Diversity and Survival of Macrofauna associated with a Non-Native Foundational Species
by
Timothy Seung-chul Lee
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Director of Dissertation: April M.H. Blakeslee, PhD
Major Department: Biology

ABSTRACT

Through globalization and increased anthropogenic movement, many non-native foundation species have established across the world. These foundation species have impacted ecological communities in myriad of ways, including but not limited to reducing native biodiversity, changing biogeochemistry, and altering physical environment. Marine macroalgae, or seaweeds, are one of the major groups of foundational species in coastal habitats throughout the world. Seaweeds play vital roles as ecosystem engineers by provide valuable habitat for macrofauna and protect their associated communities from thermal stress. Many seaweeds around the world's coastlines are also non-native, and some species have transformed native habitat by bringing novel structural complexity. The red seaweed *Gracilara vermiculophylla*, native to northwestern Pacific, has invaded much of the temperate estuarine ecosystems in Northern Hemisphere. Along the U.S. East Coast, this seaweed has physically transformed soft-sediment habitats by providing complex three-dimensional structure, thus providing refuge, shelter, and microhabitats for native macroinvertebrates. I approached this study with three chapters to understand 1) the biogeographic patterns of free-living and parasitic macroinvertebrates associated with *G. vermiculophylla* along the U.S. east coast, 2) survivability of macroinvertebrate in habitats with *G. vermiculophylla* presence coupled with rising seawater temperatures, and 3) comparisons of macroinvertebrate diversity between *G. vermiculophylla* and the native foundational species, seagrasses. For the first chapter, I sampled *G. vermiculophylla* thalli, its associated free-living and parasitic macroinvertebrates, and the abiotic environmental parameters along the U.S. east coast, from South Carolina to New Hampshire in May-August 2019, spanning across three biogeographic regions. Using Generalized Linear Mixed Model, I found that biogeographic region, site, *G. vermiculophylla* biomass, and the interaction of biogeographic region with *G. vermiculophylla* appeared in top models for free-living macroinvertebrates' response variables (abundance, richness, and diversity). For parasitic invertebrates, I found that according to Generalized Linear Models, *G. vermiculophylla* biomass was the predictor appearing top performing model for parasitic prevalence and richness, while biogeographic region was the sole predictor in a top performing model for parasitic diversity. For the second chapter, from February-March 2021 I conducted a lab experiment with *Ilyanassa obsoleta* snail on its survivability in habitats with *G. vermiculophylla* presence and rising seawater temperature, with focus on North Carolina. The eastern mudsnail *Ilyanassa obsoleta* co-occurs with *G. vermiculophylla* throughout much of the east coast, and highly abundant, making this invertebrate an ideal study organism. I tested survivability of *I. obsoleta* at three seawater temperatures (27, 32, and 36 °C) in two habitat treatments (with and without *G. vermiculophylla*) for three weeks, two trials each. I found that *I. obsoleta* mortality was greatest in 36 °C, followed by 32 and 27 °C. I also found that mortality of *I. obsoleta* was higher in *G. vermiculophylla* habitat treatment than without the seaweed, and found that infected *I. obsoleta* perished faster at higher temperatures. Furthermore, we found that oxygen diminished faster in habitats with *G. vermiculophylla* at higher temperatures, which suggests that the interaction of non-native seaweed and seawater temperature rise can possibly create anoxic conditions. For the final chapter, I collected free-living macroinvertebrates associated with the non-native foundational species (*G. vermiculophylla*) and native foundational species (seagrasses) that co-occurs in North Carolina coastlines. Seagrasses play critical role as primary producers in coastlines and provide valuable habitat for many macroinvertebrates. North Carolina coastlines are unique because it is the only region in the western Atlantic coast where two species of seagrasses, the eelgrass (*Zostera marina*) and shoalgrass (*Halodule wrightii*) co-occur. I sampled multiple replicates of *G. vermiculophylla* thalli, fronds of seagrasses, and their associated macroinvertebrates from May-July 2021. Overall, I found that macroinvertebrate abundances were higher in the non-native *G. vermiculophylla* than seagrasses. Within seagrass species comparisons, I found that macroinvertebrate richness was higher in *Z. marina* than *H. wrightii* suggesting that some seagrasses may provider habitats of higher quality. Along the U.S. East Coast, *G vermiculophylla* can accommodate diverse assemblage of macroinvertebrates. Patterns of macroinvertebrates may vary depending on the *G. vermiculophylla* biomass and biogeographic regions. However, *G. vermiculophylla* may be capable of generating habitats with low-oxygen levels with high seawater temperature, thereby potentially harming native macroinvertebrates in environments with thermal stress. Finally, *G. vermiculophylla* may accommodate greater abundances of macroinvertebrates than native foundational species, but it is important to recognize that seaweeds and seagrasses serve different roles in coastal ecosystems. As *G. vermiculophylla* continues to transform coastal habitats, it is important to continue monitoring macroinvertebrate to better understand how these communities and other organisms in the coastal food web are affected by this foundational non-native species.

Diversity and Survival of Macrofauna associated with a Non-Native Foundational Species

A Dissertation

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In Partial Fulfillment of the Requirements for the Degree

Doctor of Philosophy in Interdisciplinary Doctoral Program in Biology, Biomedicine, and Chemistry

By

Timothy Seung-chul Lee

July 2023

Director of Dissertation: April M.H. Blakeslee, PhD
Dissertation Committee Members:
Rebecca G. Asch, PhD
Rachel K. Gittman, PhD
Amy E. Fowler, PhD
Stacy A. Krueger-Hadfield, PhD

Dedication

To the loving memories of three people who have been with me every step of the way…

My father, Charles Chunil Lee (1955-2021)

Dad, you always prioritized our family's happiness above yours every minute of the day. You sacrificed so much for your children, above and beyond what I could ask for. It was so hard that you did not get to see me defend my dissertation, but I knew how supportive you were of my PhD journey, and I felt your presence in that auditorium! I still think you left way too soon, but I know that you are always cheering me on. Love you dad, and I miss you as always!

My girlfriend and soulmate, Sarah Ann Pickett (1990 – 2021)

My dear Sarah – when we first met in 2019, I knew we were soulmates. We made the best out of the time we had together. I love your adventurous spirit, your love for Jesus, and that you strived to live for God. You always thought of others before yourself, and you knew how to live every minute of your life with utmost joy, a life that we all seek. You also loved science and was incredibly supportive of my PhD goals. You continue to inspire me to live my life fully and treat every minute as a gift. Love you so much Sarah, as always, and I miss you!

My grandfather, Lee Jong-Sam (1930 – 2022)

Dear grandfather – even from so far away, you never stopped thinking of your two grandchildren here in America, and always encouraged us to work hard and that if we set our minds to our goals, we can achieve our dreams. You are a true inspiration, and I know you were watching me on my defense day! I will continue to make you proud, grandpa! I miss you!

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Lastly, I thank my savior Jesus Christ and my almighty Father for looking after me throughout my entire life. Proverbs 3:5-6: *Trust the Lord with all your heart and lean not on your own understanding; in all your ways submit to Him, and He will make your paths straight.*

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CHAPTER 1: Biogeographic patterns of community diversity associated with an introduced alga

ABSTRACT

Aims: Non-native foundation species may impact communities by altering physical environments and providing habitat. We assessed biogeographic patterns of free-living and parasitic community diversity associated with the non-native red alga *Gracilaria vermiculophylla,* which has fixed or free-floating morphs in nature. As a widespread invader, our study provides more evidence for the novel associations that may form when species establish outside native ranges.

Location: New Hampshire to South Carolina, USA.

Methods: In summer 2019, we surveyed 17 sites in 3 biogeographic regions: North of Cape Cod (NCC), Virginian Province (VP), South of Cape Hatteras (SCC). Per site, we used a random quadrat design to collect all *G. vermiculophylla* and associates into individual bags. We collected 100 *Ilyanassa obsoleta* snails for trematode diversity data. Abiotic measurements were taken per site. In the lab, macroinvertebrates were extracted from thalli and identified to lowest taxonomic level. *Ilyanassa obsoleta* were dissected to determine trematode infection. Biotic and abiotic variables were analyzed for the best sets of predictors for species richness, abundance, and diversity of associated macroinvertebrates and trematode parasites across bioregions.

Results: We found the interaction of biogeographic region and *G. vermiculophylla* biomass, *G. vermiculophylla* biomass and site, and biogeographic region to be the top models of macroinvertebrate abundance, richness, and diversity, respectively. Across sites, abundance and richness of macroinvertebrates were significantly higher in fixed versus free-floating *G.*

vermiculophylla. Trematode prevalence and richness were best explained by *G. vermiculophylla* biomass, while biogeographic region best explained diversity.

Main Conclusions: Along the U.S. East Coast, *G vermiculophylla* harbors a diverse faunal community, which is influenced by algal biomass, biogeographic region, and algal type (fixed/free-floating). Over time, the presence and spread of *G. vermiculophylla* could continue to impact macroinvertebrate structure and diversity, and future work should directly compare communities associated with *G. vermiculophylla* to other native foundation species.

KEYWORDS: Amphipod, ecosystem engineer, foundation species, *Gracilaria vermiculophylla*, *Ilyanassa obsoleta*, invasion, trematode

INTRODUCTION

Over the last several decades, biological invasions have become recognized as a major environmental and management concern due to significant impacts on biodiversity, human health, and global economies (Pimentel et al. 2001). However, for many invasive species that do not have an immediate effect on human food production, infrastructure, or health, their presence may go unnoticed or unstudied for extensive periods of time. This lag time can hinder our understanding of the potential for environmental impact, as well as the roles these species may have within recipient communities (Crooks 2005). Even among highly abundant and widespread invaders, there is often insufficient information on basic aspects of their ecology and biology in the invasive range, including novel associations that may form with native species and habitats.

This hinders our ability to effectively manage species invasions in terms of mitigating impacts of established species on native communities and in preventing future invasions (Epanchin-Niell & Liebhold 2015).

For some invasions, a species' ecological role and impact on communities could be considerable, especially if the species is habitat-forming or significantly alters the structure of existing habitats. These species are often termed "ecosystem engineers," and their introductions to novel communities may be expected to have wide-reaching influences. This is because these species alter the structural complexity and abiotic environment within an ecosystem, enhancing habitat heterogeneity and resource availability, and thereby facilitating the abundance and diversity of numerous species (Dangerfield et al. 1998, Crooks 2002). Given the intricate network of interacting species within communities, it is vital that we closely investigate organisms that play such pivotal roles on community structure and function, as changes to their population abundance can ripple through and across ecosystems, especially for broadly distributed or range expanding species (Ellison et al. 2005, Osland et al. 2013). Some ecosystem engineers, particularly plants and some algae, are referred to as "foundation species" because they provide foundational support in terms of food, shelter, and nursery grounds to associated organisms (Dayton 1972, Ellison et al. 2005, Sorte et al. 2017, Metzger et al. 2019). In aquatic systems, foundation species, like macroalgae, serve a vital role as both food and shelter to associated macroinvertebrate species, which occupy multiple trophic levels in aquatic food webs and represent pivotal components of aquatic energy flows (Umanzor et al. 2017). When changes occur to the composition and biomass of foundation species in aquatic communities, such as with species invasions, macroinvertebrate densities and diversities may also change, greatly influencing the community structure and function of invaded ecosystems (Benke 2001; Runck 2007).

In recent decades, multiple anthropogenic activities (e.g., shipping, aquaculture, food, aquaria/pets, ornamentals) have led to the intentional and unintentional introductions of numerous biota, including foundation species like macroalgae (Williams & Smith 2007, Andreakis & Schaffelke 2012, Grosholz et al. 2015). Some introduction vectors, like shipping and aquaculture, can move associated organisms vast distances to locations where they have no prior evolutionary history, thus imposing novel species interactions on invaded communities (Strauss et al. 2006). Specifically, the introductions of habitat-forming macroalgae around the world has led to significant changes in community structure and function in invaded regions due to resultant alterations in an ecosystem's structural complexity (Wernberg et al. 2004). The Northern Hemisphere, in particular, has been subject to multiple introductions of macroalgae via several introduction mechanisms over the last century (Provan et al. 2008, Minchin & Nunn 2014), with a prominent example being the East Asian red macroalga *Gracilaria vermiculophylla* (Ohmi) Papenfuss. This structurally complex foundation species has been introduced to almost every temperate coastline in the Northern Hemisphere (Krueger-Hadfield et al. 2017). On the U.S. East Coast, *G. vermiculophylla* was introduced from Japan in the mid-to-late 20th century, likely through the importation of oysters, followed by secondary vectors, such as fishing and/or boating activities (Krueger-Hadfield et al. 2017). The alga is now found from New Hampshire to Georgia. In some locations (particularly Southeast U.S.), *G. vermiculophylla* has vastly transformed soft-sediment habitats by increasing structural complexity (Byers et al. 2012). The alga's haploid-diploid life cycle, in which free-living haploid gametophytes alternate with free-living diploid tetrasporophytes, is an additional contributing factor to its community and ecosystem-level influences (Krueger-Hadfield et al. 2016, 2019). In sites with abundant hard substratum, thalli are ‘fixed’ (*sensu* Krueger-Hadfield et al. 2018) by holdfasts to hard structures, indicating sporic recruitment. In sites without abundant hard substratum, thalli drift (i.e., are ‘free-floating’), leading to tetrasporophytic dominance (Krueger-Hadfield et al. 2016). Along the U.S. East Coast, free-floating thalli are often incorporated into the tube caps of the polychaete worm *Diopatra cuprea*, thereby stabilizing free-floating thalli in these systems (Thomsen and McGlathery 2005; Kollars et al. 2016; Mott et al. 2022). Prior localized studies have also noted increases in macroinvertebrate densities of some taxonomic groups, like crustaceans, gastropods, and bivalves, in response to *G. vermiculophylla* establishment (Thomsen et al. 2007, Nyberg et al. 2009, Thomsen et al. 2013). This is presumably because the alga provides novel refuge, shelter, and structural complexity to these communities (Nyberg et al. 2009). On the other hand, *G. vermiculophylla* thalli may be a less preferred alga for herbivorous macroinvertebrates compared to native algae, such as *Ulva* sp. (Nejrup et al 2012), indicating that some grazers may utilize *G. vermiculophylla* more for its habitat-forming structure than for consumption.

On the U.S. East Coast, past comprehensive surveys have identified two major geographic barriers that delineate macroinvertebrate assemblage patterns from north to south: Cape Cod and Cape Hatteras (Engle & Summers 1999, Spalding et al. 2007, Hale 2010). *Gracilaria vermiculophylla* is found across both barriers, but to date, there are no published studies investigating biogeographic patterns of community composition and structure of associated organisms with *G. vermiculophylla*. Moreover, nothing is yet known of the alga’s potential impact on communities that are not free-living; i.e., parasite communities. Parasites are a major, but often ignored, contributor to macroinvertebrate diversity in aquatic communities and food webs (Lafferty et al. 2008). Many parasites have multi-host life cycles that form numerous links across community members (Luque et al. 2004, Santoro et al. 2020), and trophically transmitting parasites, in particular, can have a key structuring role within aquatic communities (Wood et al. 2007; Lafferty et al. 2008; Dunne et al. 2013). Co-occurring with *G. vermiculophylla* throughout much of its range, the eastern mudsnail *Ilyanassa obsoleta* (=*Tritia obsoleta*) is a highly abundant gastropod in coastal marshes (Guidone et al. 2014) and is frequently infected by several digenean trematode species that predominantly use fish or birds as definitive hosts (Curtis & Hurd 1983, Blakeslee et al. 2012, Phelan et al. 2016, Blakeslee et al. 2020). Past work has found a positive relationship between *G. vermiculophylla* presence and the abundance of resident and migratory birds, which are attracted to macroinvertebrates associated with the alga (Haram et al. 2018). Many of these birds also serve as definitive hosts to digenean trematodes (Fredensborg et al. 2006, Phelan et al. 2016, Besterman et al. 2020). Thus, increased occurrence of birds could heighten snail exposure to digenean trematode eggs from the feces of definitive bird hosts (Byers et al. 2008), possibly enhancing trematode prevalence and diversity in the first-intermediate host, *I. obsoleta*. However, to date, little is known regarding the influence that foundation species have on parasite life cycles, host transmission, and community interactions.

In our study, we aimed to characterize and establish the community composition, abundance, richness, and diversity of macroinvertebrate communities (free-living and parasitic) associated with the non-native alga, *G. vermiculophylla*, throughout much of its U.S. East coast invaded range. To do so, we surveyed 17 sites (New Hampshire to South Carolina) within three biogeographic regions for free-living macroinvertebrates associated with 'fixed' and 'free-floating' *G. vermiculophylla,* as well as trematode parasites infecting the abundant co-occurring gastropod I. obsoleta. We predicted that G. vermiculophylla would support a large diversity of macroinvertebrates throughout the study region and that biogeographic region would strongly influence the community composition of free-living and trematode species along the U.S. East Coast. Altogether, our work furthers understanding of the influence of introduced foundation species on community composition and structure in coastal soft-sediment ecosystems.

METHODS

Study System

The red macroalga *Gracilaria vermiculophylla* is tolerant to variable temperatures and salinities (Rueness 2005, Phooprong et al. 2008, Sotka et al. 2018) and has colonized coastal habitats across a wide range of latitudes (Krueger-Hadfield et al. 2017). Due to its haploid-diploid life cycle, fixed sites include all three free-living stages (haploid male and female gametophytes and diploid tetrasporophytes), but free-floating sites are overwhelmingly dominated by tetrasporophytes (Krueger-Hadfield et al. 2016).

The eastern mudsnail *Ilyanassa obsoleta* (=*Tritia obsoleta*) is a highly abundant gastropod found in coastal habitats throughout eastern North America from the Gulf of Saint Lawrence, Canada to Northern Florida, forming densities as high as 8,000 individuals/m2 (Dimon 1902, Abbott 1974, Curtis & Hurd 1983, Harmon & Allen 2018). These gastropods primarily live in soft-sediment habitats and have wide thermal and salinity tolerances, contributing to their ecological and evolutionary success (Scheltema 1965, DeLorenzo et al. 2017, Fofonoff et al. 2018). *Ilyanassa obsoleta* serves as a first intermediate host to nine species of digenean trematodes (Blakeslee et al. 2012, Phelan et al. 2016). The life cycles of these parasites typically require two to three hosts and begin when *I. obsoleta* grazes on feces of definitive hosts that contain trematode eggs (Combes et al. 1994, Rohde 2005). An infected *I.*

obsoleta is castrated and parasitized for life (Curtis 1995). Downstream second-intermediate hosts include a wide range of molluscs, crustaceans, polychaetes, and fish, and definitive hosts include fish, birds, and terrapins (Blakeslee et al. 2012, Phelan et al. 2016).

Study Sites

We identified sample sites with established *G. vermiculophylla* from previous studies (Nettleton et al. 2013, Krueger-Hadfield et al. 2017). In summer 2019, we sampled 17 East Coast sites from New Hampshire to South Carolina, capturing much of the species' introduced range and encompassing two major geographic barriers at Cape Hatteras and Cape Cod (Engle & Summers 1999, Spalding et al. 2007, Hale 2010) (Figure 1). Since summer temperatures are lagged in northern versus southern latitudes, southern sites were sampled earlier than northern sites: South Carolina (May), Virginia and Delaware (June), New York and Connecticut (July), and Rhode Island, Massachusetts, and New Hampshire (August).

Sampling of Associated Free-Living Macroinvertebrates

We sampled each site for *G. vermiculophylla* in the shallow intertidal zone while thalli were still submerged before low tide. At each site, we established a 30-meter transect tape along the water-land interface and collected all *G. vermiculophylla* clumps from within five randomly selected 0.25 m2 quadrats along the transect. We sampled environmental parameters (water temperature, salinity) using a handheld YSI Pro-1030 (Yellow Springs, OH).

We placed sealed bags of algae and water immediately into coolers and then transported them to the lab for processing. In the lab, we soaked the *G. vermiculophylla* from each replicate in a large bin filled with fresh tap water to induce osmotic shock in the associated macroinvertebrates (e.g., Blakeslee et al. 2016, Fowler et al. 2016). We then used a Fisher

Scientific™ 250 micron sieve to separate macroinvertebrates from macroalgae; upon separation, we preserved macroinvertebrates in Pharmaco™ 200 proof Ethyl Alcohol. After shaking off excess water, we weighed the thalli to obtain wet weights (g).

Following field surveys at all sites, macroinvertebrates were dyed with Rose Bengal (Gbogbo et al. 2020) and identified to the lowest possible taxonomic level using guidebooks and keys (Bousfield 1973, Johnson & Allen. 2012). Organisms were observed using a Zeiss MS Series Fixed Magnification Stereo Microscope (6x) and/or a Neatfi Elite XL HD Magnifying Lamp (5x). Gammaridean amphipods, which comprised up to 75% of the total macroinvertebrates at sites (see Results), can be difficult to identify to species level using morphology alone. We therefore classified amphipods into morphotypes and then later barcoded those morphotypes using standard DNA protocols (e.g., Blakeslee et al. 2020a). This allowed us to identify amphipods to species level by uploading our resultant sequence data for each morphotype to BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Sampling of Trematode Parasites

We collected all *I. obsoleta* at the same sites as described above, except for Provincetown, MA, where *I. obsoleta* was not found (parasite data = 16 sites). We used the same 30-meter transect tape and 0.25 m2 quadrats as above to collect snails; however, *G. vermiculophylla* and *I. obsoleta* were placed into separate bags. We counted all *I. obsoleta* per quadrat, and then randomly selected 100 snails across the five quadrats to dissect. We also counted the total number of birds by species at each site using a point-count method, while standing stationary for 10 minutes (Byers et al. 2008). Birds are common final hosts for trematode parasites. In the lab, we measured each live gastropod using digital calipers and then dissected gonad tissues under a Zeiss™ MS Series Fixed Magnification Stereo Microscope at 6x magnification. If infected, we identified the digenean trematode to species level based on its rediae/sporocyst and cercarial morphology using published images and keys and prior knowledge within the lab (Curtis & Hurd 1983, Curtis 1985, Esch et al. 2001, Blakeslee et al. 2012).

Statistical Analyses

For both free-living and parasitic communities, we used the corrected Akaike's Information Criterion (AICc) to determine which model, or sets of environmental variables, best explained the following dependent variables: abundance (free-living) or prevalence (parasite), species richness, and species diversity. A ΔAICc of 2.0 was used as a cutoff value to determine the top models. For free-living organisms, the abundances were raw counts within quadrats; the richness was the number of total species; and the diversity was quantified via the Shannon-Weiner Diversity Index. For parasites, prevalence was the proportion of infected *I. obsoleta* out of 100 randomly dissected snails per site; richness was the number of digenean species; and diversity was the Shannon-Weiner Diversity Index. AICc compares multiple models with different combinations of independent variables (Burnham & Anderson 2002). Predictors within models were based on our interest in *G. vermiculophylla* thalli as a novel habitat and other variables that were ecologically or biologically important to our system. For free-living organisms, these predictors were *G. vermiculophylla* biomass, water temperature, salinity, and biogeographic region, with site as a random effect; for parasites, the predictors were *G. vermiculophylla* biomass, water temperature, salinity, average snail count, seabird and wading bird count, and biogeographic region (Supporting Information Tables S3-5, 14-16). The biogeographic regions examined in our study were characterized by the major biogeographic breaks at Cape Cod and Cape Hatteras and the distribution of sites in our study. We called these biogeographic regions North of Cape Cod (NCC), the Virginian Province (VP), and South of Cape Hatteras (SCH) (Spalding et al. 2007). Because some biogeographic regions had more sample sites than others (Table S1), we constructed rarefaction and extrapolation curves per biogeographic region to determine the expected number of species per biogeographic region as individuals accumulated using EstimateS (v 9.1.0).

To explore which factors best explained the patterns in the communities we observed, we used Generalized Linear Mixed Models (GLMM) in R 4.2.2 (package glmmTMB) for free-living macroinvertebrates (using site as a random effect, families: abundance = Negative Binomial, richness = Poisson, diversity = Gaussian) and Generalized Linear Model (GLM) for parasites (families: prevalence = Binomial, richness = Poisson, diversity = Gaussian). For parasite analyses, pilot runs showed that including “site” as a random effect did not contribute to the models, and henceforth, we used GLM models that included fixed effects only. Due to the unevenness in detecting fixed versus free-floating ecotypes across sites, we did not have the number of replicates to analyze ecotype as a fixed effect in our analyses; as a result, we compared abundance, richness, and diversity of free-living macroinvertebrates associated with fixed and free-floating *G. vermiculophylla* thalli using two-tailed t-tests across all sites.

For free-living macroinvertebrates, we used Nonmetric Multidimensional Scaling (nMDS) to create a two-dimensional ordination plane to visually evaluate community composition and diversity among sites (Clarke & Warwick 2001). Per recommendations by Cao et al. (2001), we removed species that occurred <5% in nMDS analyses, and we used square-root transformation and Bray-Curtis Similarity (Clarke & Warwick 2001). For free-living and parasite organisms, we also conducted Similarity of Percentage (SIMPER) analyses to determine the percent each species contributed to the differences observed between biogeographic regions

(Clarke 1993, Clarke & Warwick 2001). These latter analyses and figures were created using PRIMER-e (v.7).

RESULTS

Free-Living Organisms

Across all sampled sites (Figure 1), we found 39 free-living taxa (N=10,113). Three Gammaridean amphipods (*Gammarus mucronatus*, *Ampithoe longimana*, and *Gammarus lawrencianus*) comprised >50% of all the free-living macroinvertebrates. When examining free-living diversity by bioregion, we found 13 NCC taxa (N=2,009), 28 VP taxa (N=5,550), and 20 SCH taxa (N=2,554). Rarefaction and extrapolation curves demonstrated a greater expected species richness in VP compared to NCC and SCH (Figure S1), whereby the number of macroinvertebrate species associated with *G. vermiculophylla* in VP was expected to be 31 compared to the 26 species we observed. NCC was second highest at 25 expected species versus 13 observed, while SCH reached 23 expected species versus 20 observed. Thus, greater sampling effort is predicted to reveal 5 more species in VP, 12 in NCC, and 3 in SCH. Altogether, though we were able to sample VP more extensively than the other two regions, rarefaction analyses continued to show greater expected richness in VP. Two amphipod species *G. lawrencianus* and *G. mucronatus* comprised >50% of the regional abundance in VP, while in NCC, the amphipod *A. longimana* comprised >80% of the total abundance, and in SCH, *Ilyanassa obsoleta* and *G. mucronatus* comprised >50% of the abundance (Figure 1; Table S2).

In community analyses of free-living macrofauna associated with *G. vermiculophylla* thalli across our sample sites, the interaction of biogeographic region and *G. vermiculophylla* biomass best explained the variety in the abundance of macroinvertebrates in our top performing model (i.e., lowest ΔAICc weight) with site as the random effect (Table S3); both biomass and region were significant predictors (Table S4). In linear regressions of abundance with *G. vermiculophylla* biomass grouped by biogeographic region, we found significant positive relationships for VP and SCH, and marginal significance for NCC (Figure 2A). The second best performing model for macroinvertebrate abundance included the interaction of biogeographic region and *G. vermiculophylla* biomass and water temperature, with site as the random effect (Table S3); in this case, biomass and region were once again significant predictors, but temperature was not significant (Table S5). For richness of free-living macroinvertebrates, *G. vermiculophylla* biomass and the random effect of site best explained patterns in richness (Table S6); this was significant (Table S7), with macroinvertebrate richness increasing significantly with *G. vermiculophylla* biomass (Figure 2B). For diversity, the null model, which included the random effect of site only, was the top performing model (Table S8; Figure S2). When we examined the effect of *G. vermiculophylla* type (fixed, N = 45, versus free-floating, N = 38), we found abundance and richness were both significantly higher (t = -1.99; *p* = 0.05 and t = -2.15; *p* = 0.03, respectively) in the fixed type compared to the free-floating type, while diversity did not differ significantly between the two types (t = -1.2, *p*=0.24) (Figure 3).

Finally, in nMDS plots, free-living macroinvertebrate assemblages separated by biogeographic region (Figure 4). Some replicates' assemblages in SCH differed from other replicates because they were comprised of only one or two individuals (e.g., one count of *Myrianida* spp.), a combination that was not found in other replicates. When comparing macroinvertebrate assemblages between biogeographic regions using SIMPER, three species of Gammaridean amphipods *A. longimana*, *G. mucronatus*, and *G. lawrencianus* together contributed to the greatest dissimilarity of macroinvertebrate assemblages between SCH and VP, and also between VP and NCC. When comparing SCH and VP, *G. mucronatus* and *A. longimana* also contributed to the greatest dissimilarity between the regions, with *Caprella* spp. as another important contributor to their differences (Tables S9-S11).

Trematode Parasites

Across all sample sites, we found nine digenean trematode taxa (N=183 infected *I. obsoleta* out of 1,600 dissected), with *Lepocreadium setiferoides* and *Gynaecotyla adunca* comprising >50% of the infected snails (Table S12). In NCC, *Zoogonus lasius* and *Himasthla quissetensis* comprised >50% of the infected snails, while *L. setiferoides* and *Z. lasius* comprised >50% in VP. Finally, >70% of the infected snails were parasitized by *G. adunca* in SCH (Table S12).

In GLM analyses, the model with *G. vermiculophylla* biomass alone best predicted trematode prevalence and richness (i.e., lowest ΔAICc); however, biomass was not significant in either analysis (Tables S13-S16). The second best model for trematode richness included region, and this was a significant predictor (Table S17). For trematode diversity, the model with biogeographic region alone had the lowest ΔAICc (Table S18), and this was significant (Table S19); therefore, we graphically examined diversity across the biogeographic regions, observing a trend for decreasing diversity from north to south (Figure 5). For trematode diversity, the second best model included *G. vermiculophylla* biomass, but this was not significant (Table S20). In addition, in linear regression analyses, we found trematode prevalence, richness, and diversity all demonstrated trends for declines with increasing *G. vermiculophylla* biomass; however, these trends were not significant (Figure S3).

In SIMPER analyses, we found that between SCH and VP, *Lepocreadium setiferoides*, *Gynaecotyla adunca*, and *Zoogonus lasius* were the greatest contributors to dissimilarity, with *L.*

setiferoides and *Z. lasius* having higher prevalence in VP, and *G. adunca* having higher prevalence in SCH. When comparing SCH and NCC, we found that *Himasthla quissetensis*, *Zoogonus lasius*, and *Gynaecotyla adunca* were the greatest contributors to the dissimilarity between these two regions, with *H. quissentensis* and *Z. lasius* having higher prevalence in NCC, and *G. adunca* having higher prevalence in SCH. Finally, when comparing NCC and VP, we found that *Himasthla quissetensis*, *Lepocreadium setiferoides*, and *Zoogonus lasius* were the greatest contributors to the dissimilarity between these two regions, with *L. setiferoides* having higher prevalence in VP, and *H. quissetensis* and *Z. lasius* having higher prevalence in NCC (Tables S21-S23).

DISCUSSION

Foundation species can influence ecosystem structure and function by accommodating communities of associated fauna, creating microclimates, and reducing negative impacts of severe environmental events (Ellison et al. 2005, Franssen et al. 2011, Schob et al. 2012). Non-native foundational species can also greatly impact biodiversity and community structure in novel communities by influencing the diversity of associated organisms, like macroinvertebrates in aquatic systems (Djikstra et al. 2017). Past work has shown the invasive alga *G. vermiculophylla* can provide structured habitat for a diverse array of macroinvertebrates, and in some cases, may even harbor a greater abundance of associated organisms than some native macroalgae (Thomsen et al. 2009, 2013). In addition, more localized studies, such as from the Chesapeake Bay, have indicated that *G. vermiculophylla* may promote higher survival rates of macroinvertebrates, including the commercially important blue crab when compared to other foundational species, such as seagrass (Johnston & Lipcius 2012, Wood & Lipcius 2022). Yet, even though this alga is widespread and abundant on the U.S. East Coast and has been present in some populations for multiple decades, no community surveys at the broader biogeographic scale had been completed prior to our study. This incomplete understanding of the ecological impact of *G. vermiculophylla* in its invasive U.S. East Coast range once again highlights the substantial time lags that can be present among even widespread and highly perceptible species invasions (Crooks 2005). To help address this knowledge gap, we examined the sets of environmental variables that best predicted biodiversity patterns of free-living macroinvertebrates residing in *G. vermiculophylla* and trematode parasites infecting the co-occurring snail, *I. obsoleta*, throughout much of the alga's non-native U.S. east coast range. We predicted that *G. vermiculophylla* biomass would be a key variable determining faunal patterns (abundance, diversity, and richness) on a biogeographic scale (Figure 1). Below, we discuss our results in detail and how they advance understanding of community assembly in rapidly changing coastal environments.

The Influence of *Gracilaria vermiculophylla* Biomass and Biogeographic Region on Free-Living Macroinvertebrate Diversity

We found the interaction of biogeographic region and *G. vermiculophylla* biomass influenced the abundance of macroinvertebrates associated with *G. vermiculophylla*. In particular, we found free-living biomass to be a significant predictor of free-living abundance in two biogeographic regions, VP and SCH, with a trend for NCC (Figure 2). *Gracilaria vermiculophylla* creates three-dimensional structure that can provide niche space for numerous macroinvertebrate species (Thomsen et al. 2013). Previous studies assessing macroinvertebrate assemblages in other estuarine habitats invaded by *G. vermiculophylla* also found this alga could accommodate a greater abundance and richness of macroinvertebrates than some native foundation species in the same region (Thomsen et al. 2007, Thomsen 2010, Thomsen et al. 2013). In addition, the alga has been shown to provide nursery habitat for native blue crabs (Johnson & Lipcius 2012), and there is evidence for preferential egg deposition by *I. obsoleta* (Guidone et al. 2014). Further, studies have suggested that *G. vermiculophylla* may promote higher abundances of Gammaridean amphipods compared to habitats devoid of the alga in the southeastern U.S. (Wright et al. 2014), as well as supporting greater abundance and richness of native estuarine and marsh macroinvertebrates with increasing *G. vermiculophylla* biomass in both U.S. and European waters (Nyberg et al. 2009). In a comparison of European sites (Denmark and Sweden) with four sampled sites in Virginia, Nyberg et al. (2009) found that the number and diversity of Gammaridean amphipods were vital to the differences that were observed among populations. Similar to our study, the classes Malacostraca and Gastropoda were most commonly detected in the alga (Nyberg et al. 2009).

Along the U.S. Atlantic, the coastlines of South Carolina and Georgia were historically low in biomass of structurally complex macroalgae and seagrass species before the invasion of *G. vermiculophylla* (Sandifer et al. 1980). This alga has not only dramatically transformed the appearance of these systems, but its enhanced biomass has provided novel habitat for crustaceans and gastropods (Byers et al. 2012). In fact, the native tube-building polychaete (*Diopatra cuprea*) may be a contributor to this transformation of habitat complexity in what otherwise would be soft-sediment habitats with relatively low macroalgal biomass (Byers et al. 2012, Kollars et al. 2016). These polychaetes incorporate and then anchor free-floating *G. vermiculophylla* thalli to their tubes, with a recent study suggesting that this incorporation may be preferential over other offered macrophytes (Mott et al. 2022). Thus, through this relationship between worm and alga, where the worm stabilizes free-floating *G. vermiculophylla* to the benthos, algal biomass in some systems has become enhanced, with *G. vermiculophylla* representing the dominant algal species (Thomsen et al. 2009, Byers et al. 2012, Kollars et al. 2016, Mott et al. 2022; Berke 2022).

We found biogeographic region to be another important variable driving patterns of free-living macroinvertebrate abundance along the U.S. Atlantic coast. Across this coastline, Johnson (1934) denoted four biogeographic provinces: 1) Boreal Province, which ranges from Nova Scotia to Cape Cod, 2) Virginian Province, which ranges from Cape Cod to Cape Hatteras, 3) Carolinian Province, which ranges from Cape Hatteras to Cape Canaveral, and 4) Caribbean Province, which ranges from Cape Canaveral to the Caribbean Islands. In our study, *G. vermiculophylla* crosses two biogeographic breaks at Cape Cod and Cape Hatteras; thus we analyzed diversity patterns north of Cape Cod (to New Hampshire), between Cape Cod and Cape Hatteras (i.e., Virginian Province), and south of Cape Hatteras (to South Carolina). Past work has demonstrated these provinces are often characterized by differing species assemblages. For example, Cerame-Vivas & Gray (1966) found significantly different macroinvertebrate assemblages when comparing North Carolina sites north and south of Cape Hatteras, while Coomans et al. (1962) found significantly different mollusk compositions between the Virginian Province and the Carolinian Province. Moreover, an extensive macroinvertebrate survey by Engle & Summers (1999, 2000) found Cape Cod and Cape Hatteras acted as major dispersal barriers, with macroinvertebrate composition differing significantly north and south of these geographic boundaries. Cape Cod is widely recognized as the northernmost defining ecoregion boundary of the eastern U.S., since it is the northernmost limit for many species that occur within the Virginian Province (Hale 2010). Cape Cod also acts as the southernmost limit for arctic and boreal species, particularly mollusks (Franz & Merrill 1980). Further south, Cape Hatteras has been found to reduce intraspecific gene flow, since parcels of water from the Gulf Stream traveling northward along the eastern U.S. are deflected northeast, thereby preventing homogeneity of climate across this boundary and creating different microclimates (Boehm et al. 2015). Similar to the biogeographic patterns described above, we found that communities shifted with biogeographic region in multivariate community analyses (Figure 4), suggesting that Cape Hatteras and Cape Cod also differentiate invertebrate communities associated with *G. vermiculophylla*. Moreover, we found the Virginian Province to be the biogeographic region that had the highest macroinvertebrate abundance associated with *G. vermiculophylla*, and this pattern continued to hold even following rarefaction analyses that predicted diversity as samples accumulated.

Of the associated free-living species, the most dominant taxa across all biogeographic regions were Gammaridean amphipods (Figure 1). Within the Gammarideans, two species were the most prominent: *Ampithoe longimana*, which is a grazer, and *Gammarus mucronatus*, which is a generalist (Fredette & Diaz 1986, Duffy et al. 1994, 2001). While macroinvertebrate grazers and generalists are commonly found associated with invasive *G. vermiculophylla*, past work has suggested many associated species are using this alga as habitat rather than a direct food source (Nejrup & Pedersen 2012, Wright et al. 2014). For example, in experimental trials, Weinberger et al. (2008) found that native macroinvertebrates preferred to graze on native macroalgae over *G. vermiculophylla*; however, their field surveys showed that macroinvertebrates preferred *G. vermicuophylla* as refuge over native macroalga in winter months. Alternatively, other studies have suggested that *G. vermiculophylla* could lower abundance and diversity of associated organisms (Berke 2022). For example, Keller et al. (2019) observed that during periods of time when there were super-blooms of *G. vermiculophylla*, the tube-forming polychaete *Diopatra* *cuprea* declined in abundance, possibly due to the alga limiting oxygen flow and thereby increasing anoxic conditions.

Though several studies have shown that *G. vermiculophylla* can provide habitat and shelter to associated macroinvertebrates (Weinberger et al. 2008, Nejrup & Pedersen 2012, Wright et al. 2014), the alga's life cycle may play a role in the types and abundances of the species that utilize it along the coast. In nature, differing "morphs" of *G. vermiculophylla* may be apparent at sites depending on the presence or absence of hard substratum: haploid male and female gametophytes that are 'fixed' with a holdfast to substratum and/or diploid tetrasporophytes that are 'free-floating' (Krueger-Hadfield et al. 2016). Yet it is unclear whether these types (fixed or free-floating) may attract or support different community assemblages. In our study, we found higher abundance and species richness of free-living macroinvertebrates associated with fixed thalli as compared to free-floating (Figure 3). This may be because fixed thalli possess greater genotypic and ploidy diversities than free-floating thalli, thereby promoting a greater abundance and richness of associated faunal communities (i.e., genetic diversity promotes taxonomic diversity), as predicted by Krueger-Hadfield et al. (2019). Future studies should experimentally investigate community assembly and diversity depending on the type of thallus in a more explicit way, particularly in large, expansive mudflats where *G. vermiculophylla* biomass can be substantial (Krueger-Hadfield & Ross 2022).

The Influence of *G. vermiculophylla* on Trematode Diversity in Co-occurring Snail Hosts

Though often cryptic, parasites can be strong drivers of community structure and function, as well as the evolutionary ecology of their hosts. Most studies involving food webs and community structure have only focused on connections among free-living organisms, but recent studies have demonstrated that parasites are pivotal and integral components of communities and food webs (Huxham et al. 1995, Lafferty et al. 2008; Moore et al. 2023). In our analyses of trematode communities infecting *I. obsoleta*, an abundant gastropod that co-occurs with *G. vermiculophylla* throughout our study region, we found biogeographic region alone was the best predictor of trematode diversity, with significant differences identified between the northern-most (North of Cape Cod) and southern-most (South of Cape Hatteras) biogeographic regions (Figure 5). These differences were because our southern sites were dominated by just a few trematode species, while trematode diversity was more evenly spread in the northern sites, particularly trematode species that utilize birds as final hosts. This observation was also found in a past biogeographic study of trematode parasites along the U.S. East Coast, where trematode infections in southern sites were dominated by trematode species that predominantly use fish as definitive hosts (Blakeslee et al. 2012, 2020b).

In terms of trematode prevalence and richness, *G. vermiculophylla* biomass alone was the best predictor. Yet, in contrast to our free-living data, this relationship was negative (i.e., lower prevalence and richness with higher *G. vermiculophylla* biomass), albeit these linear trends were either marginally, or not, significant. A negative relationship between *G. vermiculophylla* biomass and parasitism was contrary to our expectations because we predicted that a greater abundance, richness, and diversity of free-living hosts would also positively influence parasite prevalence, richness and diversity. Digenean trematodes have complex, multi-host life cycles that typically include two to three different hosts and alternate between trophically transmitted parasitic (from the encysted larval to the adult stage) and free-living environmental stages (the egg stage and the cercarial stage) (Rohde 2005). As such, trematodes are impacted by multiple abiotic and biotic forces during their environmental stages, as well as influences upon and by their hosts during their parasitic stages. Thus, drivers of trematode diversity in systems are likely to come from multiple sources. For example, in a prior study examining driving factors of trematode prevalence in New England in the marine snail *Littorina littorea*, Byers et al. (2008) found prevalence of infection to be primarily influenced by average snail size and bird count. In this system, all the trematode species infecting *L. littorea* use birds as final hosts; whereas, in our system, *I. obsoleta* is infected by trematodes that use birds, fish, and terrapins as final hosts (Blakeslee et al. 2020b). While we counted definitive bird hosts (waders, seabirds, and dabblers) at each site, we did not collect information on fish abundance and diversity. As a result, our understanding of the influence of host abundance on parasite diversity was more limited. Thus, the association between *I. obsoleta's* trematodes and *G. vermiculophylla* biomass is likely more complex than we were able to capture from the data we collected for our study. Future work examining native and non-native systems with *G. vermiculophylla* could design surveys and experiments with the explicit goal of determining how *G. vermiculophylla* and other factors may influence parasitic diversity.

Conclusions

Our study represents the first biogeographic examination of both free-living and parasitic communities associated with *G. vermiculophylla* in its invasive range along the U.S. Atlantic Coast. Though this alga has been present along the U.S. East Coast for multiple decades (Krueger-Hadfield et al. 2017), evidence related to its effects on associated communities had previously been based upon localized studies, thus we were missing a broader understanding of its role in this established range. Such time lags can lower our ability to respond to or predict future invasion impacts (Crooks 2005). In the case of *G. vermiculophylla*, we found the alga to be influential on the community diversity of associated macroinvertebrates, which are essential components of food webs and critical to sustaining healthy ecosystems (Luczkovich et al. 2002, Alfaro 2006, Chaplin & Valentine 2009, Medina-Contreras et al. 2020). Specifically, we discovered *G. vermiculophylla* biomass and biogeographic region to be the two main predictors driving observed patterns of abundance and richness in free-living macroinvertebrates associated with the non-native macroalga. However, the long-term effects of *G. vermiculophylla* presence in these communities need further monitoring. This study, while biogeographic in scale, was completed during one sampling season; thus future sampling across multiple years and seasons is needed to assess how free-living macroinvertebrates are shaped by *G. vermiculophylla* spatially and temporally. Moreover, parasites are an essential component of food webs and are integral to community structure, function, and diversity because they can affect overall fitness of hosts by changing their growth, mortality, nutritional requirements, and behavior (Wood et al. 2007, Park 2019). While we did not find statistically significant trends of *G. vermiculophylla* biomass on parasite prevalence or richness, we may have missed important predictor variables that would have helped us understand the potential influence that this invasive alga may be having on parasite life cycles. Moreover, examining parasite diversity in other hosts in the life cycle (e.g., birds and fish) may provide greater resolution of the impact of the alga's presence on parasite communities. As *G. vermiculophylla* continues to thrive along the U.S. east coast, it is likely that we will continue to observe changes to community structure and function in invaded systems. With continued investigation, it may be possible to generate a more comprehensive understanding of community associations with *G. vermiculophylla* that integrates both free-living and parasitic organisms.

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CHAPTER 1 FIGURES

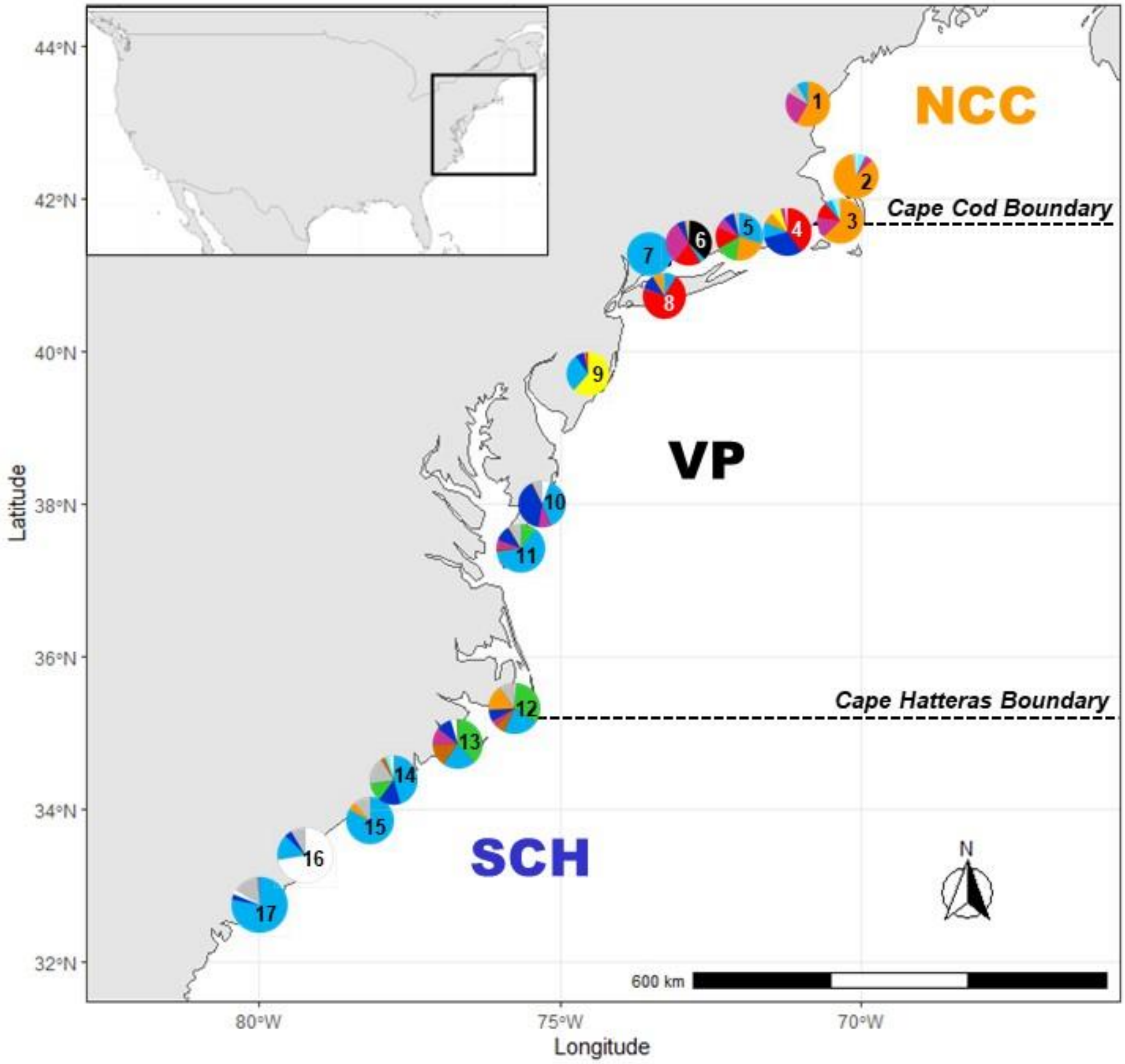

Figure 1 Map of sampled sites for May–August 2019 with ecoregion boundaries (Cape Cod and Cape Hatteras) and ecoregion labels: 1=Durham, NH; 2=Provincetown, MA; 3=Millway Beach, MA; 4=Sandy Point, RI; 5=Warwick, RI; 6=Lighthouse Point, New Haven, CT; 7=Seaside Park, Bridgeport, CT; 8=Crab Meadow, Northport, NY; 9=Tuckerton, NJ; 10=Little Toms Cove, Chincoteague, VA; 11=Quinby, VA; 12=Ocracoke Island, NC; 13=Harkers Island, NC; 14=UNCW, Wilmington, NC; 15=Fort Fisher, NC; 16=Hobcaw Barony, Georgetown, SC; 17=Fort Johnson, SC. The pie pieces represent composition of free-living macroinvertebrates for each site (light Blue = *Gammarus mucronatus*, orange = *Ampithoe longimana*, red = *Gammarus lawrencianus*, green = *Ilyanassa obsoleta*, dark blue = *Ampithoe valida*, purple = *Caprella* spp., black = *Littorina littorea*, yellow = *Idotea balthica*, brown = *Cyathura polita*, white = *Myrianida* spp., cyan blue = *Mercenaria* spp., gray = species comprising <5%. NCC=North of Cape Cod; VP=Virginian Province; SCH=South of Cape Hatteras. For the complete list of sampled sites and details, see Supplemental Information Table S1.

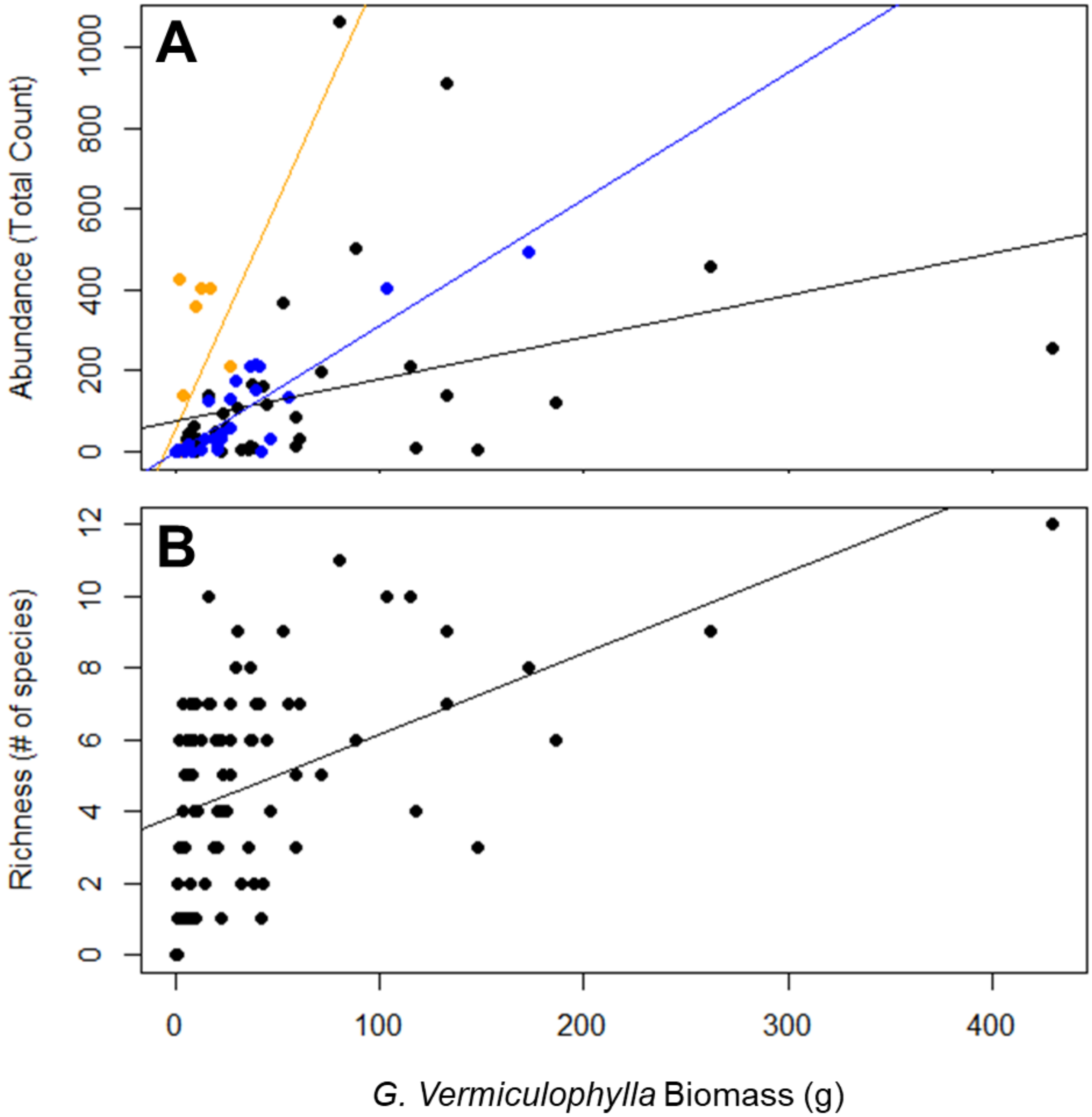

Figure 2: Scatterplots and regression lines of (A) free-living abundance by biogeographic region (orange = North of Cape Cod: $R^2 = 0.221$, $p = 0.077$, black = Virginian Province: $R^2 = 0.134$, $p < 0.022$, blue = South of Cape Hatteras: $R^2 = 0.7856$, $p < 0.001$), and (B) free-living richness ($R^2 = 0.255$, $p < 0.001$) and *G. vermiculophylla* biomass.

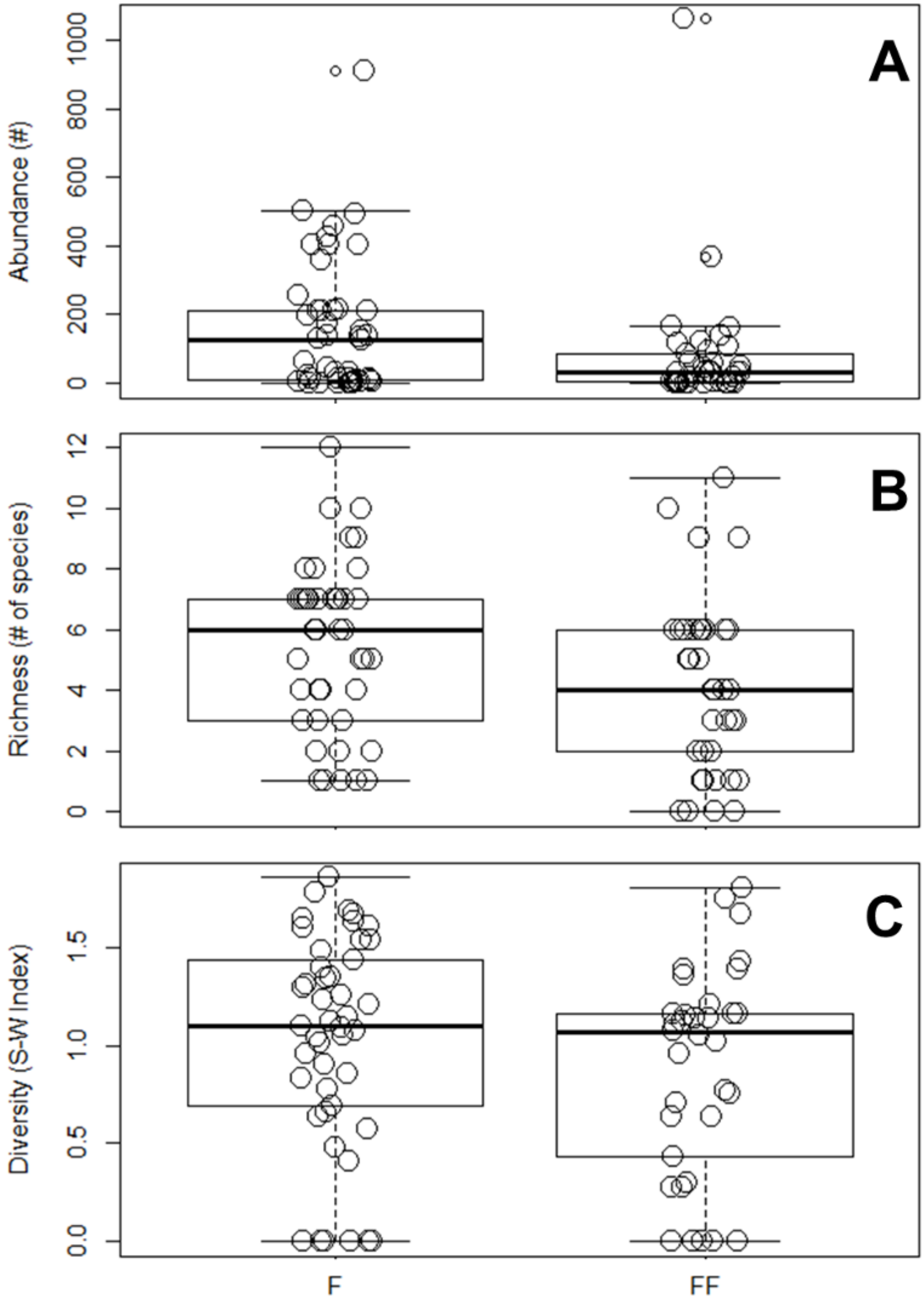

Figure 3: Boxplots of free-living (A) abundance (t = -1.99; *p* = 0.05), (B) richness (t = -2.15; *p* = 0.03), and (C) diversity (t = -1.20;*p* = 0.24) compared across *G. vermiculophylla* site types (FF = free-floating, F = fixed). Black circles represent each replicate (jittered).

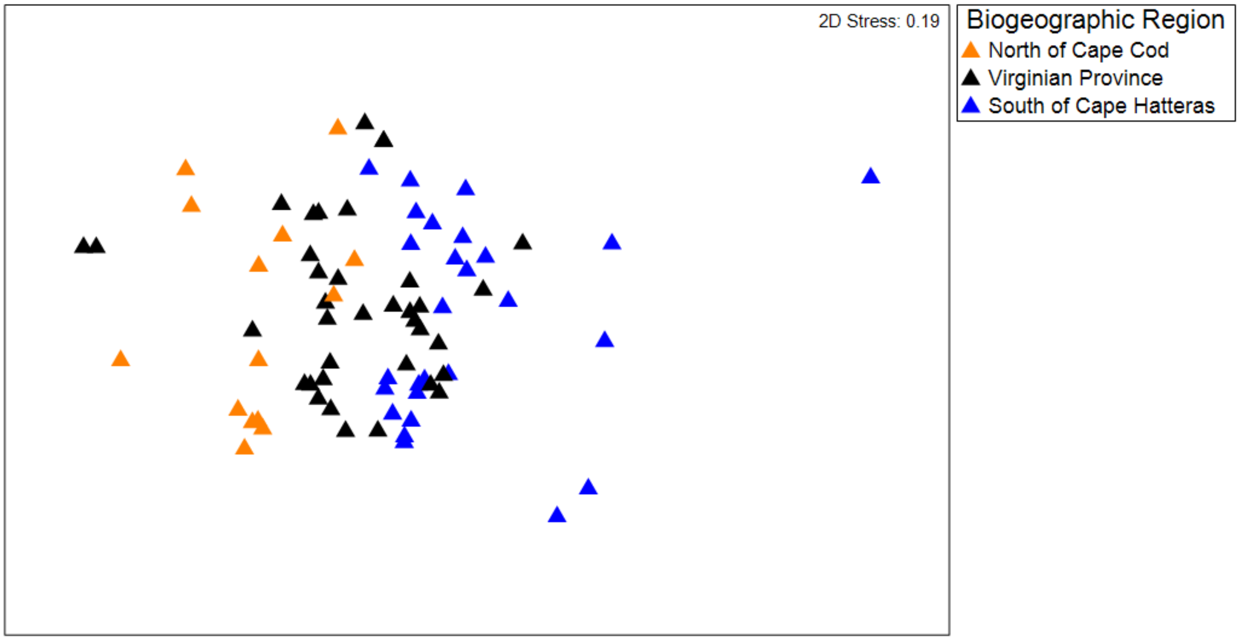

Figure 4: Non-metric multidimensional scaling plot of free-living macroinvertebrate abundances by biogeographic region. Samples that are closer to each other are more similar in terms of species composition and evenness. Species with <5% occurrence have been removed.

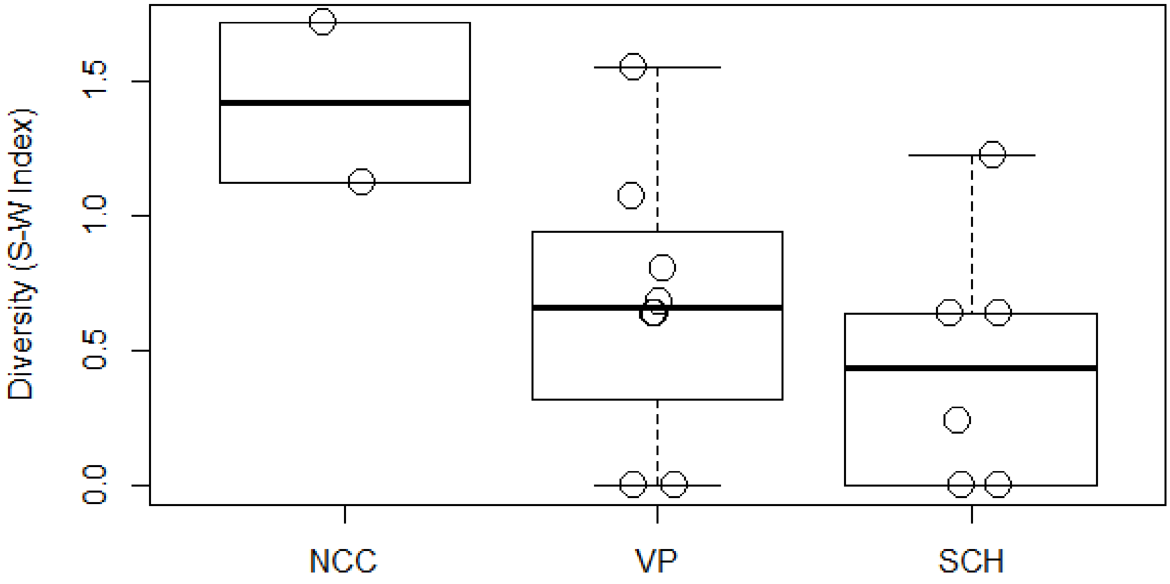

Figure 5: Boxplot of parasite diversity (Chi-sq = 4.36, *p* = 0.113). NCC = North of Cape Cod; VP = Virginian Province; SCH = South of Cape Hatteras; S-W Index = Shannon-Wiener Diversity Index. Black circles represent each replicate (jittered).

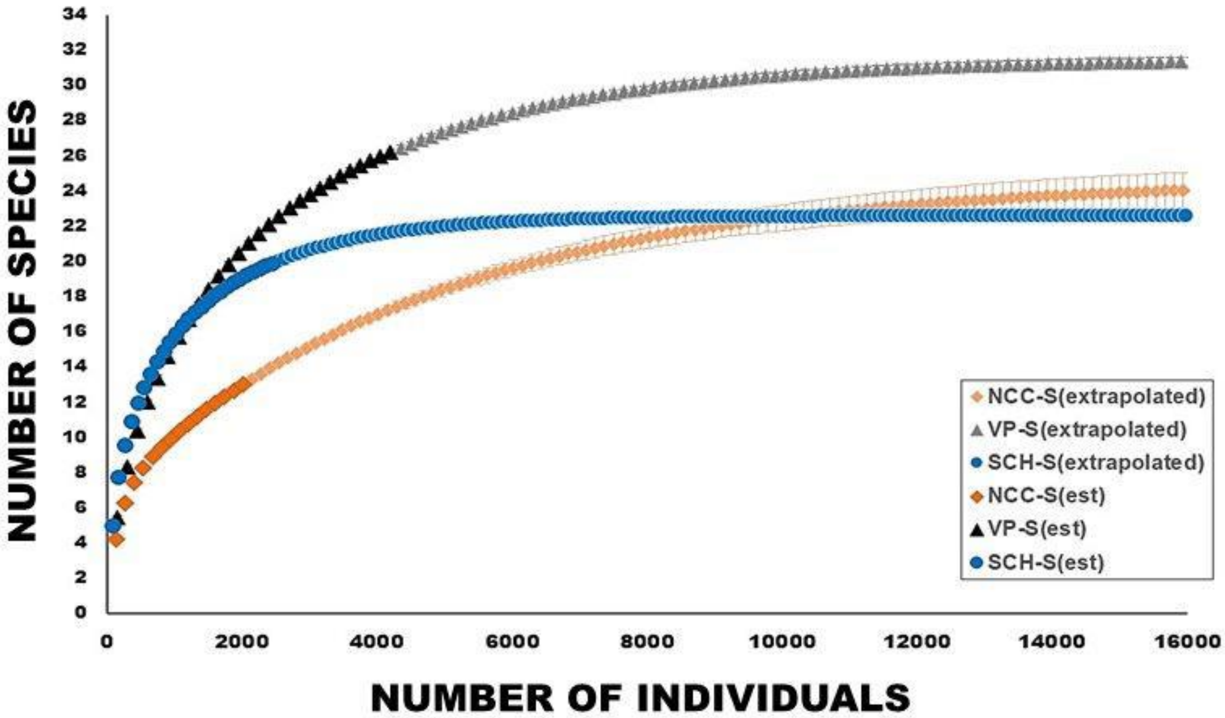

Figure S1. Rarefaction curves demonstrating observed S(est) and extrapolated S(extrapolated) species richness of associated free-living macroinvertebrates with *Gracilaria vermiculophylla* as individuals accumulate across the three biogeographic regions along the U.S. East Coast, including NCC = North of Cape Cod, VP = Virginian Province, and SCH = South of Cape Hatteras. Observed richness is in darker hues (dark orange = NCC, black = VP, and dark blue = SCH), while extrapolated richness is in lighter hues (orange = NCC, gray = VP, and blue = SCH). The extrapolation curves were arrested once all groupings had reached an asymptote in richness.

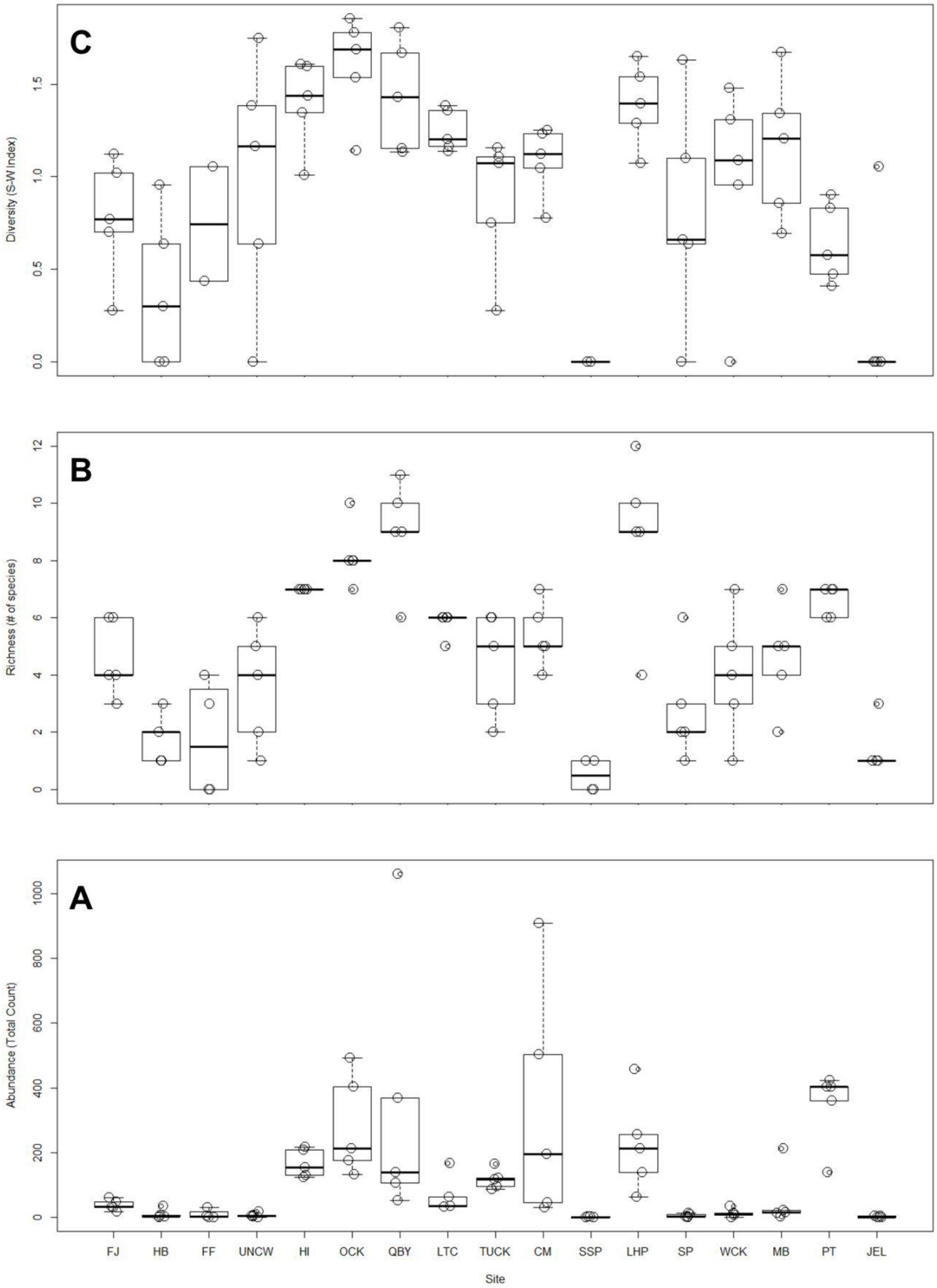

Figure S2: Boxplots of free-living (A) abundance (Chi sq = 66.194, $p < 0.001$), (B) richness (Chi sq = 62.703, $p < 0.001$), and (C) diversity (Chi sq = 46.01, $p < 0.001$) by site. FJ = Fort Johnson, HB = Hobcaw Barony, FTF = Fort Fisher, UNCW = UNC Wilmington, HI = Harkers Island, OCK = Ocracoke Island, QBY = Quinby, LTC = Little Toms Cove, TUCK = Tuckerton, CM = Crab Meadow, SSP = Seaside Park, LHP = Lighthouse Point, SP = Sandy Point, WCK = Warwick, MB = Millway Beach, PT = Provincetown, JEL = Jackson Lab (see Supporting Information Table S1 for details on sampled sites). Black circles represent each replicate (jittered).

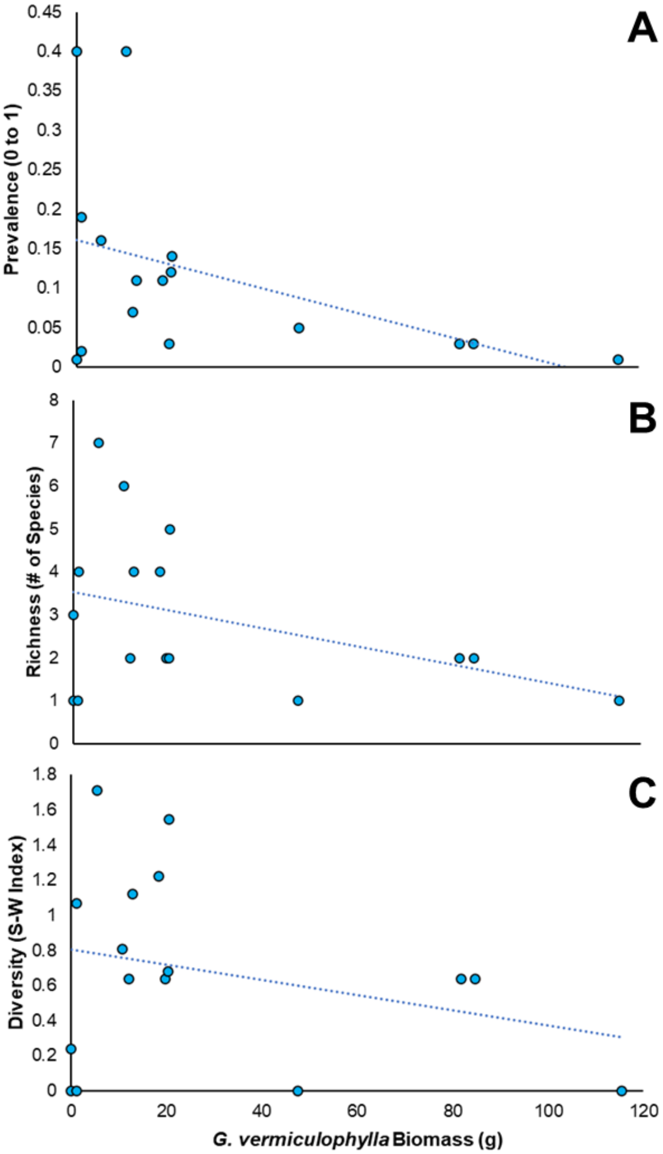

Figure S3: Scatterplots and regression lines between parasitic (A) prevalence ($R^2 = 0.1954$, $p = 0.0865$), (B) richness ($R^2 = 0.1549$, $p = 0.095$), and (C) diversity ($R^2 = 0.078$, $p = 0.295$) and *G. vermiculophylla* biomass.

CHAPTER 1 TABLES

Table S1: Sampling Locations and their site type, region, sampled date, coordinates, and water temperature. FF = free-floating, F = fixed; SCH = South of Cape Hatteras, VP = Virginian Province, NCC = North of Cape Cod.

SiAte	Site type	Region	Date	Latitude	Longitude	Water Temp (Celsius)	Sampling Personnel
Hobcaw Barony, Georgetown, SC	FF	SCH	5/20/2019	33.350536	-79.190751	31	C. Gabriel, N. Isastia, T. Lee
Fort Johnson,Charleston, SC	FF	SCH	5/21/2019	32.751103	-79.902054	32.2	C. Gabriel, N. Isastia, T. Lee
Fort Fisher, NC	FF	SCH	6/9/2019	33.958989	-77.942	27.1	T. Lee, D. Wright
Harker's Island, NC	F	SCH	6/13/2019	34.72266	-76.575727	29.2	T. Lee
Ocracoke Island, NC	F	SCH	6/17/2019	35.117566	-75.986541	30	T. Lee
UNCW Center for Marine Science, NC	FF	SCH	6/22/2019	34.140239	-77.863717	29.6	T. Lee, J. Russo, D. Wright
Little Tom's Cove, Asseteague Island, VA	FF	VP	6/25/2019	37.886791	-75.346134	24.2	A. Blakeslee, A. Fowler, T. Lee, A. Mott
Quinby Harbor, VA	FF	VP	6/26/2019	37.547747	-75.731542	26.4	A. Blakeslee, A. Fowler, T. Lee, A. Mott
Lighthouse Point, CT	F	VP	7/7/2019	41.248059	-72.904171	27.4	T. Lee
Seaside Park, CT	F	VP	7/8/2019	41.150499	-73.213405	25.3	T. Lee
Crab Meadow Beach, NY	F	VP	7/9/2019	40.928615	-73.32685	23.2	T. Lee
Little Egg Harbor/Tuckerton, NJ	FF	VP	7/10/2019	39.508973	-74.320067	28.3	T. Lee
Oakland Beach, RI	F	VP	8/2/2019	41.684452	-71.399952	29.9	T. Lee
Sandy Point, RI	F	VP	8/3/2019	41.661758	-71.409756	28.6	T. Lee
Provincetown Marina, MA	F	NCC	8/4/2019	42.050038	-70.185916	21.3	T. Lee
Jackson Lab, Durham, NH	F	NCC	8/8/2019	43.091928	-70.864512	23	T. Lee
Millway Beach, MA	F	NCC	8/9/2019	41.709342	-70.297774	26.3	T. Lee

Table S2: Proportion of free-living macroinvertebrates (cumulative >95%) out of 39 total species (N = 10,113) cumulatively, and in the North of Cape Cod (NCC), Virginian Province (VP), and South of Cape Hatteras (SCH) bioregions.

Overall (n = 10,113)		**NCC** (n = 2,009)		**VP** (n = 5,550)		**SCH** (n = 2,554)	
Taxa	**%**	**Taxa**	**%**	**Taxa**	**%**	**Taxa**	**%**
Gammarus mucronatus	22.66	*Ampithoe longimana*	81.58	*Gammarus mucronatus*	28.50	*Ilyanassa obsoleta*	31.21
Ampithoe longimana	20.39	*Caprella* spp.	6.97	*Gammarus lawrencianus*	26.27	*Gammarus mucronatus*	27.02
Gammarus lawrencianus	14.72	*Mercenaria* spp.	5.28	*Ampithoe valida*	11.06	*Ampithoe longimana*	9.16
Ilyanassa obsoleta	9.72	*Argopecten irradians*	2.19	*Caprella* spp.	8.97	*Cyathura polita*	8.54
Ampithoe valida	8.03			*Littorina littorea*	7.55	*Ampithoe valida*	7.75
Caprella spp.	7.76			*Idotea balthica*	6.61	*Caprella* spp.	5.76
Littorina littorea	4.14			*Ampithoe longimana*	3.41	*Bittium varium*	3.68
Idotea balthica	3.67			*Ilyanassa obsoleta*	3.35	*Myrianida* spp.	3.41
Cyathura polita	2.32						
Myrianida spp.	1.28						
Mercenaria spp.	1.09						

Table S3 Free-Living AICc table for Poisson GLMM testing response variable “abundance” (raw counts) with site as random effect. gvbiomass = *Gracilaria vermiculophylla* biomass (g); region = biogeographic region; salinity = seawater salinity (ppt); watertemp = seawater temperature (Celsius).

	AICc	ΔAICc	AICc Wt.	Cumulative Wt.
gvbiomass*region + site	841.67	0.00	0.45	0.45
gvbiomass*region + watertemp + site	842.95	1.28	0.24	0.68
gvbiomass + site	843.86	2.19	0.15	0.83
gvbiomass*region + watertemp + salinity + site	845.18	3.51	0.08	0.91
gvbiomass + watertemp + site	845.56	3.89	0.06	0.97
gvbiomass + region + site	848.36	6.69	0.02	0.99
gvbiomass + watertemp + region + site	850.00	8.33	0.01	1.00

Table S4: Free-Living AICc table for Poisson GLMM testing response variable “richness” (number of species) with site as a random effect. gvbiomass = *Gracilaria vermiculophylla* biomass (g); region = biogeographic region; salinity = seawater salinity (ppt); watertemp = seawater temperature (Celsius).

	AICc	ΔAICc	AICc Wt.	Cumulative Wt.
gvbiomass + site	363.28	0.00	0.73	0.73
gvbiomass + watertemp + site	365.49	2.21	0.10	0.83
NULL	367.52	4.24	0.09	0.92
gvbiomass + region + site	367.73	4.45	0.03	0.95
watertemp + site	369.67	6.39	0.03	0.98
gvbiomass + watertemp + region + site	370.05	6.76	0.01	0.99
region + site	371.65	8.37	0.01	1.00
watertemp + salinity + gvbiomass + region + site	371.84	8.56	0.01	0.99
gvbiomass*region + site	371.96	8.68	0.01	0.99
watertemp + region + site	373.91	10.63	0.00	1.00

Table S5: Free-Living AICc table for Gaussian GLMM testing response variable “diversity” (Shannon-Wiener Diversity Index) with site as random effect. gvbiomass = *Gracilaria vermiculophylla* biomass (g); region = biogeographic region with sites nested; salinity = seawater salinity (ppt); watertemp = seawater temperature (Celsius).

	AICc	ΔAICc	AICc Wt.	Cum. Wt.
NULL	113.56	0.00	0.70	0.70
gvbiomass + site	117.00	3.44	0.13	0.82
watertemp + site	117.81	4.24	0.08	0.91
region + site	118.43	4.87	0.06	0.97
gvbiomass + watertemp + site	121.56	8.00	0.01	0.98
watertemp + region + site	122.46	8.89	0.01	0.99
gvbiomass + region + site	122.51	8.95	0.01	1.00

Table S6 Model output for top performing model for free-living abundance in table S3. Formula = Abundance ~ gvbiomass*region + site. The random intercept of the site has variance = 2.434, and standard deviation of 1.56. Conditional $R^2 = 0.856$ (fixed and random variables), marginal $R^2 = 0.128$ (fixed variables only). SCH = South of Cape Hatteras, VP =Virginian Province

	β Estimate	Standard Error	z-value	p-value
Intercept	7.024	1.392	5.047	<0.001
gvbiomass	7.072	0.0002	35.437	<0.001
region (SCH)	-3.446	1.541	-2.237	0.025
region (VP)	-3.427	1.505	-2.278	0.023
gvbiomass*region (SCH)	-6.265	2.024	-3.094	0.002
gvbiomass*region (VP)	-6.553	1.988	-3.296	<0.001

Table S7: Model output for second highest performing model for free-living abundance in table S3. Formula = Abundance ~ gvbiomass*region + watertemp + site. The random intercept of the site has variance 2.22, and standard deviation of 1.49. Conditional $R^2 = 0.851$ (fixed and random variables); marginal $R^2 = 0.164$ (fixed variables only).

	β Estimate	Standard Error	z-value	p-value
Intercept	6.277	1.515	4.145	<0.001
gvbiomass	7.186	1.975	3.638	<0.001
region (SCH)	-2.13	1.896	-1.124	0.261
region (VP)	-2.807	1.567	-1.791	0.073
watertemp	-0.655	0.575	-1.139	0.255
gvbiomass*region (SCH)	-6.362	2.019	-3.151	<0.001
gvbiomass*region (VP)	-6.658	1.983	-3.357	<0.001

Table S8: Model output for highest performing model for free-living richness in table S4. Formula = Richness ~ gvbiomass + site. The random intercept of the site has variance 0.274, and standard deviation of 0.524. Conditional R^2 = 0.572 (fixed and random variables); marginal R^2 = 0.038 (fixed variables only).

	β Estimate	Standard Error	z-value	p-value
Intercept	1.408	0.141	9.979	<0.001
gvbiomass	0.140	0.054	2.614	0.009

Table S9: Model output for the second highest performing model for free-living richness in table S4. Formula = Richness ~ gvbiomass + watertemp + site. The random intercept of the site has variance 0.274, and standard deviation of 0.523. Conditional R^2 = 0.572 (fixed and random variables); marginal R^2 = 0.038 (fixed variables only).

	β Estimate	Standard Error	z-value	p-value
Intercept	1.408	0.141	9.979	<0.001
gvbiomass	0.140	0.054	2.614	0.009
watertemp	-0.008	0.141	-0.059	0.953

Table S10: Comparison of species between South of Cape Hatteras and Virginian Province based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 78.24						
	SCH	**VP**				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Gammarus mucronatus	3.14	4.3	14.76	1.15	18.87	18.87
Ampithoe valida	1.11	3.07	10.41	1.24	13.3	32.17
Gammarus lawrencianus	0	3.2	9.97	0.82	12.74	44.91
Caprella spp.	1.03	2.24	7.4	0.98	9.46	54.37
Tritia obsoleta	1.94	0.84	6.74	0.76	8.61	62.98
Myrianida spp.	1.14	0.3	5.42	0.58	6.93	69.91
Idotea balthica	0	1.25	5.05	0.41	6.45	76.37
Ampithoe longimana	0.18	1.26	4.58	0.73	5.86	82.22
Cyathura polita	1.15	0.21	3.23	0.59	4.13	86.36
Littorina littorea	0	1.14	2.74	0.38	3.51	89.87
Dyspanopeus spp.	0.16	0.48	1.86	0.57	2.38	92.25
Rimapenaeus constrictus	0.41	0	1.83	0.45	2.34	94.59
Edotia triloba	0.25	0.07	1.58	0.33	2.02	96.61

Table S11 Comparison of species between South of Cape Hatteras and North of Cape Cod based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 87.70						
	SCH	**NCC**				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarit St. Dev.	Contributi on %	Cumulati ve %
Ampithoe longimana	0.18	7.65	24.78	1.35	28.25	28.25
Gammarus mucronatus	3.14	0.89	14.29	1.03	16.29	44.55
Caprella spp.	1.03	2.4	10	1.22	11.4	55.95
Mercenaria spp.	0.05	1.85	7.08	0.88	8.07	64.03
Myrianida spp.	1.14	0.74	6.59	0.59	7.51	71.54
Tritia obsoleta	1.94	0	5.93	0.64	6.76	78.3
Ampithoe valida	1.11	0	5.35	0.66	6.1	84.4
Cyathura polita	1.15	0	3.26	0.54	3.72	88.12
Argopecten irradians	0	1.01	2.5	0.71	2.85	90.97
Rimapenaeus constrictus	0.41	0	2.15	0.45	2.45	93.42
Edotia triloba	0.25	0	1.91	0.31	2.18	95.6

Table S12 Comparison of species between Virginian Province and North of Cape Cod based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 82.46						
	VP	**NCC**				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarit St. Dev.	Contributi on %	Cumulati ve %
Ampithoe longimana	1.26	7.65	19.73	1.19	23.92	23.92
Gammarus mucronatus	4.3	0.89	11.82	1.04	14.33	38.25
Gammarus lawrencianus	3.2	0.4	9.64	0.82	11.69	49.95
Ampithoe valida	3.07	0	9.13	1.11	11.08	61.02
Caprella spp.	2.24	2.4	8.87	1.1	10.76	71.78
Mercenaria spp.	0.07	1.85	5.62	0.82	6.82	78.6
Idotea balthica	1.25	0.24	4.89	0.43	5.93	84.52
Myrianida spp.	0.3	0.74	2.65	0.79	3.21	87.73
Littorina littorea	1.14	0	2.55	0.37	3.1	90.83
Argopecten irradians	0	1.01	2.16	0.69	2.62	93.45
Tritia obsoleta	0.84	0	2.11	0.4	2.56	96.01

Table S13: Proportion of parasites (cumulative >95%) out of 9 total species (N = 183 infected *Ilyanassa obsoleta*) cumulatively, and in the North of Cape Cod (NCC), Virginian Province (VP), and South of Cape Hatteras (SCH) bioregions; n = number of *Ilyanassa obsoleta* dissected. AV = *Austrbilharzia variglandis*, DN = *Diplostomum nassa*, GA = *Gynaecotyla adunca*, HQ = *Himasthla quissetensis*, LS = *Lepocreadium setiforides*, PM = *Pleurogonius malaclemys*, ST = *Stephanostomum tenue*, ZL = *Zoogonus lasius*

Overall (n = 1,600)		**NCC (n = 200)**		**VP (n = 800)**		**SCH (n = 600)**	
Taxa	**%**	**Taxa**	**%**	**Taxa**	**%**	**Taxa**	**%**
LS	30.60	ZL	28.57	LS	48.42	GA	71.67
GA	28.42	HQ	25.00	ZL	21.05	ZL	13.33
ZL	19.67	LS	25.00	HQ	9.47	ST	5.00
HQ	9.29	ST	10.71	GA	9.47	LS	5.00
ST	4.92	AV	3.57	PM	6.32		
PM	3.28	DN	3.57	ST	3.16		

Table S14: AICc table for Binomial GLM testing response variable “prevalence” (proportion of *Ilyanassa obsoleta* infected with parasites in each site) with biogeographic region as random effect. avggvbiomass = average *Gracilaria vermiculophylla* biomass (g); region = biogeographic region (North of Cape Cod, Virginian Province, South of Cape Hatteras); salinity = seawater salinity (ppt); watertemp = seawater temperature (oC); avgsnailcount = average count of *Ilyanassa obsoleta*, seabirdwaders = total count of seabirds and wading birds.

	AICc	ΔAICc	AICc Wt.	Cumulative Wt.
avggvbiomass	8.98	0.00	0.77	0.77
region	12.00	3.02	0.17	0.94
avggvbiomass + region	15.70	6.71	0.03	0.97
avgsnailcount + avggvbiomass + seabirdwaders	15.79	6.81	0.03	1.00

Table S15: AICc table for Poisson GLM testing response variable “richness” (number of parasite species) with biogeographic region as random effect. avggvbiomass = average *G. vermiculophylla* biomass (grams); region = biogeographic region (North of Cape Cod, Virginian Province, South of Cape Hatteras); salinity = seawater salinity (ppt); watertemp = seawater temperature (Celsius); avgsnailcount = average count of *Ilyanassa obsoleta*, seabirdwaders = total count of seabirds and wading birds.

	AICc	ΔAICc	AICc Wt.	Cumulative Wt.
avggvbiomass	63.80	0.00	0.54	0.54
region	65.18	1.39	0.27	0.82
avggvbiomass + region	67.20	3.41	0.10	0.92
avgsnailcount + avggvbiomass + seabirdwaders	67.84	4.04	0.07	0.99
watertemp + salinity + region	71.70	7.91	0.01	1.00

Table S16: AICc table for Gaussian GLM testing response variable "diversity" (Shannon-Wiener Diversity Index) with biogeographic region as random effect. avggvbiomass = average *G. vermiculophylla* biomass (grams); region = biogeographic region (North of Cape Cod, Virginian Province, South of Cape Hatteras); salinity = seawater salinity (ppt); watertemp = seawater temperature (Celsius); avgsnailcount = average count of *Ilyanassa obsoleta*, seabirdwaders = total count of seabirds and wading birds.

	AICc	ΔAICc	AICc Wt.	Cumulative Wt.
region	30.96	0.00	0.54	0.54
avggvbiomass	31.89	0.93	0.34	0.34
avggvbiomass + region	34.86	3.90	0.08	0.08
avgsnailcount + avggvbiomass + seabirdwaders	37.74	6.78	0.02	0.02
watertemp*salinity + region	38.51	7.55	0.01	0.01

Table S17: Model output for top performing model in table S11. Formula = Prevalence ~ avggvbiomass.

	Β Estimate	Standard Error	z-value	p-value
Intercept	-2.234	1.004	-2.225	0.026
avggvbiomas	-0.891	1.496	-0.596	0.552

Table S18: Model output for top performing model in table S12. Formula = Richness ~ avggvbiomass.

	Β Estimate	Standard Error	z-value	p-value
Intercept	1.038	0.153	6.798	<0.001
avggvbiomass	-0.313	0.191	-1.645	0.100

Table S19: Model output for second highest performing model in table S12. Formula = Richness ~ region. SCH = South of Cape Hatteras, VP = Virginian Province

	β Estimate	Standard Error	z-value	p-value
Intercept	1.705	0.302	5.654	<0.001
region (SCH)	-0.932	0.410	-2.274	0.023
region (VP)	-0.649	0.367	-1.770	0.077

Table S20: Model output for highest performing model in table S13. Formula = Diversity ~ region. SCH = South of Cape Hatteras, VP = Virginian Province

	β Estimate	Standard Error	z-value	p-value
Intercept	1.416	0.347	4.079	0.001
region (SCH)	-0.961	0.401	-2.397	0.032
region (VP)	-0.744	0.388	-1.917	0.077

Table S21: Model output for second highest performing model in table S13. Formula = Diversity ~ avggvbiomass.

	Β Estimate	Standard Error	z-value	p-value
Intercept	0.684	0.136	5.011	<0.001
avggbiomass	-0.153	0.141	-1.088	0.295

Table S22: Comparison of parasite species in South of Cape Hatteras and Virginian Province based on Similarity of Percentage (SIMPER) results that represents >95% cumulative. GA = *Gynaecotyla adunca*, HQ = *Himasthla quissetensis*, LS = *Lepocreadium setiforides*, PM = *Pleurogonius malaclemys*, SD = *Stephanostomum dentatum*, ST = *Stephanostomum tenue*, ZL = *Zoogonus lasius*

Average dissimilarity = 72.03						
	SCH	**VP**				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
LS	0.5	1.8	16.59	1.45	23.03	23.03
GA	1.55	0.38	15.62	0.83	21.69	44.72
ZL	0.8	1.28	15.04	1.05	20.88	65.6
HQ	0.17	0.73	7.68	0.91	10.66	76.27
PM	0	0.4	6.82	0.41	9.47	85.74
ST	0.4	0.3	5.02	0.78	6.97	92.71
SD	0.24	0	3.27	0.41	4.54	97.25

Table S23: Comparison of parasite species in South of Cape Hatteras and North of Cape Cod based on Similarity of Percentage (SIMPER) results that represents >95% cumulative. AV = *Austrbilharzia variglandis*, GA = *Gynaecotyla adunca*, HQ = *Himasthla quissetensis*, LS = *Lepocreadium setiforides*, PM = *Pleurogonius malaclemys*, ST = *Stephanostomum tenue*, ZL = *Zoogonus lasius*

Average dissimilarity = 68.89						
	SCH	**NCC**				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
HQ	0.17	1.87	15.77	2.59	22.96	22.96
ZL	0.8	1.93	12.3	1.08	17.91	40.87
GA	1.55	0	10.6	0.74	15.44	56.3
LS	0.5	1.72	9.51	1.43	13.85	70.16
ST	0.4	1.21	8.51	1.57	12.39	82.54
SD	0.24	0.5	4.58	0.94	6.67	89.21
AV	0	0.5	3.71	0.93	5.4	94.6
DN	0	0.5	3.71	0.93	5.4	100

Table S24: Comparison of parasite species in North of Cape Cod and Virginian Province based on Similarity of Percentage (SIMPER) results that represents >95% cumulative. AV = *Austrbilharzia variglandis*, DN = *Diplostomum nassa*, HQ = *Himasthla quissetensis*, LS = *Lepocreadium setiforides*, PM = *Pleurogonius malaclemys*, SD = *Stephanostomum dentatum*, ST = *Stephanostomum tenue*, ZL = *Zoogonus lasius*

Average dissimilarity = 54.20						
	VP	**NCC**				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
HQ	0.73	1.87	10.48	1.26	19.33	19.33
LS	1.8	1.72	10.18	1.54	18.77	38.11
ZL	1.28	1.93	9.46	0.95	17.45	55.55
ST	0.3	1.21	8.32	1.8	15.34	70.9
PM	0.4	0	3.55	0.46	6.55	77.45
DN	0.13	0.5	3.52	0.93	6.5	83.95
AV	0.13	0.5	3.48	0.94	6.41	90.36
SD	0	0.5	3.42	0.93	6.3	96.67

CHAPTER 2: Differential survival of *Ilyanassa obsoleta* to water temperature and association with the non-native red alga *Gracilaria vermiculophylla*

HIGHLIGHTS

- Native eastern mudsnail *Ilyanassa obsoleta* co-occurs with *Gracilaria vermiculophylla* along the North Carolina coast.
- Lab experiments indicate that mortality rates of *I. obsoleta* are influenced by higher water temperature and exposure to *G. vermiculophylla*.
- In non-stressful temperatures, lab experiments demonstrated a greater number of XX laid in *G. vermiculophylla* versus bare substrate.
- *Gracilaria vermiculophylla* may create hypoxic and even anoxic conditions at high seawater temperatures; however, in cooler temperatures, *G. vermiculophylla* may provide substrate for *I. obsoleta* egg capsules.

ABSTRACT

The non-native red alga *Gracilaria vermiculophylla*, widespread along the temperate coastlines of the U.S. east coast, has a three-dimensional complex structure that provides habitat for a diverse array of macroinvertebrates. A native macroinvertebrate that co-exists with *G. vermiculophylla* in much of its Northwest Atlantic introduced range, and in our focal region of North Carolina, is the eastern mudsnail *Ilyanassa obsoleta*. Though *I. obsoleta* has wide temperature tolerances, recent increases in summer water temperatures along North Carolina coasts may enhance mortality; yet the addition of algal cover in these populations could potentially ameliorate enhanced temperature stress. In a lab setting using temperature-controlled incubators, we tested whether the presence/absence of *G. vermiculophylla* influenced snail mortality rates across a range of moderate to extreme summer temperatures over a 3-week period where one low tide was simulated daily. A subset of our snails were infected with trematode parasites, so we also analyzed whether parasitism influenced mortality at each temperature treatment. In addition, at our low temperature treatment, we noted snails laying egg capsules, and thus, we counted the number of egg capsules in *G. vermiculophylla* versus bare substrate. Our results demonstrated that *I. obsoleta* survived the longest in the lowest temperature treatment (27 ˚C), followed by the medium temperature (32 ˚C), and finally the highest temperature (36 ˚C) where all snails died within 2 days. Mortality was also greater and faster for snails in the *G. vermiculophylla* versus bare treatment. In addition, infected *I. obsoleta* died faster at higher temperatures. While we did not observe significant differences between egg deposition preferences on bare surface or on *G. vermiculophylla*, the cumulative egg count over time was higher on *G. vermiculophylla* than on bare surface. Finally, to determine whether dissolved oxygen was depressed at higher temperatures and in *G. vermiculophylla* treatments, we conducted a lab-based dissolved oxygen experiment, finding *G. vermiculophylla* to degrade faster and oxygen to significantly decline at the highest temperature treatment, thereby creating anoxic conditions. Altogether, our results demonstrate that *G. vermiculophylla* may provide beneficial habitat for *I. obsoleta* and substrate for egg laying at non-stressful temperatures, but the non-native alga could enhance anoxic conditions at high summer temperatures, resulting in enhanced faunal mortality. Future work should examine these results in field-based conditions.

KEYWORDS: Temperature, seaweed, algae, snail, survival, oxygen, *Gracilaria vermiculophylla*, *Ilyanassa obsoleta*

INTRODUCTION

Coastal marine environments are vulnerable to numerous stressors as a result of human-induced global change (Harley et al. 2006). Two main stressors include rising sea surface temperatures and the introduction of non-native species. Over the past few decades, coastal environments have been facing rapid seawater warming. Globally, over 70% of the world's coastlines are significantly warming based on a three decadal study; indeed, all coastal basins' sea surface temperatures are expected to rise by 1 ^{o}C by 2050 (Lima and Wethey 2012, Varela et al. 2023). Rising seawater temperature can change the composition of native communities, including reducing native species biodiversity, increasing species homogeneity, and enhancing spread of invasive species (Bianchi et al. 2019). Effects of rising seawater temperature not only affects free-living macro-organisms, but also microorganisms, like bacteria, phytoplankton, and parasites (Kim et al. 2020; Shodipo et al. 2020).

These ecosystems are also susceptible to the introduction of non-indigenous species via marine vectors, such as the discharge of ballast water and biofouling with vessels (Pacheco et al. 2020), aquaculture, the aquarium trade, and the transportation of live bait and seafood (Carlton and Geller 1993, Williams and Grosholz 2008, Fowler et al. 2016). Biological invasions pose major threats to native biodiversity and ecosystem function (Simberloff et al. 2013). Many invasive species have spread throughout the world's coastal habitats: examples include crustaceans such as the invasion of European green crab *Carcinus maenas* to North America which can greatly reduce populations of native infaunal organisms (Gregory and Quijon 2011), the Mediterranean Blue Mussel *Mytilus galloprovincialis* which has displaced native mussels in California (Lockwood and Somero 2011), and macroalgae such as *Caulerpa cylindracea*, which is native to western Australia but has spread throughout much of the Mediterranean Sea (Ravaglioli et al. 2022).

In many marine systems, interactions have been detected between rising seawater temperatures and species invasions, and marine algae are particularly important contributors. For example, the invasive alga *Asparagopsis armata* can release a greater volume of toxic exudates at higher seawater temperatures, and native macroinvertebrates exposed to exudates suffer increased tissue damage, weakened neurophysiology, and ultimately death (Vieira et al. 2021). Climate change is also increasing the frequency of marine heat waves, which are occurrences of unusually high seawater temperatures; studies from the rocky coastlines of UK found that these heat waves enhance the survivability of invasive *Sargassum muticum*, while negatively impacting native *Fucus serratus* and *Chondrus crispus* (Atkinson et al. 2020).

In its invasive populations on both Atlantic coasts, the red alga *Gracilaria vermiculophylla* (native to the northwestern Pacific) has been thought to induce anoxic events at high water temperatures, reducing biomass and percent cover of native algae and increasing mortality of seagrasses and invertebrates (Ramus et al. 2017, Keller et al. 2019). In contrast, *G. vermiculophylla*'s presence has also been suggested to positively influence the abundance and diversity of associated macroinvertebrates, like amphipods, due to enhanced habitat complexity as a result of the alga's three-dimensional structure (Thomsen et al. 2013, Ramus et al. 2017, Keller et al. 2019, Lee et al. in review). Studies have also indicated that *G. vermiculophylla* is associated with greater diversity and abundance of gastropods than native algae and may provide refuge, shelter, and habitat from thermal stresses (Thomsen 2010). In the western Atlantic, a common invertebrate that co-occurs with *G. vermiculophylla* in many of its non-native populations is the eastern mudsnail *Ilyanassa obsoleta* (Guidone et al. 2014). Previous studies have demonstrated that *I. obsoleta* prefers depositing eggs on *G. vermiculophylla* over native substrates (Thomsen et al. 2013, Guidone et al. 2014). To date, however, no research has investigated whether the life history and survivability of Nassariidae gastropods co-occurring with *G. vermiculophylla* may be influenced by high seawater temperatures.

We conducted a survival experiment across three seawater temperatures—representing moderate to extreme summer temperatures in coastal North Carolina—to determine whether *I. obsoleta* survivability was affected by the presence or absence of *G. vermiculophylla*. Because *I. obsoleta* is an important first-intermediate host species of digenean trematodes, we also took data on infection status to determine whether that played a role on snail survival. During our experiment, we also observed female *I. obsoleta* to lay egg capsules at our lowest temperature treatment, and therefore, we examined whether there was a preference for egg deposition on *G. vermiculophylla* versus bare substrate. Finally, to determine whether temperature and the presence of *G. vermiculophylla* affected dissolved oxygen concentrations in our treatments, we conducted a lab-based dissolved oxygen experiment. Our data help discern the influence that the combination of rising seawater temperature, invasive alga, and reduced oxygen can have on native macroinvertebrate populations in the western Atlantic.

METHODS

STUDY SYSTEM

Eastern Mudsnail *Ilyanassa obsoleta*

The eastern mudsnail *Ilyanassa obsoleta* is a highly abundant gastropod species in estuarine habitats throughout eastern North America from the Gulf of Saint Lawrence, Canada to Northern Florida, forming densities as high as 8,000 individuals/m2 (Dimon 1902, Abbott 1974, Curtis and Hurd 1983, Harmon and Allen 2018). These gastropods primarily live in soft-sediment habitats, although they have also been observed on coarser sediments such as sand, shell fragments, and eelgrass (Dimon 1902). Its ecological and evolutionary success in achieving great abundances and densities is attributed its ability of using a wide range of resources for nutrition, including detritus, carrion, and benthic microflora (Curtis and Hurd 1979, Curtis and Hurd 1981, Cranford 1988). In addition to a versatile diet, *I. obsoleta* has wide thermal (0–30 degrees Celsius) and salinity (1 –35 PSU) tolerances, which also contributes to its ecological and evolutionary success (Fofnoff et al. 2018). Their high densities and broad tolerances make *I. obsoleta* an ideal study organism for experiments on survivability and response to environmental changes (Harmon and Allen 2018). *I. obsoleta* is also an important study organism for investigating interacting biotic and abiotic stressors because they are also a first intermediate host for nine different species of digenean trematodes (Blakeslee et al. 2012). The life cycles of these parasites typically require two to three host organisms, and it begins when *I. obsoleta* grazes on feces of definitive hosts that contains eggs of parasites (Combes et al. 1994, Rhode 2005). An infected *I. obsoleta* is castrated and parasitized for life (Blakeslee et al. 2020). Parasitism alters behavior and physiology of *I. obsoleta*, as it negatively impacts their feeding rates and survivability under thermal stresses (Curtis and Hurd 1983).

Red Alga *Gracilaria vermiculophylla*

The red alga *Gracilaria vermiculophylla* (= *Agarophyton vermiculophyllum*) is native to the waters of Northwestern Pacific, from the northern Sea of Japan to the East China Sea and south towards the central Kuroshio Current (Kim et al. 2010, Krueger-Hadfield et al. 2017). *Gracilaria vermiculophylla* can tolerate wide range of environmental changes, including but not limited to extreme temperatures, low salinities, and low light exposure (Rueness 2005, Phooprong et al. 2008, Nejrup and Pedersen 2012, Sotka et al. 2018). This alga has invaded nearly every temperate coastlines in the northern hemisphere. In the eastern North America, *G. vermiculophylla* was first discovered in 1998 in Chesapeake Bay, though it is likely that the algae was present since early 20th century with the introduction of *Crassotrea gigas* (Thomsen et al. 2006, Krueger-Hadfield et al. 2017). From Virginia to Georgia, *G. vermiculophylla* have invaded primarily soft-sediment estuarine habitats (Thomsen et al. 2009, Byers et al. 2012, Ramus et al. 2017). This is differed from their native habitat, which is primarily dominated by hard sediments (Yokoya et al. 1999, Phooprong et al. 2008). In its native range, this alga is a haplodiplontic dioicious organism, which results in free-living haploid and diploid individuals. When *G. vermiculophylla* invaded these soft-sediment habitats, its haplodiplontic life cycle can became interrupted; this means that most of *G. vermiculophylla* found in introduced soft-sediment habitats are primarily diploid, free-floating, and rely on fragmentation (Krueger-Hadfield et al. 2016). Unlike other native alga in the eastern U.S., *G. vermiculophylla* grows rapidly and reach large biomasses, and tolerant to extreme environmental stresses such as high salinity and temperature (Thomsen et al. 2009). The spread of *G. vermiculophylla* in these soft-sediment coastal habitats can increase primary production, provide novel structural complexity, and increase secondary production as it provides shelter, refuge, and possibly novel substrate for macroinvertebrates (Byers et al. 2012, Guidone et al. 2014).

EXPERIMENT

We used temperature logger data we collected from Beaufort, NC, USA (Asch, pers. obs.; Blakeslee, pers. obs.) during the summers of 2019 and 2020 (May – September) to determine three temperature treatments for this study. The low temperature (21 ^{o}C) was determined based on the lowest temperature recorded continuously from May 1 – September 3, 2019 (Asch 2019: unpub. data); the medium temperature (27 ^{o}C) was determined based on the overall average temperature during the same time period (R. Asch, unpub. data); and the high temperature (32 ^{o}C) was determined based on the average peak temperature recorded from August 5-14, 2020 (A. Blakeslee, unpub. data). With these temperatures, we ran a pilot experiment in fall 2020 for two weeks, and during this experiment, we observed none of the *I. obsoleta* experienced mortality at the low temperature treatment (21 ^{o}C). While this fall 2020 pilot experiment was interrupted by the COVID-19 pandemic, we resumed the experiment in 2021. Based on our pilot data, we omitted the 21 ^{o}C treatment and reassigned three new temperature treatments: 27 ^{o}C (henceforth, “low”), 32 ^{o}C (henceforth, “medium”), and 36 ^{o}C (henceforth, “high”). This high temperature treatment was recorded as the peak temperature from the logger data deployed during our August 5-14, 2020 temperature recordings. We performed two experimental trials, as described below.

We ran our first trial from February 2-22, 2021. We used three ThermoFisher Scientific (Waltham, MA) Heratherm™ General Protocol Microbiological Incubators, one for each temperature treatment. We used lights on a 12:12 hour light cycle in each incubator. Prior to starting the experiment, on the morning of February 1st, we turned on the incubators at their appropriate temperatures (27, 32, and 36 ^{o}C) to allow them to reach their respective temperature treatments. Two days prior to running the experiment, we collected >300 *I. obsoleta* from Curtis Perry Park in Beaufort, NC, USA (Lat: 34.709731, Long: -76.632494), because prior research (Moore et al., in review) found a high abundance of snails at this site and a moderate infection prevalence. All *I. obsoleta* were immediately brought to the lab and housed in a 36.83 x 22.22 x 24.46 cm Kritter Keeper® Aquaria in 30 ppt salinity water, made from Instant Ocean® Sea Salt.

One day prior to running the experiment, we also collected *Gracilaria vermiculophylla* from the University of North Carolina Wilmington Center for Marine Science in Wilmington, NC (Lat: 34.140212, Long: -77.863701). This location was selected due to the widespread distribution of the alga in this region year-round (Freshwater et al. 2006, Lee pers. obs). We also housed *G. vermiculophylla* in a 36.83 x 22.22 x 24.46 cm Kritter Keeper® Aquaria, also with 30 ppt seawater made from Instant Ocean® Sea Salt. We selected this salinity level because it is a non-stressful salinity for both the snail and the alga.

On February 1st, 2021, we assigned *I. obsoleta* to two types of habitats for each treatment: with *G. vermiculophylla* (henceforth "algae"), and no *G. vermiculophylla* (henceforth "bare"). We assigned n = 100 *I. obsoleta* for each temperature treatment, and for each treatment, we assignment n = 50 for each habitat type (thus, N = 300 *I. obsoleta* across all treatments and habitats for this trial). We used an MroMax® 18-Grid Electronic Component Storage Box (fixed-grid) to house snails during the experiment. These storage boxes have been shown to be effective tools in invertebrate survival experiments (Tepolt et al. 2020, Blakeslee et al. 2021). Each grid housed one *I. obsoleta*, and we alternated habitat assignments such that every other grid had *G. vermiculophylla*. For grids assigned as "algae", we placed 2.5 g of *G. vermiculophylla*, and the density and structural complexity of each algal frond at this biomass was also standardized as best as possible. We used knowledge gained from our pilot study to establish this biomass and visual structural complexity in our algal treatments. We also rinsed all algal fronts in fresh water to induce osmotic shock to remove any associated organisms and macro-epiphytes prior to inclusion in the experiment (Fowler et al. 2016, Lee et al., in review). We filled each grid with 30 ppt seawater and added a single pellet of Aqueon® Cichlid Pellet in each grid as food for the snails. We then loosely covered each box with their respective lid to ensure boxes were not airtight. On the evening of February 1, 2021, we randomly assigned the 18-grid boxes with *I. obsoleta* to the three temperature treatments, such that there were 100 snails per incubator. We also placed reserves of 30 ppt seawater in each incubator to ensure that artificial seawater for water changes (described below) was always at the assigned temperature treatment.

Starting on February 2, 2021, we checked for survivability of *I. obsoleta* twice every day through February 22, 2021 (for 21 days), at 9:00 and 15:00, respectively. At each check, we recorded survivability (was *I. obsoleta* alive or not), and if a snail had died, we measured their shell length (from operculum to the spiral) and dissected their gonad tissues for trematode infection status; if parasitized, we identified to species level, using approaches by Blakeslee et al. (2012).

In order to assess the potential influence of dessication on snail mortality with and without algae, we replicated one low tide each day. To do so, we moved snails and algae to a new, dry storage compartment. At 15:00, we re-filled all the grids with 30 ppt seawater using the reserves of artificial seawater stored in each respective incubator, and fed each snail one Aqueon® Cichlid Pellet. Throughout the duration of the experiment, we checked for the structural integrity of *G. vermiculophylla* in the "algae" habitat, and if *G. vermiculophylla* was fragmenting or starting to decompose, we replaced it immediately, with the same standard biomass (2.5 g). At each mortality check, we rotated the 18-grid boxes to different shelves of the incubator. Trial 1 ended on Day 21 (February 22, 2021).

Our second 21-day trial ran from February 23 – March 15, 2021. We collected >300 new *I. obsoleta* from the same location (Curtis Perry Park, Beaufort, NC, USA) two days prior to running the second trial, and housed them using the same methodology for Trial 1. We also collected *G. vermiculophylla* a day prior to running Trial 2 from the same location housed them using the same methodology in Trial 1. For Trial 2, we re-assigned each incubator to a different temperature treatment. Otherwise, our approach in collecting data during Trial 2 was identical to Trial 1.

During Trial 1, we had observed *I. obsoleta* were depositing eggs in the low temperature treatment. As a result, during Trial 2, we took data on egg counts at every data collection period (at 9:00 and 15:00 EDT). To ensure we could obtain accurate counts without double-counting, we removed eggs off the walls of the grid boxes and/or replaced *G. vermiculophylla* if they were covered with eggs.

DISSOLVED OXYGEN DATA

At the end of our two trials, we observed low survival in high temperature treatments with algae (see *Results*). We hypothesized this may be the result of anoxic conditions induced by both the high temperature and algal degradation in this stressful environment (Bermejo et al. 2020). Though we attempted to control for algal degradation as much as possible by replacing algae during our experiment, it was clear that algae in the high temperature treatment were degrading quickly, often showing signs of bleaching. As a result, we ran two trials of a dissolved oxygen experiment using the same experimental set-up as described above (i.e., “algae” and “bare” habitats and the three temperature treatments), except in these trials we did not include snails. Each trial lasted five days (October 17-21, 2022; October 24-28, 2022). We chose a 5 day experimental period because, per our *Results,* the high temperature treatment resulted in high mortality within just a few days. We collected data twice per day, at 8:00 and 16:00. We measured dissolved oxygen using a YSI® (Yellow Springs, OH, USA) Pro20 Dissolved Oxygen Meter.

STATISTICAL ANALYSES

To analyze or survival data, we used the Cox proportional hazards model (CPHM), a model that uses number of days survived as the response variable (Cox 1972, Therneau and Grambsch 2000) to determine which sets of variables best explain survival. For our experiment, these factors included habitat type: “algae” or “bare”, temperature: “low”, “medium”, and “high”, infection status: yes or no, snail size, trial: one or two, and the following interactions: habitat*temperature, habitat*infection status, temperature*infection status. The implementation of CPHM has been used in numerous ecological studies, such as the survival of bycatch in fisheries (Depestele et al. 2014) or the effects of pollutants and climate change on freshwater invertebrates (Firmino et al. 2023). Pilot runs showed that *I. obsoleta* survival significantly differed between Trials 1 and 2. Henceforth, we ran CPHM separately for each trial. We used R 4.2.2 for all our data analyses (R Core Team 2023). For each trial, we created different sets of CPHM models with different combinations of predictors using the packages *survival* and *survminer* and ran AICc with the *aictab* function in the package *AICcmodavg* (Mazerolle 2017, R Core Team 2023) to determine which set of predictors best explained *I. obsoleta* survival. In pilot runs, we eliminated snail size as a predictor from our CPHM models, as it was not found to influence survival; this is likely because we attempted to standardize snail size as much as possible prior to running our experiment.

Based on the predictors that best explained *I. obsoleta* survival from the AICc results for each trial, we created Kaplan-Meier Curves to visualize *I. obsoleta* survival through time with temperature and habitat type as factors affecting survival in Trial 1 and temperature for Trial 2 (see *Results*). Kaplan-Meier Curves are used to visually interpret the proportion of individuals surviving over a specified time period under experimental conditions (Ranstam and Cook 2017).

We also created temperature-response curves and calculated an LT_{50} for each trial, or the temperature with 50% *I. obsoleta* mortality; this method of determining the temperature that induces 50% mortality has been implemented in numerous survival studies (e.g., Cox and Rutherford 2000).

Based on our AICc results from each trial, we then performed several univariate analyses. In Trial 1, we used a Kruskal-Wallis test (Kruskal and Wallis 1952) for overall significance, and a Bonferroni correction method (Sedgwick 2012) for pairwise comparisons to determine the effect on *I. obsoleta* survival of six categories (low-algae; low-bare; medium-algae; medium-bare; high-algae; high-bare). We also used a 2-tailed t-test to determine survivability differences between infected and uninfected *I. obsoleta*. For Trial 2, we used Kruskal-Wallis and Bonferroni correction to assess differences in *I. obsoleta* survivability among the three temperature treatments. We also analyzed egg counts in the low temperature treatment for all eggs within the "algae" versus "bare" treatment, and then within the "algae" habitat alone, we analyzed the number of eggs that were laid on the algae itself or on the wall of the container.

For dissolved oxygen, we ran series of Generalized Linear Mixed Model (GLMM) using the package *lme4* with dissolved oxygen as the response variable, and the following predictors: temperature (low, medium, and high), habitat (algae or bare), and trial (one or two), and time (AM or PM: AM = 8:00, PM = 16:00) as fixed effects, and "day" as a random effect (day 1, day 2, day 3, day 4, and day 5). We used family = Gaussian. We also ran AICc with the *aictab* function in the package *AICcmodavg* to determine which sets of predictors best explained the differences we observed in dissolved oxygen values. Based on our AICc results, we plotted the differences of dissolved oxygen between the following habitat*temperature interaction categories: low and algae, low and bare, med and algae, med and bare, high and algae, high and bare. We also used a Kruskal-Wallis test to determine the overall significance of the differences of dissolved oxygen levels across all six categories, and used a Bonferroni correction method for pairwise comparisons.

RESULTS

For Trial 1, we found that habitat*temperature interaction was the sole significant predictor (Table 1-3). Survivability of *I. obsoleta* was greater in the "low" (27 ^{o}C) temperature treatment for both habitats, with 96% and 92% surviving in "algae" and "bare" habitat, respectively, by Day 21 (Figure 1A). For the "medium" (32 ^{o}C) temperature treatment, *I. obsoleta* survival was much lower, with no snails surviving the length of the experiment in the "algae" habitat (all snails had perished by Day 7 at 15:00), and only 10% surviving in the "bare" habitat on Day 21 (Figure 1B). For the high (36 ^{o}C) temperature treatment, all *I. obsoleta* in the "algae" habitat had perished by 9:00 on Day 2, while in the "bare" habitat, all *I. obsoleta* had perished by 15:00 on Day 2 (Figure 1C). For Trial 2, we found that temperature alone was the sole significant predictor of survival (Tables 4-7), and hence we plotted Kaplan-Meier Curve for the three temperature treatments only. For the low temperature treatment, 60% of *I. obsoleta* survived by 15:00 on Day 21, while for the medium temperature, only 1% survived by 15:00 on Day 21. For the high temperature, all *I. obsoleta* perished by 9:00 on Day 2 (Figure 2).

We also calculated the LT_{50} for Trial 1 by habitat type and fitting a logistic curve, since the habitat*temperature interaction was the sole significant predictor. We found that LT_{50} for the "algae" habitat was lower (27.53 ^{o}C) than the LT_{50} for the "bare" habitat (29.62 ^{o}C)In addition, infection status was a marginally significant additive predictor in the top model, so we also calculated LT_{50} for infected (n = 20) vs. uninfected *I. obsoleta* (n = 280) and fitted logistic curve. We found that LT_{50} for infected *I. obsoleta* was lower (27.38 ^{o}C) than LT_{50} for uninfected *I.*

obsoleta (27.4 ^{o}C).In Trial 2, we calculated LT_{50} for overall *I. obsoleta* across all temperature treatments since temperature alone was the sole significant predictor in the top performing model. For Trial 2, the LT_{50} was 27.4 ^{o}C for *I. obsoleta* across all temperature treatments (Figure 3).

For univariate analyses of Trial 1's habitat*temperature interaction, we found that overall, based on the Kruskal-Wallis test, survival of *I. obsoleta* among groups were significantly different (df = 5, $p < 0.001$). Our pairwise comparisons using Bonferroni correction method showed that except for the following four pairs ($p > 0.05$), all other pairs differed in terms of *I. obsoleta* survival ($p < 0.001$): low temperature algae and low temperature bare, medium temperature algae and high temperature algae, medium temperature algae and high temperature bare, high temperature algae and high temperature bare (Figure 4). Survivability between infected vs. uninfected in trial 1 was not significant (Figure 5). For trial 2, survivability was significantly different ($p < 0.001$) between the following pairs of temperature treatments: low and medium with higher survivability in low, and low and high with also higher survivability in low (Figure 6). For Trial 2's egg counts in the "low" temperature treatment, we did not detect any significant differences ($p > 0.05$) in *I. obsoleta* egg counts for the comparisons of following two pairs: total number of eggs between "low" temperature "algae" and "low" temperature bare, and number of eggs only on algae versus eggs only on bare in "low" temperature "algae" (Figure 7); however, in the low temperature algae habitat, cumulative egg counts throughout the duration of the experiment was greater on *G. vermiculophylla* than on the grid wall (Figure 8).

For dissolved oxygen, we found that the top performing model (Tables 8-9) had temperature*habitat interaction as the sole significant predictor. Overall, based on the Kruskal-Wallis test, dissolved oxygen levels were significantly different (df = 5, $p < 0.001$). Using the

Bonferroni correction method, we found that dissolved oxygen was significantly different ($p < 0.05$) between all pairs of temperature*habitat interaction categories (with higher dissolved oxygen levels in bare habitats), except for medium*algae and high*algae (Figure 9).

DISCUSSION

Results of this study indicate that survivability of *I. obsoleta* is primarily driven by seawater temperature, though interaction with the presence of *G. vermiculophylla* can also negatively affect their survivability. Here, we discuss the differences of *I. obsoleta* survival by the following predictors: habitat type and temperature interaction, temperature ranges, infection status; we also discuss egg counts, and variations of dissolved oxygen across different habitat and temperature interactions.

Overall, survival decreased most rapidly with rising temperature (Figures 2, 3C, 6; Tables 4-7). Such observations of decreased survivability with increasing seawater temperature have been observed in experiments with other snail species, such as *Indoplanorbis exustus*, where the greatest survival was recorded at 20 degrees Celsius (Raut et al. 1992). Freshwater snails such as *Pomacea canaliculate* experienced faster growth at higher temperatures, but decrease in survival with increasing temperature; at 15 and 20 degrees Celsius, they experienced no mortality, but at 25, 30, and 35 ^{o}C they grew 2-3 mm longer than the snails at 15-20 ^{o}C, though mortality was recorded (Seuffert and Martin 2013). Parashar and Rao (1988) also observed that the freshwater planorbid snail *Gyraulus convexiusculus* also experienced the highest growth rate at the highest temperature treatment (35 ^{o}C), but experienced highest survivability in lower temperatures; according to Parashar and Rao, peak survival times differed based on life stage. Juveniles reached maximum survival rate at 30 ^{o}C, while in contrast adults reached maximum survival at

20 ^{o}C, indicating that rising seawater temperature may impact survival differently for *I. obsoleta* based on life stages.

We also found that *I. obsoleta* survival was lower and perished faster in treatments with *G. vermiculophylla*, and they perished faster in higher temperature (Figures 1, 3A, 4; Tables 1-3). Higher seawater temperature can induce algae degradation, and subsequent degradation can also make seawater anoxic; Sassi et al. (1988) found in their experiment that algal decomposition from northeast Brazilian coastal reefs can turn seawater completely devoid of oxygen in 10 hours. Bermejo et al. (2020) found in their studies of *G. vermiculophylla* blooms in their non-native habitat (estuaries of Ireland) that higher temperatures can increase stress on the seaweed, and thus increase degradation. Decomposition of *G. vermiculophylla* can increase bacterial growth, thus creating anoxic conditions (Bermejo et al. 2020). We also observed milky liquid on the water surface in medium and high temperature treatments, which is described as the bacterial growth by Bermejo et al. (2020). We found in our dissolved oxygen data collection that while the oxygen levels did not significantly differ between medium and high temperature treatments with *G. vermiculophylla*, in all temperature treatments, the oxygen levels were significantly lower in habitats with *G. vermiculophylla* than without (Figure 9, Tables 8-9). Thus, it is likely that in medium and higher temperature treatments with *G. vermiculophylla*, many *I. obsoleta* suffocated from anoxic conditions.

We also assessed survivability between infected and uninfected *I. obsoleta* in trial 1, and found that overall, the survivability does not significantly differ between two groups of infection status (Figure 5). However, through logistic regression, we observed that as temperature increases, infected *I. obsoleta* may perish faster than those that are uninfected (Figure 3B). Higher temperatures can increase parasitic egg production: for instance, trematode parasite

Ribeiroea ondatrae had four times higher fecundity in their snail host *Planorbella trivolvis* at 26 ^{o}C compared to 17 ^{o}C (Paull and Johnson 2011). Studer et al. (2010) found that in their study of trematode parasite *Maritrema novaezealandensis* and their first intermediate snail host *Zeacumantus subcarinatus* and their second intermediate host amphipod *Paracalliope novizealandiae*, the transmission of cercarial stage from first to second intermediate host was greatest at temperatures between 20-25 ^{o}C, while the transmission decreased at temperatures >30 ^{o}C, and <20 ^{o}C. Studer et al. (2010) also noted the increasing mortality of secondary intermediate host at >30 ^{o}C. This indicates that while higher temperatures may induce greater parasite fecundity, the successful transmission of trematodes with multi-host life cycle may be impacted at temperatures >30 ^{o}C due to increasing mortality of their hosts. However, we observed very few infected *I. obsoleta* in trial 1 (n=20) compared to uninfected (n = 280), and because of the low counts of infected and statistically non-significant survival difference between infected and uninfected group (Figure 5), comparing survivability of parasitized and non-parasitized *I. obsoleta* need further examination.

We observed egg counts of *I. obsoleta* in trial 2, and we did not find significant differences of egg counts between eggs only on *G. vermiculophylla*, vs. on the bare surface (Figure 7). However, we noticed that the cumulative trend of egg counts through time increased faster on *G. vermiculophylla* vs. bare surfaces (Figure 8). Guidone et al. (2014) also found that *I. obsoleta* does prefer depositing eggs on *G. vermiculophylla* vs. other substrate types, and even prefer depositing eggs on the non-native alga vs. native alga. Guidone et al. (2014) also noted that thick layers of egg masses on *G. vermiculophylla* did not hamper growth of the non-native alga thalli, while it slowed the growth of the native alga *Ceramium virgatum*. However, because of the statistically non-significant differences we found between eggs on *G. vermiculophylla* only vs. eggs on bare surfaces, we believe egg counts need further investigation. We did notice that no eggs were found in any of the medium or high temperatures. *I. obsoleta* in high temperature perished rapidly, while in medium temperature, without presence of *G. vermiculophylla*, a few individuals survived the entire duration of the experiment, though no egg were observed. Sastry (1971) found that *I. obsoleta* fecundity is caused by rising seawater temperature, but according to our experiment, an optimal temperature is required for increasing survivability and fecundity.

The presence of *G. vermiculophylla* in its non-native habitats has been known to increase abundance of native free-living macroinvertebrates compared to native foundational species, as it provides lots of space through its complex three-dimensional structure (Thomsen 2010, Thomsen et al. 2013). However, its ability of benefiting native macroinvertebrates may be hampered if their non-native habitats continue to experience seawater temperature rise. Large blooms of *G. vermiculophylla* during high seawater temperature events may induce anoxia, and thus causing major mortalities of native macroinvertebrates, including *I. obsoleta*. While we attempted our best to replicate the tides entering and leaving the system, we understand that the 18-grid boxes do not have continuous seawater flow as would a natural coastline, and will constrain and influence temperatures. However, it is possible that native macroinvertebrates associated with *G. vermiculophylla*, even during low oxygen conditions at high temperature events, may develop new behavioral responses to adapt to the changing conditions. The bivalve *Anadara trapezia*, native to Australia, has now evolved to emerge from the sediment underneath the canopies of invasive *Caulerpa taxifolia*, which can induce hypoxic conditions (Wright et al. 2010). Completely emerged *A. trapezia* survived significantly longer than those that were prevented from emerging from the sediment. The long-lasting effects of high seawater temperature and *G.*

vermiculophylla presence on native macroinvertebrate communities in the U.S. east coast need further monitoring.

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CHAPTER 2 FIGURES

FIGURES

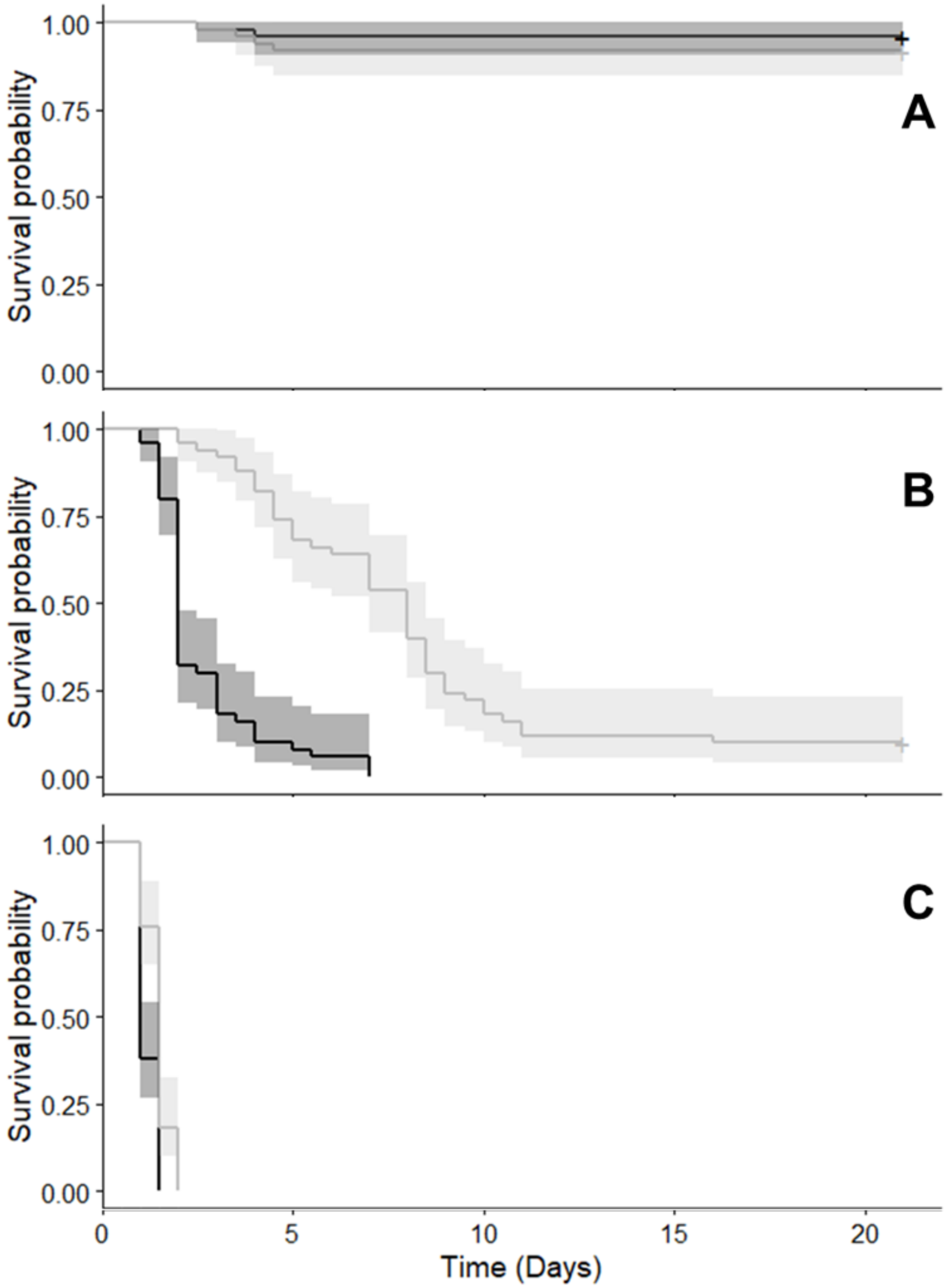

Figure 1 Survival probability of *I. obsoleta* in Trial 1 (21 days): A = “low” (27 ºC) temperature, B = “medium” temperature (32 ºC), and C = “high” temperature (36 ºC). Black = “algae”, gray = “bare”. Lines have been fitted from the Cox proportional hazard models, and shaded areas are 95% confidence intervals from the Kaplan-Meier survival analysis.

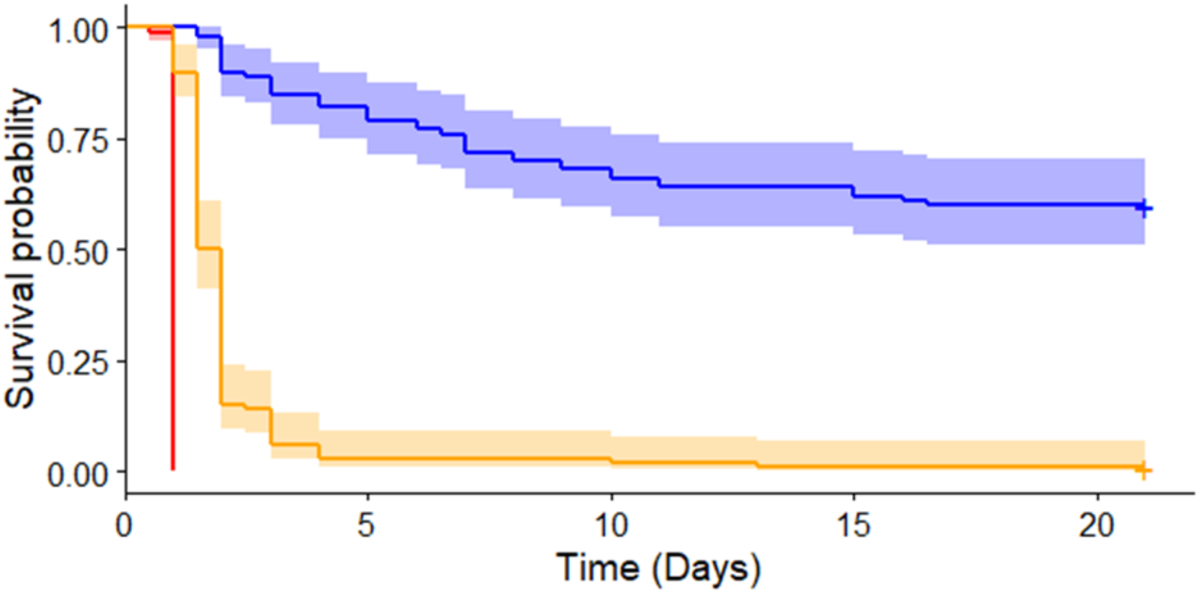

Figure 2 Survival probability of *I. obsoleta* in Trial 2 (21 days): Blue = “low” (27^{o}C) temperature treatment (32 ^{o}C), orange = “medium” temperature, and red = “high” temperature (35 ^{o}C). Lines have been fitted from the Cox proportional hazard models, and shaded areas are 95% confidence intervals from the Kaplan-Meier survival analysis.

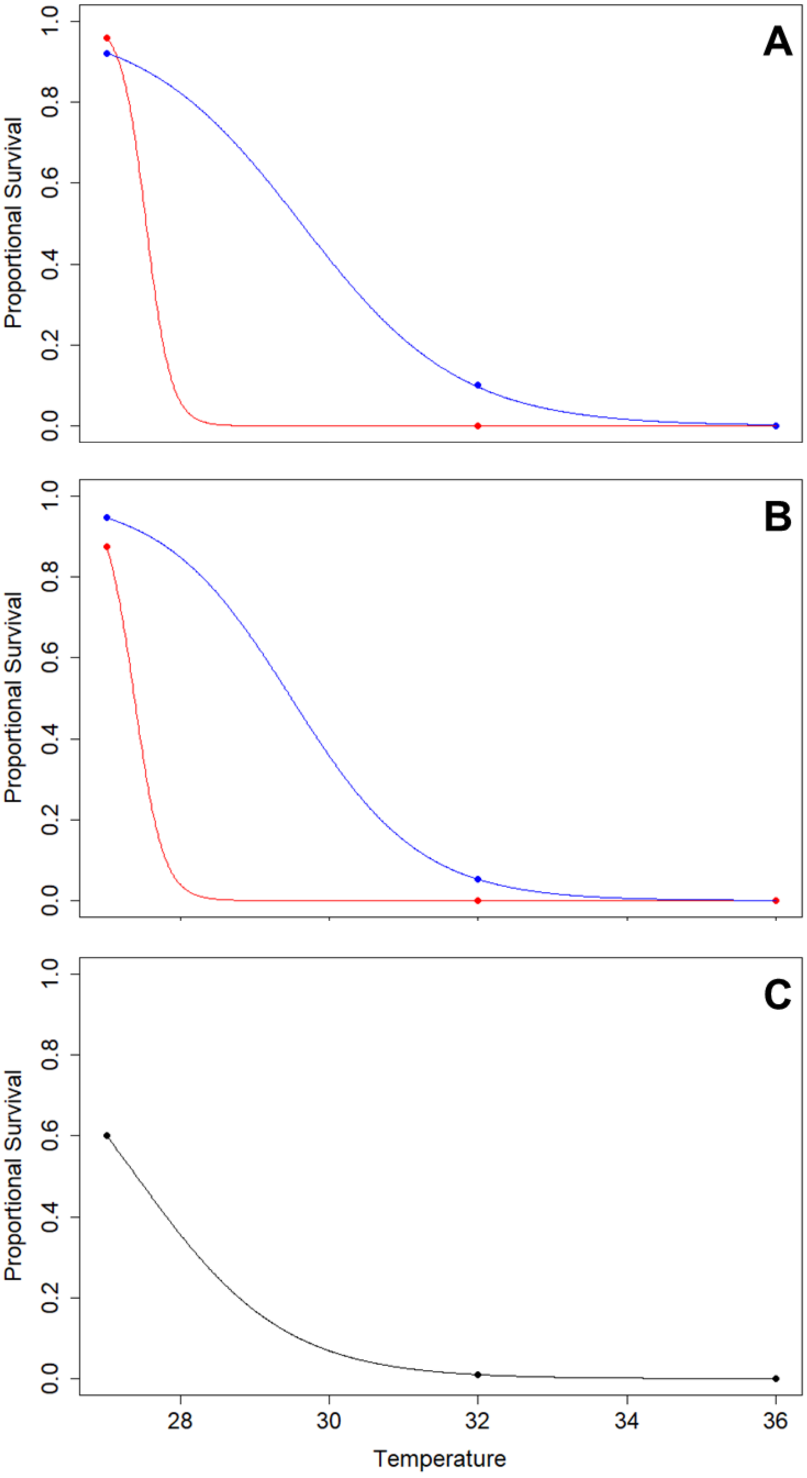

Figure 3 A) Proportional survival of *I. obsoleta* in Trial 1 (red = “algae”, blue = “bare”): LT_{50} for “algae” = 27.53 ^{o}C, LT_{50} for “bare” = 29.62 ^{o}C; B) proportional survival of *I. obsoleta* between infected (n = 20) and uninfected (n = 280) in Trial 1 (red = infected, blue = uninfected): LT_{50} for infected = 27.38 ^{o}C, LT_{50} for uninfected = 29.49 ^{o}C; C) proportional survival of overall *I. obsoleta* in Trial 2 across temperature: LT_{50} = 27.4 ^{o}C.

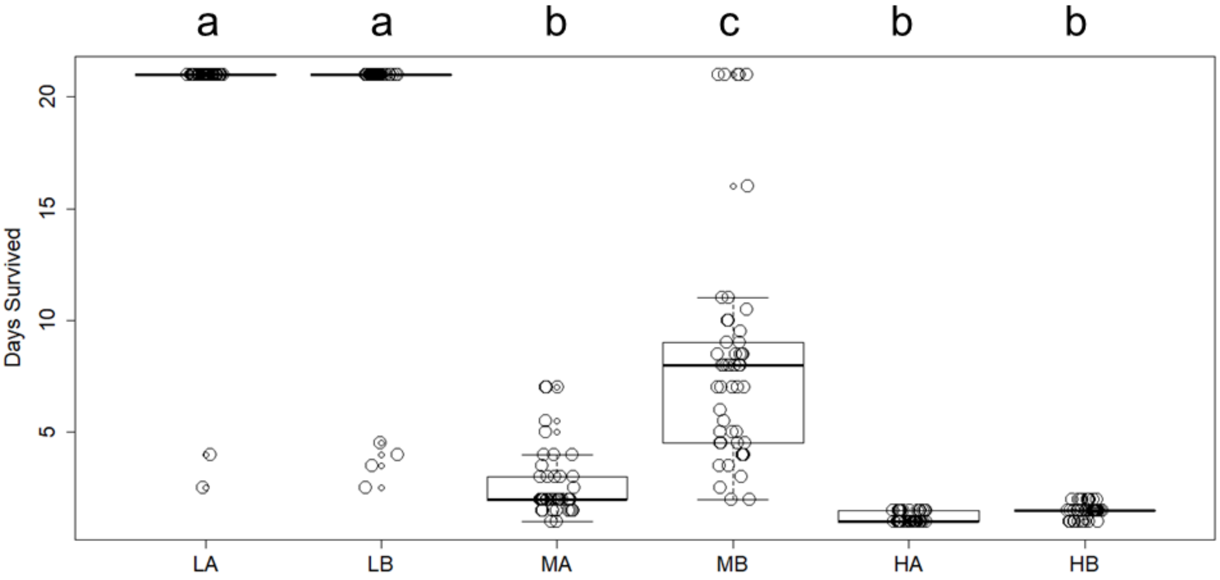

Figure 4 Trial 1's comparison of *I. obsoleta* survival by habitat and temperature interaction. LA = "low" temperature (27 ^{o}C), "algae"; LB = "low" temperature, "bare"; MA = medium temperature (36 ^{o}C), "algae"; MB = "medium" temperature, "bare"; HA = "high" temperature (36 ^{o}C), "algae"; HB = "high" temperature, "bare" (df = 5, $p < 0.001$). Same letters indicate that those categories are not statistically significant in pairwise comparisons.

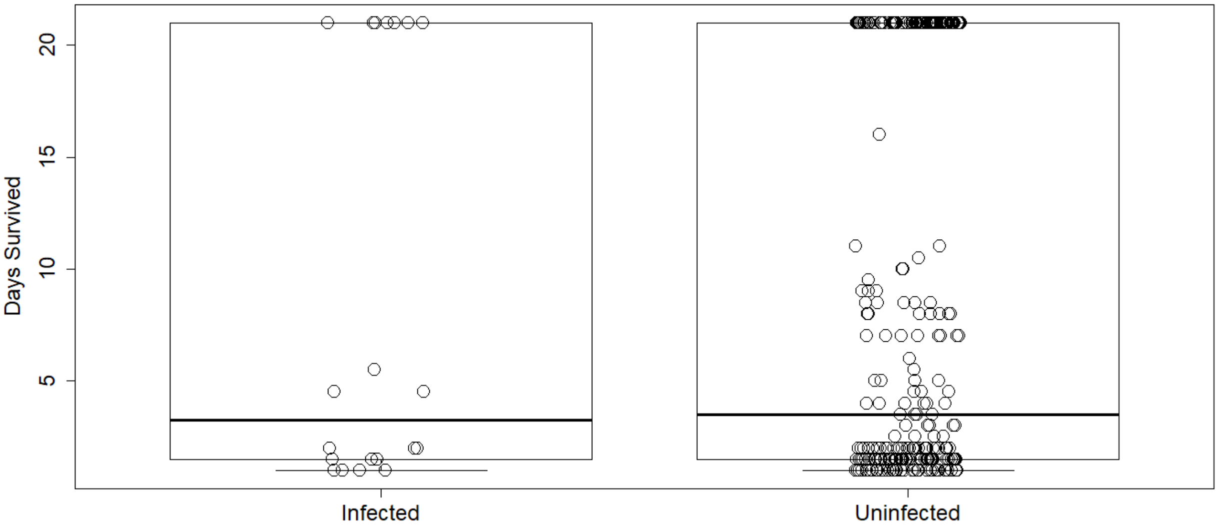

Figure 5 Trial 1's comparison of *I. obsoleta* survival (df = 21.5, $p = 0.9552$) between infected (n = 20) and uninfected (n = 280) snails.

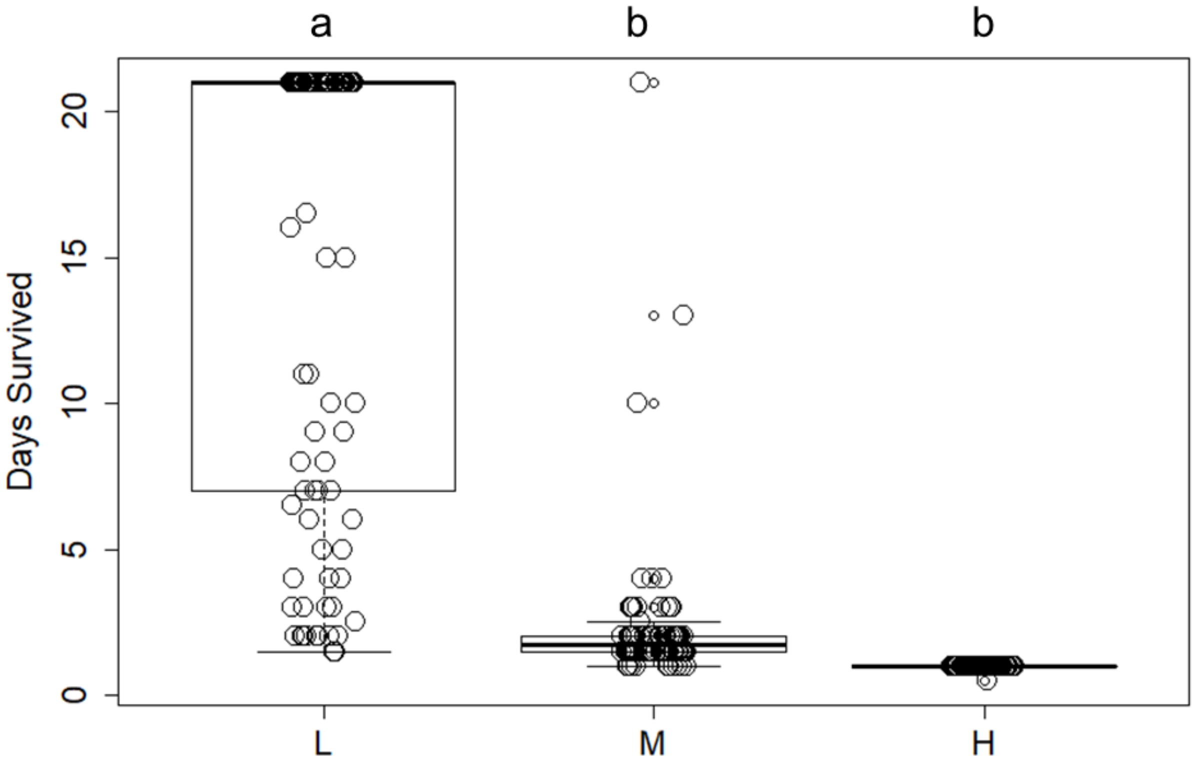

Figure 6 Comparison of *I. obsoleta* survival (df = 2, $p < 0.001$) between three temperature treatments in trial 2. L = “low” temperature (27 ºC), M = “medium” temperature (32 ºC), H = “high” temperature (36 ºC). Same letters indicate that those categories are not statistically significant in pairwise comparisons.

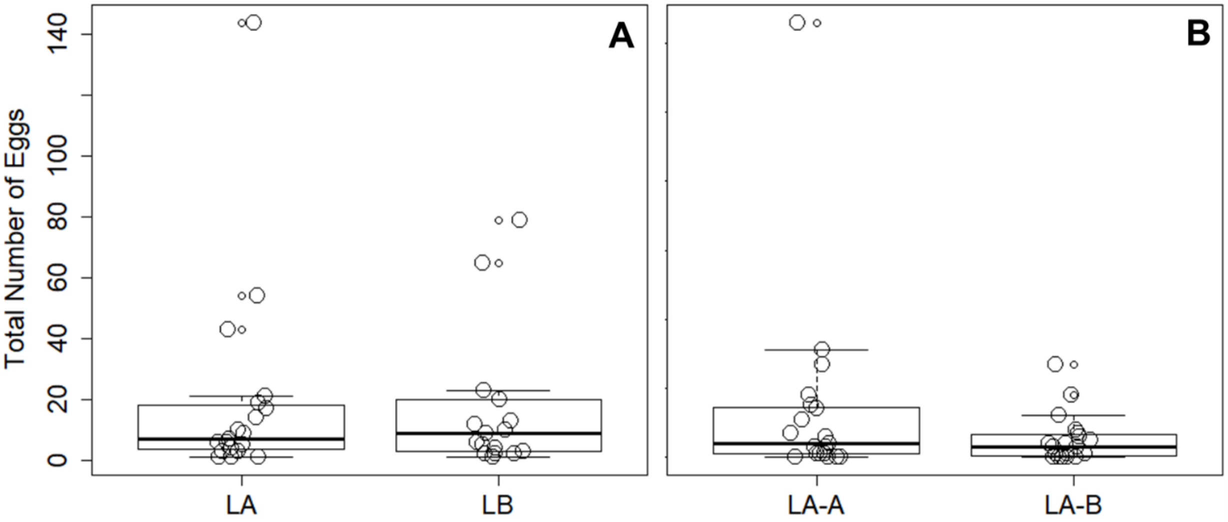

Figure 7 Trial 2 comparison of egg counts: A) LA, or the total number of eggs in "low" temperature "algae", including those on the algae and the wall, and LB, or the total number of eggs in "low" temperature "bare" habitat (t = 0.33, df = 31.559, *p* = 0.74). B) LA-A, or the total number of eggs on *G. vermiculophylla* only in "low" temperature "algae" habitat and LA-B, or the total number of eggs on the wall only in "low" temperature "algae" habitat (t = 1.3, df = 20.2, *p* = 0.20).

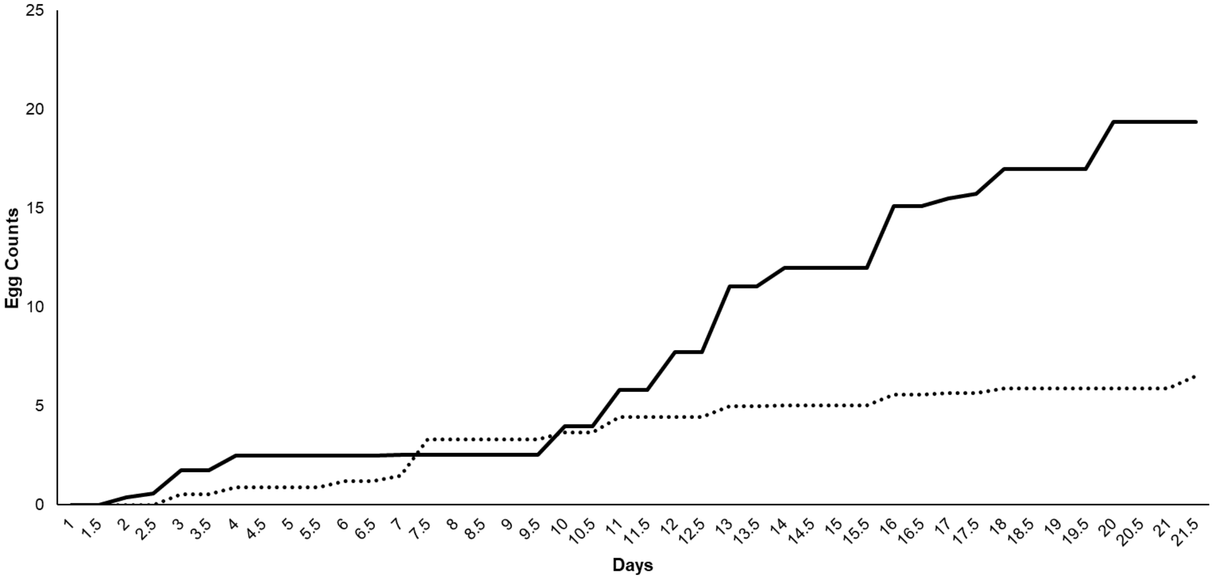

Figure 8 Cumulative egg count for Trial 2, low temperature algae habitat (solid line = eggs on *G. vermiculophylla*, dashed line = eggs on the grid wall).

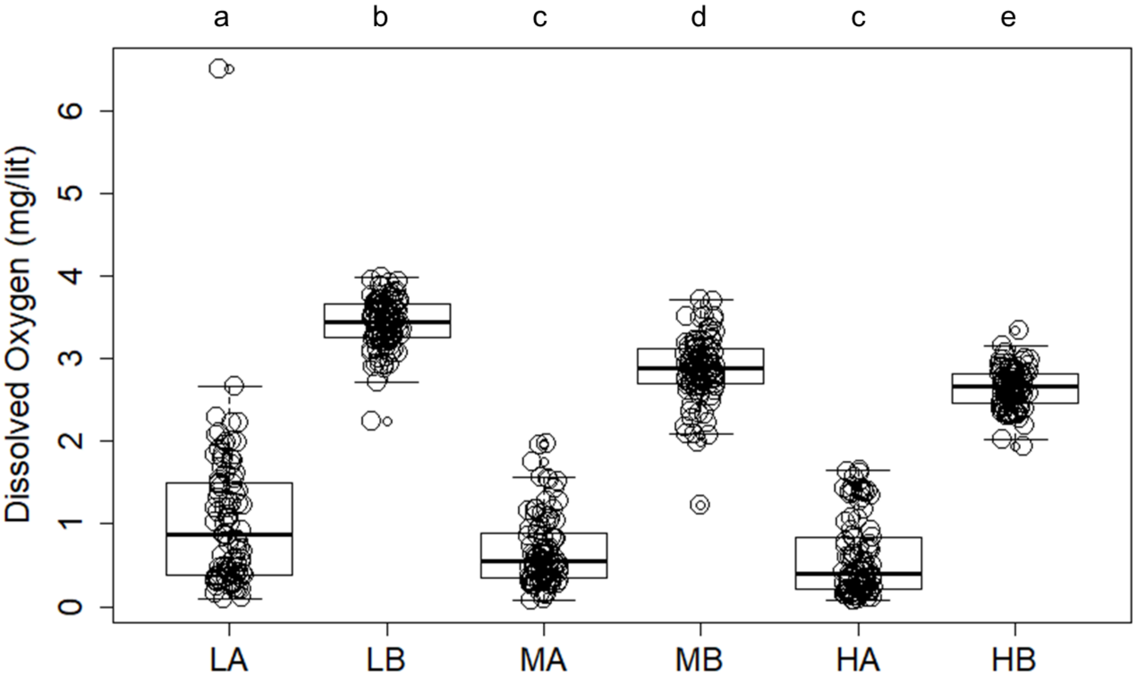

Figure 9 Comparison of dissolved oxygen by habitat and temperature interaction. LA = “low” temperature (27 ºC), “algae”; LB = "low” temperature, “bare”; MA = “medium” temperature (32 ºC), “algae”; MB = “medium” temperature, “bare”; HA = “high” temperature (36 ºC), “algae”; HB = “high” temperature, “bare” (df = 5, $p < 0.001$).Same letters indicate that those categories are not statistically significant upon pairwise comparisons.

CHAPTER 2 TABLES

Table 1 Trial 1's comparisons of *I. obsoleta* survival based on Cox proportional hazards model (CPHM) selection using AICc: temp = temperature (low, medium, high), habitat = habitat type (algae or bare), infected = infection status (yes or no).

Model	K	AICc	∆AICc	AICc Wt.	Cumulative Wt.	LL
temp*habitat + infected	6	1588.28	0	0.58	0.58	-788
temp*habitat	5	1588.95	0.67	0.42	1	-789.37
temp + habitat	3	1599.88	11.6	0	1	-796.9
temp + habitat + infected	4	1600.16	11.88	0	1	-796.01
infected*habitat + temp	6	1602.42	14.14	0	1	-795.07
temp*infected + habitat	6	1602.42	14.14	0	1	-795.07
temp	2	1664.6	76.32	0	1	-830.28
habitat	1	2109.9	521.62	0	1	-1053.94

Table 2 Summary of Table 1's CPHM model with greatest AICc weight (temp*habitat + infected): parameter estimates with standard error (S.E.) and *p*-values.

Predictor	Exp (estimate)	S.E.	*p*-value
temp (low)	0.0001972	0.7928012	< 2e-16
temp (med)	0.0704345	0.277831	< 2e-16
habitat (bare)	0.39797	0.2196899	2.74E-05
infected (yes)	1.6896345	0.2962038	0.0766
temp (low)*habitat (bare)	5.2591093	0.8931816	0.0631
temp (medium)*habitat (bare)	0.3479814	0.3381135	0.0018

Table 3 Summary of Table 1's CPHM model with the second greatest AICc weight (temp*habitat): parameter estimates with standard error (S.E.) and *p*-values.

Predictor	Exp (estimate)	S.E.	*p*-value
temp (low)	0.0002052	0.7916845	< 2e-16
temp (medium)	0.0691254	0.2781378	< 2e-16
habitat (bare)	0.3758461	0.2162347	6.02E-06
temp (low)*habitat (bare)	5.5151655	0.8926127	0.05576
temp (medium)*habitat (bare)	0.37228	0.3346082	0.00315

Table 4 Trial 2's comparisons of *I. obsoleta* survival based on CPHM selection using AICc: temp = temperature (low, medium, high), habitat = habitat type (algae or bare), infected = infection status (yes or no).

Model	K	AICc	∆AICc	AICc Wt.	Cumulative Wt.	LL
temp	2	1993.19	0	0.29	0.29	-994.58
temp + habitat	3	1993.64	0.44	0.23	0.52	-993.78
temp + habitat + infected	4	1993.79	0.6	0.22	0.74	-992.83
infected*habitat + temp	5	1995.67	2.47	0.08	0.82	-992.73
temp*infected + habitat	6	1995.79	2.6	0.08	0.9	-991.75
temp*habitat	5	1996.51	3.32	0.06	0.96	-993.16

temp*habitat + infected	6	1996.94	3.75	0.04	1	-992.33
habitat	1	2446	452.81	0	1	-1221.99

Table 5 Summary of Table 4’s CPHM model with the greatest AICc weight (temp). Parameter estimates with standard error (S.E.) and *p*-values.

Predictor	Exp (estimate)	S.E.	*p*-value
temp (low)	0.002074	0.411723	<2e-16
temp (medium)	0.025476	0.360274	<2e-16

Table 6 Summary of Table 4’s CPHM model with the second greatest AICc weight (temp + habitat). Parameter estimates with standard error (S.E.) and *p*-values.

Predictor	Exp (estimate)	S.E.	*p*-value
temp (low)	0.002016	0.413447	<2e-16
temp (medium)	0.025453	0.360297	<2e-16
habitat (bare)	0.847486	0.131064	0.207

Table 7 Summary of Table 4’s CPHM model with the third greatest AICc weight (temp + habitat + infected). Parameter estimates with standard error (S.E.) and *p*-values.

Predictor	Exp (estimate)	S.E.	*p*-value
temp (low)	0.001972	0.414012	<2e-16
temp (medium)	0.025196	0.360425	<2e-16
habitat (bare)	0.849057	0.13109	0.212
infected (yes)	1.875694	0.415292	0.13

Table 8 Comparisons of dissolved oxygen based on Generalized Linear Mixed Model (GLMM) selection using AICc, using day as the random effect (day 1, day 2, day 3, day 4, day 5); temp = temperature (low, medium, high), habitat = habitat type (algae or bare), trial (one or two).

Model	K	AICc	∆AICc	AICc Wt.	Cumulative Wt.	LL
(1|day) + temp*habitat	8	869.38	0.00	0.72	0.72	-426.57
(1|day) + temp + habitat	6	871.31	1.93	0.27	0.99	-429.58
(1|day) + temp + trial + habitat + time	8	878.99	9.61	0.01	1.00	-431.37
(1|day) + habitat	4	1009.09	139.71	0.00	1.00	-500.51
(1|day) + temp	5	1953.38	1084.00	0.00	1.00	-971.64
NULL	3	1971.19	1101.81	0.00	1.00	-982.57

Table 9 Summary of Table 8’s GLMM model with the greatest AICc weight; (1|day) + temp*habitat. Parameter estimates with Chi-square and *p*-values based on Type III Wald Chi-Square Test. Conditional R^2 = 0.849, marginal R^2 = 0.847.

Predictor	Chi-sq	df	*p*-value
(Intercept)	112.179	1	< 2.2e-16
temp	47.510	2	4.824e-11
habitat	913.879	1	< 2.2e-16
temp:habitat	12.075	2	0.002387

CHAPTER 3: Macroinvertebrate community compositions in non-native and native foundational species

ABSTRACT

Seagrasses are among the most important primary producers in coastal ecosystems. Seagrasses also provide nursery grounds and refuge from predation for many macroinvertebrates and finfish species. In terms of seagrass composition, the North Carolina coastline is unique because it is where eelgrass *Zostera marina* (southernmost limit) and shoalgrass *Halodule wrightii* (northernmost limit) co-occur. Along this coastline, these seagrasses also co-occur with the non-native red alga *Gracilaria vermiculophylla*. All three macrophytes serve as foundation species in these coastal systems and can provide habitat and nursery grounds for numerous organisms. To determine if abundance, richness, and diversity of associated macroinvertebrates differed among the non-native alga and native seagrass species, we performed surveys of the foundation species each month at four sites from May – July 2021 (austral summer). We used a randomized design to collect fronds and blades of the macrophytes from our field sites and then identified associated macroinvertebrates from each in the lab. We found that macroinvertebrate abundance was significantly higher in *G. vermiculophylla* than seagrasses, while richness was significantly higher in *Z. marina* than *H. wrightii*. Overall, macroinvertebrate abundance, richness, and diversity increased significantly with biomass of both seagrasses and *G. vermiculophylla*. We also found salinity to be an important driver of abundance and richness. These results indicate that while an introduced, non-native species, *G. vermiculophylla* provides habitat for macroinvertebrates as a novel foundation species. Continued comparison of macroinvertebrate assemblages in these foundation species is critical for our understanding of

how communities may shift through time as abundance and composition of foundation species alter with human-induced global change.

INTRODUCTION

Foundation species are habitat-forming organisms that shape ecosystem function and structure, stabilize conditions, and strongly influence associated species (Dayton 1972). Angiosperm foundation species, like trees in forests and seagrass in coastal systems, can provide habitat to a wide diversity of associated fauna, creating microclimates, and reducing negative impacts of environmental stressors (Ellison et al. 2005, Franssen et al., 2011, Schob et al., 2012). Losses of primary producer foundation species can have long-lasting and widespread impacts to ecosystems because of the degradation of their ecosystem services, including primary productivity, energy transfers, and nutrient availability (Orwig et al. 2013). Furthermore, declines in foundation species can result in losses of valuable habitat, shelter, and refuge for associated fauna (Franssen et al. 2011). In shallow estuarine habitats, seagrasses are valuable foundation species that provide vital ecosystem services such as carbon sequestration and habitat for fish and invertebrates (Thomson et al. 2015). With increasing stressors induced by climate change (e.g., salinity and temperature exceeding organismal tolerance thresholds, sea level rise), seagrasses worldwide are experiencing rapid declines, since mortality rates dramatically increased when sea surface temperatures reach 25-30 ^{o}C (Nejrup and Pedersen 2008, Thomson et al. 2015). Seagrasses are also experiencing rapid declines due to other anthropogenic drivers such as nutrient loading, increasing sedimentation, and species invasions (Short et al. 2014; Ramus et al. 2017). Loss of seagrasses can significantly reduce aquatic biodiversity, primary productivity, trophic energy transfers, and valuable habitats (Thomson et al. 2015).

Along the U.S. East Coast, the North Carolina coastline is unique in that it represents the only region with overlapping distributions of two native seagrass species, *Zostera marina* and *Halodule wrightii*. North Carolina represents the southernmost and northernmost ranges of *Z. marina* and *H. wrightii*, respectively (Thayer et al. 1984, Burkholder et al. 1994). Both seagrasses are valuable foundation species, since they provide feeding grounds, spawning habitats, and refugia for commercially important fishes and invertebrates, as well as fixing nitrogen to a usable form and sequestering organic carbon (Smith and Hayasaka 1982, Short et al. 2014, Yeager et al. 2016, Zarnoch et al. 2017). However, with climate change, *H. wrightii* is expected to become more dominant in North Carolina over *Z. marina*, since the former has a greater thermal tolerance to warmer temperatures (Fonseca et al. 1998, Biber et al. 2009). Unlike *Z. marina*, *H. wrightii* requires more access to sunlight and is therefore distributed in shallower intertidal habitats (Biber et al. 2009). Much of previous seagrass restoration efforts in North Carolina have focused on *Z. marina* (Kenworthy et al. 1980, Fonseca et al. 1990), but as sea surface temperatures rise, *Z. marina* may not endure in the region. Therefore, it is important to understand if expanding tracts of *H. wrightii* will provide adequate ecosystem resources for economically and ecologically critical faunal communities.

In addition to thermal stress, another potential threat to seagrasses is the presence of non-native primary producing species. Invasions by the non-native alga *Gracilaria vermiculophylla* could be influencing seagrass populations and their associated fauna in the region (Ramus et al. 2017). This structurally complex alga, native to northeastern Japan, was introduced throughout temperate coastal habitats of the Northern Hemisphere (Krueger-Hadfield et al. 2017, 2018). Based on previous studies assessing impacts on *Z. marina*, *G. vermiculophylla* in Atlantic estuaries can enhance *Z. marina* mortality through reductions in metabolism and growth as a result of generating high sulfide levels in the water (Martinez-Luscher and Holmer 2010, Hoffle et al. 2011). Though not native, *G. vermiculophylla* is described as a foundation species in the western Atlantic because in some cases it can promote and facilitate biodiversity by forming novel habitats (Thomsen et al. 2013). For example, *G. vermiculophylla* can increase epifaunal richness and abundance, nursery richness and abundance, and multifunctionality (Ramus et al. 2017). However, as a potential novel foundation species, it remains unclear how analogous the fauna associated with *G. vermiculophylla* could be to other native foundation species (seagrasses, oyster reefs, salt marshes) that have declined in many southeastern estuaries in supporting community diversity and structure (Jackson et al. 2001, Gedan and Silliman 2009, Lotze 2010, Zu Ermgasesn et al. 2012, Ramus et al. 2017).

To date, no studies have compared the faunal compositions among the two native seagrasses, *H. wrightii* and *Z. marina*, and the novel, non-native alga, *G. vermiculophylla*. Since all three species co-occur along North Carolina's coasts, it is critical to understand how each of these species accommodates faunal abundance, richness, and diversity, and where there are redundancies or major differences in the communities they support. So far, one previous study compared faunal compositions between *G. vermiculophylla* and seagrasses; Thomsen et al.'s (2013) study in Odense Fjord (Denmark), macroinvertebrate abundance, richness, and diversity were all significantly higher in *G. vermiculophylla* than *Z. marina* because *G. vermiculophylla* has more interstitial space in its three-dimensional structure than *Z. marina*. Based on these results, we compared associated fauna in *G. vermiculophylla* with that of the native seagrass species to determine whether we would observe similar results in the western Atlantic.

METHODS

STUDY SYSTEM

Red Alga *Gracilaria vermiculophylla*

The red alga *Gracilaria vermiculophylla* is native to the waters of Northwestern Pacific, from the northern Sea of Japan to the East China Sea and south towards the central Kuroshio Current (Kim et al. 2010, Hammann et al. 2013, Krueger-Hadfield et al. 2017). *Gracilaria vermiculophylla* can tolerate wide range of environmental changes, including but not limited to extreme temperatures, low salinities, and low light exposure (Rueness 2005, Phooprong et al. 2008, Nejrup and Pedersen 2012, Sotka et al. 2018). This alga has invaded nearly every temperate estuary in the Northern Hemisphere. In the eastern North America, *G. vermiculophylla* was first discovered in 1998 in Chesapeake Bay, though it is much more likely that the algae was present since the early 20th century with the introduction of *Crassostrea gigas* (Thomsen et al. 2006, Krueger-Hadfield et al. 2017). From Virginia to Georgia, *G. vermiculophylla* have invaded primarily soft-sediment estuarine habitats (Thomsen et al. 2009, Byers et al. 2012, Ramus et al. 2017). In its native range, this alga is a haploid-diploid dioicious organism, which results in free-living haploid and diploid individuals. When *G. vermiculophylla* invaded some soft-sediment habitats, its haplodiplontic life cycle became interrupted; this means that in these habitats, most of *G. vermiculophylla* were primarily diploid, free-floating, and rely on fragmentation (Krueger-Hadfield et al. 2016). However, in the coastlines of Bogue Sound in North Carolina, like in their native habitats, *G. vermiculophylla* are primarily fixed via holdfast to hard surfaces (Lee, pers. obs.). *Gracilaria vermiculophylla* grows rapidly and reach large biomasses, and tolerant to extreme environmental stresses such as high salinity and temperature (Thomsen et al. 2009). The spread of *G. vermiculophylla* in these soft-sediment coastal habitats can increase primary production, provide novel structural complexity, and increase secondary production as it provides shelter, refuge, and even novel substrate for macroinvertebrates (Thomsen 2010, Byers et al. 2012, Guidone et al. 2014).

Seagrasses (*Zostera marina, Halodule wrightii, Ruppia maritama*)

Zostera marina is one of the most critical foundational species in shallow coastline habitats. It provides valuable habitat and nursery grounds for associated species of fish and invertebrates, is a major contributor of primary productivity, sediment stabilization, nutrient fixation, and carbon sequestration (Franssen et al. 2011, Bostrom et al. 2014, Lefcheck et al. 2017). In North Carolina, *Z. marina* contributes >60% of total primary production (Adams 1976). *Zostera marina* can convert N_2 to a usable form contributing to nutrient accessibility to other organisms (Zarnoch et al. 2017). *Zostera marina* can also sequester carbon. This ecological function is critical, since the food web in *Z. marina* dominated habitats draws most of its carbon from these foundation species (McConnaughey and McRoy 1979). *Zostera marina* is prone to biofouling by epiphytes, such as algae. In turn, this provides habitat and food for many grazing macroinvertebrates (Jephson et al. 2008). Along the U.S. East Coast, the shorelines of North Carolina are the southernmost limit of *Z. marina*. Water temperatures in this region during the summers can exceed 30 ˚C, which can lead to their seasonal mortalities, since *Z. marina* thrives at an optimal thermal range of 10-20 ˚C (Thayer et al. 1984, Nejrup and Pedersen 2008, Combs 2021).

Halodule wrightii has a range in western Atlantic that extends from southern Brazil to North Carolina, USA (Short et al. 2010, Digiantonio et al. 2010). This makes North Carolina unique, as it is the only state where *Z. marina* and *H. wrightii* co-occur. Micheli et al. (2008) predicted that with increasing seawater temperature and high mortalities of *Z. marina* in the summers in North Carolina, *H. wrightii* will eventually dominate these habitats. Donaher et al. (2021) observed that in North Carolina, *Z. marina* was the dominant seagrass until July. *H. wrightii* experiences greatest growth in June (Thayer et al. 1984). Also present and co-occurring with *H. wrightii* is *Ruppia maritima* (Micheli et al. 2008, Donaher et al. 2021), another seagrass species that is morphologically similar to *H. wrightii* and considered to have lower habitat value than *Z. marina* (Burkholder et al. 1994). The seagrass beds of North Carolina are often mixed with *Z. marina*, *H. wrightii*, and *R. maritama* (Voigt and Eggleston 2023). *R. maritama* beds are often utilized by blue crab *Callinectes sapidus*, suggesting that all seagrasses have the potential to provide valuable habitat for commercially important species (Voigt and Eggleston 2023). However, Micheli et al. (2008) found that seagrass beds dominated by *H. wrightii* have lower abundance and diversity of macroinvertebrates than *Z. marina*, which indicates concern for estuarine biodiversity associated with seagrasses as *Z. marina* continues to decline.

DATA COLLECTION

We collected *G. vermiculophylla* and seagrasses at four locations in the Beaufort, North Carolina region of North Carolina, USA from May-July 2021 (Figure 1, Table 1). These sites (CMAST = Center for Marine Sciences and Technology, IMS = UNC Institute of Marine Sciences, HIBR = Harkers Island Boat Ramp, HIPB = Harkers Island Public Beach) were selected based on summer 2019 and 2020 surveys and site scouting (Lee et al. 2023: submitted, Lee: unpub.) to determine where *G. vermiculophylla* and seagrasses co-occur. We surveyed each site three times (once every month, from May – July). At all sites, *G. vermiculophylla* was in the intertidal zone (sometimes completely emerged), while seagrasses were mostly in the shallow subtidal zone.

At each site, we set out a 30-meter transect along the tract of shoreline where *G. vermiculophylla* occurred and used a random number generator to select five random numbers (between 1-30). Each of these five numbers represented the marker (e.g. 2 = 2 meters) on the transect tape, and thus each marker was our replicate (n = five replicates of *G. vermiculophylla* at each site in each month). We used a 0.25 m^2 quadrat adjacent to the transect tape on these appropriate markers to obtain percent cover of *G. vermiculophylla*. We bagged each replicate's *G. vermiculophylla* in Ziploc bags with seawater as quickly as possible to minimize the loss of organisms. We also recorded depth of the seawater if the *G. vermiculophylla* thalli. We then established transect tapes that were perpendicular from each *G. vermiculophylla* replicate and walked perpendicularly until we found the first presence of seagrass, and noted the distance from the *G. vermiculophylla* to the nearest perpendicular replicate of seagrass. We also used 0.25 m^2 quadrat to obtain percent cover of seagrass, and used scissors to cut all the blades of seagrass (or aboveground biomass), which were also bagged in Ziploc bags with seawater as quickly as possible. We also noted the depth of the seagrass (if the seagrasses were not emerged), and the seagrass type (Z = *Zostera marina* only, HR = *Halodule wrightii* and *Ruppia maritama,* ZHR = *Z. marina* and *Halodule wrightii* and *Ruppia maritama*). Since *H. wrightii* and *R. maritama* co-occur in these habitats and are difficult distinguish from each other morphologically (Burkholder et al. 1994, Gittman, pers. comm.), we grouped these two species together when categorizing seagrass types. Thus, we collected n = five replicates of seagrasses in each site at each month. We also noted air temperature, and recorded water temperature, salinity, and dissolved oxygen using a handheld YSI Pro-1030 (Yellow Springs, OH).

Upon bringing the replicates of *G. vermiculophylla* and seagrasses to the lab, we immediately submerged them in fresh water to induce osmotic shock, since this method has been proven effective at removing associated macroinvertebrates from algal samples (Blakeslee et al. 2016, Fowler et al. 2016). We then used a Fisher Scientific™ 250 µm sieve to separate macroinvertebrates from *G. vermiculophylla* and seagrasses; upon separation, we preserved macroinvertebrates in Pharmaco™ 200 proof Ethyl Alcohol. After shaking off excess water, we weighed the thalli to obtain wet weights (g). We also dried each replicate's *G. vermiculophylla* and seagrasses in the drying oven for two weeks to obtain dry weights.

We dyed all *G. vermiculophylla* and seagrass replicates' macroinvertebrates with Rose Bengal (Gbogbo et al. 2020) and identified them to the lowest possible taxonomic level using different guidebooks and keys pertaining to the region (Bousfield 1973, Johnson and Allen. 2012). We observed macroinvertebrates using a Zeiss MS Series Fixed Magnification Stereo Microscope (6x) and/or a Neatfi Elite XL HD Magnifying Lamp (5x). Gammaridean amphipods, which comprised majority of the macroinvertebrates (see Results), can be difficult to identify to species level using morphology alone. We used pre-existing knowledge from classifying amphipods into morphotypes and then later barcoding those morphotypes using standard DNA barcoding protocols (e.g., Blakeslee et al. 2020, Lee et al. 2023: *submitted*). This allowed us to identify amphipods more precisely to the species level.

STATISTICAL ANALYSES

We used Generalized Linear Mixed Models (GLMM), an approach that is best used for non-normal data with random effects (Bolker et al. 2009), to determine which sets of predictors best explain the following responses: abundance (total raw count of macroinvertebrates), richness (total number of species), and diversity (Shannon-Wiener Diversity Index).

The predictors were site (random effect: see Table 1 for complete list of sites) and all the following variables were fixed effects: month = May, June, July, GV or SG = *G. vermiculophylla* or seagrass, seagrass type = GV (for *G. vermiculophylla*), Z, HR, ZHR, wet weight (g), dry weight (g), % cover, distance (the distance from *G. vermiculophylla*'s replicate to the nearest seagrass replicate, in meters), depth (m), air temperature (˚C), water temperature (˚C), oxygen (mg/lit), and salinity (ppt).

We initially conducted autocorrelation tests between every pair of continuous variables to determine which sets of variables were to be used for GLMM. We removed dry weights, since we found that these had high correlation with wet weight ($R^2 = 0.75$, $p < 0.001$). In addition, we also removed % cover, since these had high autocorrelation with wet weight (*G. vermiculophylla*: $R^2 = 0.79$, *p*, < 0.001, seagrass: $R^2 = 0.6$, $p < 0.001$). We also removed air temperature, since upon initial correlation tests with abundance, richness, and diversity, we did not see any significant correlations ($p > 0.05$). We also removed distance (the distance from the *G. vermiculophylla* to the nearest perpendicular seagrass replicate), since we did not find five replicates of *G. vermiculophylla* during every month at every site (e.g. HIPB in June had only two replicates of *G. vermiculophylla*, and for July, CMAST, HIPB, and IMS had no *G. vermiculophylla* replicates); by removing replicates without distance, too many samples were removed, resulting in problems with GLMM with low replicate counts. For distance, we also observed that none of the *G. vermiculophylla*'s or seagrasses' abundance, richness, and diversity were significantly correlated with the distance, except for richness associated with *G. vermiculophylla* ($R^2 = 0.32$, $p = 0.038$).

We also removed seagrass type, since there were uneven number of replicates (Z: n = 27, HR: n = 18, ZHR: n = 18), and uneven distribution across sites and months. Our initial runs using Bonferroni's correction method to determine if abundance, richness, and diversity varied across the seagrass types showed no significance ($p > 0.05$) between every pairwise comparison, except for richness (GV and HR, HR and Z: $p < 0.05$).

Thus, the final predictors we used for GLMM were site (random effect), month, wet weight, GV or SG, depth, water temp, oxygen, and salinity. We created different sets of GLMM (family = negative binomial for abundance, gaussian for richness, and diversity) with different combinations of predictors and interactions using package *lme4* (Wang et al. 2022). All models were checked for overdispersion (Bolker 2010). We ran AICc with the *aictab* function in the package *AICcmodavg* (Mazerolle 2017, R Core Team 2023) to determine which set of predictors best explained abundance, richness, and diversity. Based on our AICc results for each response variable, we then conducted different sets of univariate analyses (Pearson's Correlation Test, Kruskal-Wallis Test, Two-Tailed T-test, and pairwise comparisons using Bonferroni correction: see Results). We used R 4.2.2 for all our GLMM, AICc, and univariate data analyses (R Core Team 2023).

We also used Nonmetric Multidimensional Scaling (nMDS) to create two-dimensional ordination plane to visually evaluate macroinvertebrate community compositions across the following categories: months, sites, GV or SG, and seagrass type (Clarke and Warwick 2001). We also removed species from the nMDS matrix that occurred <5% prior to running nMDS per recommendations by Cao et al. (2001). All the macroinvertebrate data in the nMDS matrix were square-root transformed and a Bray-Curtis Similarity matrix was calculated (Clarke and Warwick 2001). We also conducted Similarity of Percentage (SIMPER) analyses to determine the percent of each species contributed to the differences observed between months, sites, and seagrass type; since SIMPER requires at least three categories, we did not conduct this test for the GV or SG (*G. vermiculophylla* or seagrass) category (Clarke 1993, Clarke and Warwick 2001).

RESULTS

Overall, we found N = 3,330 macroinvertebrates across 19 species, with *Mitrella lunata* and *Ampithoe longimana* comprising >50% abundance (Table 2). When comparing abundance between the macroalga (*G. vermiculophylla*) and seagrass, we found that *M. lunata* was also the most abundant invertebrate in both groups. Gammaridean amphipods *Ampithoe valida* and *A. longimana* were second most abundant in each group respectively (Table 3). When comparing abundances across months, in May, we found that Gammaridean amphipods (*A. longimana* and *A. valida*) were most abundant (comprising >60%), while in June, we found that *M. lunata* comprised >60%, and in July, *Bittium varium* and *A. longimana* together comprised >60% (Table 4). When comparing abundances between sites, for CMAST, we found that *A. longimana* and *A. valida* comprised >60% of the total abundance, while at HIBR, *M. lunata* comprised >50% of the total abundance. At HIPB, three amphipod species (*A. longimana, A. valida*, and *G. mucronatus*) together comprised >60% of the total abundance, while at IMS, *A. longimana* and *M. lunata* together comprised >50% of the total abundance (Table 5). Finally, when comparing abundances between seagrass types, we found that at HR, *A. longimana* and *B. varium* together comprised >60%, while at Z, *M. lunata* and *B. varium* together comprised >60%. However, at ZHR, two Gammaridean amphipods (*A. longimana* and *G. mucronatus*) together comprised >60% (Table 6).

For macroinvertebrate abundance, we found that the model that held the greatest Akaike weight (=0.99) was site (random effect), month interacting with salinity, GV or SG, and wet weight (Tables 7-8). Using Kruskal-Wallis test, we found that macroinvertebrate abundance differed significantly between all four sites overall (df = 3, $p < 0.001$), while Bonferroni's correction method showed that the following pairs of sites had significantly different abundances ($p < 0.05$): CMAST and HIBR, HIBR and HIPB, and HIBR and IMS (Figure 2). We found that correlations between salinity and abundance were only significantly related ($R^2 = 0.53$, $p < 0.001$) for the month of June (Figure 3). We also found that abundance significantly differed (df = 61.184, $p = 0.02$) between *G. vermiculophylla* and seagrasses (Figure 4). Finally, we found that wet weight (or biomass of *G. vermiculophylla* or seagrass) had significant positive correlation ($R^2 = 0.46$, $p < 0.001$) with abundance (Figure 5).

For macroinvertebrate richness, we found that the three models (site: random effect, month, wet weight, salinity; site: random effect, month*salinity, GV or SG, wet weight; site: random effect, month, GV or SG, wet weight, salinity) contributed ~0.7 weight cumulatively (Tables 9-12). We found that overall, richness differed across all sites (df = 3, $p = 0.002$), and pairwise comparisons were significant ($p<0.05$) for the following pairs: CMAST and HIBR, CMAST and HIPB (Figure 6). We also found that correlation between salinity and richness was significant ($R^2 = 0.75$, $p < 0.001$) for only the month of June (Figure 7). We did not find significant differences ($p > 0.05$) in richness between *G. vermiculophylla* and seagrass (Figure 8), but we found that wet weight (or biomass of *G. vermiculophylla* or seagrass) had significantly positive correlation ($R^2 = 0.36$, $p < 0.001$) with richness (Figure 9).

For macroinvertebrate diversity, we found that the following three models contributed >0.7 weight overall: null model (random effect, or site, only); site + wet weight; site + month (Tables 13-16). We did not find any significant differences of diversity across all four sites, and no significant differences with any pairwise comparisons (Figure 10). We found that wet weight (or biomass of *G. vermiculophylla* or seagrass) had significant positive correlation ($R^2 = 0.23$, p

= 0.02) with diversity (Figure 11). Finally, we found that diversity differed significantly across months (df = 2, *p* = 0.01); pairwise comparisons showed that diversity was significantly different between May and June only (Figure 12).

We also compared macroinvertebrate abundance, richness, and diversity between *G. vermiculophylla* and three seagrass types (Z, HR, and ZHR). We did not find any significant differences between any pairwise comparisons, except for the following: *G. vermiculophylla* vs. HR in abundance (*p* = 0.02), and *G. vermiculophylla* vs. HR (*p* = 0.01) and HR vs. Z in richness (*p* = 0.009). Through the Kruskal-Wallis test, we found that abundance (df = 3, *p* = 0.004) and richness (df = 3, *p* = 0.008) were overall significantly different (Figures 13-14).

When doing pair-wise comparisons using SIMPER, for months, we found that between May and June, the three species that contributed to the greatest dissimilarity were *M. lunata*, *A. longimana*, and *A. valida* (Table 17). For May andand July, *A. longimana*, *A. valida*, and *B. varium* contributed to the greatest dissimilarity of cumulative >50% (Table 18). For June and July, *M. lunata* and *B. varium* contributed to >50% cumulative dissimilarity (Table 19). For sites, between HIBR and IMS, *M. lunata*, *B. varium*, and *A. longimana* together contributed >50% dissimilarity (Table 20). These three species also contributed to the dissimilarity (>50%) for comparison between HIBR and CMAST (Table 21). For comparison between IMS andand CMAST, the three amphipods (*A. longimana*, *A. valida*, and *G. mucronatus*) contributed cumulative >50% dissimilarity (Table 22). For HIBR andand HIPB, the three species that contributed cumulative >50% dissimilarity were the same species that contributed to the dissimilarity between HIBR andand IMS (Table 23). For IMS andand HIPB however, four species altogether contributed >50% cumulative dissimilarity; they were the three species of Gammaridean amphipods (*A. longimana*, *A. valida*, *G. mucronatus*), and *M. lunata* (Table 24).

Finally, for the comparison of CMAST andand HIPB, three species of Gammaridean amphipods (*A. longimana*, *A. valida*, *G. mucronatus*) and *B. varium* contributed >60% of the cumulative dissimilarity (Table 25). For the comparisons of seagrass type, between Z and HR, two gastropods (*B. varium* and *M. lunata*) and *A. longimana* contributed >50% cumulative dissimilarity (Table 26). For Z andand *G. vermiculophylla*, *M. lunata*, *A. valida*, and *A. longimana* together contributed >50% dissimilarity (Table 27). For HR andand *G. vermiculophylla, A. valida, A. longimana*, and *M. lunata* together contributed >50% dissimilarity (Table 28). For Z andand ZHR, *M. lunata*, *A. longimana*, and *B. varium* contributed cumulative 50% dissimilarity (Table 29). For HR and ZHR, *A. longimana*, *G. mucronatus*, and *B. varium* contributed cumulative >50% of dissimilarity (Table 30). Finally, for *G. vermiculophylla* and ZHR, we found that *A. valida*, *A. longimana*, and *M. lunata* contribute cumulative >50% dissimilarity (Table 31).

Upon plotting each replicate's abundance on nMDS ordination plane, we found that for seagrass type, there did not appear to be differences in species compositions between different seagrass groups (Figure 15). However, much of the seagrass samples appear to be more similar with each other than with *G. vermiculophylla* (Figure 16). For the month, we noticed that replicates from the month of May were quite similar in terms of species compositions, while there was more overlap between replicates from June and July (Figure 17). Finally, for sites, it appeared that many replicates' species compositions did not differ between sites (Figure 18).

DISCUSSION

Across all sites, we found that the most abundant macroinvertebrates in both *G. vermiculophylla* and the two seagrasses overall were the three Gammaridean amphipods (*A.*

longimana, *A. valida*, *G. mucronatus*) and two gastropods (*M. lunata*, *B. varium*). These species were also the major contributors to the pairwise dissimilarity comparisons by SIMPER (Tables 17-31). All these species have been found to be strongly associated with seagrasses, although past studies indicated that abundances of *Gammarus* and *Bittium* sp. were more strongly correlated with shape of seagrasses, while *Ampithoe* and *Mitrella* sp. responded more significantly to the epiphytes on the seagrass (Schneider and Mann 1991a, 1991b). The gastropods *M. lunata* and *B. varium* are epiphytic grazers that can be found in large abundances on seagrasses (Moore and Wetzel 2000). Past studies have indicated that *M. lunata* also use macroalgae as source of food by grazing not on the macroalga, but on its epiphytes; seaweeds with gastropods present were found to be free of biofouling and overgrowth (Stachowitz and Whitlatch 2005). The three most abundant Gammaridean amphipod species found throughout this study (*A. longimana*, *A. valida*, and *G. mucronatus*) are grazers.

However, seaweeds, including *G. vermiculophylla*, can release toxic compounds upon being grazed as a defense mechanism (Hammann et al. 2016). Duffy and Hay (1994) found in their study on effects of chemical defense released by alga *Dictyota menstrualis* that *A. longimana* was most tolerant to chemical defenses, while *A. valida* and *G. mucronatus* were quickly deterred and avoided the alga. This suggests that macroinvertebrates like *A. longimana*, when given availability of two foundational species (*G. vermiculophylla* or seagrass), can utilize either habitat as refuges from fish predation (Duffy and Hay 1994). However, we found greater abundance of *A. valida* than *A. longimana* in *G. vermiculophylla* in our study (Table 3). Bippus et al. (2018) found that *A. valida* will graze on *G. vermiculophylla* thalli, and that wounding of the thalli does not appear to change the thalli palatability, suggesting that *A. valida* is adapting to the anti-herbivory defense mechanisms. The gastropod *Bittium varium*, a common grazer, is one of the most abundant epifauna on seagrasses, with greater abundance present on seagrass than on alga (Virnstein et al. 1987), which also aligns with our result of higher abundance of *B. varium* in seagrass habitat (Table 3).

We found that abundance of macroinvertebrates differed significantly between *G. vermiculophylla* and seagrass (Figure 4). Studies comparing seagrass *Z. marina* and *G. vermiculophylla* in its non-native habitat in European coasts also found that abundances of native macroinvertebrates were higher in *G. vermiculophylla*, and the abundances of macroinvertebrates increased with *G. vermiculophylla* biomass (Thomsen 2010, Thomsen et al. 2013). We also found that abundance overall was significantly higher in *G. vermiculophylla* than seagrasses. Heck and Wetstone (1977) found that aboveground plant biomass of seagrasses, which is what we measured, is significantly correlated with increasing number of macroinvertebrates and richness. We also found that in the month of June, salinity was positively correlated with abundance (Figure 3). Blake and Duffy (2010) found that different species of macroinvertebrate respond differently to salinity fluctuations, with Gammaridean amphipods such as *Elasmopus levis* responded negatively to lower salinities, while *G. mucronatus* responded positively or remained neutral to freshwater input. Furthermore, salinity was recognized as a main driver of differentiating biodiversity between seagrass meadows (Whippo et al. 2018). Our study could benefit from long-term observations of changes of salinity in the system, but it was constrained by the presence of *G. vermiculophylla*; as previously noted by Freshwater et al. (2006), *G. vermiculophylla* in North Carolina’s coastlines were most prevalent from May-July, which is also what we noticed during our initial scouting of sites in summer 2020 and for the duration of this study. Repeated monitoring across multiple years may indicate how macroinvertebrate abundances between *G. vermiculophylla* and seagrasses are changing with varying salinities.

For macroinvertebrate richness, we found that it had positive significant correlation with salinity, but only for the month of July. Thomsen et al. (2007) found in their experiment that activity of grazers on *G. vermiculophylla* was highest at their highest salinity treatment, which was 34 PSU. Similarly, Yamada et al. (2007) found that at Akkeshi-ko estuary in Japan, macroinvertebrate richness of seagrass meadows was positively correlated with salinity. During our duration of the study, the salinities we observed in July ranged from 28.4 – 29.6 ppt, which is lower than the optimal salinity at the highest grazer and salinity interaction recorded by Thomsen et al. (2007). July was also the month when we noticed a major decline of *G. vermiculophylla* thalli presence in our sites, since only one site (HIBR) was recorded having *G. vermiculophylla* by then. As a result, it is possible that salinity and richness correlation is more reflective of invertebrates associated with seagrasses rather than the non-native seaweed. As Heck and Wetstone (1977), Thomsen (2010), and Thomsen et al. (2013) found, richness of macroinvertebrates does increase with biomass of aboveground seagrass and biomass of *G. vermiculophylla* thallus. While we did not find significant differences of richness between *G. vermiculophylla* and seagrass, further monitoring may be necessary to understand how macroinvertebrate richness will shift as *G. vermiculophylla* continues to thrive in the system.

We found that diversity had positively significant correlation with biomass (Figure 11). Thomsen et al. (2013) also found positive correlation between *G. vermiculophylla* biomass and diversity, and Nordlund and Gullstrom (2013) found that seagrasses that are not disturbed or damaged (hence, lower aboveground biomass) by anthropogenic activities can support greater macroinvertebrate densities, richness, and biomass. Our results indicated that diversity did differ significantly across months, with the greatest diversity in May (Figure 12). This could be attributed to the biomass of *G. vermiculophylla* thalli available to sample in May. While all sites in May had enough *G. vermiculophylla* to fulfill five replicates per site, in June, HIPB had only enough *G. vermiculophylla* to fulfill two replicates. This decline of *G. vermiculophylla* could cause the significant decline of diversity from May to June. Repeated annual monitoring when *G. vermiculophylla* is present in the region may be necessary to understand how macroinvertebrate diversity shifts as *G. vermiculophylla* availability changes.

We also compared abundance, richness, and diversity between different types of seagrasses (Z, ZHR, and HR: see Methods for full names of the abbreviations). We did not find any significant differences in any of the pairwise comparisons, except for richness, where Z and HR were significantly different (Figure 14), with Z exhibiting greater richness than HR. North Carolina's coastlines are where *Z. marina* and *H. wrightii* overlap; Micheli et al. (2008) found that both abundance and diversity of macroinvertebrates were lower in seagrass habitats dominated by *H. wrightii* than *Z. marina*. Both densities and biomasses of *Z. marina* declined significantly in North Carolina since the mid-1980s (Micheli et al. 2008). If *H. wrightii* continues to spread while *Z. marina* experiences decline, there may be rippling effects to the entire ecosystem, since lower macroinvertebrate abundances can reduce prey for other secondary and tertiary consumers. However, we also noticed that abundance and richness were significantly higher in *G. vermiculophylla* than the seagrass type HR (Figures 13-14). This suggests that even if *Z. marina* continues to decline, *G. vermiculophylla* can possibly maintain high macroinvertebrate diversity and abundance by serving as alternate structure-forming foundational species, though it may not stabilize sediments like segrasses.

So far, comparisons of macroinvertebrates between *G. vermiculophylla* and seagrasses indicate that while abundances are overall greater in *G. vermiculophylla*, other patterns need further exploration. At areas where *G. vermiculophylla* is non-native, they pose concerns for native *Z. marina*, as past experiments indicated that greater *G. vermiculophylla* biomass can reduce net leaf photosynthesis of seagrasses (Martinez-Luscher and Holmer 2010). Hoffle et al. (2011) found that in contrast, *G. vermiculophylla* has marginal effect on the growth and survival of *Z. marina*; even at the highest temperature (27 ˚C), while the overall growth and survival of *Z. marina* declined, it was not significant (Hoffle et al. 2011). In our sites, we observed that tracts of *G. vermiculophylla* are at higher tidal elevations at intertidal zones, whereas seagrass tracts were in lower shoreline elevations, often in subtidal zones. We did not notice any overlap between *G. vermiculophylla* and seagrasses, which suggest that so far, *G. vermiculophylla* is maintaining its niche without encroaching into seagrass habitats in this region. It is entirely possible that *G. vermiculophylla* can benefit the system by providing additional structural complexity and habitats for macroinvertebrates as *Z. marina* continues to decline. It is critical to note, however, that *G. vermiculophylla* cannot replace the functional role of seagrasses, as seagrasses are critical macrophytes that can stabilize sediments and prevent erosion (Christianen et al. 2013) Furthermore, since *G. vermiculophylla* in this region are constrained during summer months (Lee, pers. ob., Lee 2023 et al.), multi-year monitoring may be necessary to understand how the non-native seaweed and native seagrasses will interact to maintain epifaunal biodiversity.

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CHAPTER 3 FIGURES

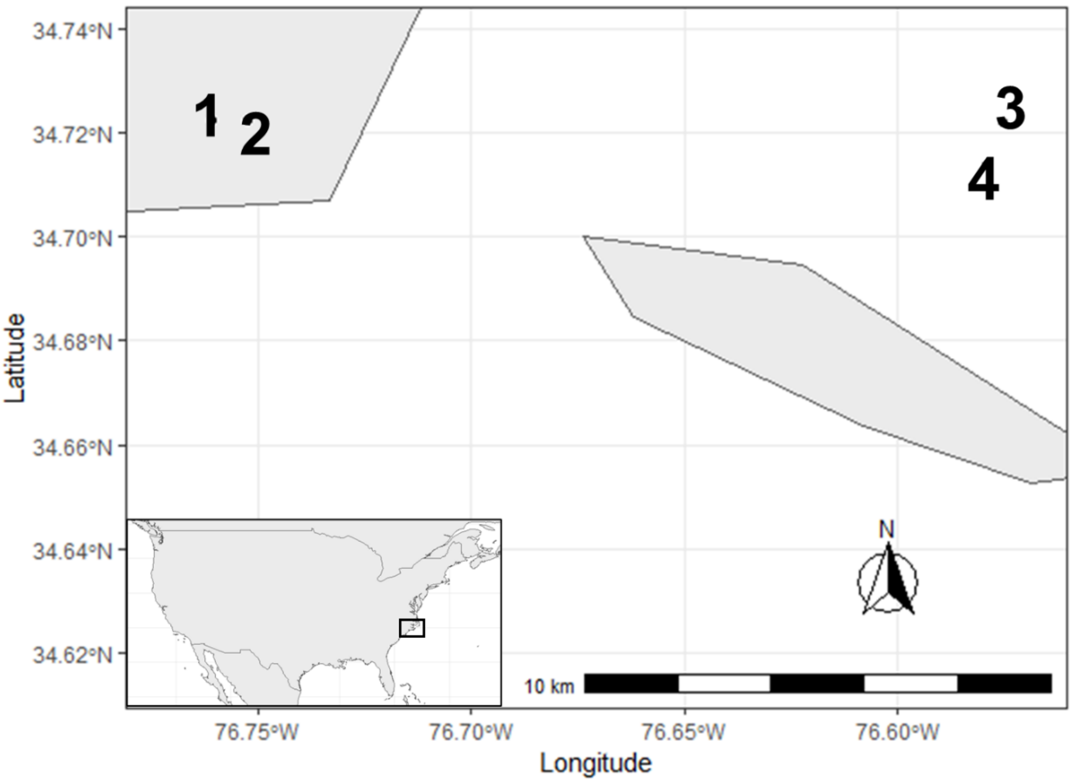

Figure 1 Map of sampled sites. 1 = CMAST, 2 = IMS, 3 = HIBR, 4 = HIPB. See Table 1 for full names of the site abbreviations.

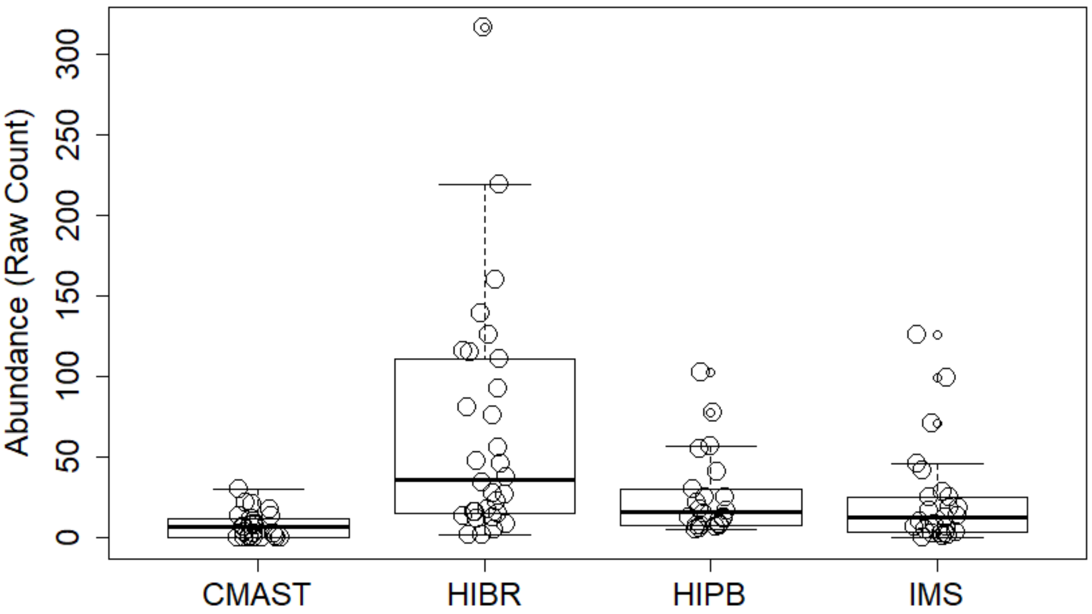

Figure 2 Comparisons of macroinvertebrate raw counts between sites (Kruskal-Wallis: df = 3, $p < 0.001$). Pairwise comparisons using Bonferroni's Correction Method were significant ($p < 0.005$) between the following pairs: CMAST and HIBR, HIBR and HIPB, and HIBR and IMS (for full list of site names, refer to table 1).

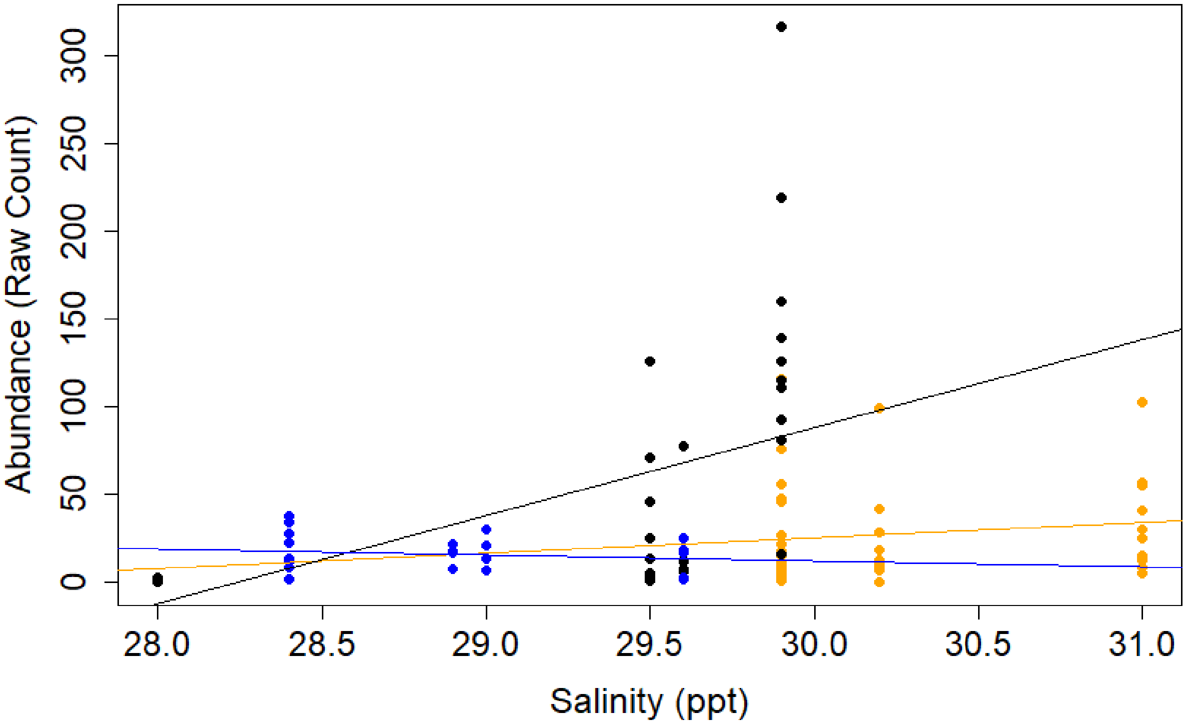

Figure 3 Correlations between salinity and abundance for May (orange: $R^2 = 0.14$, $p = 0.4$), June (black: $R^2 = 0.53$, $p < 0.001$), and July (blue: $R^2 = -0.15$, $p = 0.47$).

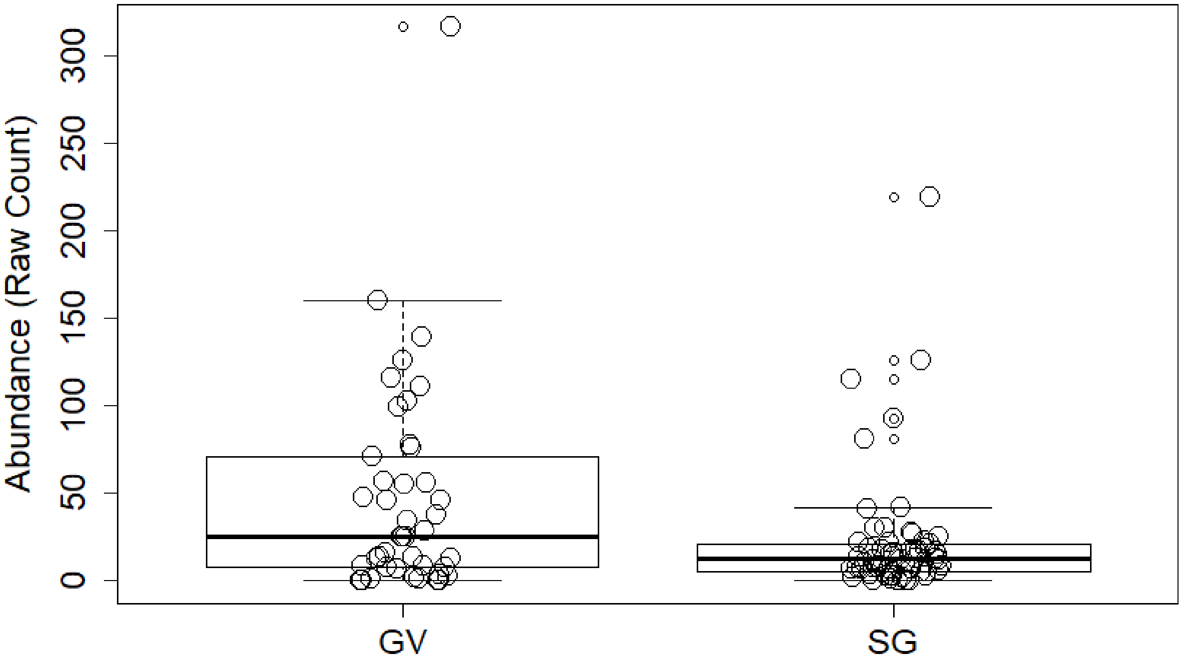

Figure 4 Comparison of macroinvertebrate counts between GV (*Gracilaria vermiculophylla*) and SG (seagrass). Df = 61.184, p = 0.02.

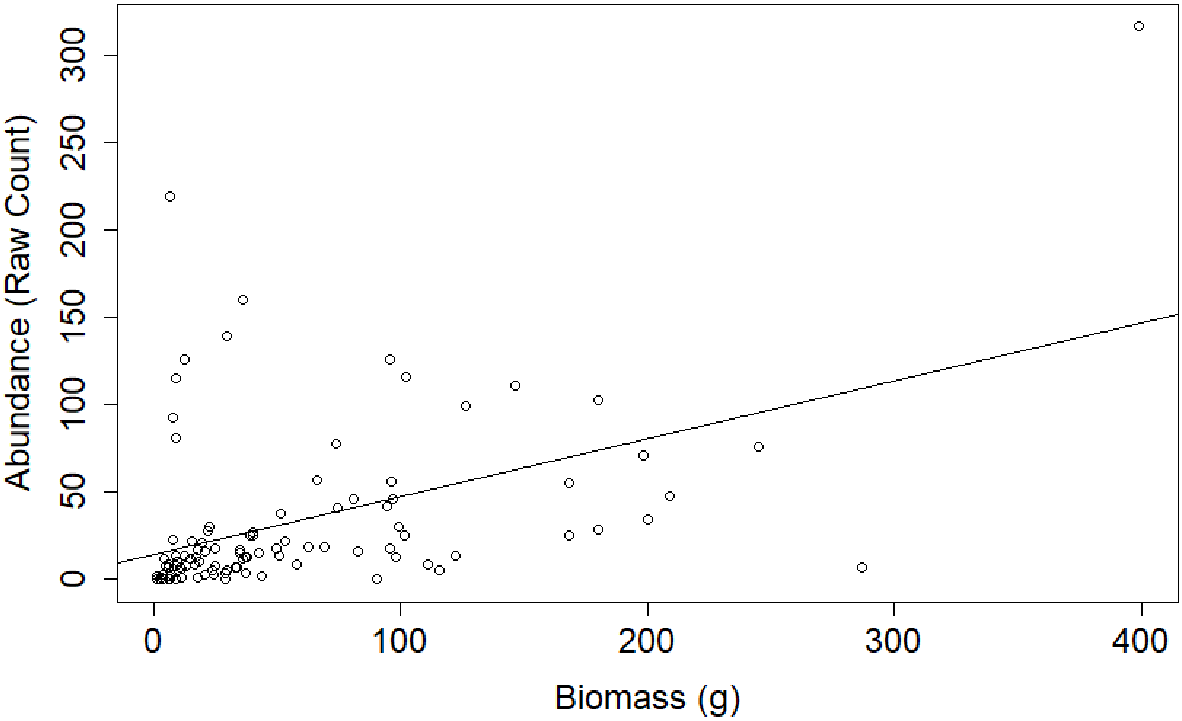

Figure 5 Correlation between biomass (of seagrass and *Gracilaria vermiculophylla*) and abundance of macroinvertebrates ($R^2 = 0.46$, $p < 0.001$).

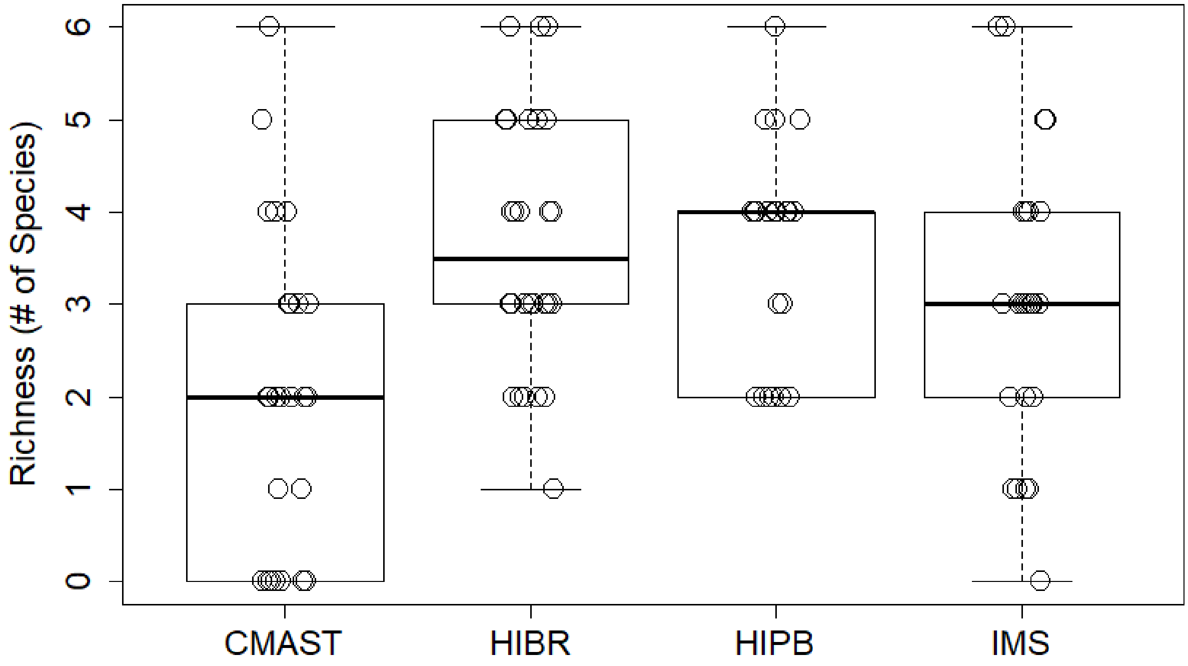

Figure 6 Comparisons of macroinvertebrate richness between sites (Kruskal-Wallis: df = 3, *p* = 0.002). Pairwise comparisons using Bonferroni's Correction Method were significant (*p* < 0.005) between the following pairs: CMAST and HIBR, CMAST and HIPB (for full list of site names, refer to table 1).

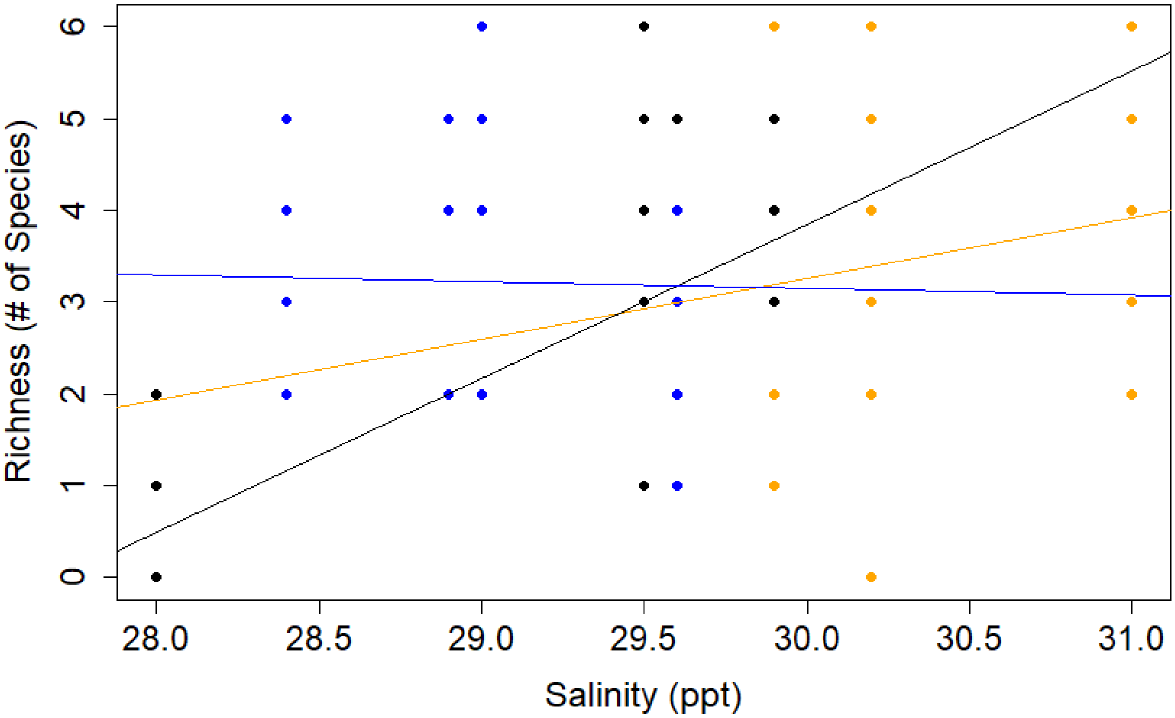

Figure 7 Correlation between salinity and richness (number of species) by month. May = orange ($R^2 = 0.21$, $p = 0.2$), June = black ($R^2 = 0.75$, $p < 0.001$), July = blue ($R^2 = -0.02$, $p = 0.91$).

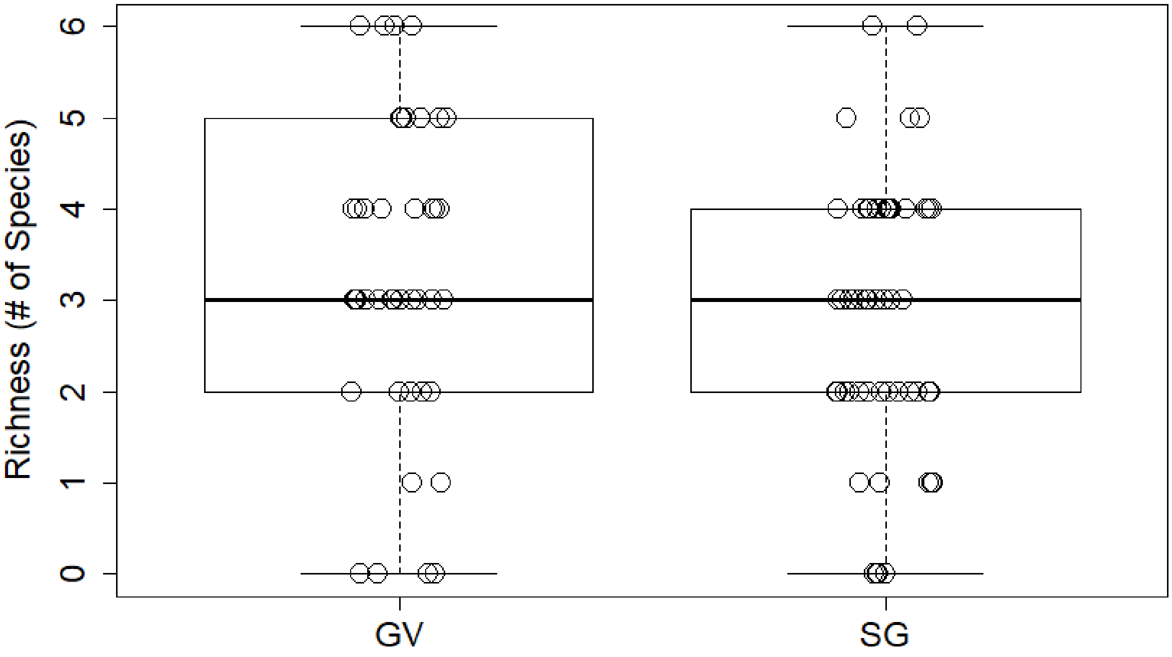

Figure 8 Comparison of macroinvertebrate richness between GV (*Gracilaria vermiculophylla*) and SG (seagrass). Df = 80.926, p = 0.19.

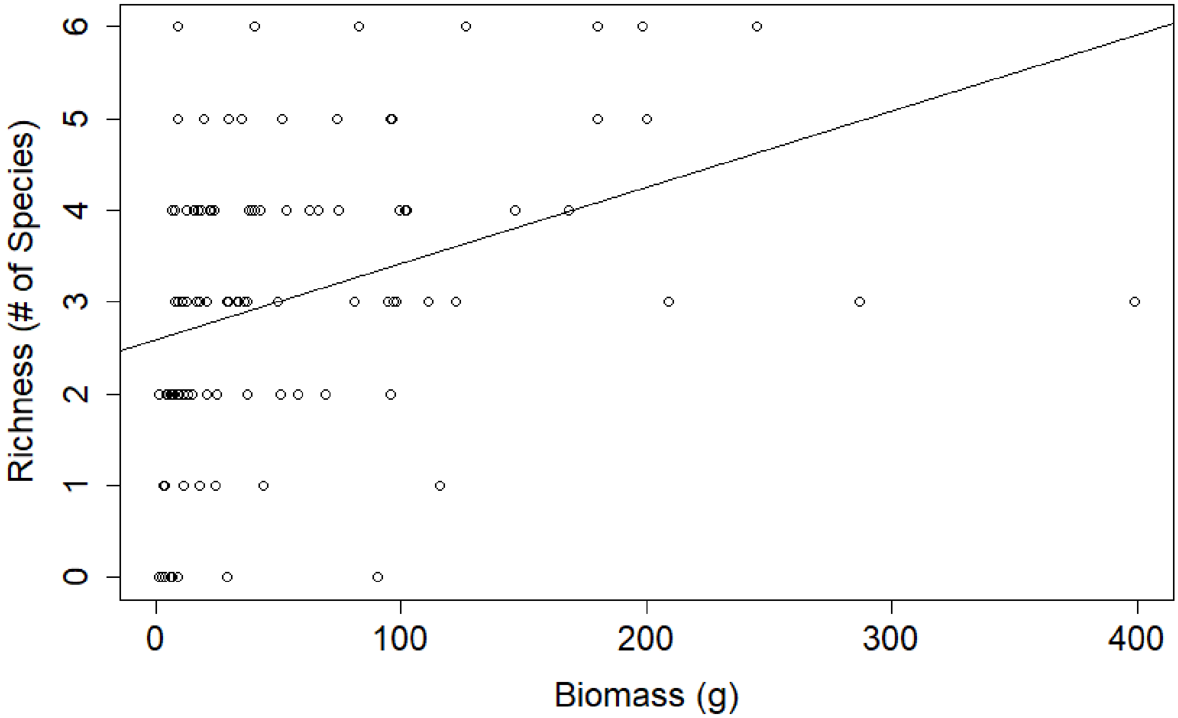

Figure 9 Correlation between biomass (of seagrass and *Gracilaria vermiculophylla*) and richness of macroinvertebrates ($R^2 = 0.36$, $p < 0.001$).

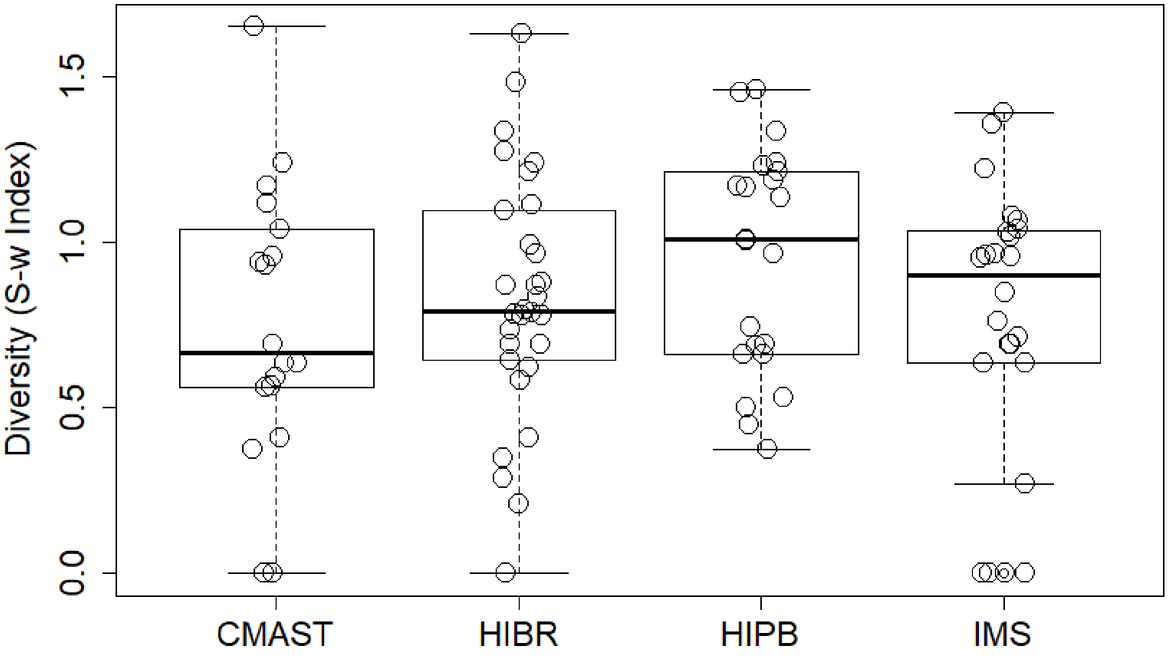

Figure 10 Comparisons of macroinvertebrate diversity (S-W Index = Shannon-Wiener Diversity Index) between sites (Kruskal-Wallis: df = 3, *p* = 0.37). Pairwise comparisons using Bonferroni's Correction Method were not significant (*p* > 0.005) for any pairs.

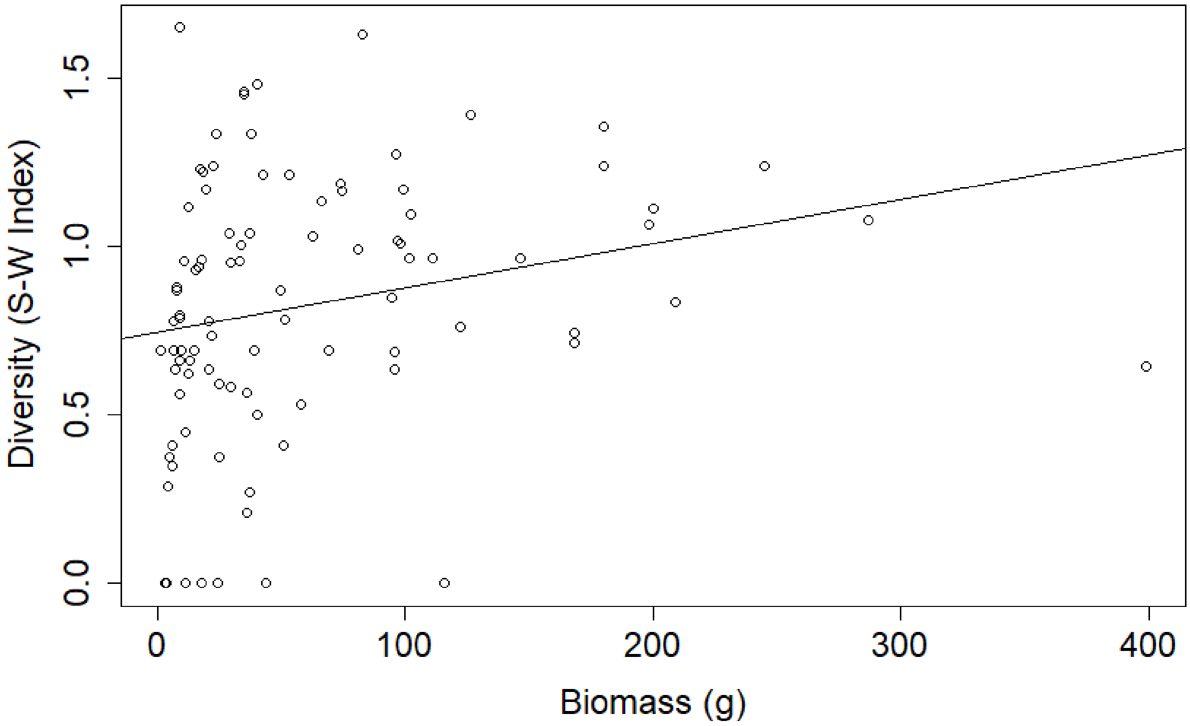

Figure 11 Correlation between biomass (of seagrass and *Gracilaria vermiculophylla*) and diversity of macroinvertebrates ($R^2 = 0.23$, $p = 0.02$).

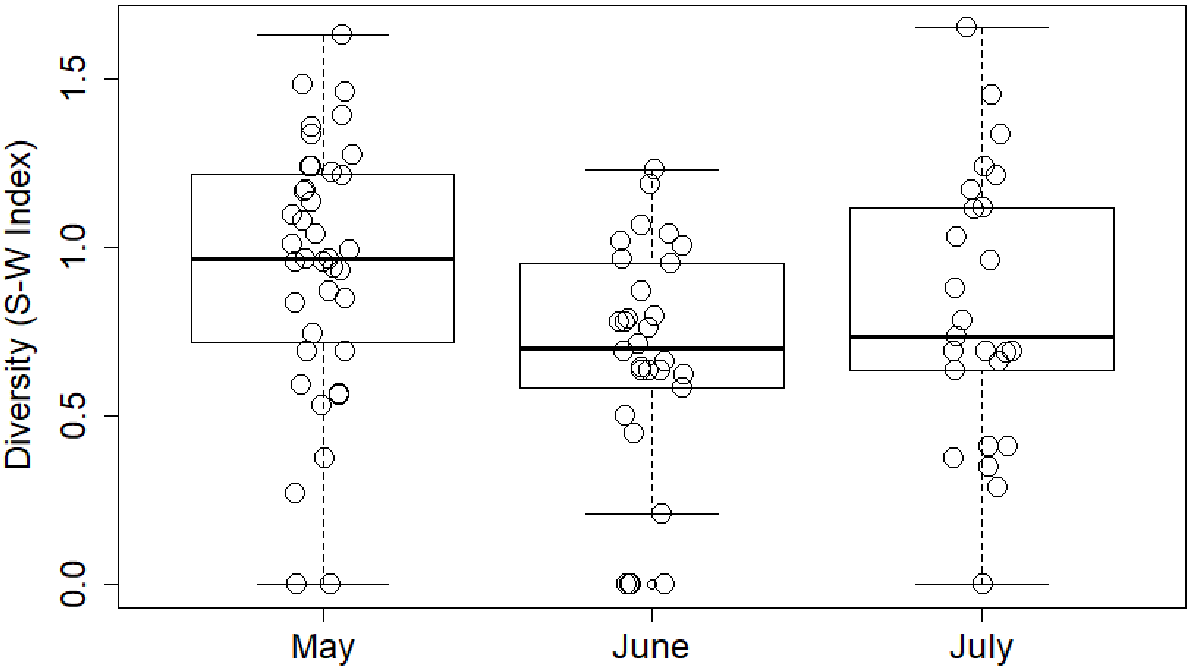

Figure 12 Comparisons of macroinvertebrate diversity (S-W Index = Shannon-Wiener Diversity Index) between months (Kruskal-Wallis: df = 2, $p = 0.01$). Pairwise comparisons using Bonferroni's Correction Method were significant ($p < 0.005$) for May and June only.

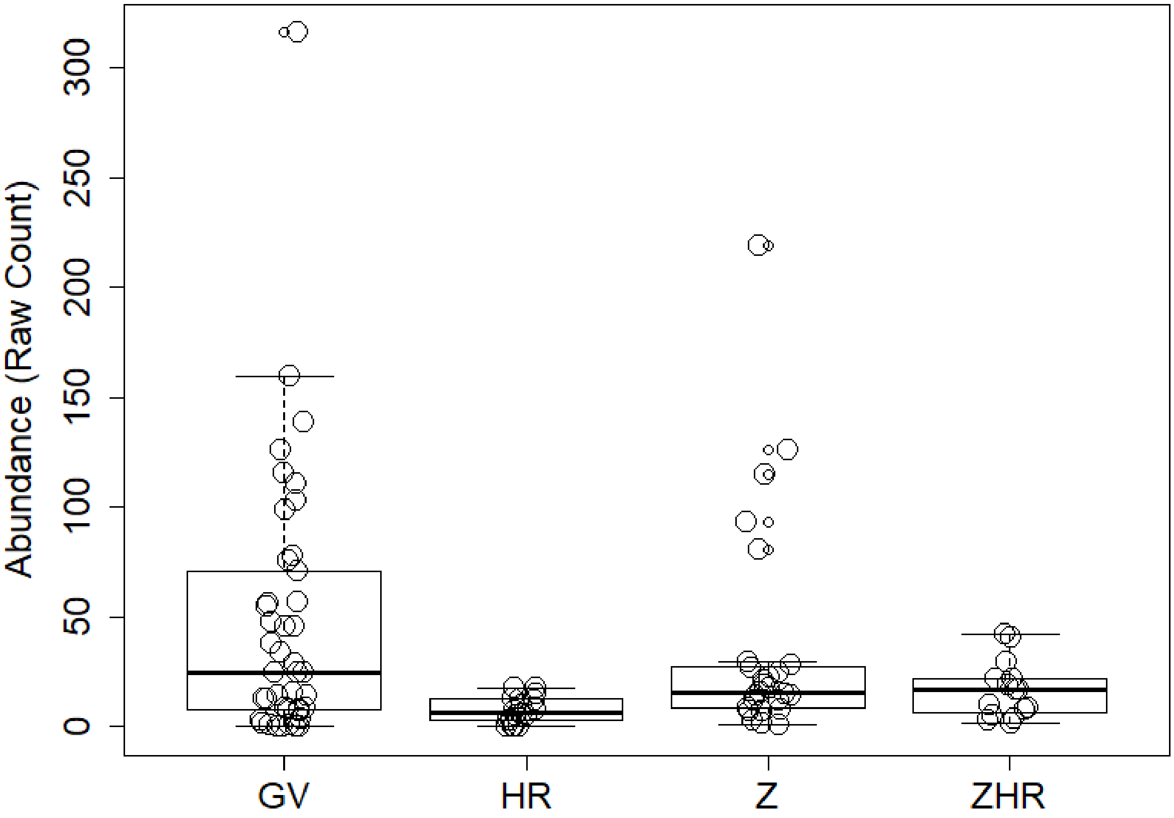

Figure 13 Comparisons of macroinvertebrate abundance between GV (*G. vermiculophylla*) and seagrass types (Kruskal-Wallis: df = 3, $p = 0.004$). See Methods for full names of the seagrass types. Pairwise comparisons using Bonferroni's Correction Method was significant ($p < 0.005$) for GV and HR only.

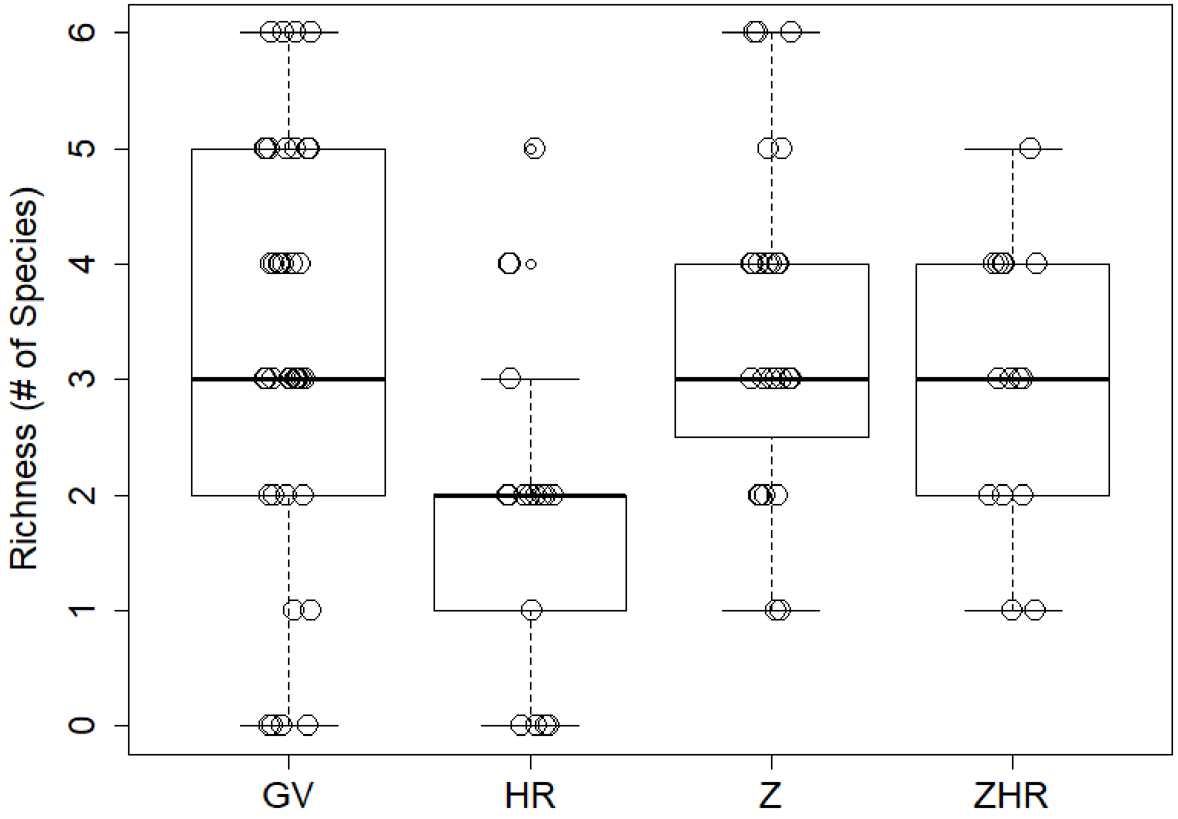

Figure 14 Comparisons of macroinvertebrate richness between GV (*G. vermiculophylla*) and seagrass types (Kruskal-Wallis: df = 3, $p = 0.008$). See Methods for full names of the seagrass types. Pairwise comparisons using Bonferroni's Correction Method were significant ($p < 0.005$) for GV and HR and HR and Z.

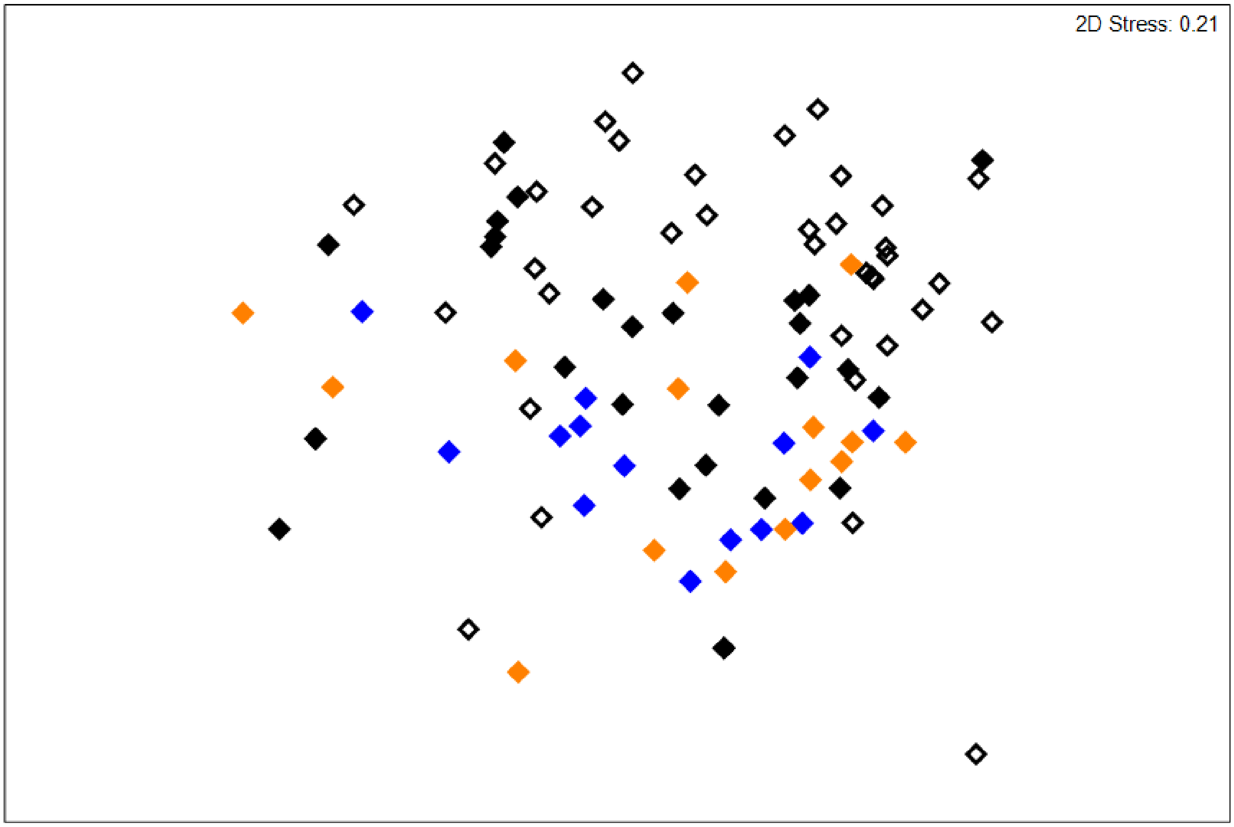

Figure 15 Non-metric multidimensional scaling of macroinvertebrate abundances by seagrass type. Samples that are closer to each other are more similar in terms of species composition and evenness. Solid black diamond = *Zostera marina*, solid blue diamond = *Halodule wrightii and Ruppia maritama*, solid orange diamond = *Zostera marina* + *Halodule wrightii and Ruppia maritama*, open black diamond = *Gracilaria vermiculophylla*.

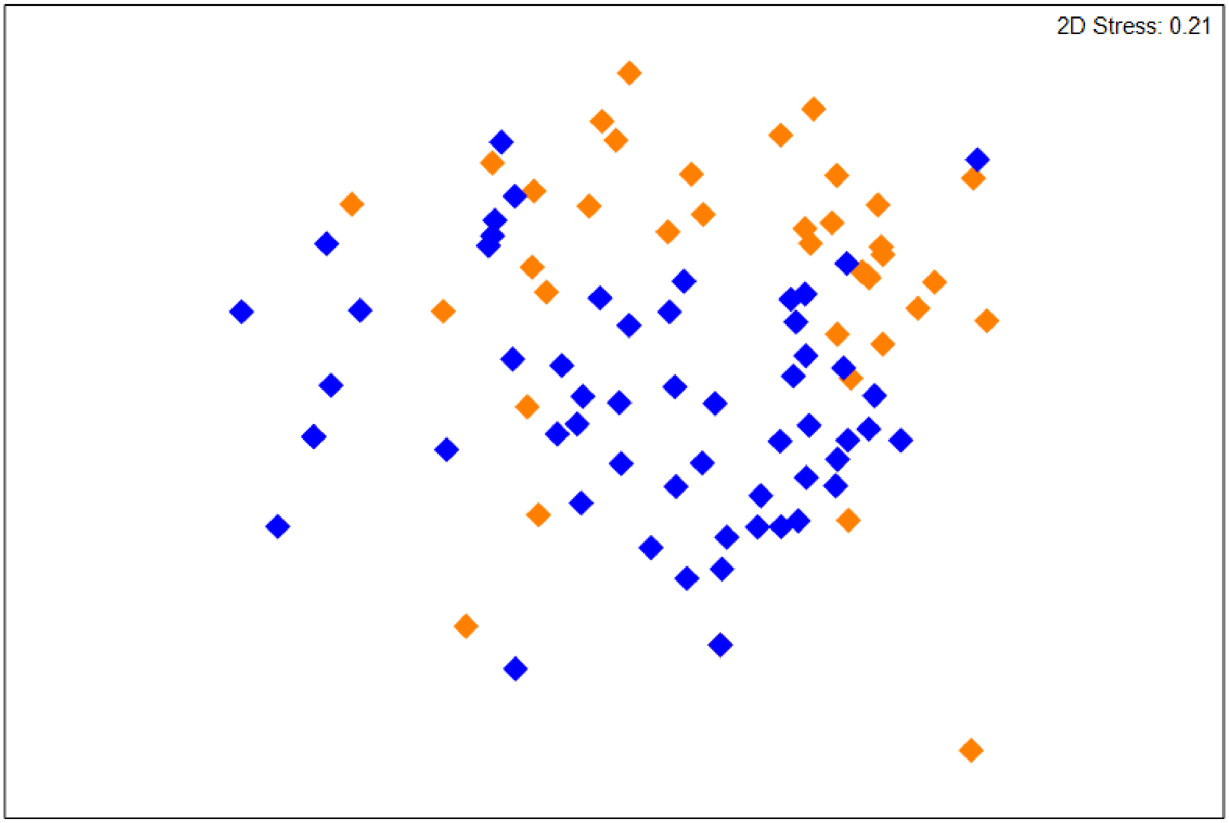

Figure 16 Non-metric multidimensional scaling of macroinvertebrate abundances by seagrass or seaweed category. Samples that are closer to each other are more similar in terms of species composition and evenness. Blue diamond = seagrass, orange diamond = seaweed, or *G. vermiculophylla*.

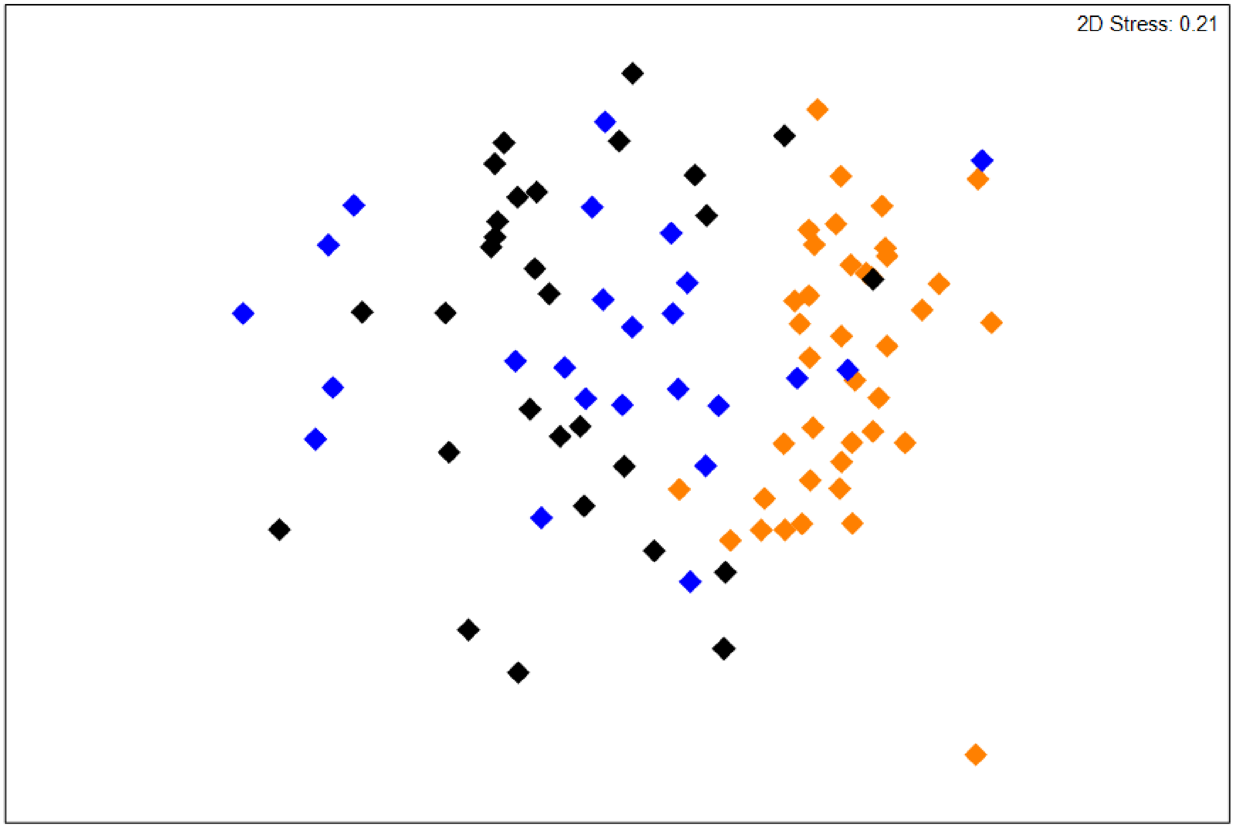

Figure 17 Non-metric multidimensional scaling of macroinvertebrate abundances by month. Samples that are closer to each other are more similar in terms of species composition and evenness. Orange diamond = May, black diamond = June, blue diamond = July.

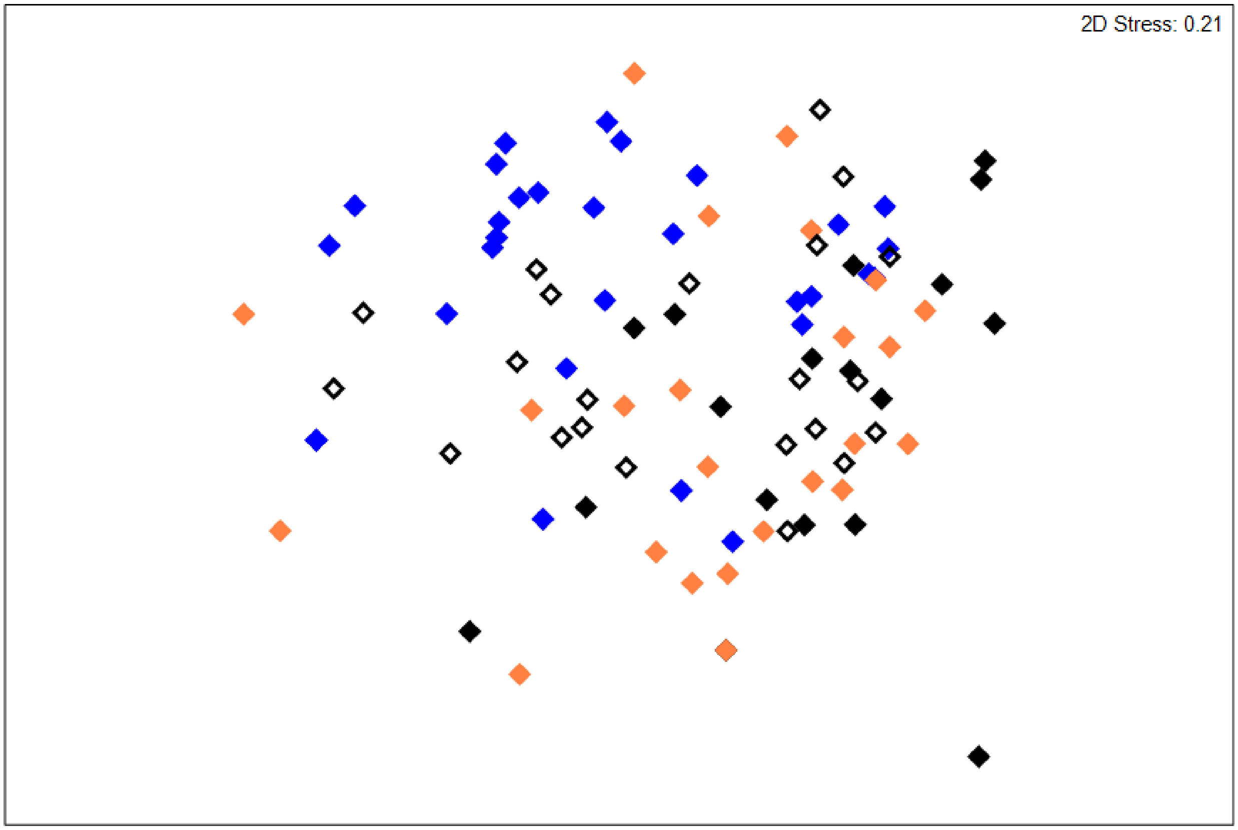

Figure 18 Non-metric multidimensional scaling of macroinvertebrate abundances by sites. Samples that are closer to each other are more similar in terms of species composition and evenness. Solid black diamond = CMAST, solid orange diamond = IMS, solid blue diamond = HIBR, open black diamond = HIPB.

CHAPTER 3 TABLES

Table 1 Sampling locations and their dates, coordinates, water temperature (CMAST = Center for Marine Sciences and Technology, IMS = UNC Institute of Marine Sciences, HIBR = Harkers Island Boat Ramp, HIPB = Harkers Island Public Beach).

Site	Dates	Latitude	Longitude	Water Temp (Celsius)	Sampling Personnel
CMAST	5/26/2021	34.722383	-76.760757	30.4	T. Lee
	6/26/2021	34.722383	-76.760757	30.7	T. Lee, L. Oliveira
	7/19/2021	34.722383	-76.760757	27	T. Lee, L. Oliveira
IMS	5/26/2021	34.722381	-76.752395	28.1	T. Lee
	6/26/2021	34.722381	-76.752395	27.3	T. Lee, L. Oliveira
	7/19/2021	34.722381	-76.752395	27.5	T. Lee, L. Oliveira
HIBR	5/24/2021	34.722262	-76.575331	32.1	T. Lee, J. Spoon, N. Spoon, C. Spoon, L. Spoon
	6/25/2021	34.722262	-76.575331	26.3	T. Lee, L. Oliveira
	7/18/2021	34.722262	-76.575331	27.8	T. Lee, L. Oliveira
HIPB	5/27/2021	34.713983	-76.578948	28.9	T. Lee
	6/25/2021	34.713983	-76.578948	28.1	T. Lee, L. Oliveira
	7/18/2021	34.713983	-76.578948	29.3	T. Lee, L. Oliveira

Table 2 Proportion of macroinvertebrates overall that comprise cumulative >95% out of 19 total species (N = 3,330).

N = 3,330	
Taxa	**%**
Mitrella lunata	38.11
Ampithoe longimana	19.28
Bittium varium	14.62
Ampithoe valida	13.39
Gammarus mucronatus	8.53
Myrianida sp.	2.01

Table 3 Proportion of macroinvertebrates between *Gracilaria vermiculophylla* and seagrass that comprise cumulative >95% in each group.

Gracilaria vermiculophylla (n = 2001)		**Seagrass** (n = 1,329)	
Taxa	**%**	**Taxa**	**%**
Mitrella lunata	39.43	*Mitrella lunata*	36.12
Ampithoe valida	19.04	*Ampithoe longimana*	22.65
Ampithoe longimana	17.04	*Bittium varium*	20.69
Bittium varium	10.59	*Gammarus mucronatus*	6.09
Gammarus mucronatus	10.14	*Ampithoe valida*	4.89
		Caprella sp.	3.46
		Myrianida sp.	3.39

Table 4 Proportion of macroinvertebrates between months that comprise cumulative >95% in each group.

May (n = 1,110)		**June** (n = 1,830)		**July** (n = 390)	
Taxa	**%**	**Taxa**	**%**	**Taxa**	**%**
Ampithoe longimana	37.48	*Mitrella lunata*	66.67	*Bittium varium*	36.67
Ampithoe valida	28.74	*Bittium varium*	18.63	*Ampithoe longimana*	28.72
Gammarus mucronatus	23.78	*Ampithoe longimana*	6.23	*Mitrella lunata*	10.51
Caprella sp.	4.95	*Ampithoe valida*	5.19	*Ampithoe valida*	8.21
Mercenaria sp.	1.71			*Myrianida* sp.	6.41
				Cyathura polita	3.85
				Gammarus mucronatus	2.31

Table 5 Proportion of macroinvertebrates between sites that comprise cumulative >95% in each group. See Table 1 for full names of the sites.

CMAST (n = 192)		**HIBR** (n = 1,964)		**HIPB** (n = 575)		**IMS** (n = 599)	
Taxa	**%**	**Taxa**	**%**	**Taxa**	**%**	**Taxa**	**%**
Ampithoe longimana	38.54	*Mitrella lunata*	51.27	*Ampithoe longimana*	25.39	*Ampithoe longimana*	30.05
Ampithoe valida	27.60	*Bittium varium*	20.37	*Ampithoe valida*	19.48	*Mitrella lunata*	28.05
Mitrella lunata	8.85	*Ampithoe longimana*	12.32	*Gammarus mucronatus*	17.22	*Ampithoe valida*	18.03
Gammarus mucronatus	7.81	*Ampithoe valida*	8.81	*Mitrella lunata*	13.39	*Gammarus mucronatus*	14.69
Caprella sp.	6.77	*Gammarus mucronatus*	4.18	*Bittium varium*	12.70	*Caprella* sp.	2.84
Myrianida sp.	6.25			*Caprella* sp.	6.09	*Myrianida* sp.	2.34
				Myrianida sp.	4.17		

Table 6 Proportion of macroinvertebrates between seagrass types that comprise cumulative >95% in each group. HR = *Halodule wrightii* and *Ruppia maritama*, Z = *Zostera marina*, ZHR = *Zostera marina* and *Halodule wrightii* and *Ruppia maritama*.

Gracilaria vermiculophylla (n = 2001)		**HR** (n = 136)		**Z** (n = 942)		**ZHR** (n = 251)	
Taxa	**%**	**Taxa**	**%**	**Taxa**	**%**	**Taxa**	**%**
Mitrella lunata	39.43	*Ampithoe longimana*	49.26	*Mitrella lunata*	47.45	*Ampithoe longimana*	34.26
Ampithoe valida	19.04	*Bittium varium*	22.06	*Bittium varium*	24.73	*Gammarus mucronatus*	25.90
Ampithoe longimana	17.04	*Caprella* sp.	8.82	*Ampithoe longimana*	15.71	*Caprella* sp.	11.55
Bittium varium	10.59	*Myrianida* sp.	5.15	*Ampithoe valida*	4.14	*Mitrella lunata*	10.76

Gammarus mucronatus	10.14	*Ampithoe valida*	4.41	*Myrianida* sp.	3.29	*Ampithoe valida*	7.97
		Mitrella lunata	4.41			*Bittium varium*	4.78
		Gammarus mucronatus	2.94				

Table 7 AICc table for Negative Binomial GLMM testing response variable “abundance” (total number of invertebrates) with site as random effect. Month = May, June, July; gvorsg = *G. vermiculophylla* or seagrass; wet = wet weight, or biomass (g).

	K	AICc	ΔAICc	AICc Wt.	Cumulative Wt.	LL
(1|site) + month*salinity + gvorsg + wet	10	822.08	0	0.99	0.99	-399.83
(1|site) + month + gvorsg + wet + salinity	8	833.72	11.63	0	0.99	-408.08
(1|site) + month + wet + salinity	7	833.89	11.81	0	0.99	-409.35
(1|site) + month + gvorsg + wet + depth + watertemp + salinity	10	834.7	12.61	0	0.99	-406.14
(1|site) + month + gvorsg + wet + depth + watertemp + oxygen + salinity	11	835.26	13.17	0	1	-405.16
(1|site) + month*gvorsg + wet + salinity	10	835.81	13.73	0	1	-406.7
(1|site) + month + gvorsg*salinity + wet	9	835.91	13.82	0	1	-407.98
(1|site) + month + gvorsg + wet + watertemp + salinity	11	836.02	13.94	0	1	-405.54
(1|site) + month + gvorsg*wet + salinity	9	836.11	14.02	0	1	-408.07
(1|site) + month*wet + gvorsg + salinity	10	838.02	15.94	0	1	-407.8
(1|site) + month + salinity	6	848.76	26.67	0	1	-417.94
(1|site) + wet + salinity	5	857.55	35.46	0	1	-423.46
(1|site) + salinity	4	865.1	43.01	0	1	-428.34
(1|site) + wet	4	872.48	50.4	0	1	-432.03
(1|site) + month + wet	6	874.2	52.11	0	1	-430.66
NULL	3	884.28	62.2	0	1	-439.02
(1|site) + month	5	885.47	63.39	0	1	-437.42

Table 8 Model output for the top performing model for abundance in Table 7. Formula = Abundance ~ (1|site) + month*salinity + gvorsg + wet (gvorsg = *G. vermiculophylla* or seagrass; wet = wet weight, or biomass in grams). Random intercept of the site has variance = 0.072, and standard deviation = 0.27. Conditional R^2 = 0.785, Marginal R^2 = 0.753.

	β Estimate	Standard Error	z-value	p-value
(Intercept)	3.452025	0.432285	7.986	1.40E-15
month (June)	0.147399	0.410822	0.359	0.71975
month (May)	-0.612181	0.501645	-1.22	0.22233
salinity	0.442454	0.398326	1.111	0.26666
gvorsg (seagrass)	-0.373012	0.224145	-1.664	0.09608

wet	0.352156	0.130724	2.694	0.00706
month (June)*salinity	1.594488	0.513956	3.102	0.00192
month (May)*salinity	-0.001838	0.451861	-0.004	0.99675

Table 9 AICc table for Gaussian GLMM testing response variable “richness” (total number of species) with site as random effect. Month = May, June, July; gvorsg = *G. vermiculophylla* or seagrass; wet = wet weight, or biomass (g).

	K	AICc	ΔAICc	AICc Wt.	Cumulative Wt.	LL
(1|site) + month + wet + salinity	7	362.37	0	0.39	0.39	-173.59
(1|site) + month*salinity + gvorsg + wet	10	363.89	1.52	0.18	0.57	-170.74
(1|site) + month + gvorsg + wet + salinity	8	364.64	2.27	0.12	0.7	-173.55
(1|site) + month + salinity	6	365.66	3.29	0.08	0.77	-176.39
(1|site) + month*gvorsg + wet + salinity	10	365.75	3.39	0.07	0.84	-171.67
(1|site) + month + gvorsg*wet + salinity	9	366.36	3.99	0.05	0.9	-173.2
(1|site) + month + gvorsg*salinity	9	367.61	5.24	0.03	0.92	-173.83
(1|site) + month + gvorsg + wet + watertemp + salinity	9	367.95	5.58	0.02	0.95	-174
(1|site) + month + gvorsg + wet + depth + watertemp + oxygen + salinity	11	368.72	6.35	0.02	0.96	-171.89
(1|site) + wet + salinity	5	369.39	7.03	0.01	0.98	-179.38
(1|site) + month*wet + gvorsg + salinity	10	369.44	7.08	0.01	0.99	-173.51
(1|site) + salinity	4	370.29	7.92	0.01	0.99	-180.94
(1|site) + month + gvorsg + wet + depth + watertem + salinity	10	371.18	8.82	0	1	-174.38
(1|site) + month + wet	6	375.42	13.06	0	1	-181.27
(1|site) + wet	4	375.44	13.07	0	1	-183.51
(1|site) + month	5	378.86	16.49	0	1	-184.12
NULL	3	380.59	18.23	0	1	-187.17

Table 10 Model output for the top performing model for richness in Table 9. Formula = Richness ~ (1|site) + month + wet + salinity (wet = wet weight, or biomass in grams). Parameter estimates with Chi-square and *p*-values based on Type III Wald Chi-Square Test. Random intercept of the site has variance = 0.12, and standard deviation = 0.35. Conditional R^2 = 0.362, Marginal R^2 = 0.316.

	Chi-sq	df	p-value
(Intercept)	125.829	1	< 2.2e-16
month	12.421	2	0.002008

wet	7.915	1	0.004903
salinity	18.097	1	2.10E-05

Table 11 Model output for the second highest performing model for richness in Table 9. Formula = Richness ~ (1|site) + month*salinity + gvorsg + wet (wet = wet weight, or biomass in grams, gvorsg = seagrass or *G. vermiculophylla*). Parameter estimates with Chi-square and *p*-values based on Type III Wald Chi-Square Test. Random intercept of the site has variance = 0.013, and standard deviation = 0.11. Conditional R^2 = 0.358, Marginal R^2 = 0.353.

	Chi-sq	df	p-value
(Intercept)	38.4075	1	5.74E-10
month	0.8282	2	0.66093
salinity	0.0425	1	0.83674
gvorsg	0.4177	1	0.51807
wet	4.4573	1	0.03475
month:salinity	6.0915	2	0.04756

Table 12 Model output for the third highest performing model for richness in Table 9. Formula = Richness ~ (1|site) + month + gvorsg + wet salinity (wet = wet weight, or biomass in grams, gvorsg = seagrass or *G. vermiculophylla*). Parameter estimates with Chi-square and *p*-values based on Type III Wald Chi-Square Test. Random intercept of the site has variance = 0.113, and standard deviation = 0.336. Conditional R^2 = 0.358, Marginal R^2 = 0.316.

	Chi-sq	df	p-value
(Intercept)	92.7462	1	< 2.2e-16
month	12.9441	2	0.001546
gvorsg	0.557	1	0.45547
wet	4.2661	1	0.03888
salinity	18.6505	1	1.57E-05

Table 13 AICc table for Gaussian GLMM testing response variable "diversity" (Shannon-Wiener Diversity Index) with site as random effect. Month = May, June, July; gvorsg = *G. vermiculophylla* or seagrass; wet = wet weight, or biomass (g).

	K	AICc	ΔAICc	AICc Wt.	Cumulative Wt.	LL
NULL	3	99.27	0	0.46	0.46	-46.5
(1|site) + wet	4	100.76	1.49	0.22	0.69	-46.15
(1|site) + month	5	101.02	1.76	0.19	0.88	-45.17
(1|site) + month + wet	6	103.96	4.7	0.04	0.92	-45.5
(1|site) + month + salinity	6	104.37	5.1	0.04	0.96	-45.7
(1|site) + wet + salinity	5	104.48	5.22	0.03	0.99	-46.9

(1|site) + month + wet + salinity	7	107.98	8.72	0.01	1	-46.34
(1|site) + month + gvorsg + wet + salinity	8	113.19	13.92	0	1	-47.75
(1|site) + month + gvorsg*wet + salinity	9	117.22	17.95	0	1	-48.54
(1|site) + month + gvorsg*salinity + wet	9	118.76	19.5	0	1	-49.31
(1|site) + month + gvorsg + wet + watertemp + salinity	9	118.93	19.67	0	1	-49.4
(1|site) + month*gvorsg + wet + salinity	10	120.66	21.39	0	1	-49
(1|site) + month*salinity + gvorsg + wet	10	121.77	22.51	0	1	-49.56
(1|site) + month*wet + gvorsg + salinity	10	123.19	23.93	0	1	-50.27
(1|site) + month + gvorsg + wet + depth + watertemp + salinity	10	125.42	26.15	0	1	-51.38
(1|site) + month + gvorsg + wet + depth + watertemp + oxygen + salinity	11	129.83	30.57	0	1	-52.31

Table 14 Model output for the highest performing model for diversity in Table 13. Formula = Diversity ~ (1|site). Parameter estimates with Chi-square and *p*-values based on Type III Wald Chi-Square Test. Random intercept of the site has variance = 0.0007, and standard deviation = 0.026. Conditional R^2 = 0.005, Marginal R^2 = 0.000.

	Chi-sq	df	p-value
(Intercept)	381.21	1	< 2.2e-16

Table 15 Model output for the second highest performing model for diversity in Table 13. Formula = Diversity ~ (1|site) + wet (wet = wet weight, or biomass in grams). Parameter estimates with Chi-square and *p*-values based on Type III Wald Chi-Square Test. Random intercept of the site has variance = 0.0015, and standard deviation = 0.039. Conditional R^2 = 0.066, Marginal R^2 = 0.056.

	Chi-sq	df	p-value
(Intercept)	354.5041	1	< 2e-16
wet	5.4269	1	0.01983

Table 16 Model output for the third highest performing model for diversity in Table 13. Formula = Diversity ~ (1|site) + month. Parameter estimates with Chi-square and *p*-values based on Type III Wald Chi-Square Test. Random intercept of the site has variance = 0.0022, and standard deviation = 0.047. Conditional R^2 = 0.102, Marginal R^2 = 0.087.

	Chi-sq	df	p-value
(Intercept)	108.8558	1	< 2e-16
month	8.9495	2	0.01139

Table 17 Comparison of macroinvertebrate abundance between May and June based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 79.01						
	May	June				

Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Mitrella lunata	0.13	4.28	18.52	1.05	23.44	23.44
Ampithoe longimana	2.72	1.48	12.54	1.23	15.88	39.31
Ampithoe valida	2.05	0.86	12.45	1.04	15.76	55.07
Bittium varium	0.06	2.16	10.71	0.96	13.55	68.63
Gammarus mucronatus	1.94	0.17	10.64	1.13	13.46	82.09
Caprella sp.	0.75	0.18	5.45	0.77	6.9	88.99
Myrianida sp.	0.27	0.18	3.69	0.78	4.67	93.66
Mercenaria sp.	0.3	0.18	1.83	0.46	2.32	95.98

Table 18 Comparison of macroinvertebrate abundance between May and July based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 74.73						
	May	July				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Ampithoe longimana	2.72	1.62	14.38	1.3	19.24	19.24
Ampithoe valida	2.05	0.69	12.83	1.14	17.17	36.41
Bittium varium	0.06	1.75	12.7	1.08	16.99	53.4
Gammarus mucronatus	1.94	0.26	11.59	1.25	15.51	68.91
Caprella sp.	0.75	0.06	5.85	0.82	7.83	76.74
Mitrella lunata	0.13	0.63	4.95	0.6	6.63	83.37
Myrianida sp.	0.27	0.58	4.35	0.83	5.82	89.18
Cyathura polita	0.2	0.33	3.16	0.61	4.23	93.41
Mercenaria sp.	0.3	0.04	2.04	0.49	2.73	96.15

Table 19 Comparison of macroinvertebrate abundance between June and July based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 72.21						
	June	July				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %

Mitrella lunata	4.28	0.63	21.02	1.21	29.11	29.11
Bittium varium	2.16	1.75	15.36	1.24	21.28	50.39
Ampithoe longimana	1.48	1.62	11.32	1.11	15.68	66.06
Ampithoe valida	0.86	0.69	7.63	0.79	10.57	76.63
Myrianida sp.	0.55	0.58	5.33	0.89	7.38	84.01
Cyathura polita	0.13	0.33	2.69	0.51	3.73	87.74
Gammarus mucronatus	0.17	0.26	2.64	0.53	3.66	91.4
Caprella sp.	0.18	0.06	1.95	0.38	2.7	94.1
Litopenaeus setiferus	0.03	0.19	1.35	0.42	1.88	95.98

Table 20 Comparison of macroinvertebrate abundance between HIBR and IMS based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 74.53						
	HIBR	IMS				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Mitrella lunata	3.24	1.08	16.38	0.89	21.98	21.98
Bittium varium	2.5	0.3	14	1.08	18.78	40.76
Ampithoe longimana	2.18	2.16	12.14	1.17	16.29	57.05
Ampithoe valida	1.53	1.14	10	0.99	13.42	70.47
Gammarus mucronatus	0.81	1.19	8.05	0.94	10.8	81.27
Cyathura polita	0.57	0.08	3.66	0.7	4.91	86.18
Myrianida sp.	0.42	0.37	3.48	0.76	4.66	90.84
Caprella sp.	0	0.44	2.38	0.53	3.2	94.04
Mercenaria sp.	0.32	0.08	2.33	0.5	3.12	97.16

Table 21 Comparison of macroinvertebrate abundance between HIBR and CMAST based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 76.82						
	HIBR	CMAST				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Mitrella lunata	3.24	0.37	16.07	0.83	20.92	20.92

Bittium varium	2.5	0.17	15.4	1.09	20.05	40.97
Ampithoe longimana	2.18	1.58	12.35	1.17	16.08	57.05
Ampithoe valida	1.53	1.22	11.31	1.03	14.73	71.78
Gammarus mucronatus	0.81	0.5	6.25	0.83	8.14	79.91
Caprella sp.	0	0.54	4.13	0.68	5.38	85.29
Cyathura polita	0.57	0.06	3.95	0.71	5.15	90.44
Myrianida sp.	0.42	0.37	3.74	0.77	4.87	95.31

Table 22 Comparison of macroinvertebrate abundance between IMS and CMAST based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 69.94						
	IMS	CMAST				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Ampithoe longimana	2.16	1.58	15.77	1.27	22.55	22.55
Ampithoe valida	1.14	1.22	13.71	1.03	19.6	42.15
Gammarus mucronatus	1.19	0.5	10.43	0.99	14.92	57.06
Mitrella lunata	1.08	0.37	8.82	0.66	12.62	69.68
Caprella sp.	0.44	0.54	6.76	0.84	9.66	79.34
Myrianida sp.	0.37	0.37	4.4	0.72	6.29	85.63
Bittium varium	0.3	0.17	4.05	0.53	5.79	91.43
Cyathura polita	0.08	0.06	1.27	0.32	1.82	93.25
Litopenaeus setiferus	0.04	0.08	0.92	0.3	1.32	94.56
Boonea seminuda	0.08	0.06	0.9	0.36	1.29	95.85

Table 23 Comparison of macroinvertebrate abundance between HIBR and HIPB based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 70.55						
	HIBR	HIPB				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Mitrella lunata	3.24	0.89	15.23	0.91	21.59	21.59
Bittium varium	2.5	1.17	12.9	1.27	18.28	39.87

Ampithoe longimana	2.18	2.05	10.48	1.19	14.85	54.72
Ampithoe valida	1.53	1.26	9.9	1.02	14.03	68.75
Gammarus mucronatus	0.81	1.16	7.18	0.91	10.18	78.93
Caprella sp.	0	0.72	4.02	0.67	5.7	84.63
Myrianida sp.	0.42	0.62	4	0.87	5.67	90.3
Cyathura polita	0.57	0	3.21	0.7	4.55	94.85
Mercenaria sp.	0.32	0.11	2.19	0.53	3.1	97.95

Table 24 Comparison of macroinvertebrate abundance between IMS and HIPB based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 69.26						
	IMS	HIPB				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Ampithoe longimana	2.16	2.05	12.73	1.36	18.38	18.38
Ampithoe valida	1.14	1.26	10.78	0.94	15.57	33.95
Gammarus mucronatus	1.19	1.16	10.24	1.06	14.79	48.74
Mitrella lunata	1.08	0.89	9.7	0.76	14.01	62.75
Bittium varium	0.3	1.17	9.47	0.83	13.67	76.42
Caprella sp.	0.44	0.72	5.95	0.82	8.6	85.02
Myrianida sp.	0.37	0.62	4.99	0.88	7.21	92.23
Litopenaeus setiferus	0.04	0.16	1.15	0.41	1.66	93.89
Mercenaria sp.	0.08	0.11	1.14	0.4	1.65	95.54

Table 25 Comparison of macroinvertebrate abundance between CMAST and HIPB based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 69.72						
	CMAST	HIPB				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Ampithoe longimana	1.58	2.05	13.18	1.34	18.91	18.91
Ampithoe valida	1.22	1.26	12.73	1.1	18.26	37.17

Bittium varium	0.17	1.17	10.41	0.83	14.93	52.1
Gammarus mucronatus	0.5	1.16	8.85	0.97	12.69	64.79
Mitrella lunata	0.37	0.89	7.92	0.68	11.36	76.15
Caprella sp.	0.54	0.72	7.04	0.98	10.1	86.26
Myrianida sp.	0.37	0.62	5.52	0.89	7.91	94.17
Litopenaeus setiferus	0.08	0.16	1.48	0.43	2.13	96.3

Table 26 Comparison of macroinvertebrate abundance between Z and HR based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 68.22						
	Z	HR				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Bittium varium	1.73	0.92	14.09	1.15	20.65	20.65
Ampithoe longimana	1.96	1.93	12.97	1.07	19.01	39.67
Mitrella lunata	2.01	0.17	11.96	0.74	17.53	57.2
Ampithoe valida	0.74	0.23	7.39	0.75	10.83	68.04
Myrianida sp.	0.71	0.37	5.86	0.95	8.59	76.62
Caprella sp.	0.16	0.48	4.56	0.69	6.68	83.3
Gammarus mucronatus	0.28	0.2	3.38	0.6	4.95	88.25
Cyathura polita	0.25	0.07	2.51	0.52	3.68	91.93
Mercenaria sp.	0.25	0.07	2.36	0.47	3.45	95.38

Table 27 Comparison of macroinvertebrate abundance between Z and GV based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 72.78						
	Z	GV				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Mitrella lunata	2.01	2.14	14.8	0.87	20.33	20.33
Ampithoe valida	0.74	2.47	12.43	1.17	17.08	37.42
Ampithoe longimana	1.96	2.13	12	1.28	16.48	53.9
Bittium varium	1.73	1.18	11.32	0.95	15.55	69.45

Gammarus mucronatus	0.28	1.5	8.13	0.98	11.17	80.62
Myrianida sp.	0.71	0.32	4.27	0.87	5.87	86.49
Caprella sp.	0.16	0.35	2.98	0.58	4.1	90.59
Cyathura polita	0.25	0.32	2.71	0.61	3.73	94.32
Mercenaria sp.	0.25	0.14	1.93	0.47	2.66	96.97

Table 28 Comparison of macroinvertebrate abundance between HR and GV based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 75.30						
	HR	GV				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Ampithoe valida	0.23	2.47	15.32	1.29	20.35	20.35
Ampithoe longimana	1.93	2.13	13.97	1.34	18.55	38.9
Mitrella lunata	0.17	2.14	11.19	0.64	14.86	53.76
Bittium varium	0.92	1.18	11.14	0.92	14.79	68.55
Gammarus mucronatus	0.2	1.5	9.48	1.02	12.59	81.14
Caprella sp.	0.48	0.35	4.92	0.74	6.54	87.68
Myrianida sp.	0.37	0.32	3.75	0.71	4.98	92.67
Cyathura polita	0.07	0.32	2.59	0.51	3.44	96.11

Table 29 Comparison of macroinvertebrate abundance between Z and ZHR based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 71.91						
	Z	ZHR				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Mitrella lunata	2.01	0.76	13.06	0.91	18.17	18.17
Ampithoe longimana	1.96	2	12.46	1.09	17.32	35.49
Bittium varium	1.73	0.44	11.25	0.97	15.64	51.13
Gammarus mucronatus	0.28	1.35	9.25	0.92	12.86	63.99
Ampithoe valida	0.74	0.41	7.38	0.75	10.27	74.26
Caprella sp.	0.16	0.79	5.92	0.77	8.23	82.49

Myrianida sp.	0.71	0.34	5.17	0.93	7.19	89.68
Mercenaria sp.	0.25	0.07	2.34	0.47	3.25	92.93
Cyathura polita	0.25	0	1.75	0.47	2.44	95.36

Table 30 Comparison of macroinvertebrate abundance between HR and ZHR based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 66.29						
	HR	ZHR				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Ampithoe longimana	1.93	2	13.82	1.17	20.84	20.84
Gammarus mucronatus	0.2	1.35	10.77	0.94	16.25	37.09
Bittium varium	0.92	0.44	10.22	0.93	15.41	52.51
Caprella sp.	0.48	0.79	8.15	0.9	12.3	64.8
Mitrella lunata	0.17	0.76	7.85	0.7	11.84	76.64
Ampithoe valida	0.23	0.41	5.05	0.52	7.61	84.25
Myrianida sp.	0.37	0.34	4.84	0.8	7.31	91.56
Mercenaria sp.	0.07	0.07	1.3	0.36	1.96	93.52
Litopenaeus setiferus	0	0.16	1.21	0.38	1.83	95.35

Table 31 Comparison of macroinvertebrate abundance between GV and ZHR based on Similarity of Percentage (SIMPER) results that represents >95% cumulative.

Average dissimilarity = 73.43						
	GV	ZHR				
Species	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	Dissimilarity St. Dev.	Contribution %	Cumulative %
Ampithoe valida	2.47	0.41	14.21	1.22	19.35	19.35
Ampithoe longimana	2.13	2	12.88	1.32	17.54	36.89
Mitrella lunata	2.14	0.76	12.57	0.8	17.12	54.02
Gammarus mucronatus	1.5	1.35	10.92	1.13	14.87	68.88
Bittium varium	1.18	0.44	8.48	0.77	11.55	80.43
Caprella sp.	0.35	0.79	5.8	0.81	7.89	88.33
Myrianida sp.	0.32	0.34	3.22	0.7	4.38	92.71

Cyathura polita	0.32	0	1.91	0.45	2.6	95.31