spp.* (crustacean, n = 2,963), calanoid copepods (crustacean, n = 1,106), and amphipods (crustacean, n = 580) made up most of the identified seagrass-associated taxa across all habitat surveys (Fig. 23). *Bittium spp.* was present at all habitat surveys except for three: OH_Mar_2025, IMS_Apr_2025, and JL_Apr_2025. On the level of a single habitat survey, the maximum abundance of *Bittium spp.* observed was 1,446 individuals at site MM in June 2024. This habitat survey also reported the highest abundance of all seagrass-associated taxa (n = 1,579), with the community composition made up of *Bittium spp.*, amphipods, bivalves, calanoid copepods, *Palaemon spp.*, foraminifera, isopods, nematodes, ostracods, and polychaetes. The most abundant non-grazers were bivalves (n = 373 total), foraminifera (n = 341), and nematodes (n = 330) across all habitat surveys.

The four most abundant taxonomic groups were all mesograzers and either crustaceans or molluscs, supporting my hypothesis (**H4**) that these taxonomic groups would make up most of the identified seagrass-associated taxa. This is due to *Bittium spp.* being significantly more abundant than any other taxonomic group. This is likely because these gastropods are dominant members in epifaunal communities in habitats made up of seagrass, algae, sand, and gravel with populations that range from the Gulf of St. Lawrence to Texas depending on the specific species (Knopf 1981; Abbott and Morris 1995). Some species of this genus have been documented to achieve densities of 200 individuals per gram of *Z. marina* (Marsh 1976), allowing these gastropods to mediate the growth of epibionts like periphyton and diatoms on seagrass substrates (van Montfrans et al. 1982).

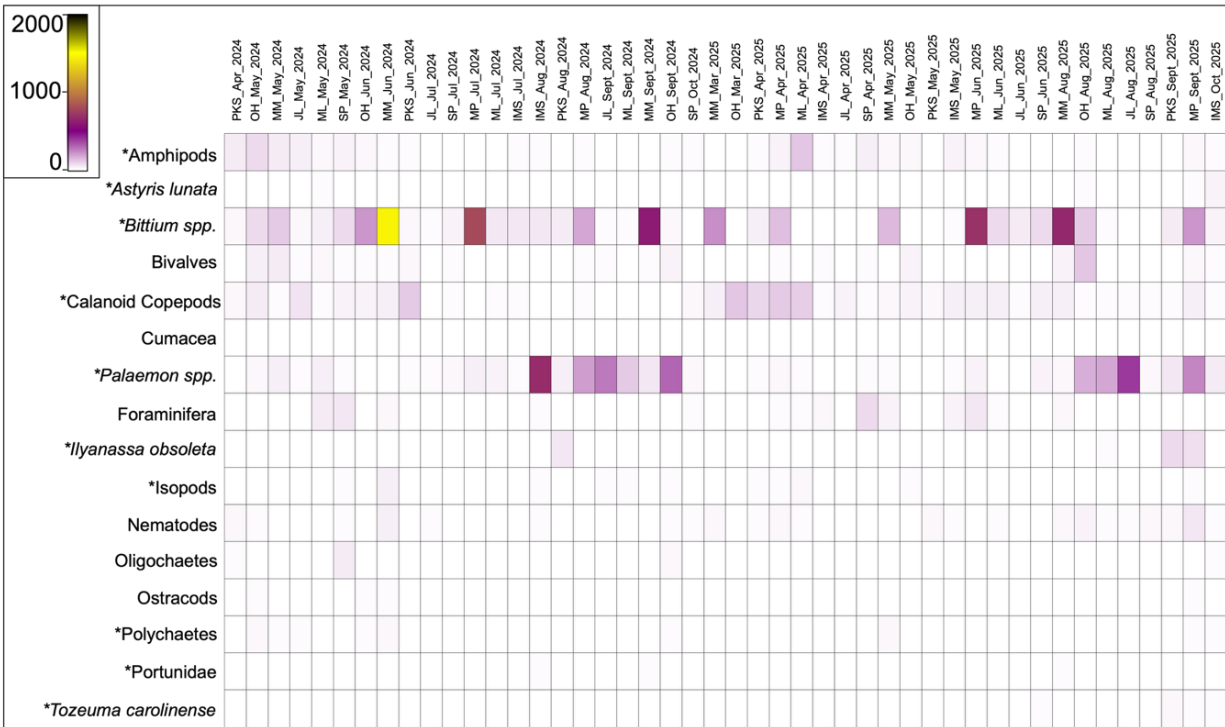


Figure 23. Shade plot visualizing raw seagrass-associated taxa abundance contribution to community similarity across all habitat surveys. Rows represent taxonomic group and columns represent habitat surveys sorted consecutively by month (abbreviated) and year. Taxonomic groups led by an asterisk were identified as mesograzers, while groups with no asterisk were identified as non-grazers. Shading intensity reflects the raw abundance counts of each taxonomic group to within-group similarity, with darker shades indicating higher abundance. Only taxonomic groups showing up to 70% cumulative similarity are shown.

Patterns in the abundance between taxonomic groups were observed (Fig. 24). Overall diversity of seagrass-associated taxa diversity was visualized in PCoA plots. Much like the analyses on trematode diversity, this multivariate analysis largely supported the monthly separation of the data versus a site-by-site comparison, as month-to-month comparisons helped highlight the four most abundant taxonomic groups. A main PERMANOVA indicated that the assemblages of seagrass taxa between months were significantly different ($F = 3.0033$, $p = 0.001$). The unconstrained ordination by PCoA of taxa diversity demonstrated some probable relationships among taxonomic groups from month to month. SIMPER analyses revealed that the average dissimilarity of abundance between all month comparisons was 54.76%, with

contributions led by *Bittium spp.*, *Palaemon spp.*, calanoid copepods, amphipods, foraminifera, and bivalves in highly various percentages. Points in the PCoA clustering positively in the direction of *Bittium spp.* on the PC1 axis are appearing to be associated with months of March (< 88.32 % contribution), May (17.25%), June (40.41%), July (32.57%) and August (19.52%) (Fig. 24). However, higher abundance of *Bittium spp.* in May shaped the assemblage of taxa diversity, as abundance of *Bittium spp.* in May, when paired with the other months in SIMPER and pairwise PERMANOVA analyses, showed significant dissimilarity ($p < 0.05$). For example, average dissimilarity of taxa between May and June was 46.38%, with *Bittium spp.* contributing 30.37% (Jun; $p = 0.07$). Similarly, average dissimilarity between May and July was 50.11% and between May and August was 53.97%, with *Bittium spp.* contributing 25.25% (Jul; $p = 0.007$) and 16.95% (Aug; $p = 0.001$), respectively.

No efforts were made to identify what epiphytes, periphyton, detritus, microalgae, or macroalgae comprised the epibionts present on sampled *H. wrightii* blades. As grazers are known to use different modes of feeding depending on the different foods available, it is possible that abundance of specific grazers is determined by the type of epibiont material present (Jernakoff et al. 1996; Jaschinski and Sommer 2008). For instance, Jernakoff et al. (1996) reviewed how many groups of crustaceans are omnivorous, and adaptations of grazing mechanisms not only fluctuate with availability. Harpacticoid copepods, feed primarily on periphyton in comparison to amphipods that consume macroalgae, microalgae in periphyton, and detritus, or to decapods (i.e., *Palaemon spp.*) that feed on seagrass tissue in addition to algae. Multiple studies also acknowledge the influence of fish predation on the populations of mesograzers, leading to fluctuation in epibionts (Jernakoff et al. 1996; Coull 1999; Duffy and Hay 2000; Moore and Hovel 2010; Beerman et al. 2018), or the influence of abiotic variables

such as salinity and water temperature (Coull 1999). Regardless of additional biological controls, diversity of seagrass-associated taxa of coastal North Carolina has been assessed repeatedly in multiple habitats other than beds of *H. wrightii*; yet with yearly declines of seagrass bed densities, the value of monitoring mesograzers increases with them being dominant primary consumers in seagrass beds (van Montfrans 1982). As competition from fast-growing epiphytes threatens seagrass growth, seagrass bed health could depend on suppression of epibionts, yet there is still much unknown about community impacts mesograzers can have.

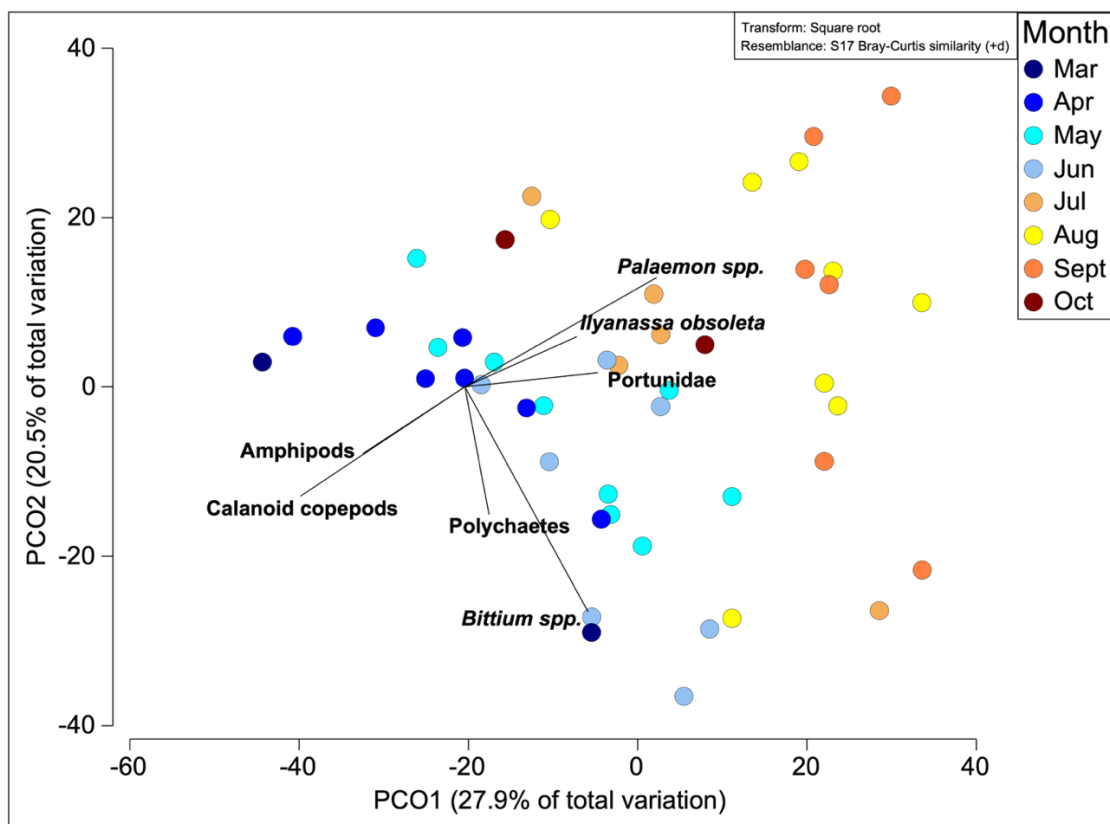


Figure 24. Principal coordinate analysis showing an unconstrained ordination plot in a 2-dimensional space using Bray-Curtis similarity (+1 dummy variable) to assess square-root transformed seagrass-associated taxa by month. Each individual symbol represents one habitat survey ($n = 46$). Months are differentiated by color. The overlay vectors are based Pearson correlation greater than 0.4. Axes represent principal coordinates PCOs 1 (27.9%) and 2 (20.5%), explaining 48.4% of the variation.

Patterns of the observed taxa can be considered on scales as small as a single shoot of seagrass to different bodies of water as habitat sampling took place on the site level and between two sounds. Within varying spatial scales, patterns of overall mesograzer composition can be followed down to an individual taxonomic group. These patterns can be observed further in Figure 25 with nMDS plots factored by month. Larger bubble sizes indicate greater abundance of that taxonomic group. With colors indicating different months and the raw abundances of the four most abundant taxonomic groups (amphipods, *Bittium spp.*, calanoid copepods, and *Palaemon spp.*), change in community composition in *H. wrightii* beds over time can be visualized. Two of the crustacean groups, amphipods, and copepods, were more abundant in the months of March (Mar) and April (Apr), months of which are considered spring in temperate zones (Fig. 25). This is in comparison to *Bittium spp.* and *Palaemon spp.* being more abundant in the summer months of June (Jun), July (Jul), and August (Aug). This further supports that there was a shift in which taxonomic group of mesograzers most dominant in seagrass beds over time, and there is likely seasonal change in community diversity. Similar to the trends observed in trematode diversity, patterns of diversity in seagrass-associated taxa are likely due to changes on the community scale within each seagrass habitat and/or between Bogue Sound and the Straits. However, changes in seagrass-associated taxa are more likely associated with food availability, nutrient concentration, and pressure from predators, as previously discussed.

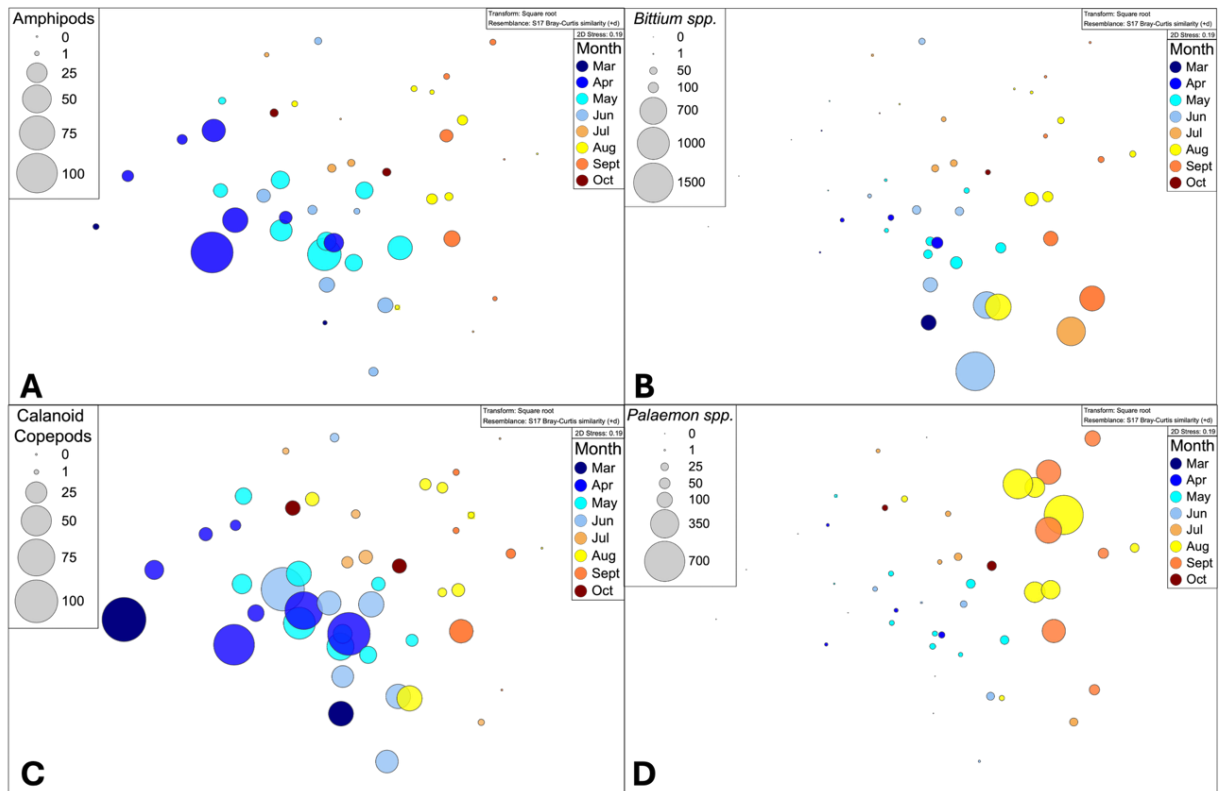


Figure 25. Four panels of non-metric multidimensional scaling bubble plots using the Bray-Curtis similarity (+1 dummy variable) to assess the changes in seagrass-associated taxa abundance of the four most abundant taxonomic groups at all habitat surveys in Bogue Sound and the Straits by month. Each panel is associated with one taxonomic group: (A) amphipods, (B) *Bittium* spp., (C) calanoid copepods, and (D) *Palaemon* spp. An individual bubble represents one habitat survey ($n = 46$). The bubble size indicates the number of individuals found per habitat survey.

Another important consideration is that we observed a correlation between infection prevalence and the abundance of seagrass-associated taxa in beds of *H. wrightii* (Fig. 16). Infection prevalence was positively correlated with avg. mesograzers abundance ($\rho = 0.31$) and minimally correlated with avg. non-grazer abundance ($\rho = 0.18$). Avg. non-grazer abundance was more strongly correlated with structural habitat components, specifically avg. shoot density ($\rho = 0.46$). Given that we observed high abundance of multiple taxonomic groups, grazers and non-grazers alike, we can conclude that this diversity adds to the trophic complexity in seagrass

beds in addition to the presence of trematode diversity in *I. obsoleta*. This supports our hypothesis and previous findings of parasite diversity correlating with overall biodiversity across taxonomic groups (Christoffersen et al. 2012; Wood and Johnson 2016; Moore et al. 2020; Moore et al. 2023; Moore et al. 2024). Further, the strongest biotic correlations with avg. epibiont biomass were avg. mesograzer abundance and avg. non-grazer abundance besides avg. shoot density, as previously mentioned in the discussion of Figure 17. Due to correlations between seagrass-associated taxa and infection prevalence, there is additional support for how community structure and diversity are key components of epibiont biomass in beds of *H. wrightii*.

CONCLUSIONS AND SIGNIFICANCE

These results primarily display the diversity of not only parasitic trematodes on a habitat level, but also in the effects and relationships shared between parasites and the structural elements of soft-sediment ecosystems. Different forms of assessment regarding trematode presence in populations of *I. obsoleta* provide unique insight to direct and indirect effects from a large parasite component community. With distinct changes in trematode species richness diverging between temperature ranges, this data is informative of what trematode life cycle diversity could reveal in the sense of biodiversity and, further, how this specific component community could signal changes in ecosystem health and structure. The distinction of bird-using versus fish-using parasites and their different biotic drivers only continues to support how intricate food webs can be and the necessity for parasites to be included. Furthermore, while infection prevalence did not have any strong correlation with epibiont biomass on *H. wrightii*, my data reveals biodiversity in other forms (e.g., non-grazer abundance, mesograzer abundance) is correlated to the structure of *H. wrightii* beds. Therefore, it is important to use methods that

help assess the everchanging composition of seagrass-associated taxa beyond the taxa identified in this work, as measuring diversity on any scale assists with understanding how communities coexist.

Moreover, the complexity of seagrass habitats provided opportunity for data collection on an assortment of abiotic and biotic variables beyond trematodes, with each offering a quantitative approach to how parasitism was or was not associated. This is explicitly seen in the strong abiotic and biotic correlations between epibiont biomass and the observed variables in addition to infection prevalence. As such, these results indicate a need for further investigation on the connections between ecosystem engineers like *I. obsoleta* and its parasite component community as they and seagrasses of North Carolina endure the ever-changing environment.

CHAPTER TWO

INTRODUCTION

Soft sediment intertidal systems such as mudflats create coastal structure, nutrient cycling, and enhanced living space and food for hundreds of species including myriad infaunal and epifaunal organisms (Best and Stachowicz 2012; Hovel et al. 2002). Yet, intertidal mudflats are becoming more increasingly vulnerable to climate change, terrestrial runoff, powerful storms, alteration from tidal barriers, and pollution, dramatically modifying population and community dynamics (Flowers et al. 2024; Norris et al. 2022). This, of course, threatens *Ilyanassa obsoleta*, its parasite community, and the contribution this host-parasite provides in this ecosystem's structure. As macroconsumers and ecosystem engineers, uninfected *I. obsoleta* can alter both the abundance of benthic organisms and benthic productivity via disturbance generated through bioturbation and direct consumption, reducing abundance and distribution of benthic macroalgae (i.e. chlorophyll-*a*) but contributing to nutrient cycling through excrement (Kelaher et al. 2003; Pace et al. 1979; Sousa 1991). Due to the ability of *I. obsoleta* to gain nutrition from multiple sources, these mud snails, in addition to sediment mixing and grazing, return significant quantities of carbon to sediment surfaces in forms of trail and fecal mucus (Cranford 1988). Invertebrate burial behavior also can contribute to sediment oxygen uptake, and as temperatures rise, molluscs generally can rework sediment surfaces more intensely, releasing ammonium and nitrate from the sediment to the overlying water (Zhang et al. 2013). Nutrient cycling by *I. obsoleta* clearly contributes to ecosystem structure; however, little research shows how parasites indirectly influence mud snail behaviors driving such nutrient changes. In addition, parasitic trematode diversity can contribute to biodiversity and production of an ecosystem and acknowledging how parasitism alters trophic cascades in food webs is vital (Christoffersen et al. 2012), as previously stated in Chapter 1.

Because *I. obsoleta* can alter the structure of their constituent communities through their grazing in seagrass systems, parasites that alter ecologically influential behaviors may have cascading indirect effects on the community structure and functioning of mudflats. In this chapter, I aimed to investigate the indirect consequences of parasite diversity within *I. obsoleta* on specific components of mudflat functioning and structure: species richness of meiofauna and infauna and nutrient concentrations within sediment (chlorophyll-*a*, organic carbon). This was addressed in the form of a series of field-based treatments set in a highly productive mudflat environment with treatments based on species-specific parasitic infection in *I. obsoleta*. As little research shows how parasites influence *I. obsoleta* behaviors (i.e. consumption, movement) driving such changes, it is worth investigating how *I. obsoleta* contributes to mudflat productivity and biodiversity while infected by its parasite component community. With that, this research addresses the following question:

Research Question: How does species-specific infection within *I. obsoleta* affect productivity and biodiversity properties within mudflat ecosystems?

I hypothesized that *Lepocreadium setiferoides* (LS) would have greater physiological effect on *I. obsoleta* than *Zoogonus lasius* (ZL), and that differences in trematode size and physiological impact are the likely mechanisms behind trait-mediated indirect effects upon mudflat ecosystem structure (meiofaunal diversity, abundance per meiofaunal taxonomic group, benthic chlorophyll-*a* concentration, organic carbon concentration). Despite similar life cycles and both using fish species as definitive hosts (Altman 2010; Curtis 2007), this acknowledges the trematodes' size and ability to cause varying degrees of tissue damage to snail's gonad-digestive gland complex and overall changes in host consumption rates (Clausen et al. 2008; Lauckner 1987; Magalhães et al. 2020; Morton 2018; Wood et al. 2007). Therefore, I expect larger

trematodes to directly cause greater amounts of physiological effect and behavioral change on or within their hosts, resulting in greater levels of indirect effects on surrounding nutrient concentrations in sediment through increased or decreased consumption behavior. LS is a digenean fluke (Miller and Northup 1926; Stunkard 1972), producing actively feeding rediae and cercariae capable of altering protein metabolism when its host is exposed to carrion (Cheng et al. 1983; Curtis 1985). This physiological response can also come with varying degrees of tissue damage to the snail's gonads (Cheng et al. 1983). Rediae possess a mouth, pharynx, and primitive gut, allowing trematodes with this life stage to directly consume host tissue through tearing and swallowing (Esch et al. 2002; Kuris 1990). On the other hand, ZL is a plagiorchiid digenean producing sporocysts which appear to be less capable of causing tissue damage (Rossiter and Sukhdeo 2012; Stambaugh and McDermott 1969; Stunkard 1938). Lacking a mouth and gut, these sporocysts, a life stage that once mature and contains cercariae, absorb nutrients from the host's body such as oxygen and carbohydrates (i.e. presents higher metabolic needs) (Kuris 1990; Magalhães et al. 2020). Further, I also hypothesized that mudflat treatments with greater abundance of meiofaunal taxa would coincide with treatments containing infected snails, specifically LS and ZL, resulting in a positive correlation between parasite diversity and meiofaunal diversity. With parasite presence contributing to trophic complexity, it correlates with greater overall biodiversity across taxonomic groups (Christoffersen et al. 2012; Moore et al. 2020; Moore et al. 2023; Moore et al. 2024; Wood and Johnson 2016), like Research Question 4 in Chapter 1. Overall, due to each trematode's different capabilities of internally damaging and manipulating *I. obsoleta* regarding life stages and physiological impact, I expected parasites such as LS to cause greater differences in indirect effects onto mudflat systems in comparison to ZL as well as uninfected snails.

MATERIALS AND METHODS

Study Site

As previously mentioned in Chapter 1, the Albemarle-Pamlico Sound ecosystem supports acres of estuarine waters that are made up of soft sediment ecosystems such as intertidal mudflats (Albemarle-Pamlico National Estuary Partnership 2012). I sought out a highly productive mudflat within the Albemarle-Pamlico Sound that supported not only strong populations of *I. obsoleta* but also was easily accessible by foot for regular monitoring and maintenance of the field experiment. I chose to utilize the intertidal ecosystems along the shoreline of the Theodore Roosevelt State Natural Area (THRO) in Pine Knoll Shores, NC through its connection with the North Carolina Aquarium at Pine Knoll Shores. THRO is a 290-acre parcel on Bogue Sound that is managed and protected by the Aquarium and Fort Macon State Park (Buchanan 2023). Locations for the experiment's deployment were scouted in February 2025 prior to any pilot experiments, sampling, or deployment. A total of six experimental locations were scouted and then narrowed to three locations listed in Table 6. Due to our experiment being organized by infection treatment, replicates per treatment were divided into the three locations and organized in rectangular grids. Therefore, the experimental locations will be hereafter referred to as grids.

GPS coordinates and elevations of each grid were collected with a Trimble R10 GNSS system. Grid numbers in Figure 26 correlate with numbers in Table 5. Grid location along the shoreline was dictated by no obstructions by any installments (i.e. docks, seawalls, artificial reef structures, man-made materials), seagrass beds, oyster populations, or mouths of intertidal creeks to prevent sediment accumulation in or around the experiment. I also considered avoidance of other ongoing research projects at PKS regarding space availability for the grids. Distributing the

experiment into three grids instead of one was to accommodate for other ongoing installments of research projects as to not cause disturbance or skew results, as well as to provide statistical support in case replicates from one grid were lost or manipulated.

Table 5. Grid locations in the Theodore Roosevelt State Natural Area. This table lists each grid's number that corresponds with latitude and longitude coordinates and the size of the grid.

Grid number	Coordinates	Size (hectares)
1	34°69'83"N 76°83'56"W	0.002292
2	34°69'83"N 76°83'61"W	0.002292
3	34°69'80"N 76°83'64"W	0.002292

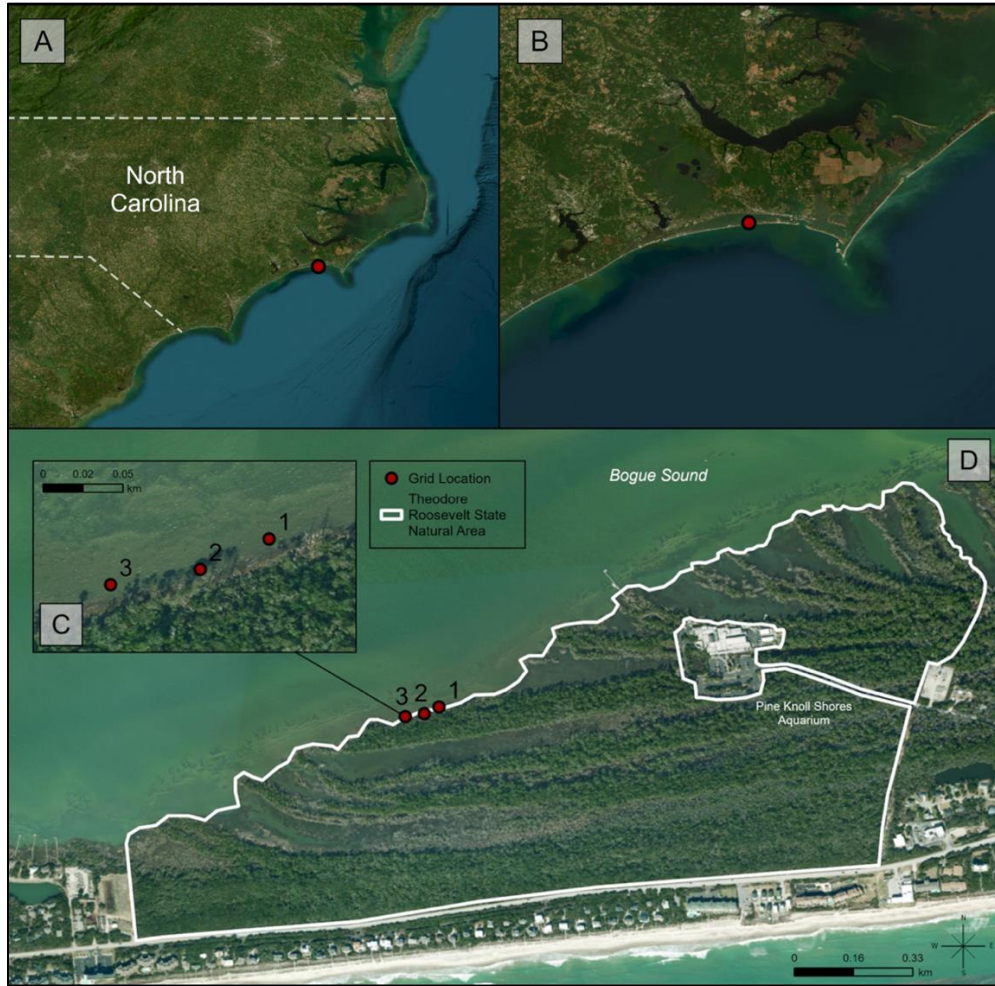


Figure 26. Map of grid locations. (A) The general location of all three grids within Theodore Roosevelt State Natural Area are indicated by the red circle in North Carolina. (B) Grid locations in relation to Bogue Sound and the North Carolinian coastline. (C) Specific grid locations (1-3) are depicted with individual red circles. Numbers associated with red circles correspond with grid numbers in Table 6, as well as placement of treatment replicates in each grid. (D) The extent of the Theodore Roosevelt State Natural Area (white boundary) in relation to the North Carolina Aquarium at Pine Knoll Shores.

Experimental Design

This was a 7-week field experiment occurring from 22 July 2025 to 1 November 2025 with a pilot experiment from mid-March 2025 to 6 April 2025. All data collection occurred at seven evenly spaced timepoints from July to November, with the first round of data collection on 22 July considered time-zero and initial site conditions. Timepoints were evenly spaced to occur bi-weekly, with only slight deviations from this strict pattern occurring due to weather delays: 22

July, 4 August, 18 August, 4 September, 19 September, 4 October, 15 October, 1 November. Each round of data collection occurred at low tides (-0.3-0.1 ft). Water temperature (°C) and salinity (ppt) were recorded at each timepoint using a YSI probe. Salinity, temperature, and light data loggers were also attached to enclosure exteriors to record more frequent fluctuations in abiotic data. Additionally, every replicate per grid was checked for snail density, snail mortality, and enclosure durability and structural integrity per timepoint. If damage to enclosures was present (i.e. holes), repairs were made on-site. No enclosure was replaced throughout the duration of the experiment. Bi-weekly timepoint data collection also involved the replacement of dead snails with live snails to maintain infection prevalence per treatment. To accommodate for this, snail collection and cercarial shedding in snails had to be ongoing post deployment (see below in Snail Collection, Shedding, and Dissection). During each timepoint, the number of dead or missing snails was recorded, as well as what treatments those snails were associated with. Deployment of the enclosures with initial sampling took place on 22 July, while deployment of all snails took place on 23 July. Removal of all snails, enclosures, and remaining data was conducted on 1 November 2025 with all samples and snails brought back to East Carolina University Life Sciences and Biotechnology Building (LSBB) for processing.

Pilot Experiment

Before establishing the number of treatments and replicates needed, I conducted a pilot study testing enclosure design, snail marking techniques, and nutrient sample collection. Three enclosures (0.5-m diameter) were constructed using 5-mm mesh galvanized wire and were supported by two wooden stakes. Enclosures were sunk 10-cm into the sediment, with one placed at each grid location (Fig. 26). Each enclosure contained 20 snails marked with dots of colored nail polish on their shells to test if the color would be identifiable over a long period of

time. Nail polish color would be associated with infection status of the snail (i.e. uninfected versus infected with LS). Snail shells were dried gently with towels prior to marking them with polish. Placement of the enclosures at the then-estimated grid locations was to determine if a grid location needed to be reassessed. Grid locations did not deviate from chosen coordinates (Fig. 26; Table 5). Methods of enclosure structure, use of nail polish, and containment of snails proved effective, but I decided to make the enclosure smaller to allow more ease in finding all snails per enclosure and when revisiting all enclosures at each timepoint.

Snail Collection, Shedding, and Dissection

To determine the indirect effects of trematode component community structure on mudflat ecosystem structure, I collected snails from highly productive mudflats that supported populations of *I. obsoleta* with high infection prevalence and species richness. Sites were chosen based on data collected in Chapter 1 on trematode species diversity across Bogue Sound and the Straits in the 2024 habitat surveys, as well as previous surveys done in Nadzam et al. (in prep) and random sampling where infection prevalence was seen as high as 48-78% (n = 100 snails). From March through July 2025, snails were collected from all sites noted in Figure 27 with the greatest percentage of them originating from Miramar, Pine Knoll Shores, and Rachel Carson Reserve due to high infection prevalences of LS and ZL. Snails smaller than 12 mm were not collected due to not only infection being more likely in larger (i.e. older) snails that have been exposed longer to parasites in their habitats, but also snail gonad development needs to occur for infection to be successful (Lafferty 1993; Blakeslee et al. 2012). All snails were transported back to LSBB for processing.

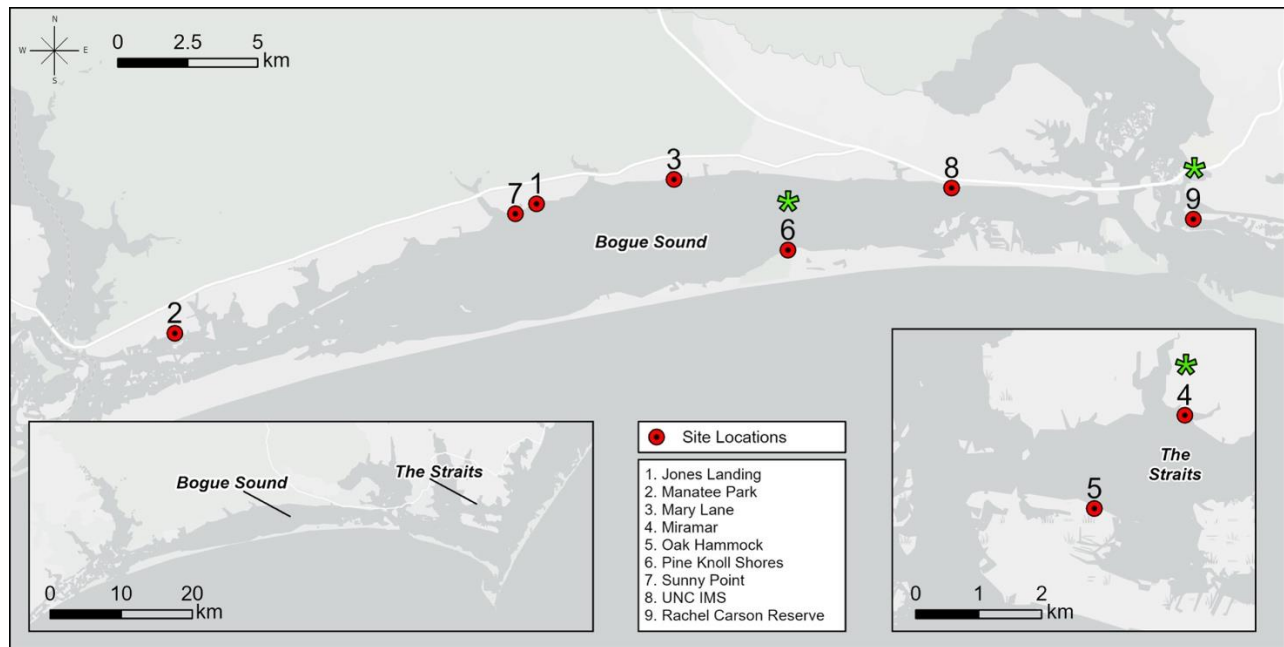


Figure 27. Map of sampling sites for *Ilyanassa obsoleta*. This map shows site locations within the extent of Bogue Sound and the Straits of North Carolina. Sites are depicted with red markers and numbers based on their location and name ($n = 9$). Green asterisks indicate the three sites where the greatest quantities of snails were collected for the experiment: Miramar (4), Pine Knoll Shores (6), and Rachel Carson Reserve (9).

Processing of snails included identifying prevalence of trematode species within each sample population through a proven trematode larval release method, also known as cercarial shedding, that reliably identifies infection to species level (Morton 2018). Snails with coinfections were not used. Snails with no shed cercariae were identified as uninfected. Upon identifying infection status, snail shells were dried and marked with a dot of colored nail polish to distinguish infection status by a specific parasite species or no infection. Once marked, snails were fed with algal discs and housed in aerated aquaria (30 ppt) at LSBB and in large free-flowing tanks with running seawater at the Duke University Marine Lab (DUML). Use of the free-flowing tanks was to accommodate not only for the space needed to keep the snails alive until deployment, but to also minimize longer transport of snails to PKS. To obtain large quantities of snails infected with specific species of trematodes, I collected and processed a total

of 18,500 snails to establish and maintain our treatment design and replicates. Any extra live snails not deployed in enclosures were released. Post enclosure deployment on 22 July, all marked snails were organized into labeled mesh bags at DUMML and kept in the free-flowing tanks. Mesh bag labels corresponded to enclosure number. This provided simple deposition of marked snails into the correct enclosures and treatments on 23 July.

Based on cercarial shedding, I established the following eight treatments (n = number of replicates): uninfected (U) (n = 20); (2) caged-control (CC) (no snails) (n = 9); uncaged-control (UC) (no snails) (n = 9); 30% LS (n = 20); 30% ZL (n = 4); 30% HQ (n = 6); 30% ST (n = 8); 30% SD (n = 2). Logistical constraints on infected-snail collection prevented inclusion of additional replicates for species of ZL, HQ, ST, and SD. 30% prevalence falls within observed infection from initial surveys and a naturally occurring high prevalence. Enclosures were monitored to ensure constant snail densities (n = 30/enclosure). This led to a total of 1,800 snails used in the field experiment. Caged controls isolated the effect of mud snail absence within enclosures, while the uncaged control allowed us to assess caging artifacts on the response variables.

Upon the experiment's removal on 1 November, all snails were collected from each enclosure and placed into the numbered mesh bags used for deployment. Snails were transported back to LSBB in large 5-gallon buckets of seawater with air stones and bubblers. All bags of snails were then housed in a large cooler of seawater (30 ppt) with air stones and bubblers. Snails were dissected to within a week of being removed from the field. I recorded any changes of infection status per snail, snail size (mm), and sex, as well as nail polish color on the snail shell that indicated original infection status, presence of specific trematode life stages, snail eggs,

coinfections, and presence of additional fauna in or on the snail (i.e. barnacles, ciliates, nematodes).

Enclosure Construction

Enclosure design was modified from the pilot and a previous model (Nadzam et al. *in prep*), using 5-mm mesh galvanized wire to create 0.05-m² circular cages bound with zip-ties. Each enclosure was wrapped in a ribbon of copper flashing 1-inch from the top to prevent snail escape and predator interference. The top 2-cm of each enclosure's wall was also folded inwards at a 45° angle to also assist with snail escape prevention. A blue zip-tie was added 10-cm up from the base of the enclosure to mark how far the enclosure was to be sunk into the mud for stability. Each enclosure was supported by two wooden stakes on opposite sides and three galvanized garden staples evenly spaced around the enclosure before being sunk into the mud. The number of enclosures was based on the number of replicates possible per treatment and the number of snails infected with specific species when identified in the shedding protocol. A total of 78 enclosures were constructed. I attached galvanized metal field tags to each enclosure that were stamped with an enclosure number and code identifying what grid it was in, what treatment it represented, and what replicate it was (ex. 1 G1-U-1 = enclosure number 1, grid 1, uninfected treatment, replicate 1). A strip of orange flagging tape with the enclosure number written on the strip was also tied to each enclosure in case the metal tags became fouled over time and were unreadable. Enclosures were distributed between the three grids with each grid set in a randomized scheme. Schematics of treatment and enclosure layout per grid were kept as keys in case tags were lost and to assist with sample collection. Enclosure spacing was set at 0.5-m. Elevation and coordinates of each enclosure's location were recorded using a Trimble R10 GNSS system on 22 July.

Field Sampling & Laboratory Processing

All data collection occurred at eight timepoints with the first as time-zero. Data collected at time-zero is referred to as initial site conditions. Sampling was set to align with higher productivity in summer months (Mallin et al. 2000), followed by seasonal changes into the fall regarding meiofaunal diversity and nutrient levels.

Data was downloaded from the salinity, temperature, and light data loggers and added to the master datasheets containing all abiotic and biotic data for the experiment. Upon retrieving the data from the salinity logger at the experiment's end, I noticed the salinity dramatically decreased to zero in late July and the logger stopped recording. Error likely occurred when the logger was no longer submerged in water or the sensor malfunctioned. Salinity data came from point-data recordings with a YSI probe at each timepoint. Light and water temperature data was successfully downloaded and culled to focus on the specific dates that timepoint data was collected and to not include the hours at which the loggers were sitting at room temperature or in transit. On each timepoint, light data was culled further specifically to 12 hours each day to reflect the natural sunrise and sunset (~ 7:00 AM – ~7:00 PM), with the exception of timepoints on 22 July (11:24 AM – 7:24 PM) and 1 November (7:24 AM – 3:24 PM) where light was culled to eight hours, as these were our experiment deployment and removal dates.

Regarding nutrient metrics (chlorophyll-a and organic carbon), sampling occurred at every timepoint except 15 October, where sampling was hindered by severe weather. Only snail density and infection prevalence per enclosure was recorded for this timepoint. All nutrient samples were collected in pre-weighed and labeled 50-ml polypropylene tubes and transported back to LSBB post sampling on ice. Sample tubes for chlorophyll-a (chl-a) were covered in light-blocking tape to prevent further photosynthesis post sampling. Tube labels entailed sample

date, enclosure number, and treatment code. I collected 15-ml of surface sediment (depth = 3 cm, n = 1 per enclosure) from each enclosure's center in the taped tubes for chlorophyll. Chl-*a* samples were stored in a -20°C freezer for no longer than two weeks before processing. After thawing, chl-*a* analysis was conducted using a Trilogy Laboratory-Turner Designs fluorometer to calculate average chl-*a* concentrations (mg/m²) per treatment at ECU Environmental Research Laboratory (ERL) with provided standard operating procedures (SOPs) (i.e. UMCES 2024, 2025). Additional 15-ml samples (depth = 3 cm, n = 1 per enclosure) were collected from each enclosure's center to measure organic carbon concentration (%). Samples were stored in a -20°C freezer for no longer than two weeks before processing. After thawing, samples were dried at 105°C in a drying oven for 72 hours and ashed in a muffle furnace at 500°C before processing via Loss of Ignition (LOI) (%) protocol for organic carbon concentration (%) at the ERL. LOI protocol was provided by the ERL with methodology and calculations derived from Allen (1989, 15-16), Hirota and Szyper (1976), Nelson and Sommers (1996), and Heiri et al. (2001).

In continuation, the sampling of meiofauna took place at four timepoints: time-zero (22 July), 18 August, 4 October, 1 November. Cores were only collected four times due to diversity unlikely to change as frequently in comparison to nutrient levels. 8-cm cores (n = 3 per enclosure) were collected using plastic sediment corers and homogenized in plastic Ziploc bags. Sample bags were pre-labeled with sample date, enclosure number, enclosure code, and were each given a Write-in-the-Rain paper tag with the same information. Samples were transported back to LSBB on ice and stored in cold rooms set at 3.5 °C until filtering. Sample filtering occurred within 48 hours of sample collection. Each sample was filtered through a 63-micron sieve in the lab, then divided in half into labeled plastic sample containers. Labels consisted of

site name (i.e. PKS), sample date, enclosure number, and enclosure code. Samples were preserved with 70% ethanol until processing.

Meiofauna samples underwent a two-week preserving, sub-sampling, and decanting protocol with methods derived from Armenteros et al. (2008), Coull (1999), Dalman et al. (2023), Joint et al. (1982), and Torres-Martínez and Riera (2024). Meiofauna will be sorted by to the lowest taxonomic group possible to calculate average species abundance per replicate. All organisms will be identified using guidebooks and keys. Each sample was drained of its ethanol over a 3-inch diameter, 50- μ m sieve, then spread over a white tray. This was done to identify any noticeable or large organisms in the sample and ensure they were not missed before subsampling. 50 g of the sample was then gathered, weighed, and put back into the labeled plastic container (Armenteros et al. 2008; Dalman et al 2023). Subsampling each meiofauna core sample was done to ensure sample size was universal and to not provide opportunity for bias on meiofaunal diversity between samples. The new sample was then preserved in a 70% ethanol solution with Rose Bengal dye for at least 96 hours. After this period, each sample's meiofauna was extracted agitation and decantation in tap water (Armenteros et al. 2008; Joint et al. 1982). Post drainage of the Rose Bengal solution over a 10-inch diameter, 63- μ m sieve, all sediment and meiofauna were poured into a 1-L beaker. Approximately 950 ml of tap water was added into the beaker and stirred with a glass stirring rod for 60 seconds. I decanted the supernatant over the 63- μ m sieve just before sediment reached the edge of the beaker. The decanting procedure was repeated 10 times per sample. The remaining material left in the sieve was then sorted for meiofaunal diversity and taxonomic group abundance. All samples were sorted and observed under a Zeiss compound scope (0.63-5.0X). Images were taken of organisms to assist with identification using a Jenoptik Gaphax camera attached to the scope.

FUTURE DIRECTIONS

To complete all data collection and processing, I aim to finish sorting the remaining meiofaunal samples in summer 2026. All further data filtering and statistical tests are to be conducted in R and Primer 7. In Primer, I will analyze and visualize biodiversity changes over time and by treatment with PERMANOVA and nMDS (Moore et al. 2023). I will conduct a series of GLMMs via package *glmmTMB* with response variables (chlorophyll-*a*, organic carbon, meiofaunal abundance), fixed predictors (treatment, temperature, salinity, sampling event, light, grid), and enclosure ID as a random effect (Moore et al. 2023). GLMMs will likely be constructed with a logit-link function (i.e. logistic regression) (Potter et al. 2026), followed by a fixed effect with a strong AICc set on the x-axis much like those conducted in Chapter 1. The assemblages of meiofaunal diversity per treatment and grid will also be visualized in forms of multivariate analysis such as nMDS plots and PCoAs in combination with PERMANOVAs, SIMPERs, and ANOSIMs.

CLOSING REMARKS

Investigating parasites, let alone the diversity of them within a single host, and how they drive ecosystem structure and functioning is a difficult task. As our data indicated, there is a continuous list of variables to consider when it comes to the coexistence between parasites and their ecosystems, but parasites like trematodes must adapt to these variables to achieve successful transmission and reproduction. It is this navigation through complex environments like seagrasses and mudflats that draws our attention and drives future assessment. The abundant eastern mud snail (*Ilyanassa obsoleta*) provided a unique opportunity to assess relationships between elements of soft-sediment intertidal ecosystems and parasitic infection. More specifically, the results here add to the growing literature on parasite-host networks containing multiple species of parasites, and how we understand *I. obsoleta* to be an ecosystem engineer. Despite being commonly left out of food webs and trophic levels, trematode diversity, let alone parasite diversity, and their associated life cycles are essential to understanding ecological processes in these systems, as they can be fascinating indicators of ecosystem health and biodiversity.

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