# EFFECTS OF AN INTRODUCED PRIMARY PRODUCER ON TROPHIC INTERACTIONS IN ESTUARIES OF THE SOUTHEASTERN USA

by

LINSEY E. HARAM

(Under the Direction of James E. Byers)

## ABSTRACT

Invasive species are one of the leading drivers of global ecological change. In particular, the introduction of primary producers can have profound effects on recipient communities, as these species, once established, can spread quickly, can affect multiple energy and nutrient pathways, and can acts as ecosystem engineers that modify or generate habitats. The recent invasion of the red macroalga, *Gracilaria vermiculophylla*, provides an opportunity to investigate the effects of a prominent non-native primary producer on the estuaries of the southeastern U.S.A. In this region, mudflat habitats were previously devoid of macroalgal primary production. However, since its introduction, *G. vermiculophylla* has transformed the estuarine ecosystems into a patchwork of macroalgal beds. *G. vermiculophylla* presents a novel basal resource as well as habitat type, and its effects on the local trophic structure and native species behaviors are unknown. Thus, this dissertation investigates the direct and indirect impacts of the non-native *G. vermiculophylla* on the trophic interactions of native consumers in estuaries of South Carolina and Georgia. This research aims to: 1) Determine how *Gracilaria vermiculophylla* directly alters trophic interactions of the southeastern mudflats; 2) Determine the indirect effects of *G. vermiculophylla*'s novel structure on the foraging behaviors of native species. As ecosystems continue to change in response to anthropogenic activities, insights into how introduced species alter community dynamics and native species behavioral responses are essential to predict the outcomes of future species introductions.

INDEX WORDS: Invasive species, macroalgae, ecosystem engineer, trophic interactions, mixed effects, foraging behavior, habitat selection, nutrient content

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# DEDICATION

With gratitude and humility, I dedicate this dissertation to my family, who fostered my love of science from an early age and supported my academic endeavors with love and patience. I also dedicate this document to my high school and undergraduate mentors. I could not have achieved my goals without their encouragement and wisdom. Finally, I dedicate my dissertation to the dozens of students that I taught and mentored during this process. Their enthusiasm and curiosity for the natural world was a daily source of inspiration.

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

Humans have altered their environments for thousands of years, recently leading to marked and accelerated global change (Vitousek et al. 1996, 1997; Ricciardi 2007; Hulme 2009; Jackson and Hobbs 2009). One major driver of rapid ecological change is the introduction of invasive species via human-mediated activities, such as the global shipping industry (Carlton 1989; Ricciardi 2007; Hulme 2009; Richardson and Gaertner 2013). With well over one hundred thousand introduced species identified across all continents and oceans, invasive species are one of the leading causes of global biodiversity loss (Vitousek 1990; Vitousek et al. 1997; Pimentel et al. 2000, 2001). Invasive species often proliferate in concert with other anthropogenic stressors, such as habitat degradation or non-point source pollution, making the management of invaded ecosystems logistically and economically challenging (Vitousek et al. 1996; Pimentel et al. 2000, 2001; Occhipinti-Ambrogi and Savini 2003). Furthermore, invasive species can transform recipient ecosystems, creating regime shifts and novel ecosystems, that are difficult to reverse (Hobbs et al. 2006; Lindenmayer et al. 2008; Kennedy et al. 2013; Morse et al. 2014). The greatest impacts of invasive species often result from the provision of a novel role, such as nitrogen-fixation or an additional trophic level, within their recipient ecosystems (Parker et al. 1999).

Introduced primary producers can be particularly transformative because they can influence multiple energy and nutrient pathways and can ecologically engineer their recipient ecosystems (Richardson et al. 2000; Berke 2010; Richardson and Gaertner 2013). Primary producer quality and quantity mediates ecosystem function through processes such as secondary production, nutrient cycling, and decomposition (Cebrian 1999). Non-native primary producers can increase the diversity and abundance of primary resources for native consumers in both live-tissue based and detritus based trophic pathways (Rodriguez et al. 2006). However, primary producer introductions can also create mismatches between the quality of food (often measured by the presence of essential nutrients/minerals and inhibitory secondary metabolites) and the nutritional and energetic requirements of native consumers, leading to dead-ends in the trophic structure (Bishop and Kelaher 2008). Alteration in the quality and quantity of primary resources can also change ecosystem function. For instance, introduced primary producers with stoichiometric ratios (C:N) that differ from those of native primary producers can alter the quality of available detritus and consequently accelerate or decelerate decomposition rates (Hladyz et al 2009; Taylor et al. 2010). Such changes in basal resources caused by the introduction of non-native primary producers can in turn cause shifts in consumer populations (Cross et al 2006; Bishop and Kelaher 2007, 2008). For example, the invasion of macroalgae, *Caulerpa taxifolia*, into estuarine soft-sediment communities decreased detritivore abundance, but increased predator abundance (Taylor et al. 2010). Such alterations to the recipient community can fundamentally alter the ecosystem's trophic structure, making a community-level understanding of the effects of introduced primary producers essential to predicting the net outcome of invasions.

Introduced primary producers can further affect secondary production and trophic interactions when invasive primary producers alter the native habitat's physical structure. Ecosystem engineers are organisms that generate, modify or maintain habitat and that alter resource availability for other organisms in the community (Jones et al. 1994, 1997; Crooks

2002; Wright and Jones 2006). The creation or alteration of physical habitat structure can lead to ecosystem-level changes to abiotic conditions, such as sediment composition, that influence community composition (Crooks 2002; Wallentinus and Nyberg 2007). Increased physical structure often leads to shifts in prey density to areas with greater structural complexity (Bially and MacIsaac 2000; Rodriguez 2006; Sousa et al. 2009), as structure can increase refugia for native fauna (Gotceitas and Colgan 1989; Schwindt et al. 2012; Bruschetti et al. 2009; Wright et al. 2014) and thus increase overall prey/predator biomass (Thayer et al. 1997; Rodriguez 2006). However, structure can negatively impact secondary production by decreasing predator foraging efficiency, leading to decreases in predator biomass (Crowder and Cooper 1982; Robinson and Holmes 1984; Thayer et al. 1997; Levi and Francour 2004).

Because invasive species rarely exist in isolation of other human-mediated disturbances, it is essential to assess the effects of invasive species in combination with other impacts (Cebrian 1999). One environmental stressor that directly affects the success of primary producers is nutrient pollution from human activities. Since the onset of nutrient-loading from agricultural practices and human waste, eutrophication of aquatic and marine systems has caused changes in the species composition and function of these ecosystems (Kates et al. 1990; Vitousek et al. 1997; Chapin et al. 2011; O’Gorman et al. 2012). Increased availability of phosphorus and nitrogen can increase primary productivity and biomass accumulation (Vitousek and Howarth, 1991; Valiela et al. 1997; Chapin et al. 2011), thus altering the rates and pathways of nutrient cycling and loss (Tilman 1987; Berendse et al. 1993; Aber et al. 1995). Nutrient pollution can impact the quality of primary producers by increasing nutrient content within the tissue, making them more attractive to herbivores (Valiela et al. 1997). Increased nutrients can also counteract the presence of chemical defenses, as heightened nutritional content may override negative effects on consumer fitness (Cruz-Rivera and Hay 2003). Furthermore, interspecific differences in nutrient uptake by primary producers can lead to patchy distributions of nutrient-rich basal resources. Thus, understanding how the quality of an introduced primary producer alters its effect on trophic interactions may illuminate patchy patterns of species interactions that may result from an introduced primary producer growing in concert with nutrient pollution.

## Study System

The aim of this dissertation is to identify the effects of an introduced species that is both a primary producer and ecosystem engineer on the estuarine ecosystems of the southeastern U.S.A. The recent biological invasion of estuaries in Georgia and South Carolina by a red seaweed, *Gracilaria vermiculophylla*, provided an opportunity to assess the impacts of a novel introduced species on the direct and indirect trophic interactions of multiple consumer guilds. Native to Japan, *G. vermiculophylla* has established on the east and west coasts of North America and on the Atlantic, Mediterranean and Baltic coasts of Europe (reviewed in Hu and Juan 2014). *G. vermiculophylla* was introduced to Europe and North America through the importation of Japanese oysters (Kim et al. 2010; Krueger-Hadfield et al. 2017). In *G. vermiculophylla*'s non-native range, the seaweed is considered invasive, demonstrating competitive advantage over co-occurring macrophytes (Thomsen et al. 2006; Weinberger et al. 2008; Martínez-Lüscher and Holmer 2010; Nettleton et al. 2013), tolerance of transport (Nyberg and Wallentinus 2009), and prolific expansion due to its unique reproductive strategy, as the macroalgae can produce both sexually and asexually through vegetative fragmentation (Nyberg and Wallentinus 2005; Thomsen and McGlathery 2005) and does not require attachment to substrate to grow and reproduce.

In the estuaries of the southeastern U.S.A., *G. vermiculophylla* is a novel species and has properties of a transformative invader (Richardson et al. 2010). Prior to introduction, salt marsh cordgrass (*Spartina alterniflora*) detritus and microalgae were the main sources of carbon and nutrients in these ecosystems, as the estuaries of Georgia and South Carolina were devoid of macroalgae and macroalgal detritus (Haines 1977; Currin et al. 1995). However, *G. vermiculophylla* now proliferates in the region due to its tolerance to a wide range of abiotic conditions, such as sedimentation (Thomsen and McGlathery 2007), water temperature (Yokoya et al. 1999; Raikar et al. 2001; Rueness 2005; Abreu et al. 2011a), salinity (Yokoya et al. 1999; Rueness 2005; Weinberger et al. 2008; Kim et al. 2012; Nejrup and Pedersen 2012), nitrogen (Thomsen and McGlathery 2007; Abreu et al. 2011a,b), UV radiation (Roleda et al. 2012) and light availability (Thomsen and McGlathery 2007; Weinberger et al. 2008). Furthermore, its expansion has been facilitated by the native tube worm, *Diopatra cuprea*, which preferentially decorates its tube with the non-native seaweed to attract its amphipod prey (Thomsen et al. 2005; Byers et al. 2012; Berke 2012; Kollars et al. 2016). In the low intertidal zone of southeastern estuaries, *G. vermiculophylla* grows quickly, increasing up to 200% of its biomass in eight weeks, and decomposes rapidly, losing up to 79% of its biomass within ten days of burial in the sediment (Byers et al. 2012). The altered availability of live and decaying primary production could alter essential ecosystem functions and properties, such as nutrient cycling and trophic structure. Moreover, *G. vermiculophylla* could serve as link between anthropogenic nutrient pollution in the pelagic zone and nutrients in the benthos as it readily uptakes nitrate and ammonium (Tyler and McGlathery 2006; Abreu et al. 2011a,b).

Along the Georgia and southern South Carolina coasts, *G. vermiculophylla* now serves as important habitat for many small epifaunal invertebrates. Its highly-branched structure supports high abundances of epibenthic invertebrates, including amphipods, gastropods, and crabs (Byers et al. 2012). The structure provided by *G. vermiculophylla* reduces desiccation stress (Wright et al. 2014) and increases protection from predators for these invertebrates (Bishop and Byers 2015). *G. vermiculophylla* is also preferentially used as oviposition sites for the common mud snail *Tritia obsoleta* (Newton 2013). Despite the high frequency and positive association of many native invertebrates with *G. vermiculophylla*, few studies have investigated the direct and indirect role of *G. vermiculophylla* within the trophic structure of this region.

## Summary of Chapters

Using a mixed-method approach, including the use of regional and local surveys and use of laboratory and *in situ* experiments, this dissertation explores the role of *G. vermiculophylla* as a contributor to and modifier of trophic interactions. The purpose of this research is to determine the overall effect of the introduction on key biotic factors in the invaded estuaries.

Chapter Two focuses on what native species consume the non-native seaweed and how their consumption is altered by heightened nutrient content. Because *G. vermiculophylla* represents a novel source of primary production in these ecosystem that lack abundant seaweed resources, understanding how native secondary consumers respond to the non-native resource is essential in estimating the overall positive or negative effect of *G. vermiculophylla* on these ecosystems. Additionally, because *G. vermiculophylla* readily absorbs excess nitrogen in the environment, identifying the role of nutrient content in the consumption of the seaweed provides a more complete understanding of the species' role in estuarine trophic structure. We found that native consumers have varied consumptive responses to the introduced basal resource, as the mud snail *Tritia obsoleta* and the mud crab *Eurypanopeus depressus* both preferred a native seaweed to the non-native *G. vermiculophylla*. However, the native fish *Lagodon rhomboides* greatly preferred the non-native seaweed, showing potential differences in consumption between trophic guilds. Additionally, *L. rhomboides* preferred to consume nutrient-enriched G. vermiculophylla, while the native amphipod, *Ampithoe valida*, showed similar consumption across enriched and non-enriched seaweed. Thus, this chapter reports the first finding of consumption of *G. vermiculophylla* by fish and elucidates potential differences in consumption between trophic levels within a nutrient availability context.

The third and fourth chapters investigate how *G. vermiculophylla*'s novel structure impacts the foraging behavior of two important predator guilds: shorebirds and excavating foragers. Shorebirds and other seabirds are fundamental cogs in the wheel of estuarine ecosystems, locally limiting invertebrate populations (Schneider and Harrington 1981; Steinmetz et al. 2003) and transferring energy and nutrients across habitat types (e.g., Anderson and Polis 1999). Similarly, excavating predators, particularly blue crabs (*Callinectes sapidus*.), limit infaunal invertebrate populations and distributions (Woodin 1978, 1981; Hines et al. 1990), are important links in estuarine food webs to higher trophic levels (Winemiller et al. 2007; Oakley et al. 2014; Byers et al. 2017), and improve ecosystem function through bioturbation of marine sediments (Woodin 1978; Hines et al. 1990; Cadée 2001).

In the third chapter, we found that shorebird species that are often grouped as the same trophic guild, respond to *G. vermiculophylla* differently, with some shorebirds utilizing the seaweed as a foraging resource, while other shorebirds avoid the seaweed's structure entirely. This research represents the first investigation of the effect of *G. vermiculophylla* on shorebird habitat preference and foraging behavior and provides a comprehensive view of the entire shorebird community. The fourth chapter builds on the third chapter, by targeting another important predator guild and takes it a step further by investigating how the non-native seaweed affects the foraging success of excavating predators. In this chapter, we found that *G. vermiculophylla*'s structure does not inhibit the foraging behavior or success of *C. sapidus* in both the laboratory and field. Both chapters three and four provide essential information about the indirect effect of *G. vermiculophylla* on trophic interactions in the invaded estuaries.

The estuaries of Georgia and South Carolina are some of the most productive ecosystems in the world, supporting a plethora of biodiversity, natural resources, and ecosystem services, which are beneficial to humans (Haines 1977; Lotze and Milewski 2004). Despite the potential for direct and indirect effects of *G. vermiculophylla* on trophic interactions of the southeastern estuarine ecosystems, little research has investigated how the seaweed acts as a novel basal resource and how its physical structure alters trophic interaction between large predators and their small epibenthic and infaunal prey. This dissertation contributes to the study of invasive species by further determining how introduced organisms affect trophic interactions of native species in recipient communities and puts it into a behavioral ecology context.

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# CHAPTER 2

# EFFECTS OF NOVEL, NON-NATIVE DETRITUS ON DECOMPOSITION AND INVERTEBRATE COMMUNITY ASSEMBLAGE[1]

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## Abstract

The majority of organic material from primary producers enters the detrital pathway, which is the foundation of many trophic structures across biomes. The introduction of a non-native primary producer can fundamentally alter the structure and function of an ecosystem due to alterations in the identity of available detrital resources. The salt marsh estuaries of the southeastern U.S.A. are detritus-based ecosystems, relying on the annual input of detritus from the primary macrophytic species, *Spartina alterniflora*. However, the relatively recent introduction of a novel primary producer, the red macroalga *Gracilaria vermiculophylla*, provides the opportunity to measure the effect of non-native basal resources on the detrital pathway. Two *in situ* experiments were conducted to determine how the novel primary producer impacts 1) decomposition and 2) invertebrate colonization of dead vegetative matter (i.e., wrack) in comparison to the native *S. alterniflora*. We found that *G. vermiculophylla* decomposes more rapidly and harbors a significantly different assemblage of epibenthic invertebrates than the native macrophyte. Thus, *G. vermiculophylla* may provide a complementary source of detritus and structure for invertebrates within the salt marsh ecosystem.

## Introduction

The trophic structure of an ecosystem is mandated by the transfer and cycling of energy and minerals (Chapin et al. 2011). In most ecosystems, the majority of energy and nutrients originate from detritus pathways rather than grazer pathways; relatively little material from live primary producers is consumed by grazers, leading to large contributions of decaying plant material to the detritus pool (Teal 1962; Odum and de la Cruz 1963; Mann 1972; Fisher and Likens 1973; Dickinson and Pugh 1974; Mann 1988). As a result, the detritus pool drives overall productivity and animal diversity for many ecosystems (Willoughby 1974; Chapin et al. 2011). The quantity and quality of materials cycling within an ecosystem further shapes the community composition and trophic structure of an ecosystem (Chapin et al. 2011). Thus, inputs of detritus into a system will have different effects on ecosystem structure and function given the specific identity of the detrital source, which is particularly important when considering the introduction of non-native primary producers into recipient ecosystems.

Across ecosystem types, the introduction of invasive species can have far-reaching consequences that may lead to alterations in ecosystem structure and function (Vitousek et al. 1997; Crooks 2002). One mechanism of such alterations is the introduction of novel primary production into the detrital pathway. Detritus identity determines how a resource is utilized by consumers and how it cycles within the ecosystem, as primary producers harbor inter- and intraspecific differences in traits, such as C:N:P content, lability of nutrients, structural compounds and chemical compounds. In particular, detrital differences in nutritional quality, refractory compounds, and herbivore defenses make certain resources more or less attractive to micro- and macroconsumers (Valiela et al. 1985; Bishop and Kelaher 2008). Causing changes to not only identity/quality of detritus, but also to the amount of biomass entering the detrital pathway, non-native primary producers can alter the degree to which consumers utilize detrital resources and shift the composition and distribution of detritivorous guilds (Rodil et al. 2008; Maerz et al. 2010; Taylor et al. 2010; Krumhansl and Scheibling 2012; Vázquez-Luis et al. 2012; Bishop and Kelaher 2013a,b; MacKenzie et al. 2013).

Detrital identity can also affect biogeochemical properties and processes in an ecosystem (Williams and Gray 1974; Valiela et al. 1985; Perkins et al. 2011). If a non-native primary producer's identity is largely different from native counterparts, the invasive species can alter processes and characteristics that define the function and structure of an ecosystem's detrital pathway, such as rates of decomposition, nutrient mineralization, nitrogen-fixation and soil and water quality (Gordon 1998; Ehrenfeld 2003; Alonso et al. 2010; MacKenzie et al. 2013). For example, litter from invasive terrestrial plant species generally hasten decomposition rates and the release of nitrogen from decaying material, though these patterns are highly context dependent and exceptions exist (Ehrenfeld 2003; Allison and Vitousek 2004; Ashton et al. 2005). Furthermore, alterations of detrital quality and quantity by introduced primary producers can impact storage and cycling of carbon and other minerals in the detrital pathway (Ehrenfeld 2003). These alterations can in turn impact community structure, as the new abiotic conditions and cycling characteristics may favor certain invertebrate species over others (Cardoso et al. 2004). Thus, alterations to the identity of detrital resources by introduced species can have extensive impacts on ecosystem properties and processes and should be more broadly investigated (Bishop and Kelaher 2008; Taylor et al. 2010).

The salt marsh estuary ecosystem is a classic example of a detritus-based ecosystem (Odum 1963). In estuarine salt marshes, there are three primary sources of detritus: vascular macrophytes (most often in the form of *Spartina spp.*), algae (both micro- and macroalgae) and, to a much lesser extent, animal material (deriving from feces and dead tissue). Saltmarsh plant communities are characterized by high levels of primary productivity contributed predominantly by vascular plants. Across its range, the saltmarsh cordgrass *Spartina alterniflora* generates aboveground biomass estimated between 550 and 2000 g $m^{-2}$ $year^{-2}$ (dry weight) (Marinucci 1982). Despite the vast expanse of *S. alterniflora* primary production, less than 5% of that live production is consumed and assimilated by grazers (Teal 1962) due to high structural lignocellulose content that requires microbial conditioning prior to consumption (Findley and Tenore 1982; Anesio et al. 2003). *Spartina* decomposition and integration into the detrital pathway is a long process, taking over a year to completely break down (Marinucci 1982; Valiela et al 1985; Mann 1988).

With much of the primary productivity held in slowly degraded vascular macrophytes, macroalgae can be an essential carbon and nutrient pool in coastal and estuarine ecosystems (Mann 1988; Polis and Hurd 1996). Algae often harbor less structural, fibrous material and more nutrients than vascular marsh macrophytes, making algal biomass easier for detritivores to degrade and assimilate (Mann 1988; Adin and Riera 2002). Macroalgae can link marine and terrestrial systems, with macroalgal wrack (detritus stranded in the high tide region of the intertidal zone) serving as food subsidies for primary consumers that live in the littoral and supralittoral ecotone and maintaining cross-boundary faunal communities (Polis and Hurd 1996; Polis et al. 1997; Catenazzi and Donnelly 2007; Ince et al. 2007; Rodil et al. 2008; Gonçalves and Marque 2011). Not only can macroalgal detritus affect overall secondary production, it can also dictate the community structure and composition of macrofauna (McGwynne et al. 1988; Ford et al. 1999; Dugan et al. 2003; Cardoso et al. 2004; Olabarria et al. 2007; Rodil et al. 2008; Urban-Malinga and Burska 2009; Olabarria et al. 2010; Piovia-Scott et al. 2011, 2013;

MacMillan and Quijón 2012; Orr et al. 2014; Duggins et al. 2016). However, because of high turbidity and low hard substrata for attachment, macroalgae have traditionally been in very low abundance in many estuaries of Georgia and South Carolina (Byers et al. 2012). Thus, although they are more degradable, their low abundance has limited their overall role.

### *Study System*

Invasive macroalgae are altering recipient marine and estuarine ecosystems as they continue to expand globally (Schaffelke et al. 2006; Williams and Smith 2007; Thomsen et al. 2016). Negative responses to introduced seaweeds by native fauna have been documented (e.g., Rodil et al. 2008; Taylor et al. 2010; Bishop and Kelaher 2013a,b). However, introduced macroalgae can have mixed effects on native species and processes within a recipient ecosystem (e.g., Rossi et al. 2009; Olabarria et al. 2010). For example, an invasive marine macroalga may positively influence subsidies to low productivity areas, serving as a novel food source for primary consumers and allowing for niche diversification (e.g. Rossi et al. 2010; Bishop and Kelaher 2013b). The introduction of invasive macroalgae may also enhance availability of previously limiting nutrients (Rossi et al. 2011). Furthermore, invasive macroalgae, such as *Enteromorpha intestinalsis*, can alter abiotic conditions, creating beneficial habitats for some native species (Cardoso et al. 2004).

A recent invasion scenario in the South Atlantic Bight of the southeastern United States provides an opportunity to investigate the effects of a novel, non-native primary producer on a historically detritus-based ecosystem. *Gracilaria vermiculophylla*, a red macroalga originating from Japan, has colonized much of Europe as well as the length of the U.S. East Coast from

Georgia to New Hampshire (Kim et al., 2010; Rueness, 2005; Krueger-Hadfield et al. 2017). In the southeastern U.S., this species creates an especially interesting invasion scenario. Prior to the introduction of *G. vermiculophylla* in the 1990s to early 2000s, the estuarine mudflats of southern South Carolina and Georgia were devoid of macroalgal beds, making *S. alterniflora* the primary macrophytic detrital input in this detritus-based system (Haines 1977; Teal, 1962). *G. vermiculophylla* now presents a novel source of detrital inputs of fundamentally different identity from *S. alterniflora* (Byers et al 2012). In contrast to the slowly decomposed *S. alterniflora*, *G. vermiculophylla* rapidly produces biomass (up to 200% net increase in 8 weeks) and even more rapidly decomposes (79% loss of biomass upon burial in 10 days) (Byers et al. 2012). As a novel source of detritus, *G. vermiculophylla* has the potential to drastically change ecosystem processes and community composition in southeastern estuaries.

Given the importance of the detrital pathway in marine and estuarine ecosystems, more research is required to determine the effects of novel, non-native primary producers on these systems. The objective of this study was to determine how detrital inputs of a novel, non-native species alter mudflat-salt marsh ecotone communities and ecosystem processes in comparison to native *S. alterniflora*. We used two *in situ* experiments to assess differences in decomposition rates and invertebrate colonization of the non-native and native wrack. We hypothesized that *G. vermiculophylla* wrack would decompose faster than *S. alterniflora*. Given rapid breakdown, and the presence of secondary compounds, we further hypothesized that the non-native *G. vermiculophylla* wrack would be colonized by fewer invertebrates than native *S. alterniflora* wrack. However, *G. vermiculophylla* is more structurally complex, which could counteract these effects.

## Methods

The following experiments were conducted at the Skidaway Institute of Oceanography on Skidaway Island, Georgia, U.S.A, in June and July 2015 within the mudflat-saltmarsh ecotone of the Wilmington River estuary (31°57'43.88"N, 81° 0'46.00"W). All *G. vermiculophylla* and *S. alterniflora* were collected from the same site. The site selected is often free of both *G. vermiculophylla* and *S. alterniflora* wrack, reducing the potential for immigration of extraneous natural wrack into experimental arrays. We placed both experiments in the *S. alterniflora* – mudflat ecotone, where the low intertidal mudflat habitat abuts the lowest elevation edge of the *S. alterniflora* stands that predominate in the upper intertidal zone. This ecotone habitat supports a diversity of invertebrate taxa that exhibit cross-boundary distributions, such as the highly abundant amphipod, *Gammarus mucronatus* (Wright et al. 2014).

### *Decomposition*

To examine the differences in decomposition rate between *G. vermiculophylla* and *S. alterniflora,* we conducted a factorial *in situ* experiment that measured biomass loss over one month, from June 20, 2015 to July 20, 2015. After collection from the field, we rinsed *G. vermiculophylla* and *S. alterniflora* with filtered seawater and defaunated them by hand. We then spun them in a salad spinner for 30 seconds and weighed them for wet biomass (g). We weighed approximately 25 g of either species, recorded the weight for initial biomass, placed it in a ~3.8 L Blue Hawk® fine-mesh paint strainer bag, and closed it with a zip tie. To obtain an estimate of the initial dry mass of all replicates, we separately collected ten samples of ~25 g of each species from the site, rinsed them, spun them and weighed them for wet biomass (g). We then dried them at 60°C until consistent dry mass (g) was achieved (at least two days). From this we calculated the average wet:dry biomass ratio for each species.

To simulate the ways in which wrack can be deposited and decomposed, we crossed the detritus species treatments with two deposition treatments, whereby half of the bags were buried just below the surface of the sediment and the other half were secured on the sediment surface. We tripled the number of replicates of each treatment in order to sample at three time intervals through the experimental duration. We placed the bags in a blocked design 0.5 m into the *S. alterniflora*-mudflat ecotone, parallel to the water line and spaced at least 1.5 m apart. The bags were secured on the sediment surface or were buried within the top 10 cm of the mud, depending on treatment. Within each of seven blocks, we placed three replicates of each of the four treatment combinations, using a random number generator to determine the order of replicates. We placed the blocks at least 1.5 m apart. One bag from each treatment combination was collected from each of the seven blocks on days 10, 20, and 30. After collection, contents of mesh bags were cleaned of sediment, weighed for biomass (wm g) and dried at 60°C until consistent dry mass (g) was achieved. One bag was lost in the *G. vermiculophylla* surface and burial treatments, resulting in a sample size of six; while two bags were lost in both the *S. alterniflora* surface and burial treatments, resulting in a sample size of five.

From the dry mass, we calculated the percentage of dry mass remaining (%DMR) for each replicate. Percent DMR was also used to calculate the decomposition constant (*k*) by first taking the log of %DMR for each day and then running a linear regression across all days per block (Conover et al 2016). *k* is equivalent to the slope of the regression.

All analyses were completed in R 3.4.0 (R Core Team 2017). We analyzed *k* using a non-parametric Kruskal-Wallis test, due to unequal variances between treatments as determined visually using the ‘qqnorm’ function and a Shapiro-Wilk test ($\alpha = 0.05$) (‘stats’ package; R Core Team 2017). For post-hoc analysis, we used a Dunn test (package ‘FSA’; Ogle 2017), with p-values adjusted according to the Benjamini-Hochberg method (Zar 2010; Mangiafico 2015). To determine temporal differences in biomass loss between the two species, we also analyzed %DMR using this method.

### *Invertebrate Colonization*

To determine how *G. vermiculophylla* alters the community structure of macroinvertebrates colonizing wrack, we conducted an *in situ* litterbag experiment from June 6, 2015 to June 16, 2015. We again tested the effect of detritus species using non-native *G. vermiculophylla* and native *S. alterniflora*. We also included a control treatment that did not contain either macrophyte to account for any artifacts in colonization that may occur due to the structure of the litterbags.

After rinsing *G. vermiculophylla* and *S. alterniflora* with filtered seawater and removing invertebrates, we weighed ~25 g wm of each detritus type, recorded the weight for initial biomass and placed it in plastic mesh litterbags. The litterbags were 144 $cm^2$ with 0.5 cm mesh, sewn closed using monofilament. A larger mesh size was used in this experiment to allow invertebrates to move freely. We secured twenty bags of each species treatment 0.5 m into the *Spartina*-mudflat ecotone on the sediment surface with galvanized steel garden staples, using a blocked design as described above. We collected half of the bags on day five (n = 10) and the remaining bags on day ten (n = 10). Upon collection, we returned the bags to the laboratory and rinsed the contents into a 250 µm sieve. Invertebrates were preserved in 80% ethanol until they were later counted and identified to the lowest taxonomic group possible under a dissecting scope. For crabs, we kept adults, juveniles, and megalopae separate to increase resolution of age classes. We dried the remaining macrophyte biomass in a drying oven at 60°C until it reached constant weight and weighed it for dry mass (g) remaining.

We measured diversity using species richness, Shannon-Weaver diversity index, and Pielou's evenness for each replicate. Species richness was calculated as the number of species present in each replicate. We calculated the Shannon-Weaver diversity index (SDI) using the 'diversity' function in R ('vegan' package; Okansen et al. 2017). We then calculated Pielou's evenness using the SDI value for each replicate according to methods detailed in Okansen (2017). Because these data did not meet parametric assumptions, we analyzed differences between treatments for each diversity measure using a non-parametric Kruskal Wallis test and a post-hoc Dunn test as in the decomposition analysis.

We used a multivariate approach to analyze invertebrate colonization (accounting for invertebrate identity and abundance) as a function of detritus species and day collected. Invertebrate abundance was analyzed across the community and for separate species using the 'manyglm' function (package 'mvabund'; Wang et al. 2012) with a negative binomial distribution. Only organisms with over five individuals across the site were analyzed (resulting in the exclusion of 11 individuals). The mvabund package allows for model-based analysis of community abundance data and is superior to distance-based methods because it has greater power and allows for analysis of differences in treatment blocks, identification of species-level effects, and prediction of abundances of each species (Wang et al. 2012). To account for differences in macrophyte biomass decomposition between the treatments, we offset our models by the remaining *G. vermiculophylla* or *S. alterniflora* dry biomass (g) present in each replicate upon collection. Because the control litterbags did not contain macrophyte biomass a dummy value of 1 g was added to the remaining biomass of each replicates to allow for analysis of the data with an offset across treatments. We conducted the analysis both with and without the control to isolate the effect of the experimental treatments while assessing the effect of the control. Block was included as a fixed effect, however it was excluded from the final analysis because it did not account for significant variance in the model.

## Results

### *Decomposition*

We detected a significant difference in the decomposition constant (*k*) across wrack species treatments (Kruskal Wallis: $\chi^2 = 16.46$, df = 3, $p < 0.001$). Neither wrack species showed a significant difference in *k* between surface deposition or burial (Fig. 1a; Table 1). However, *G. vermiculophylla* had a greater *k* for both the surface deposition and burial treatments than *S. alterniflora* (Fig. 2.1a; Table 2.1).

The percent dry mass remaining was also significantly different among treatments for all three time points (Kruskal Wallis: Day 10 - $\chi^2 = 17.47$, df = 3, $p < 0.001$; Day 20 - $\chi^2 = 17.14$, df = 3, $p < 0.001$; Day 30 - $\chi^2 = 17.05$, df = 3, $p < 0.001$). By day 30, on average, *G. vermiculophylla* lost greater than 80% of its biomass in both the surface and burial deposition treatments, while *S. alterniflora* lost ~50% (Fig. 2.1b; Table 2.1).

### *Invertebrate Colonization of Wrack*

Species richness was significantly different across treatments, with significantly greater species richness in the *G. vermiculophylla* and *S. alterniflora* treatments than in the control (Kruskal Wallis: $\chi^2 = 32.50$, df = 2, $p < 0.001$; Table 2.2). We found similar significantly different results for the Shannon-Weaver diversity index across treatments, with the control having a significantly lower diversity index value than the experimental treatments (Kruskal Wallis: $\chi^2 = 29.14$, df = 2, $p < 0.001$; Table 2). Finally, no significant difference in species evenness was detected among treatments (Kruskal Wallis: $\chi^2 = 1.96$, df = 2, p = 0.38; Table 2).

For the multivariate analysis of invertebrate colonization, invertebrate abundance significantly varied across treatments (GLM: dev = 190.20, p = 0.001; Table 2.3) and collection days (GLM: dev = 48.05, p = 0.001; Table 2.3), with a significant interaction (GLM: dev = 25.93, p = 0.05). The control drove the interaction, as it was far from significant when comparing only *S. alterniflora* to *G. vermiculophylla* (GLM: dev = 6.07, p = 0.53; Table 2.3).

The amphipod, *Ampithoe valida*, was the most abundant species, accounting for 41.33% of the 888 invertebrates evaluated. Significantly more *A. valida* colonized the *G. vermiculophylla* bags than the *S. alterniflora* or control bags, with no significant difference occurring over time (Fig. 2.2a; Table 2.4 and 2.5a,b). Another amphipod, *Gammarus mucronatus*, was also highly abundant (30.74%) and significantly different among treatments (Fig. 2.3b; Table 2.4 and 2.5a,b), with increasing density over time (Table 2.5b). Crabs, at juvenile and megalopa stages, together accounted for 17.34% of all individuals and demonstrated significantly greater colonization *in G. vermiculophylla* than *S. alterniflora* (Fig. 2.3c,d; Table 2.4 and 2.5a,b). Only three adult mud crabs, *Eurypanopeus depressus*, were present across treatments and thus were not included in the analysis. Finally, the polychaete, *Alitta succinea*, which accounted for 6.76% of individuals, showed significantly different colonization between wrack species treatments and days (Table 2.5a,b), with more polychaetes present in *G. vermiculophylla* and increasing over time (Fig. 2.3e; Table 2.4). The remaining species were present in low densities and had variable responses to both treatment and collection day (Fig. 2.3f-i; Table 2.4 and 2.5a,b).

## Discussion

Novel sources of primary production can have profound effects on recipient ecosystems, as detritus identity determines fundamental ecosystem functions (e.g., Rossi et al. 2011; Cacabelos et al. 2012; Salvaterra et al. 2013) and structure (e.g., Rodil et al. 2008; Deudero et al. 2011; Drouin et al. 2011; Salvaterra et al. 2013). The non-native macroalga, *G. vermiculophylla*, contributes substantial amounts of primary production in southeastern estuaries (Byers et al. 2012). However, as seen in our decomposition experiment, *G. vermiculophylla* is an ephemeral wrack species, providing short pulses of organic material to the salt marsh ecosystem rather than the long press provided by *S. alterniflora*. Thus, *G. vermiculophylla* production may introduce a complementary source of primary production.

As we hypothesized, *G. vermiculophylla* decomposed more rapidly than *S. alterniflora* in both the buried and surface treatments. *S. alterniflora* is also known to decompose slowly in other regions where it has been examined. For example, *S. alterniflora* decomposition measured using litterbags in Massachusetts rendered ~ 50% decrease in mass and *k* of 0.003 ± 0.0003 SE (White and Howes 1994). The rapid biomass loss of buried *G. vermiculophylla* was in line with previous literature that found that *G. vermiculophylla* decays within two weeks belowground (Byers et al. 2012). However, our observed rate of decay was much slower than that observed in Rhode Island during the same time of year, with a mean *k* of ~ 0.4 (Conover et al. 2016). The observed disparity may be due to the method of seaweed preparation, as Conover et al. (2016) froze their *G. vermiculophylla* samples prior to deployment, while we did not. In Georgia and South Carolina, freezing is not a common source of decay for macrophytes; rather, high tide stranding and burial present more realistic modes of decomposition.

Several factors affect decomposition rates in these systems, including abiotic conditions, invertebrate activity, nutrient enrichment, content of fibrous/refractory material, and chemical defenses (Valiela and Teal 1979; Valiela et al. 1985; Mann 1988). Differences in decomposition observed between *G. vermiculophylla* and *S. alterniflora* are likely due in large part to the high content of refractory carbon and nitrogen present in *S. alterniflora* (White and Howes 1994). *G. vermiculophylla*'s relatively rapid decomposition could alter carbon and nutrient cycling of invaded estuaries because *G. vermiculophylla* contains relatively high concentrations of nitrogen (between 2.0% ± 0.1 SE and 3.7% ± 0.2 SE observed in Virginia; Tyler and McGlathry 2006) and mediates nitrogen cycling between the sediment and water column while both alive and decomposing (Abreu et al. 2011a,b; Tyler and McGlathery 2006; Gonzalez et al. 2013). In Virginia, live *G. vermiculophylla* mats release on average 0.24 g N $m^{-2}$ $d^{-1}$ to the water column (Tyler and McGlathery 2006). Furthermore, a conspecific, *Gracilaria verrucosa*, which is abundant in coastal Florida, releases ~6 g N $m^{-2}$ $d^{-1}$ as it decays, contributing a significant amount of nitrogen to the system (Hanisak 1993). This contrasts with *S. alterniflora*, which harbors a low nitrogen concentration (less than 1% dry mass during the summer in Georgia; Hopkinson and Schubauer 1984) and slowly loses nitrogen during the decomposition process (White and Howes 1994), with an estimated 14.4 g N $m^{-2}$ lost annually (Hopkinson and Schubauer 1984). Thus, *G. vermiculophylla* may present a pulse of highly concentrated nitrogen for microbial and invertebrate detritivores.

*G. vermiculophylla* wrack also attracted greater invertebrate abundances than *S. alterniflora* and had a significantly positive affect on community assemblage. The magnitude of the non-native seaweed's colonization effect varied by invertebrate taxonomic group. For example, amphipods were the most abundant organisms that colonized the wrack species, collectively composing over 70% of the invertebrate community. The abundance of amphipods relative to other organisms, and their preference for colonizing *G. vermiculophylla*, reflects similar relationships observed between amphipods and the non-native seaweed observed in lower tidal zones of mudflats; however, *G. mucronatus* dominates the mudflat community (Byers et al. 2012; Wright et al. 2014), while *A. valida* was the most abundant amphipod in the mudflat – saltmarsh ecotone, suggesting potential zonation of these species. Similar responses of crabs and the polychaete *A. succinea* suggest that *G. vermiculophylla* provides a novel resource that is attractive across invertebrate species, though the mechanism of attraction remains unclear.

Possible mechanisms of greater invertebrate colonization on *G. vermiculophylla* may be a combination of benefits from its provision of novel physical structure or food, but currently the evidence points to structure being more influential (Wright et al. 2014; L. Haram, *in prep*). *G. vermiculophylla*'s structure provides environmental refugia for epibenthic invertebrates, such as protecting amphipods from desiccation (Wright et al. 2014) – an important refuge for invertebrates given that the salt marsh ecotone is exposed to high air temperatures and UV radiation for multiple hours during low tide in the summer. *G. vermiculophylla* structure also protects amphipods and mud crabs from predation (Wright et al. 2014; Bishop and Byers 2015). *S. alterniflora*, while not preferred over *G. vermiculophylla*, still attracted a sizeable invertebrate community likely due to its refugia quality. For example, crabs preferentially utilize *S. alterniflora* wrack over other structural estuarine species due to its high value as habitat (R. Smith, *in prep*). Furthermore, the utilization of invasive macroalgae by native epibenthic communities has been observed in other ecosystems. For example, *Sargassum muticum* provides comparable habitat to native macroalgae for native epifaunal invertebrates of the coast of Spain, particularly when the native seaweeds are of similar morphology and chemical makeup (Viejo

1999). *S. muticum* also provisions new habitat for some invertebrates with differences in structure contributing to differences in epifaunal assemblages between the invasive and native seaweeds (Cacabelos et al. 2010; Gestoso et al. 2010). Furthermore, the generation of structurally complex habitat by non-native seaweeds can support greater densities of invertebrates, as has been observed on mudflats invaded by *G. vermiculophylla* (Byers et al. 2012; Wright et al. 2014).

In addition to novel structure, *G. vermiculophylla* may serve as a novel trophic resource for invertebrates in southeastern salt marsh ecosystems where native primary producer diversity is low. In fact, some of the organisms colonizing *G. vermiculophylla*, including the amphipod *A. valida* and the mud crab *E. depressus*, do utilize *G. vermiculophylla* for food (Haram et al. *in prep*); while others, namely the amphipod *G. mucronatus* and mud snail *T. obsoleta*, do not consume *G. vermiculophylla* (Wright et al. 2014; Haram et al. *in prep*). Thus, *G. vermiculophylla*'s value as wrack may result from both its role as novel food and structure.

In conclusion, *G. vermiculophylla* may provide a complementary source of detritus in southeastern salt marshes. *G. vermiculophylla* provides a quick pulse of detrital nutrients and organic matter compared to the usual steady release of these materials provided by *S. alterniflora*, altering fundamental ecosystem functions and properties in this recipient system. The differences in structure between *G. vermiculophylla* and *S. alterniflora* also diversify habitat for invertebrates that rely on macrophytic wrack for refuge in the upper intertidal. Despite the seemingly enhancing effect of *G. vermiculophylla*, further research should investigate how density of *G. vermiculophylla* deposition impacts ecosystem function and community structure. Dense algal mats may have different outcomes on decomposition rates, nutrient cycling, and invertebrate community assemblage (e.g., Bishop and Kelaher 2013b).

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## Tables

Table 2.1. Mean and standard error (± SE) of the decay constant (*k*) and percent dry mass remaining (% DMR) for *G. vermiculophylla* (n = 6) and *S. alterniflora* (n = 5) wrack that was experimentally placed on the sediment surface or buried. Average % DMR is present for each of three collection days: 10, 20, and 30. These data were analyzed using Kruskal Wallis tests with Dunn post-hoc tests in R. Significant differences are not represented in the table (see Fig. 1).

| Species | Deposition | Decay Constant (*k*) | | % DMR Day 10 | | % DMR Day 20 | | % DMR Day 30 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| *Gracilaria* | Surface | 0.029 | 0.004 | 53.57 | 2.34 | 26.29 | 3.53 | 17.09 | 4.33 |
| | Buried | 0.045 | 0.007 | 20.24 | 2.09 | 11.66 | 3.42 | 6.46 | 2.26 |
| *Spartina* | Surface | 0.010 | 0.001 | 63.93 | 1.32 | 51.12 | 1.41 | 49.92 | 3.55 |
| | Buried | 0.010 | 0.001 | 52.62 | 2.29 | 47.82 | 1.57 | 48.88 | 4.2 |

Table 2.2. Mean and standard error (± SE) of diversity indices across wrack species treatments [control (empty bag), *G. vermiculophylla*, and *S. alterniflora*]. Diversity indices include species richness, Shannon-Weaver diversity index, and Pielou's species evenness and were analyzed using Kruskal Wallis tests with Dunn post-hoc test in R. Due to the insignificant effect of day of collection, litterbags were combined across days for sample sizes of 20 per *G. vermiculophylla* and control treatments and of 18 per *S. alterniflora* treatment (due to loss in the field). Superscript letters indicate statistical differences ($p < 0.05$).

| Treatment | Species Richness | | Shannon Diversity Index | | Species Evenness | |
|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE |
| Control | $1.0^a$ | 0.3 | $0.25^a$ | 0.09 | $0.39^a$ | 0.13 |
| *Gracilaria* | $3.9^b$ | 0.2 | $1.10^b$ | 0.07 | $0.77^a$ | 0.04 |
| *Spartina* | $3.8^b$ | 0.3 | $1.12^b$ | 0.07 | $0.78^a$ | 0.03 |

Table 2.3. Analysis of deviance results for the multivariate generalized linear regression analysis of invertebrate community colonization across wrack species treatments (*G. vermiculophylla* or *S. alterniflora*) and collection days (5 and 10). Results are presented for analyses of treatments with and without the control. Bold text indicates significant differences (p < 0.05), while bold and italicized text indicates trends (0.05 < p > 0.1). These data were analyzed using the mvabund package in R.

| | With Control Treatment | | | Without Control Treatment | | |
|---|---|---|---|---|---|---|
| | Res. Df | Deviance | p | Res. Df | Deviance | p |
| (Intercept) | 56 | | | 36 | | |
| Treatment | **54** | **157.91** | **0.001** | **35** | **168.78** | **0.001** |
| Day Collected | **53** | **46.63** | **0.001** | **34** | **48.08** | **0.001** |
| Interaction | ***51*** | ***24.77*** | ***0.07*** | 33 | 6.07 | 0.52 |

Table 2.4. Mean density of invertebrates (per g of remaining wrack) that colonized *G. vermiculophylla* versus *S. alterniflora* wrack. Densities were calculated as the average number of individuals that colonized each litterbag by the remaining dry biomass (g) of the respective wrack species. Wrack treatments included *G. vermiculophylla* (n = 10), *S. alterniflora* (n = 9), and a control (n = 10), which consisted of empty litterbags. Litterbags were collected on days 5 and 10 to assess colonization over time. Statistical differences are not represented in the table (see Table 2.5).

| Day Collected | Wrack Treatment | *Ampithoe valida* | | *Gammarus mucronatus* | | Juvenile Crab | | Megalopae Crab | | *Alitta succinea* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Five | Control | 0.4 | 0.22 | 0.6 | 0.43 | 0 | 0 | 0.3 | 0.15 | 0.2 | 0.13 |
| | *Gracilaria* | 4.75 | 1.07 | 2.52 | 0.99 | 0.61 | 0.19 | 0.6 | 0.12 | 0.25 | 0.09 |
| | *Spartina* | 2.34 | 0.77 | 0.72 | 0.18 | 0.52 | 0.12 | 0.27 | 0.07 | 0.15 | 0.08 |
| Ten | Control | 0 | 0 | 0.2 | 0.13 | 0.3 | 0.3 | 0.2 | 0.13 | 0 | 0 |
| | *Gracilaria* | 3.93 | 1.12 | 4.43 | 2.49 | 1.38 | 0.39 | 0.5 | 0.26 | 1.2 | 0.22 |
| | *Spartina* | 0.52 | 0.19 | 1.46 | 0.37 | 0.35 | 0.16 | 0.34 | 0.11 | 0.4 | 0.1 |

| Day Collected | Wrack Treatment | *Isopoda* | | Other Polychaetes | | *Tritia obsoleta* | | Larval Gastropod | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Five | Control | 0.2 | 0.13 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Gracilaria* | 0.14 | 0.11 | 0 | 0 | 0 | 0 | 0.14 | 0.08 |
| | *Spartina* | 0.15 | 0.06 | 0 | 0 | 0 | 0 | 0.02 | 0.02 |
| Ten | Control | 0 | 0 | 0.3 | 0.21 | 0.1 | 0.1 | 0 | 0 |
| | *Gracilaria* | 0.05 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.05 | 0.05 |
| | *Spartina* | 0 | 0 | 0.08 | 0.06 | 0.09 | 0.07 | 0 | 0 |

Table 2.5. Analysis of deviance tables for univariate generalized linear models of individual invertebrate species that colonized *G. vermiculophylla* (n = 10) versus *S. alterniflora* (n = 9) litterbags across collection days (5 and 10), (a) with and (b) without the litterbag control treatment (n =10). Treatment effects were largely maintained when the bag control treatment was excluded, suggesting that significant differences in invertebrate colonization between *G. vermiculophylla* and *S. alterniflora* occurred. These data were analyzed using the mvabund package in R.

Univariate Tests
(With Control Treatment)

| | *Ampithoe valida* | | *Gammarus mucronatus* | | Crab (Juvenile) | | Crab (Megalopae) | | *Alitta succinea* | | Isopod | | Polychaete (Other) | | *Tritia obsoleta* | | Gastropod (Larvae) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p |
| Treatment | **46.24** | **0.001** | **33.53** | **0.001** | **24.65** | **0.001** | **18.5** | **0.002** | **22.98** | **0.001** | 2.52 | 0.77 | 1.02 | 0.79 | 1.84 | 0.79 | 6.63 | 0.19 |
| Day Collected | **10.62** | **0.02** | 0.35 | 0.83 | 0.32 | 0.831 | 0.76 | 0.78 | **9.58** | **0.04** | ***8.07*** | ***0.06*** | ***7.96*** | ***0.06*** | ***6.32*** | ***0.09*** | 2.66 | 0.44 |
| Interaction | 6.179 | 0.35 | 2.65 | 0.71 | 5.82 | 0.346 | 1.55 | 0.71 | 6.16 | 0.35 | 2.05 | 0.71 | 0 | 0.82 | 0 | 0.82 | 0.36 | 0.71 |

Univariate Tests
(Without Control Treatment)

| | *Ampithoe valida* | | *Gammarus mucronatus* | | Crab (Juvenile) | | Crab (Megalopae) | | *Alitta succinea* | | Isopod | | Polychaete (Other) | | *Tritia obsoleta* | | Gastropod (Larvae) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p | Dev | p |
| Treatment | **37.9** | **0.001** | **33.41** | **0.001** | **30.01** | **0.001** | **28.51** | **0.001** | **20.58** | **0.001** | ***5.92*** | ***0.08*** | 0.363 | 0.7 | 0.69 | 0.7 | **11.41** | **0.02** |
| Day Collected | 0.35 | 0.61 | **8.79** | **0.04** | 3.78 | 0.27 | 1.28 | 0.61 | **16.88** | **0.001** | 2.5 | 0.44 | ***6.35*** | ***0.08*** | ***7.18*** | ***0.06*** | 1 | 0.61 |
| Interaction | 1.63 | 0.77 | 0.49 | 0.86 | 1.16 | 0.78 | 1.17 | 0.78 | 0 | 0.99 | 1.43 | 0.77 | 0 | 0.99 | 0 | 0.99 | 0.19 | 0.86 |

## Figures

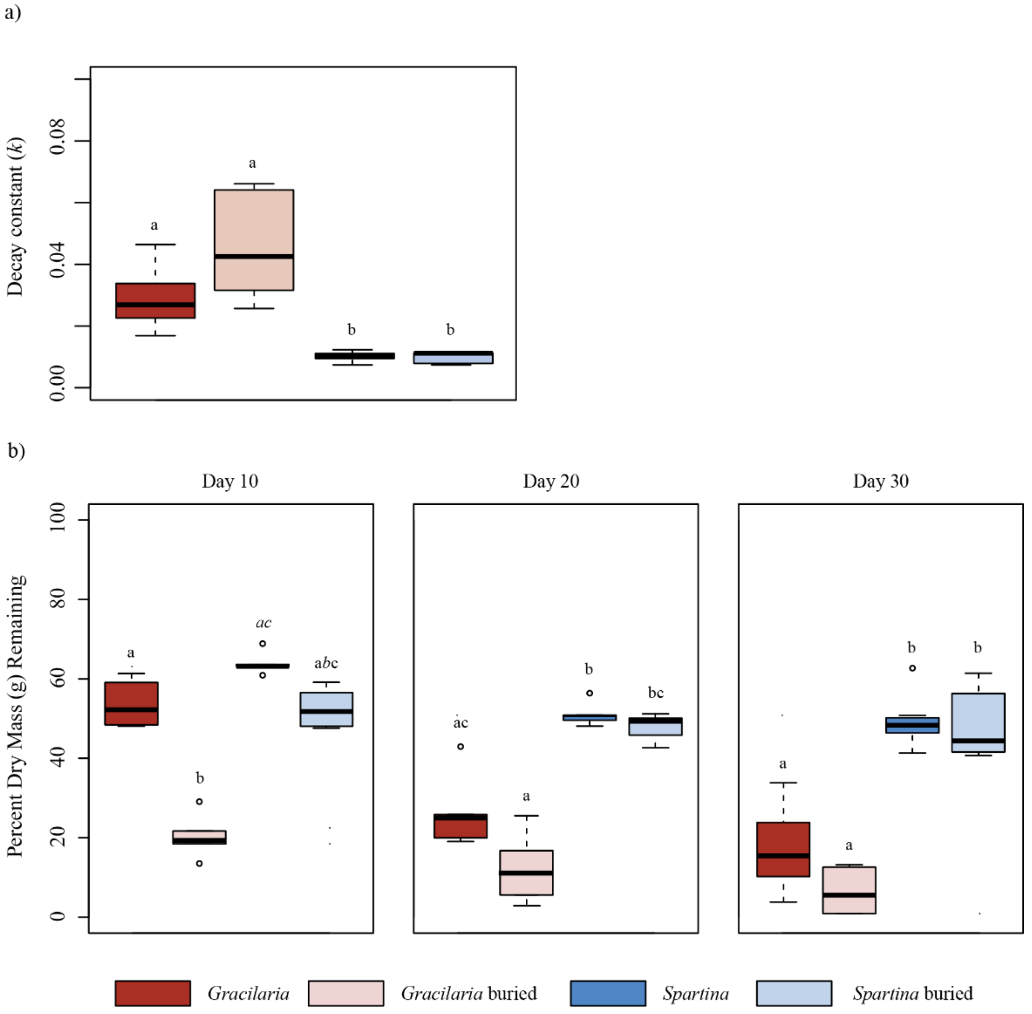

Figure 2.1. a) Median decay constant (*k*) across wrack treatments: *G. vermiculophylla* (n = 6), buried *G. vermiculophylla* (n = 6), *S. alterniflora* (n = 5), and buried *S. alterniflora* (n = 5). b) Median percentage of dry mass (g) remaining across wrack treatments (*G. vermiculophylla*, *G. vermiculophylla* buried, *S. alterniflora*, and *S. alterniflora* buried) for each collection day (10, 20, 30). Letters indicate statistical differences between treatments ($p < 0.05$), which were evaluated across treatments per collection day using Kruskal Wallis tests with Dunn post-hoc tests. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

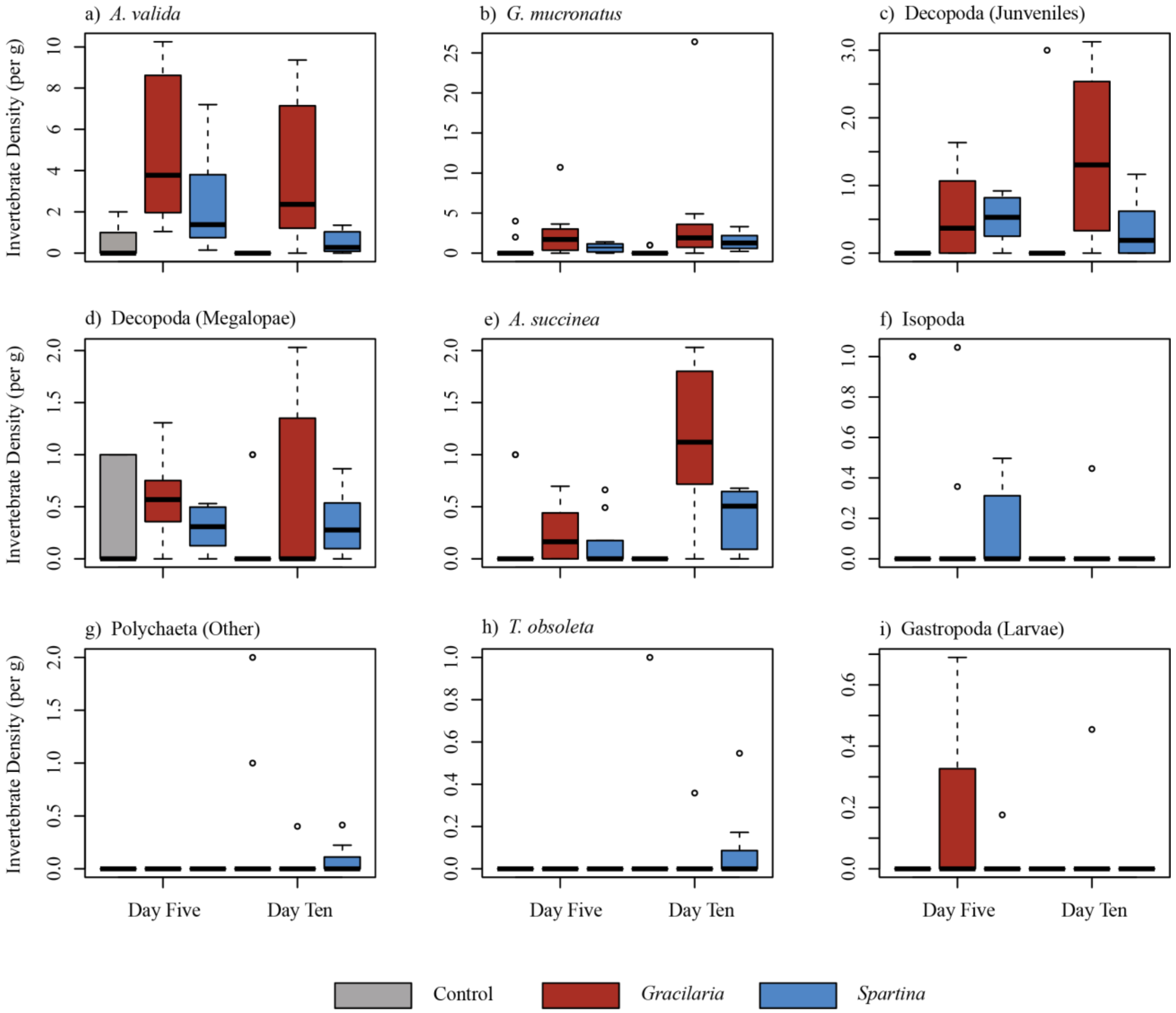

Figure 2.2. Median invertebrate density (per g of remaining wrack) of invertebrates across wrack treatments (control [n =10], *G. vermiculophylla* [n = 10], and *S. alterniflora* [n = 9]) and collection days (5 and 10). Invertebrate species or groups with over 5 individuals across the colonization experiment are presented. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values. Statistical differences are not indicated on the figure (see Table 2.5).

# CHAPTER 3

# NON-UNIFORM USE OF A NOVEL, NON-NATIVE SEAWEED BY NATIVE SPECIES[2]

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[2] Haram, L., K. Kinney, E. Sotka, and J. Byers. To be submitted to *Biological Invasions*.

## Abstract

With over 350 non-native and cryptic species, invasive seaweeds are of global concern due to their abilities to fundamentally alter the structure and productivity of marine food webs. Introduced seaweeds can outcompete native macrophytes and may lack top-down control by native herbivores, thus decreasing available food resources for native consumers. However, often little is known about the identity of native herbivores; their relative rates of consumption of introduced primary producers; and how their consumption may be mediated by variable nutrient content of the introduced seaweed. Throughout estuaries along the southeastern USA coast, the introduced red seaweed, *Gracilaria vermiculophylla*, has transformed unvegetated mudflats into a patchwork of non-native seaweed beds. Here, we used a series of laboratory feeding assays to assess how G. *vermiculophylla* is utilized as a novel food resource by two trophic guilds (marine invertebrates and fish) in estuaries of the Georgia coast. We also used feeding assays and a supplementary *in situ* experiment to test the role of nutrient enrichment in the seaweeds' tissues in determining its consumption. We found that *G. vermiculophylla* was not readily consumed by the mud snail *Tritia obsoleta* and the mud crab *Eurypanopeus despressus* but that it was rapidly eaten and even preferred over native seaweed *Ulva lactuca* by pinfish *Lagodon rhomboides*. Furthermore, we found that nutrient-enrichment of *G. vermiculophylla* led to differential consumption in the two trophic guilds, with amphipods *Ampithoe valida* consuming similar amounts of enriched and non-enriched seaweed and pinfish consuming twice as much enriched seaweed. Thus, the divergent responses of two trophic guilds to a novel, non-native seaweed highlight the importance of evaluating multiple trophic guilds when determining the role of introduced species in recipient communities. Given that herbivore identity and nutrient content of the seaweed mediated both its consumption in the laboratory and breakdown in the field, site

specific patterns of herbivore population dynamics and environmental conditions could lead to patchy effects of the introduced seaweed on energy cycling and food web structure.

## Introduction

Across ecosystem types, one of the proposed reasons for rapid expansion of introduced primary producers is the lack of top-down control by native herbivores (Keane and Crawley 2002; Callaway and Ridenour 2004; Thomsen et al. 2016). A group of primary producers that is of global concern are non-native seaweeds, which have been introduced to all major coastal regions through human activities (Williams and Smith 2007; Thomsen et al. 2016). While some non-native seaweeds appear to fit this pattern of escape from native herbivore control (e.g., Gollan and Wright 2006; Cummings and Williamson 2008; Monteiro et al. 2009; Cacabelos et al. 2010; Wright et al. 2014), many marine generalists consume a wide range of resources, including non-native seaweeds (e.g., Sumi and Schiebling 2005, Cummings and Williamson 2008, Britton-Simmons et al. 2011; Thomsen 2016). The incorporation of non-native seaweeds into the diets of native grazers can reduce competition among grazers, alleviate stress from dwindling native resources, and provide more nutrient-rich resources for native consumers in recipient communities (Nejrup et al. 2012). In fact, in nearshore regions where native macroalgal production is naturally low, non-native macroalgae may benefit native consumers by increasing the diversity and abundance of basal resources (Rodriguez et al. 2006). However, if native herbivores cannot consume non-native seaweeds, introduced seaweeds left unchecked can outcompete native primary producers and further reduce the availability of food resources. Thus, understanding the foraging preferences of native grazers is fundamental to determining the effects of non-native seaweeds on recipient ecosystems’ trophic structures and in predicting their future spread.

The interaction of herbivore identity and seaweed traits are important determinants of trophic incorporation of non-native seaweeds. Herbivore preferences for food resources are dictated by resource traits, including chemical defenses, nutritional quality, and availability (Nicotri 1980; Hay 1991, 1996; Van Alstyne et al. 2009; Duarte et al. 2010; Duarte et al. 2011; Lastra et al. 2014a; Jiménez et al. 2015), due to herbivores' varied nutritional requirements (Elser et al. 2000; Sterner and Elser 2002) and tolerances to chemical defenses (Inderjit et al. 2006; Jormalainen and Honkanen 2008). Seaweeds are particularly plastic in their responses to biotic and abiotic conditions (Pelletreau and Targett 2008), and as a result a single population can express an array of chemical, nutritional, and structural traits. For example, despite their ubiquity in marine systems, chemical defenses can vary within a single seaweed population, and even within an individual, based on genetic variation, environmental conditions, and herbivore pressure (Hay 1996; Hay and Fenical 1996; Van Alstyne et al. 2007; Jormalainen and Honkanen 2008). Furthermore, the chemical defenses and nutritional quality of macroalgae can be linked, as nutrient availability can drive tradeoffs in growth and generation of secondary compounds in seaweeds (Stefels 2000; Pavia and Toth 2008). Though seaweed traits exist on a continuum in nature, few studies have investigated how differences in variable non-native seaweed traits, such as C:N content, determine herbivory by native grazers.

The responses of native herbivores to an introduced primary producer can vary within and among trophic guilds. Commonly, marine invertebrate grazers are the focus of invasive seaweed-herbivore interaction studies, yet herbivorous fish can shape seaweed communities (e.g., Ojeda and Munoz 1999) and can effectively serve as biological controls of introduced seaweeds (e.g., Tomas et al. 2011). For example, herbivorous fish can reduce the biomass of invasive *Caulerpa taxifolia* in degraded seagrass beds, potentially slowing the seaweed's spread (Caronni et al. 2014). Despite the fundamental differences in size, mobility, and nutrient requirements between herbivorous invertebrates and fish, few studies have simultaneously tested the feeding preferences of these trophic guilds with non-native seaweeds. Therefore, our current understanding of native and non-native plant-herbivore interactions may underestimate the incorporation of non-native primary producers into marine trophic structures.

The recent invasion of the South Atlantic Bight, U.S.A. by the rhodophyte, *Gracilaria vermiculophylla*, provides an opportunity to investigate the role of a novel, non-native basal resource in native herbivore diets across different trophic levels. *G. vermiculophylla* was first documented in North Carolina in the early 2000s (Freshwater et al. 2006) and was likely cryptically introduced in Georgia around that time; though, the first record of the non-native seaweed in Georgia did not occur until nearly a decade later (Byers et al. 2012). Its rapid spread, tolerance to harsh environmental conditions, lack of competition, and mutualistic relationship with the native tubeworm, *Diopatra cuprea*, have led to its current dominance on intertidal mudflats throughout the Southeast (Thomsen and McGlathery 2007; Byers et al. 2012; Kollars et al. 2016). In much of *G. vermiculophylla*'s invasive range, native submerged aquatic vegetation, such as seaweeds and seagrasses, are diverse and abundant. However, the estuarine region of Georgia consists of low production and diversity of native seaweeds, likely due to high turbidity, high sedimentation of soft sediments, and a general scarcity of hard substrate needed for attachment. In this area, *G. vermiculophylla*'s novel structure is an important habitat resource for epifaunal invertebrates, harboring greater densities than the previously bare mudflat (Byers et al. 2012; Wright et al. 2014). Despite the prevalence of *G. vermiculophylla* in southeastern estuaries and its role as preferred habitat for many herbivorous species, little is known about the seaweed's role as a novel food source for native, generalist consumers.

Prior to the introduction of *G. vermiculophylla*, primary productivity in southeastern estuaries originated from the detritus of *Spartina alterniflora* stands (Teal 1962) and from benthic and planktonic microalgae (Mann 1988). As seaweeds can provide more readily digestible and more nutrient rich primary production than co-occurring marine vascular plants (Mann 1988; Hay and Steinberg 1992; Duarte et al. 2010), the novel, non-native seaweed may be an attractive resource for generalist consumers in these ecosystems. In fact, *G. vermiculophylla* is known for its fast absorption and incorporation of nitrogen into its tissues, making *G. vermiculophylla* a potentially nutritious resource (Abreu et al. 2011a,b; Gorman et al. 2017). However, previous work suggests that high levels of chemical defenses in *G. vermiculophylla* tissue deter native invertebrate herbivores (Nylund et al. 2011; Nejrup et al. 2012; Rempt et al. 2012; Hammann et al. 2013, 2016), though some species consume *G. vermiculophylla* despite the presence of inhibitory compounds (Weinberger et al. 2008). Furthermore, these trophic interactions can be nuanced, with seasonality and diversity of native resources affecting consumption of the non-native seaweed (Weinberger et al. 2008; Nejrup et al. 2012).

To investigate how *G. vermiculophylla* is utilized by generalist consumers in southeastern estuaries, we conducted laboratory feeding assays across two trophic guilds. For the epifaunal invertebrate guild, we tested the mud crab *Eurypanopeus depressus*, the mud snail *Tritia obsoleta*, and the amphipod *Ampithoe valida*. These macroinvertebrates are commonly associated with *G. vermiculophylla* on intertidal mudflats in Georgia (Byers et al. 2012; Bishop and Byers 2015; L Haram, unpublished data) and are known to consume algae and other plant material (MacDonald 1982; Cruz-Rivera and Hay 2000; Lohrer et al. 2000; Giannotti and McGlathery 2001; Douglass et al. 2011; Reynolds et al. 2012). For the fish guild, we selected the native pinfish *Lagodon rhomboides*. This species is one of the most abundant bait fish in estuarine waters of the southeastern U.S. (Stoner 1980). They also experience an ontogenetic dietary shift as they mature, with older fish consuming more macrophytes (Stoner 1980;

Winemiller et al. 2007). Due to their high abundance and their mixed diets, *L. rhomboides* are responsible for shaping epifaunal communities and organic matter cycling through substantial predation on plant material and epifaunal invertebrates during peak seasonal abundances in the spring and summer (Adams 1976; Nelson 1979; Stoner 1980), making them a possible pathway for *G. vermiculophylla* into the estuarine trophic structure. Given that our selected consumers are generalists, we hypothesized that the native species would eat *G. vermiculophylla* in both choice and no-choice assays.

In addition to determining which of these abundant native generalists consume *G. vermiculophylla*, we assessed how nutrient enrichment of the seaweed affects consumption, again using laboratory feeding assays. We also assessed how nutrient enrichment of *G. vermiculophylla* attracts native epifauna, namely amphipods, using an *in situ* wrack colonization experiment. We hypothesized that higher nutrient content would make the non-native resource more attractive to native herbivores, thus increasing consumption and colonization by generalist grazers across experiments. Our laboratory assays and *in situ* experiment provide insight into how *G. vermiculophylla* is integrated into the food webs of southeastern estuaries and identify potential sources of biological control for the non-native seaweed.

## Methods

### *Site description*

All feeding experiments were conducted in a flow-through seawater system at the Skidaway Institute of Oceanography in Savannah, Georgia. We also conducted a complementary *in situ* experiment at Priest Landing, Savannah, Georgia (31°57'43.88"N, 81° 0'46.00"W). Invertebrates and seaweed were collected from a single site (Priest Landing) to reduce variability in consumer condition and seaweed quality. However, *Ulva lactuca*, the native, low-abundance seaweed was collected from one site at the Grice Marine Laboratory in Charleston, South Carolina (32°45'3.93"N, 79°54'5.85"W) in 2014 due to very low abundances in Savannah, Georgia. All seaweeds were defaunated prior to use by rinsing the tissue with filtered seawater and removing the remaining invertebrates and eggs by hand. *L. rhomboides* were collected from the Skidaway and Wilmington Rivers.

### *Native vs. Invasive Seaweed Feeding Assays*

**Epifaunal Invertebrate Consumer.** To test if the selected generalists consume the introduced seaweed when native food resources are not available, we conducted a no-choice feeding assay. We paired this with a choice assay to determine if consumption of the introduced seaweed was consistent when multiple resources were available. Using *E. depressus* and *T. obsoleta*, we conducted the epifaunal invertebrate choice and no-choice feeding assays in July 2014. For each assay, we collected all invertebrates from the mid-intertidal region of Priest Landing. We haphazardly collected large *T. obsoleta* individuals (10 - 18 mm) from the mudflat. Snails were not sexed due to the difficulty of non-destructively distinguishing between sexes. We collected *E. depressus* through an oyster harvest method – oyster clumps were harvested from Priest Landing and shucked, and *E. depressus* were removed. We kept only juvenile *E. depressus* (9 - 11 mm) with both claws intact for the experiment. Snails and crabs were housed separately in plastic containers with mesh sides in a flow-through seawater system prior to the assays.

*E. depressus* and *T. obsoleta* assays were conducted simultaneously with ten replicates of each treatment. A single invertebrate was housed in an individual container (3.81 cm diameter x ~12 cm depth, PVC tubing with 0.5 mm mesh secured to the openings) that allowed for seawater circulation. For the no-choice assays, we placed ~0.5 g wet mass (wm) of either *G. vermiculophylla* or *U. lactuca* in each container. All macroalgae were spun in a salad spinner for 30 seconds before weighing to remove excess water and standardize wet biomass measurements. For the choice assays, ~0.5 g wm of both *G. vermiculophylla* and *U. lactuca* were placed simultaneously into the container of each *T. obsoleta* and *E. depressus*. We included paired autogenic controls (n = 10 for each seaweed) of the same quantity of each seaweed species, held in consumer-free containers, to account for natural variability in macroalgal biomass due to photosynthesis and decomposition of the macroalgae during the experiment (Sotka and Hay 2002). The assays ran for seven days, at which point the remaining macroalgae in each container were removed, spun and weighed to measure the remaining wet biomass (g wm).

**Fish Consumer.** For the fish trophic guild, we assayed *L. rhomboides* in August 2012. We collected *L. rhomboides* from the Skidaway River using baited traps that were checked every 24 hours. All fish used in the assays were over 9 cm in length. During the feeding assays, one *L. rhomboides* was placed in a plastic container (0.7 m x 0.4 m x 0.3 m depth) within a flow-through seawater system. We divided each container in half with plastic Vexar® mesh (1 mm) and each container received one of three treatments: *G. vermiculophylla* (no choice), *U. lactuca* (no-choice), or *G. vermiculophylla* and *U. lactuca* (choice). We placed one fish on one side of the container; the opposite side contained an autogenic control. The position of the fish and autogenic controls were alternated per replicate. For all replicates, we threaded the seaweed through 9 cm of twisted nylon rope and attached it to a weight to keep the seaweed in place. While in holding, the fish were fed shrimp pellets *ad libidum*. Prior to the initiation of the experiment, we withheld food for 24 hours during acclimation. We conducted the no-choice assay first, offering each fish ~1.0 g of either *U. lactuca* or *G. vermiculophylla* (n = 4). Once the treatments were in place, we ran the experiment for 72 hours. However, because of rapid consumption, we stopped the assay after 48 hours, at which point we removed the seaweed, spun it for 30 seconds to remove excess water, and weighed the remaining wet biomass (g). Due to the limited number of fish collected, the same fish were used in the choice assays. For the choice assay, we offered each fish a total of ~2.0 g of seaweed (~1.0 g of each seaweed species) (n = 8) and otherwise followed the methods of the no-choice assay.

**Data Analysis.** In all assays, the response variable, consumed biomass (g wm), was calculated for each seaweed species using the following equation: $T_i (C_f / C_i) - T_f$, where $T_i$ and $T_f$ represent the initial (i) and final (f) biomass of seaweed offered to consumers and $C_i$ and $C_f$ represent that of the paired autogenic control (Sotka and Hay 2002). For all data presented in this study, we determined data normality, and therefore the appropriateness of parametric analyses, by visually assessing our distributions with the ‘qqnorm’ function (package ‘stats’) and by running Shapiro-Wilk tests ($\alpha = 0.05$) with the ‘shapiro.test’ function (package ‘stats’). All data were analyzed in R 3.4.0 (R Core Team 2017).

For the epifaunal invertebrate experiment, the response variable, consumed biomass (g wm), was not normally distributed for both the choice and no-choice assays. Therefore, we used non-parametric analyses, which do not assume normal distributions or equal variances. We analyzed the choice assays using Wilcoxon Signed Rank tests due to the paired nature of the data. However, we analyzed the no-choice invertebrate feeding assays with Wilcoxon Rank Sum tests because independent consumers were used for each replicate and therefore were not paired. For the fish consumer experiment, the distribution of consumed biomass (g wm) met parametric assumptions. Thus, we analyzed our fish consumer choice assay using a paired t-test and our no-choice assay with a Welch’s two sample t-test (package ‘stats’).

### *Nutrient Enrichment Assays*

**Epifaunal Invertebrate Consumer.** To investigate the role of nutrient content on consumption by invertebrates, we used *A. valida*, which are known to readily consume *Gracilaria* sp. (Reynolds et al. 2012; Scheinberg 2015; E. Sotka, unpublished data), for no-choice assays. In June 2016, we collected *A. valida* from Priest Landing by gathering *G. vermiculophylla* from the *Spartina*-mudflat ecotone and rinsing it in filtered seawater to dislodge associated epifaunal invertebrates. We then identified the dislodged amphipods to species under a dissecting scope. Both adult males and females (4.5 – 12 mm in length) were used for the experiment. We housed individual *A. valida* in plastic Reditainer® cups (~164 mL) that we filled with 150 mL of filtered seawater (~31 psu) and capped with plastic lids. We punctured the lids with ~1 mm holes to reduce evaporation while allowing for oxygen flow. Food was withheld from *A. valida* for 48 hours prior to initiation of the experiment. Each amphipod was offered ~0.50 g wm of one of the three *G. vermiculophylla* treatments detailed below (n = 12). We spun the seaweed for 30 seconds prior to weighing. Autogenic controls were included in separate containers without an amphipod for each replicate. The experiment ran for seven days, and every other day we extracted 50 mL of water from each container, targeting amphipod waste that accumulated on the bottom, and replaced it with fresh, filtered seawater.

We used three treatments to determine the effect of nutrient content on amphipod feeding: nutrient-enriched, non-enriched, and freshly-collected *G. vermiculophylla*. The freshly-collected treatment allowed us to assess whether growing the other treatments in laboratory conditions altered consumption by the grazers. For the enriched and non-enriched treatments, ~200 g wm of *G. vermiculophylla* were collected from the field, rinsed in filtered seawater, and manually defaunated. We placed ~100 g of *G. vermiculophylla* in two clear, plastic Sterilite®

bins (42.5 cm x 30 cm x 17.8 cm depth), with 14 L of aerated, filtered seawater. The seaweed was grown under Phillips® T8 32-watt daylight deluxe bulbs (6500 K, 2850 lumens) set to a 16:8 hr light:dark cycle to mimic natural summer conditions for ten days before initiation of the feeding trials. Deionized water was added to each bin daily to maintain consistent salinity (~31 psu). For the nutrient-enriched treatment, one bin of *G. vermiculophylla* was treated with a solution of 1.0 g of $NH_4Cl$, 1.5 g of $NaNO_3$, and 0.15 g of $Na_2HPO_4$ six times over the course of the growth period. The combined use of ammonium, nitrate, and phosphorus was adapted from methods in Abreu et al. (2011b) and previous pilot studies. Our nutrient-enriched method increased nitrogen content from ~2% N (C:N ~ 14) to ~6% N (C:N ~ 5) (see Methods in Appendix A), which mimics levels of percent nitrogen found in *G. vermiculophylla* in highly urbanized estuaries (Gorman et al. 2017; L. Haram, unpublished data). The freshly-collected treatment consisted of *G. vermiculophylla* collected from the field at Priest Landing, rinsed, and defaunated on the same day that the experiment began.

**Fish Consumer.** We collected adult *L. rhomboides* (9 – 12.5 cm) from Wilmington River, Georgia in August 2014 using baited traps that were checked every 24 hours. In the laboratory, prior to the start of the experiment, individuals were housed together (up to three per container) in ~28 L filtered aquaria. In addition to a recirculating filter, each aquarium was aerated and seawater was maintained at ~35 psu. Nutrient quality in the aquaria was monitored daily using an API® Saltwater Master Test Kit. The fish were fed shrimp pellets daily *ad libitum* and a 25% water change was completed every other day.

During the feeding assays, each *L. rhomboides* was placed in a plastic container (0.7 m x 0.4 m x 0.3 m depth) within a flow-through seawater system. After allowing 24 hours for the fish to acclimate, each container received one of three treatments [enriched *G. vermiculophylla* (no-choice), non-enriched *G. vermiculophylla* (no-choice), or enriched and non-enriched *G. vermiculophylla* (choice)] either with or without a fish. A freshly-collected *G. vermiculophylla* treatment was not used in this experiment as in the *A. valida* assay because it was conducted two years prior to the invertebrate experiment and its results served as an impetus for including the treatment in the later amphipod experiment.

*G. vermiculophylla* was grown in the laboratory prior to the feeding trial using the same method as described in the *Nutrient Enrichment Assay* 'Epifaunal Invertebrate Consumer' section. Fish were offered ~1.0 g of seaweed in the no-choice assay and a total of ~2.0 g of seaweed (1 g of each treatment) in the choice assay. For all replicates, the seaweed was threaded through 9 cm of twisted nylon rope and attached to a weight to keep the seaweed in place. Each trial ran for three days, when the seaweed was removed, spun for 30 seconds, and weighed for final biomass (g wm). In this experiment, we allowed the fish to eat for three days rather than two days (as in *Native vs. Invasive Seaweed Feeding Assay* 'Fish Consumer') because this experiment was conducted late in the fall when seawater temperatures are lower and fish consumption rates were depressed. Due to constraints in the number of flow-through containers available, we were only able to run up to two replicates of each treatment simultaneously. Unlike the previous *L. rhomboides* feeding trials, each fish received all treatments over the course of the experiment. The sequence of treatments was randomized per fish, and food was withheld for 24 hours before each trial. Two iterations were completed from November to December 2014 for a total of ten replicates of each treatment per assay.

**Data Analysis.** For all assays, we calculated the consumption of seaweed biomass (g wm) and assessed data normality using the same methods as in the *Native vs. Invasive Seaweed Feeding Assays* experiments. For the epifaunal invertebrate consumer experiment, we analyzed consumed biomass (g wm) as a function of nutrient treatment using a One-Way ANOVA (package 'stats'). For the fish consumer experiment, we analyzed consumed biomass (g wm) as a function of nutrient treatment using paired t-tests (package 'stats') for both the no-choice and choice assays, as the same individual fish was used as a single replicate for each assay type.

### *In Situ Colonization of Nutrient-Enriched Seaweed*

To more realistically determine how differences in seaweed nutrient content alter the attraction of native generalist herbivores, we assessed the effect of nutrient content on invertebrate colonization of stranded *G. vermiculophylla* (i.e., seaweed wrack) in the field. We tested two treatments: enriched *G. vermiculophylla* and non-enriched *G. vermiculophylla*. We placed 25 g wm of *G. vermiculophylla* in plastic mesh litterbags (50 mm mesh, sewn closed using monofilament) and secured the litterbags 0.5 m into the *Spartina*-mudflat ecotone using a galvanized steel garden staple. In October 2014, we deployed ten bags and collected half of them on day five and the other half on day eleven. We returned the bags to the laboratory, rinsed the contents into a 250 µm sieve, and weighed the remaining *G. vermiculophylla* (g wm). Invertebrates were preserved in 80% ethanol until they were later counted and identified to species under a dissecting scope. On day 11, two litterbags from the enriched treatment and one litterbag from the non-enriched treatment were dislodged and lost. We repeated the experiment in August 2015 to account for potential seasonal variation, while increasing our replication (n = 7) and including a litterbag control without seaweed. We collected all litterbags on day 10 and followed the same procedures as in the October 2014 iteration. Two litterbags were lost from the control treatment, for a final sample size of five.

**Data Analysis.** For each seasonal iteration (October 2014 and August 2015) of the experiment, we analyzed amphipod colonization (number of amphipods per litterbag) as a function of nutrient enrichment and day collected using a generalized linear model with a negative binomial distribution, due to overdispersion (package 'MASS', Venables and Ripley 2002). We also included block as a fixed effect. To account for any differences in biomass of *G. vermiculophylla* between the treatments that may have occurred over the experimental duration, we offset our amphipod models with the remaining *G. vermiculophylla* biomass (g wm) present in each litterbag upon collection. For the August iteration, a dummy value of 1 was added to the remaining biomass of all litterbags to allow for analysis with an offset across all treatments, including the control. In August, we only collected litterbags on day 10, so "day collected" was excluded as a predictor variable from both analyses.

In a second set of analyses, we determined the effect of nutrient enrichment on *G. vermiculophylla* biomass (due to growth or herbivory/decay) in the field. We analyzed the percentage of *G. vermiculophylla* biomass (g wm) remaining upon collection as a function of treatment and day collected using a linear regression (package 'lme4').

## Results

### *Native vs. Non-Native Feeding Assays*

**Epifaunal Invertebrate Consumer.** In the no-choice feeding assays, *T. obsoleta* consumed significantly more *U. lactuca* than *G. vermiculophylla* (Wilcoxon rank sum test: W = 64.5, p = 0.04; Table 3.1; Fig. 3.1a). However, *E. depressus* consumed similar amounts of *U. lactuca* and *G. vermiculophylla* (Wilcoxon rank sum test: W = 42, p = 0.60; Table 3.1; Fig. 3.1a). In the choice feeding assays, *T. obsoleta* consumed significantly more native *U. lactuca* biomass than non-native *G. vermiculophylla* biomass (Wilcoxon signed rank test: V = 42, p = 0.02; Table 3.1; Fig. 3.1b). Similarly, *E. depressus* consumed significantly more *U. lactuca* biomass than *G. vermiculophylla* biomass (Wilcoxon signed rank test: V = 55, $p < 0.01$; Table 3.1; Fig. 3.1b).

**Fish Consumer.** In the no-choice feeding assays, *L. rhomboides* ate slightly more *G. vermiculophylla* than *U. lactuca*, though their consumption was not significantly different between the treatments (Welch's t-test: $t_{4.18} = -1.01$, p = 0.37; Table 3.1; Fig. 3.2a). In the choice feeding assays, *L. rhomboides* ate significantly more *G. vermiculophylla* than *U. lactuca* (paired t-test: $t_7 = -4.51$, $p < 0.01$; Table 3.1; Fig. 3.2b).

*Nutrient Enrichment Assays*

**Epifaunal Invertebrate Consumer.** *A. valida* consumed similar amounts of non-enriched *G. vermiculophylla* (0.03 ± 0.03 g wm, mean ± SE) as enriched (0.05 ± 0.03 g wm) or freshly-collected (0.03 ± 0.02 g wm) *G. vermiculophylla*, with no significant difference among the treatments (One-Way ANOVA: $F_{2,31} = 0.32$, p = 0.73; Fig. 3.3). The lack of difference between the laboratory grown treatments and the freshly-collected treatments, suggests that the laboratory conditions did not affect amphipod feeding.

**Fish Consumer.** In the no-choice feeding assays, *L. rhomboides* consumed similar amounts of *G. vermiculophylla* in the enriched (0.46 ± 0.12 g wm, mean ± SE) and non-enriched (0.39 ± 0.11 g wm) treatments over the three-day assays (paired t-test: $t_9 = 0.48$, p = 0.65; Fig. 3.4a). However, in the choice feeding assay, *L. rhomboides* consumed more of the enriched *G. vermiculophylla*, eating 0.31 g wm (± 0.04) of the enriched seaweed vs. 0.13 g wm (± 0.04) of the non-enriched seaweed (paired t-test: $t_9 = 2.90$, p = 0.02; Fig. 3.4b).

*In Situ Colonization of Nutrient-Enriched Seaweed*

For October 2014, we found an interactive effect of treatment and day collected on amphipod density (per g wm of *G. vermiculophylla*) (GLM: interaction - $X^2_1 = 13.02$, $p < 0.001$). On both collection days, amphipods were on average over twice as abundant in the non-enriched treatment than the nutrient-enriched treatment, with abundance increasing over time (Table 3.2; Fig. 3.5a). For percentage of *G. vermiculophylla* biomass remaining, we found a significant additive effect of treatment and day collected (LM: $F_{2,14} = 10.65$, $p = 0.002$, $R^2 = 0.55$). Significant differences were detected between treatments, with nutrient-enriched *G. vermiculophylla* maintaining greater biomass (LM: $F_{1,14} = 14.31$, $p = 0.002$; Table 3.2; Fig. 3.5a). The percent remaining biomass was also significantly different between collection days, with biomass decreasing over time (LM: $F_{1,13} = 5.62$, $p = 0.03$; Table 3.2; Fig. 3.5a).

In August 2015, amphipod abundance was significantly different among treatments (GLM: treatment – $X^2_1 = 10.52$, $p = 0.001$). A significant difference was maintained when the experimental treatments were analyzed in isolation of the control, with more amphipods present in the non-enriched treatment (GLM: $X^2_1 = 10.52$, $p = 0.001$; Table 3.2; Fig. 3.5b). The percentage of *G. vermiculophylla* biomass remaining was not significantly different between treatments (LM: $F_{1,11} = 0.08$, $p = 0.78$, $R^2 = 0.26$; Table 3.2; Fig. 3.5b). Overall, greater decay and/or consumption occurred in the August trial than in the October trial, with well over half of the *G. vermiculophylla* biomass lost within 10 days of deployment (Table 3.2).

## Discussion

Understanding how an introduced primary producer is incorporated into a recipient food web is fundamental in predicting how the non-native species will shape ecosystem structure and function. Here, both consumer identity and seaweed nutrient content determined the fate of non-native *G. vermiculophylla* within feeding assays and an *in situ* experiment. In the absence of a native seaweed (*U. lactuca*), at least two species, *E. depressus* and *L. rhomboides,* will consume *G. vermiculophylla*. Differences between the consumer guilds manifested in *L. rhomboides* strong preference for the non-native seaweed over the native seaweed and in the fish's preference for nutrient-enriched seaweed. Thus, we provided insights about how *G. vermiculophylla* may be incorporated into the estuarine food webs of the Southeast USA.

Seaweeds are important resources for generalist consumers in nearshore marine systems; however, non-native seaweeds can be less useful to native consumers given mismatched palatability. Though *G. vermiculophylla* provides a novel and abundant resource, the common invertebrates, *T. obsoleta* and *E. depressus*, preferred native *U. lactuca* over the introduced *G. vermiculophylla* when given a choice. Within the same system, another common epifaunal invertebrate, the amphipod *Gammarus mucronatus*, similarly prefers to eat native *Spartina alterniflora* detritus to *G. vermiculophylla* (Wright et al. 2014). Our results also reflect those of studies conducted in other regions of the *G. vermiculophylla* invasive range, in which native herbivores preferred native seaweeds over the introduced seaweed (Weinberger et al. 2008; Nejrup et al. 2012; Hu and Juan 2014) – a pattern attributed to high concentrations of wound-activated and inducible chemical defenses produced by the invasive populations of *G. vermiculophylla* (Nylund et al. 2011; Hamman et al. 2013). Similarly, across seaweed taxa, previous studies that documented lower consumption of non-native seaweeds in comparison to native seaweeds by local, herbivorous invertebrates have attributed these disparities to differing identities and/or concentrations of secondary compounds in non-native species' tissues (e.g., Critchley et al. 1986, Britton-Simmons 2004, Monteiro et al. 2009, Enge et al. 2012; Nejrup et al. 2012; Sagerman et al. 2014; Schwartz et al. 2016). Though *U. lactuca* is chemically defended, the native seaweed produces secondary compounds that are qualitatively different from that of *G. vermiculophylla*. For instance, *U. lactuca* produces dimethylsulphoniopropionate (DMSP) (Stefels 2000; Van Alstyne and Puglisi 2007), while *G. vermiculophylla* produces oxylipins (Nylund et al. 2011; Rempt et al. 2012). Given their sympatry with *U. lactuca*, the studied invertebrates may have evolved tolerances to *U. lactuca*'s chemical defense compounds, making the native seaweed a more palatable resource (Sotka and Hay 2002). In fact, DMSP and its biproducts can promote consumption by some native invertebrate consumers (Van Alstyne and Puglisi 2007). Thus, to fully understand the nature of the invertebrates' preferences for the native seaweed, a suite of feeding assays that isolate the chemical differences between *U. lactuca* and *G. vermiculophylla* should be conducted.

In contrast to the invertebrates, pinfish *L. rhomboides* preferred to consume *G. vermiculophylla* over the native *U. lactuca*. Though this result has not previously been observed in our system, studies of other non-native seaweeds have found that non-native primary producers can benefit some herbivores or, at least, can be non-discriminately incorporated into herbivores' diets (e.g., Rossi et al. 2009; Cacabelos et al. 2010; Bulleri and Malquori 2015). Not only did *L. rhomboides* prefer *G. vermiculophylla*, they also rapidly consumed large amounts of the seaweed. Additionally, previous research has documented rapid grazing of *Gracilaria* spp. by herbivorous fish in comparison to brown and green seaweeds (Ganesan et al. 2006). Thus, their rapid consumption of *G. vermiculophylla* may point to pinfish as a possible means of population control for the non-native seaweed.

In addition to the observed differences in consumption of native versus non-native seaweeds between consumer types, we observed differences in the role of nutrient content. In no-choice trials, amphipod *A. valida* and pinfish *L. rhomboides* consumed similar amounts of nutrient-enriched versus non-enriched *G. vermiculophylla*. However, *L. rhomboides* consumed significantly more enriched seaweed when presented with a choice between non-enriched and enriched *G. vermiculophylla*. Though it was not logistically possible to conduct a choice trial with the amphipod consumers to test for a preference, our no-choice results suggest that nutrient enrichment is not an important determinant of amphipod consumption of *G. vermiculophylla*. When coupled with our finding of higher amphipod colonization of non-enriched versus nutrient-enriched *G. vermiculophylla* during the fall and summer, our results suggest contrasting patterns of utilization/consumption by amphipods and pinfish, with pinfish exhibiting a preference for nutrient-enrichment.

One possible explanation of the difference between *A. valida* and *L. rhomboides* consumption of nutrient-enriched resources may lie in differences in nutritional requirements between the consumer species. Herbivores are generally nutrient limited, and thus the nutrient content of primary producers can dictate herbivore food selection (Mattson 1980; Hay 1991; Sterner and Hessen 1994; Elser et al. 2001; Fink and Von Elert 2006; Kraufvelin et al. 2006). However, organisms require different amounts of energy and nutrients based on their specific physiological constraints (Sterner and Elser 2000; Sterner and Hessen 1994). For example, herbivorous fishes must process large amounts of carbon to meet their nitrogen and/or phosphorus requirements (Hay 1991; Hood et al. 2005), suggesting that our additions of nitrogen and phosphorus may have improved the nutritional value for *L. rhomboides*. In fact, *L. rhomboides* have exhibited similar nutrient-driven consumption in seagrass beds, *Halodule wrightii* (Heck et al. 2006), further illustrating the importance of high-nutrient food items in pinfish diets. While the nutrients enhanced in our study appear to stimulate *L. rhomboides* feeding, they simply may not limit *A. valida* due to its small body size and generalist feeding strategy (Sterner and Hessen 1994). In fact, the conspecific *A. longimana* similarly does not select seaweed based on nutritional value, as compensatory feeding behavior allows the amphipod to make up for lower nutritional values (Cruz-Rivera and Hay 2001).

A second explanation relates to the relationship between nutrient enrichment and the production of secondary chemical compounds in seaweed tissue. Nutrient availability can control the production of chemical defenses (Stefels 2000). For instance, DMSP content can be inversely related to nitrogen availability in the environment (Stefels 2000), while terpenoid metabolites can increase with nitrogen enrichment (Cronin and Hay 1996a). If the concentrations of secondary compounds in *G. vermiculophylla* are linked to nutrient availability, the potential benefit of increased nutrient content for invertebrates may be outweighed by deterrence. Thus, our results suggest that the suite of herbivores present and the nutrient conditions within a recipient ecosystem may be important drivers in the incorporation of non-native seaweeds into recipient food webs. Further investigation is needed to clarify the ecological mechanisms (i.e., herbivore nutrient limitation or chemical defenses) behind the observed differences in consumption of nutrient-enriched *G. vermiculophylla* between consumer types.

In addition to the role of consumer identity and primary producer nutrient content, it is also essential to consider how the interaction of both biotic and abiotic conditions may alter the incorporation of non-natives into recipient food webs. For example, *A. valida*'s consistent consumption of *G. vermiculophylla* in the laboratory feeding assays was not reflected in the field across seasons. In fact, amphipods colonized non-enriched seaweed more readily than the enriched seaweed. The differences in seaweed biomass loss across season provide some insights into possible explanations of the amphipod colonization pattern. In October, the enriched seaweed maintained its biomass better than the non-enriched seaweed, while in August both treatments underwent substantial, but similar, biomass loss. Seasonal differences in temperature, UV radiation, and consumer abundances may account for the observed differences. For example, the rapid biomass loss of both enrichment treatments in August may have been driven by elevated abiotic stressors known to affect seaweed growth and decomposition, such as high air temperatures, high UV conditions, and increased desiccation (Davison and Pearson 1996), which are characteristic of the upper intertidal zone during the late summer in southeastern estuaries. Additionally, seaweeds can become more attractive to grazers as decomposition progresses due to the breakdown of deterrent compounds and the increase in organic and mineral contents (Hay 1996; Cronin and Hay 1996b; Pennings et al. 2000; Eereveld et al. 2013; Lastra et al. 2015). Another explanation relates to the antimicrobial activity expressed by many seaweed species across taxa (e.g., González del Val et al. 2001; Cox et al. 2010); as some anti-herbivore defense compounds, such as phenolics, also exhibit anti-microbial activity (Hay 1991), nutrient availability may additionally alter the production of antimicrobial compounds and thus alter rates of seaweed decay, though this mechanism has not been well investigated. Given that seasonal changes in abiotic and biotic conditions drive many patterns in estuarine ecosystems, including decomposition and consumer abundance, seasonal control of non-native seaweeds by native consumers and abiotic conditions warrants further investigation.

While the present study increases our knowledge of which species consume *G. vermiculophylla*, we do not know the long-term effects of seaweed consumption on native species fitness. Indeed, consumption of non-native seaweeds has been linked to degraded metabolic and physiological conditions of both native invertebrate and fish grazers (e.g., Scheibling and Anthony 2001; Gollan and Wright 2006; Lyons and Scheibling 2007; Felline et al. 2012). For instance, the invasive seaweed *Caulerpa racemosa* causes reduced growth in sea urchins, *Paracentrotus lividus* (Tomas et al. 2011) and cellular and physiological alterations in white sea bream, *Diplodus targus* (Felline et al. 2012), which may lead to reduced fitness and stocks over the long-term. Much like *L. rhomboides* in the present study, *D. targus* and *P. lividus* prefer *C. racemosa* to native seaweeds and other invasive seaweeds. For these native Mediterranean herbivores, their preferences of the invasive seaweed demonstrate a mismatch between preference and fitness consequences that elucidates an ecological trap for the native herbivores. This is a critical scenario to consider for *L. rhomboides* and *G. vermiculophylla*. *G. vermiculophylla*'s novelty to southeastern estuaries may have subtle, long-term ecological consequences for native consumers that have not been studied to date. Therefore, without research on the fitness consequences of *G. vermiculophylla* consumption, we cannot determine the overall positive or negative effect of the novel resource within these ecosystems.

## Conclusion

Despite the potential of *G. vermiculophylla* to provide a novel, abundant primary resource within southeastern estuaries, we observed varied utilization of the introduced seaweed depending on consumer identity and seaweed nutrient content. Nonetheless, the mixed effects of *G. vermiculophylla* on grazer consumption and attraction highlights the need for more in-depth research on trophic interactions with non-native seaweeds globally. Furthermore, assessing the consumption of non-native seaweeds across an array of consumer guilds, macroalgal traits, and seasons will provide a more realistic illustration of seaweed-herbivore interactions. Given that exotic seaweed species continued to be introduced across the world, resulting in millions of dollars in damages annually (Schaffelke and Hewitt 2007), more fine-tuned analysis of trophic dynamics is necessary to predict and control the spread of nuisance non-native seaweeds.

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## Tables

Table 3.1. Mean and standard error (± SE) of seaweed biomass consumption (g wm) for three native herbivores in both 'choice' and 'no-choice' feeding assays.

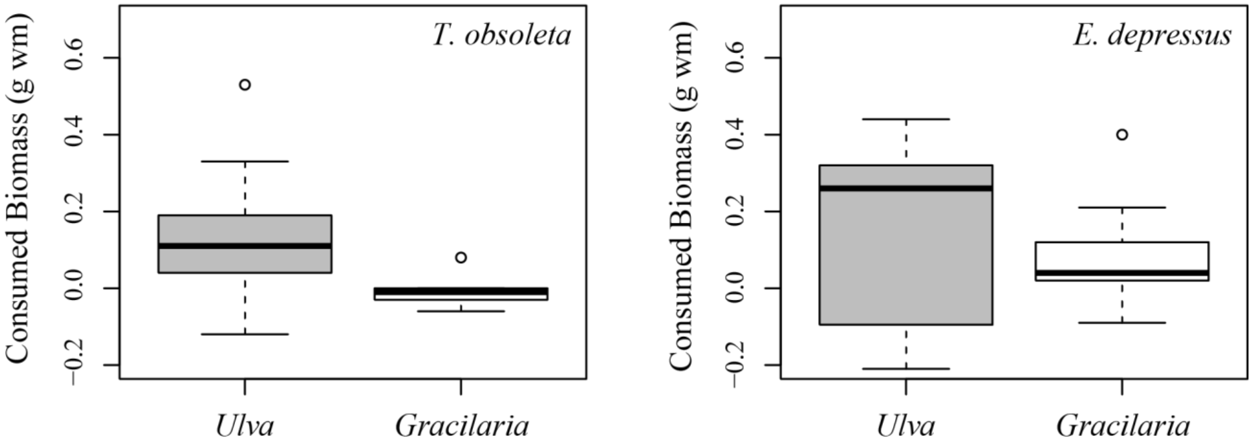
| **Feeding Assay** | **Herbivore Species** | **Seaweed Species** | **Consumption (g wm)** | |
|---|---|---|---|---|
| | | | **Mean** | **± SE** |
| Choice Assay | *T. obsoleta* | *G. vermiculophylla* | 0.01 | 0.02 |
| | | *U. lactuca* | 0.13 | 0.04 |
| | *E. depressus* | *G. vermiculophylla* | 0.09 | 0.04 |
| | | *U. lactuca* | 0.32 | 0.06 |
| | *L. rhomboides* | *G. vermiculophylla* | 0.68 | 0.11 |
| | | *U. lactuca* | 0.07 | 0.1 |
| No Choice Assay | *T. obsoleta* | *G. vermiculophylla* | -0.01 | 0.01 |
| | | *U. lactuca* | 0.14 | 0.07 |
| | *E. depressus* | *G. vermiculophylla* | 0.09 | 0.05 |
| | | *U. lactuca* | 0.15 | 0.09 |
| | *L. rhomboides* | *G. vermiculophylla* | 0.72 | 0.26 |
| | | *U. lactuca* | 0.44 | 0.12 |

Table 3.2. Mean and standard error (± SE) of the density of total amphipods/g wm *Gracilaria*, the density of *Ampithoe valida*/g wm *Gracilaria*, and the percentage of *G. vermiculophylla* biomass remaining across experimental treatments and collection days during two iterations of an *in situ* invertebrate colonization experiment.

| **Month** | **Treatment** | **Day Collected** | **All Amphipods** | | ***Ampithoe valida*** | | **% Biomass Remaining** | |
|---|---|---|---|---|---|---|---|---|
| | | | **Mean** | **± SE** | **Mean** | **± SE** | **Mean** | **± SE** |
| October 2014 | Enriched | 5 | 0.2 | 0.0 | 0.1 | 0.0 | 96.26 | 3.15 |
| | Non-Enriched | 5 | 1.2 | 0.1 | 0.9 | 0.1 | 80.73 | 0.98 |
| | Enriched | 11 | 0.5 | 0.2 | 0.5 | 0.2 | 86.54 | 2.37 |
| | Non-Enriched | 11 | 1.9 | 0.2 | 1.5 | 0.2 | 70.48 | 6.41 |
| August 2015 | Enriched | 10 | 0.3 | 0.1 | 0.3 | 0.1 | 22.2 | 6.11 |
| | Non-Enriched | 10 | 0.8 | 0.3 | 0.6 | 0.3 | 24.07 | 4.93 |

## Figures

a) No-Choice Assay



b) Choice Assay

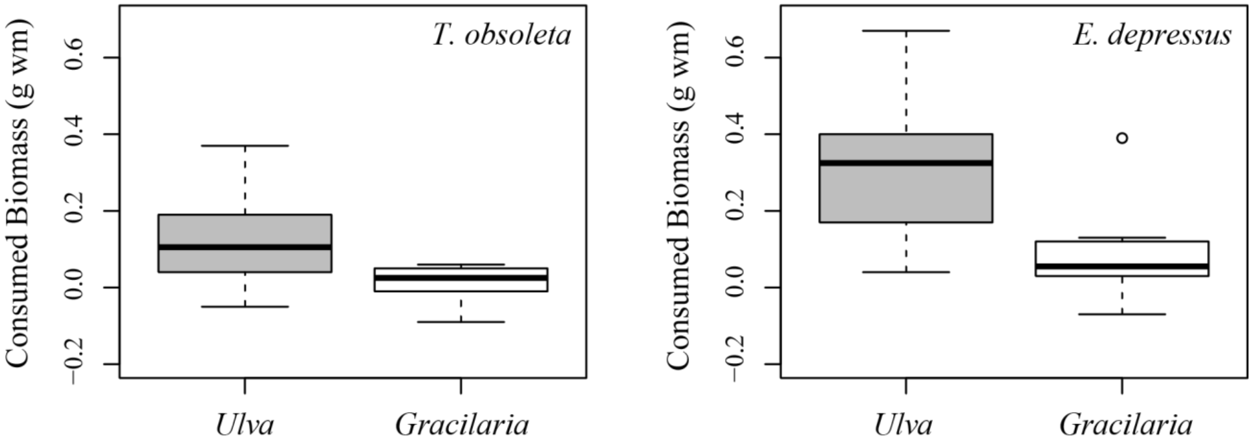


Figure 3.1. Amount of biomass (g wm) of native (*Ulva lactuca*) and non-native (*Gracilaria vermiculophylla*) seaweed consumed by native *Tritia obsoleta* and *Eurypanopeus depressus* in (a) no-choice (n = 10) and (b) choice (n = 10) feeding assays. In the no-choice feeding assays, the consumers were offered ~0.5 g of one seaweed species; in the choice feeding trials, invertebrate consumers were offered ~0.5 g of each seaweed species. Assays ran for seven days.

We analyzed the choice assays using Wilcoxon Signed Rank tests and the no-choice assays using Wilcoxon Rank Sum tests. Grey represents the *U. lactuca* treatment, and white represents the *G. vermiculophylla* treatment. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

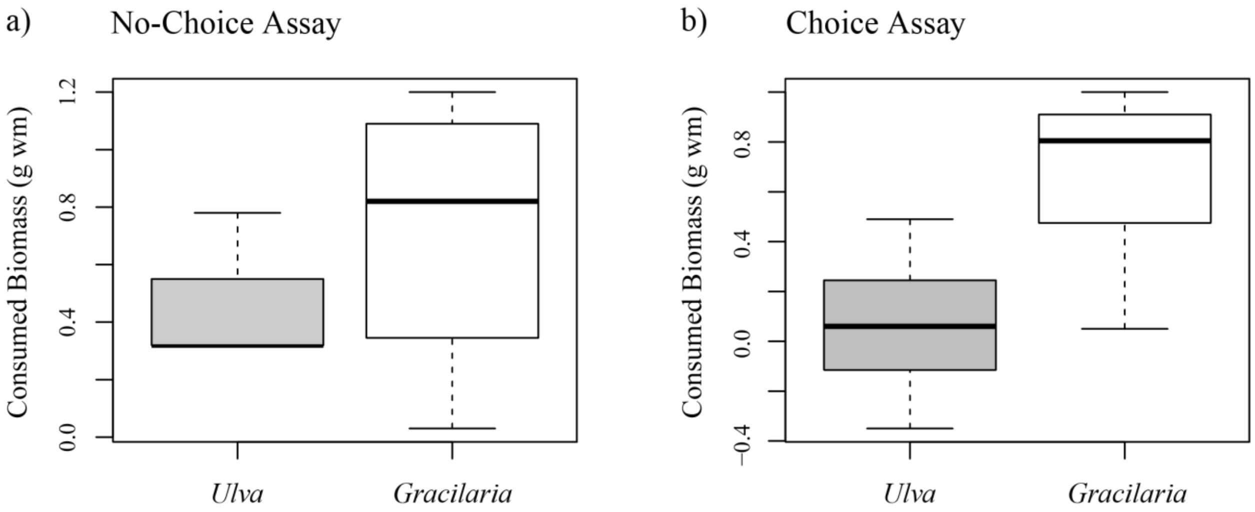


Figure 3.2. Amount of biomass (g wm) of native (*Ulva lactuca*) and non-native (*Gracilaria vermiculophylla*) seaweed consumed by pinfish, *Lagodon rhomboides*, in the (a) no-choice (n = 4) and in the (b) choice (n = 8) feeding assays. In the no-choice feeding assays, the fish were offered ~1.0 g of one seaweed species; while in the choice feeding trials, fish were offered ~1.0 g of each species. Assays ran for 2 days. We analyzed the choice assay using a paired t-test and the non-choice assay using a Welch's two-sample t-test. Grey represents the *U. lactuca* treatment, and white represents the *G. vermiculophylla* treatment. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

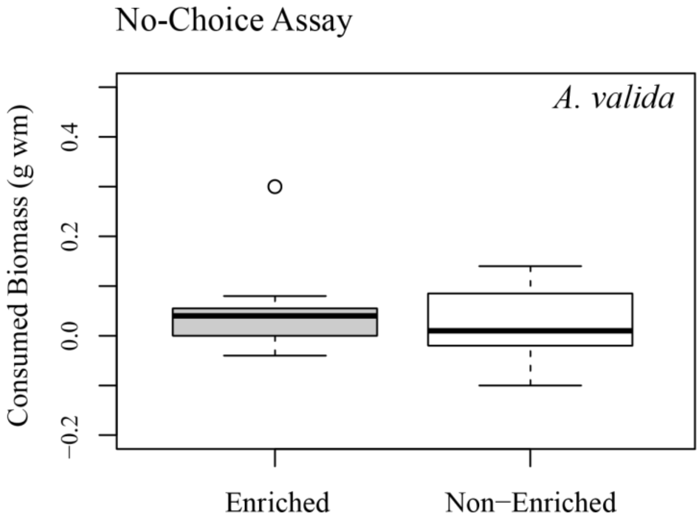


Figure 3.3. Amount of *Gracilaria vermiculophylla* biomass consumed (g wm) by amphipods, *Ampithoe valida*. *G. vermiculophylla* treatments were nutrient-enriched (n = 12) and non-enriched (n = 11). Amphipods were offered ~0.5 g of *G. vermiculophylla*. Assays ran for 7 days. Data were analyzed using ANOVA. Grey represents the nutrient-enriched treatment, and white represents the non-enriched treatment. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

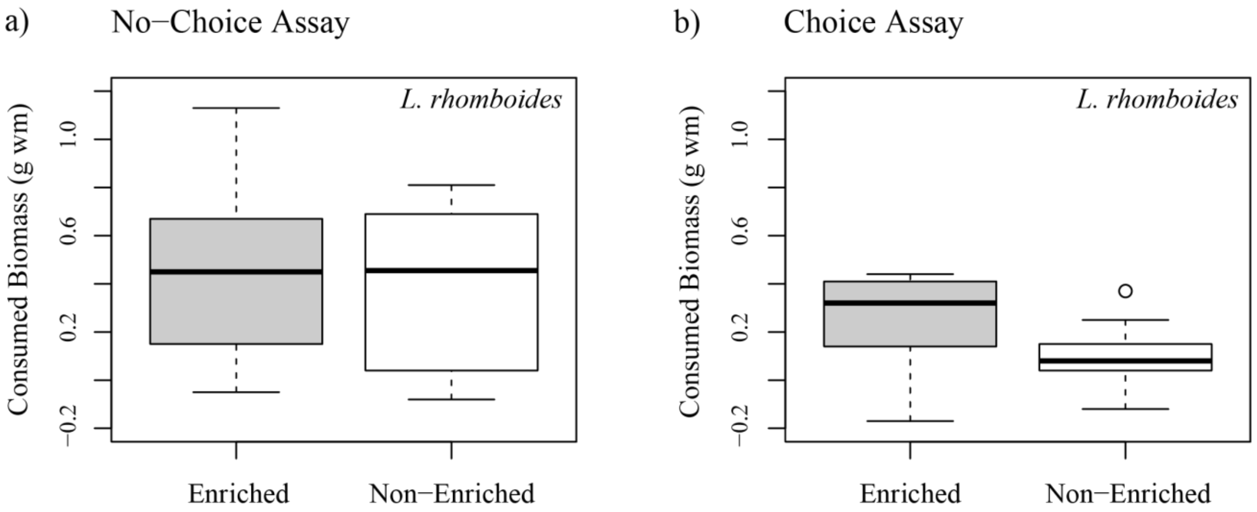
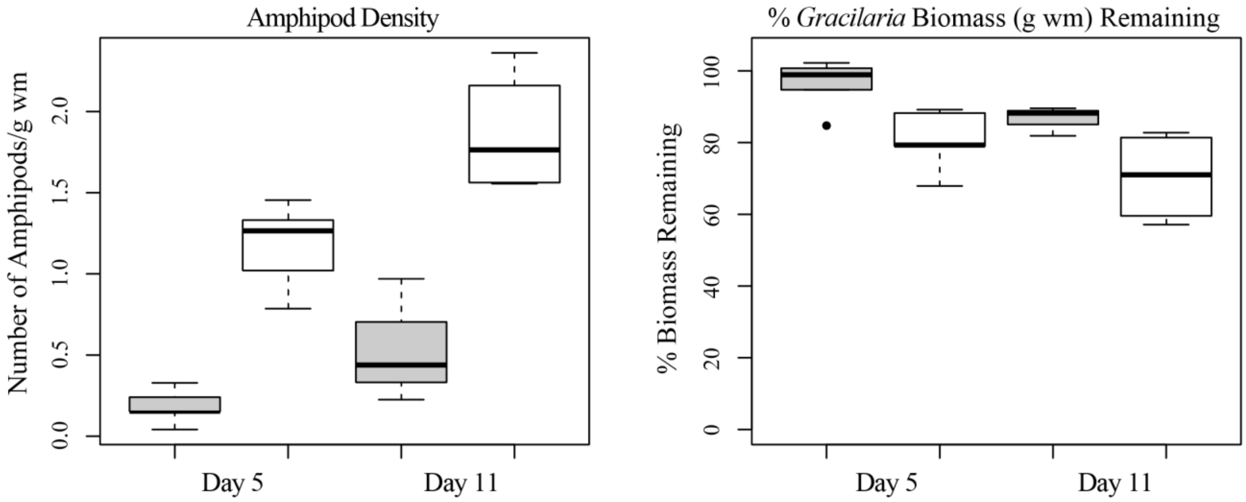


Figure 3.4. Amount of *Gracilaria vermiculophylla* biomass (g wm) consumed by *Lagodon rhomboides* during (a) no-choice and (b) choice feeding assays. *G. vermiculophylla* treatments were nutrient-enriched (n = 10) and non-enriched (n = 10). In the no-choice feeding assays, the consumers were offered ~1.0 g of one enrichment treatment; while in the choice feeding assay, fish were offered ~1.0 g of each enrichment treatment. Assays ran for 3 days. Data were analyzed using paired t-tests. Grey represents the nutrient-enriched treatment, and white represents the non-enriched treatment. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

a) October 2014



b) August 2015

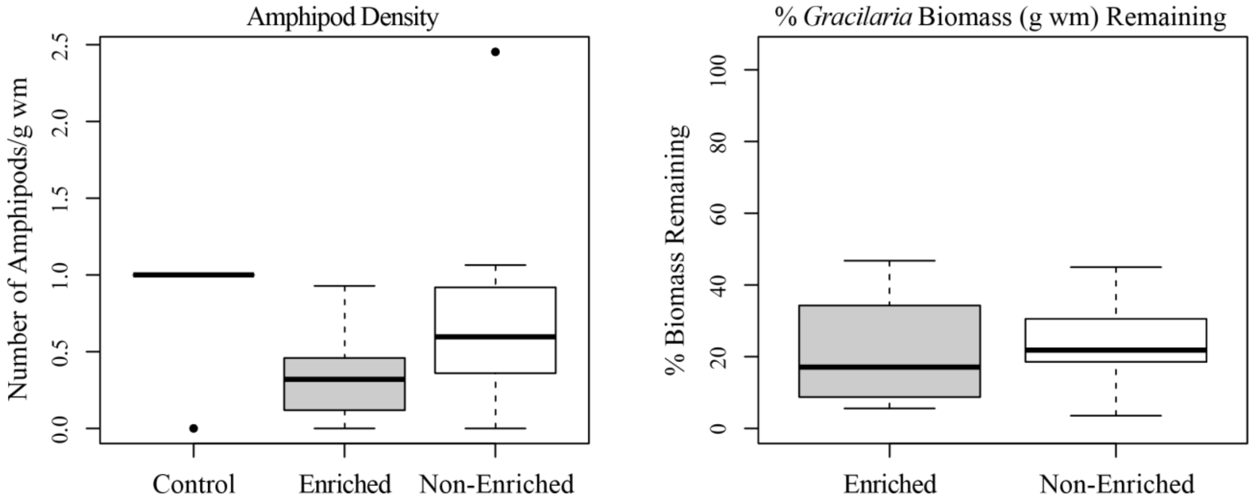


Figure 3.5. Colonization by amphipods and breakdown of *Gracilaria vermiculophylla* from an *in situ* litterbag experiment in (a) October 2014 and (b) August 2015. In October (a), amphipod density per gram of final *Gracilaria* biomass (g wm) (left panel) and percentage of *Gracilaria* biomass remaining (right panel) were measured after 5 and 11 days for two treatments: nutrient-enriched (n = 5) and non-enriched (n = 3 and 4, respectively). In August (b), amphipod density per gram of final *Gracilaria* biomass (g wm) (left panel) and percentage of *Gracilaria* biomass

remaining (right panel) were measured after 10 days for three treatments: nutrient-enriched (n = 14), non-enriched (n =14), and control (n = 8). Amphipod density was analyzed using generalized linear regression with a negative binomial distribution, while % remaining biomass was analyzed using simple linear regression. Grey represents the nutrient-enriched treatment, and white represents the non-enriched treatment. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

# CHAPTER 4

# MIXED EFFECTS OF AN INTRODUCED ECOSYSTEM ENGINEER ON THE FORAGING BEHAVIOR AND HABITAT SELECTION OF PREDATORS[3]

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[3] Haram, L., K. Kinney, E. Sotka, and J. Byers. In Review at *Ecology*.

## Abstract

Invasive ecosystem engineers both positively and negatively affect their recipient ecosystems by generating novel habitats. Many studies have focused on alterations to ecosystem properties and to native species diversity and abundance caused by invasive engineers; however, relatively few studies have documented the extent to which behavior of native species is affected. The red seaweed, *Gracilaria vermiculophylla* (Rhodophyta), invaded estuaries of the southeastern United States within the last few decades and now provides abundant aboveground vegetative cover on intertidal mudflats that were historically devoid of seaweeds. We hypothesized that *G. vermiculophylla* may affect the foraging of native shorebirds positively for birds that forage on seaweed-associated invertebrates or negatively for birds that less effectively forage on or within the sediment covered with seaweed. Visual surveys of mudflats (>1 ha) with or without *G. vermiculophylla* revealed that more shorebirds occurred on mudflats with *G. vermiculophylla*. This increased density was consistent across 7 of 8 species, with the one exception being the semipalmated plover (*Charadrius semipalmatus*). However, other abiotic and biotic factors were also significant predictors of shorebird density. At smaller spatial scales (200 $m^2$ and <1 $m^2$), experimental removals and additions of *G. vermiculophylla* and focal observations showed there was greater variation in behavioral response to *G. vermiculophylla* among bird species; some birds preferentially foraged on bare mud (e.g., *C. semipalmatus*), some preferentially foraged on *G. vermiculophylla* (e.g., *Arenaria interpres*), and some showed no preference (e.g., *Tringa semipalmata*). Thus, while the presence of the invasive ecosystem engineer on a mudflat may have attracted greater numbers of the generalist predators, these bird species differed in their behavioral responses at the smaller spatial scales that affect their foraging. Our research

illuminates the need to account for species identity, individual behavior, and scale when predicting impacts of invasive species on native communities.

## Introduction

Invasive species are one of the leading threats to global and local biodiversity (Vitousek et al. 1996); yet, their effects are often nuanced, affecting native species both negatively and positively by altering resource availability and quality, trophic interactions, and disturbance regimes (e.g., Straube et al. 2009; Byers et al. 2010; Simberloff 2011; Simberloff et al. 2013; Pintor and Byers 2015). Invasive species can have particularly far-reaching effects if they are ecosystem engineers – organisms that directly or indirectly regulate the availability of resources through physical state changes within an ecosystem (Jones et al. 1994, 1997; Crooks 2002). Their control of food and habitat resources makes invasive ecosystem engineers fundamental determinants of the diversity and abundances of native species (e.g., Di Tomaso 1998; Grosholz et al. 2009; Villamagna and Murphy 2010; DeVore and Maerz 2014).

Ecosystem engineers can disproportionately affect community structure, as these organisms influence both bottom-up and top-down controls. The effects of an invasive ecosystem engineer may be even more conspicuous because the invasive engineer may exert mixed effects through a variety of mechanisms that can ultimately transform whole ecosystems as the system settles into a new equilibrium (Byers et al. 2010). Despite the potential for negative consequences of invasive ecosystem engineers during system transformation, they can positively affect native community members through generation of habitat (e.g., Gribben et al. 2013; DeVore and Maerz 2014; Wright et al. 2014). Furthermore, the relative contribution of negative and positive effects is dependent on the context in which the ecosystem engineer is introduced. For example, in southeastern Appalachian forests, the invasion of herbaceous understory communities by Japanese stilt grass (*Microstegium vinineum*) has mixed effects on some native community members. The increased habitat complexity reduces arthropod density (Simoa et al.

2010) on the forest floor, but increases habitat availability for predatory spiders (DeVore and Maerz 2014). Both effects reduce populations of amphibians (DeVore and Maerz 2014). Thus, studies of community-level interactions are important when identifying the potential mixed effects of invasive ecosystem engineers.

Positive effects of invasive ecosystem engineers often result when the habitat that they provision is different in kind from the structure that is naturally available (Crooks 2002). For instance, novel ecosystem engineers may provide new habitat structure that shifts prey distributions to areas with greater structural complexity (Tanner 2011). In fact, a review of facilitation of native species by invasive species identified habitat modification, specifically the creation of novel habitat, as the most frequently cited mechanism for these facilitative interactions (Rodriguez 2006). Furthermore, the introduction of structure by an invasive species could be more pronounced if structure is a limiting factor in the community. Such alterations in habitat quality and subsequent bolstering of native species may have cascading effects on an ecosystem's trophic structure. For instance, zebra mussels, *Driessena polymorpha*, which provide novel structure and refuge in the benthic zones of freshwater lakes, house exponentially greater numbers of invertebrates than *D. polymorpha*-free substrate (Bially and MacIsaac 2000; Rodriguez 2006; Sousa et al. 2009). The greater abundance of food items in the invaded areas has led to increased growth rates of benthic consumers (Thayer et al. 1997; Rodriguez 2006). Not only can habitat modification lead to changes in overall prey abundance, it can also cause predators to change their preferences for foraging habitat. For example, the invasive tubeworm, *Ficopomatus enigmaticus*, creates reef structures that attract greater densities of invertebrates and increases shorebird foraging in the novel habitat (Schwindt et al. 2001; Bruschetti et al. 2009). Such positive interactions are not necessarily the norm, and these interactions and subsequent impacts are likely to be highly dependent on scale, history of invasion, and local community dynamics (Jones et al. 1997).

Another consideration when assessing how an ecosystem engineer may influence a recipient environment is how resident species distinctively respond to novel structure. For example, native species with different ecological niches may perceive the environmental modifications by invasive ecosystem engineers differently, suggesting the possibility of mixed effects across a community (e.g., Crooks 1998). Distinguished alterations of species behaviors can in turn lead to shifts in species interaction strengths for fundamental processes, like predation. Because predator identity and diversity are known to determine trophic structure by altering behavior and abundances of lower trophic levels (Bruno and O'Connor 2005; O'Connor et al. 2008), predator species reacting differently to an invasive ecosystem engineer could create effects that cascade through the food web. Thus, when assessing the effect of an invasive ecosystem engineer on native communities, it is important to recognize that species and individuals may respond distinctively. Additionally, determining the effects of invasive species on multiple predator species could reveal whether niche differentiation or behavioral differences produce divergent responses among a suite of predators that utilize the same prey resources. Here we examine the responses of multiple native species within the same trophic level to a ubiquitous introduced ecosystem engineer. Such comparisons may elucidate the degree of variation in overall response by the native community and may help to predict responses of individual species based on their foraging ecology and ecological roles within the community.

### *Research System and Questions*

A recent ecosystem engineer invasion in the southeastern United States provides an opportunity to investigate the role these species play on recipient communities, specifically the foraging patterns of multiple predator species within the same trophic level. *Gracilaria vermiculophylla*, a red seaweed from the coast of Japan, has invaded many coastal habitats in Europe and North America (Thomsen et al. 2009; Kim et al. 2010; Krueger-Hadfield et al. 2017). Since the early 2000s, the invasive seaweed has considerably altered estuaries of Georgia by creating novel habitat on the previously bare mudflats (Byers et al. 2012). This system was largely devoid of macroalgae due to high turbidity and lack of hard substrate for algal attachment. The alga is able to anchor on mudflats due to an association with native tube building polychaete worms, *Diopatra cuprea*, that attach the alga to their tubes (Thomsen and McGlathery 2005; Berke 2012; Byers et al. 2012; Kollars et al. 2016). Increased habitat structure and more amenable abiotic conditions (reduced surface temperatures and desiccation stress during low tide) created by *G. vermiculophylla* have increased abundance of epifaunal invertebrates and shifted many of their distributions from bare mudflats to those colonized by the invasive seaweed (Byers et al. 2012; Wright et al. 2014; Bishop and Byers 2015).

In these estuaries, migratory shorebirds are important predators and are pivotal in regulating macroinvertebrate abundances (Schneider and Harrington 1981; Steinmetz et al. 2003). Mudflat ecosystems provide shorebirds with high quality, often preferred, foraging habitat (Burger et al. 1977; Lorenço et al. 2015). Furthermore, the estuaries of the southeastern U.S. serve as important stopover and over-wintering sites for many shorebird species that migrate between the Arctic to the tropics using the Atlantic Flyway. The most common shorebird species found in the southeast during the peak migratory season (April-May) are dunlin (*Calidris* *alpine*), semipalmated plover (*Charadrius semipalmatus*), western sandpiper (*Calidris mauri*), least sandpiper (*Calidris minutilla*), semipalmated sandpiper (*Calidris pusilla*), ruddy turnstone (*Arenaria interpres*), willet (*Tringa semipalmata*), black-bellied plover (*Pluvialis squatarola*), and short-billed dowitcher (*Limnodromus griseus*) (Tomkins 1965; Stinson 1980; Harrington 2008; Rose and Nol 2010; L. Haram, *personal observation*). Shorebirds form dense aggregations of mixed species that forage on intertidal mudflats during low tide. Niche differentiation in these aggregations is achieved in part because each species has distinct foraging strategies (visual, tactile, or mixed) and feeding morphologies (body and bill size and shape) to catch epifaunal, epibenthic, and/or infaunal invertebrates in estuarine systems (Mouristen 1994; Nettleship 2000; Jehlr et al. 2001; Elner et al.2005; Nebel et al. 2005; Hicklin and Gratto-Trevor 2010; Rose and Nol 2010; Lowther et al. 2011; Nol and Blanken 2014; Poole et al. 2016).

Shorebird species and community composition are non-randomly distributed within estuarine habitats. The abundance, biomass, and availability of prey are crucial predictors of shorebird communities on intertidal mudflats (Dugan et al. 2003; Spruzen et al. 2008; VanDusen et al. 2012), with shorebirds known to forage in areas with greater prey availability (Fraser et al. 2010). Across microhabitats and entire mudflats, prey availability is constrained directly by abiotic factors, such as sediment composition and tidal cycle (Burger et al. 1977; VanDusen et al. 2012). Presence of vegetation on mudflats also influences shorebird distributions due to its effects on macroinvertebrate abundance and shorebird foraging efficiency. Increased benthic structure provided by submerged macrophytes, such as seaweeds, seagrasses, and reef-forming fauna, can increase macroinvertebrate abundances (Spruzen et al. 2008; Bruschetti et al. 2009), possibly leading to greater densities of birds utilizing invaded mudflats. Macrophytic wrack subsidies to low productivity beaches can also increase availability of prey items for shorebirds, with positive relationships observed between standing crop of wrack and shorebird abundances (Dugan et al. 2003). However, dense macrophyte coverage on a mudflat may inhibit shorebird foraging, likely through interference with tactile and visual detection of prey, smothering of resources, and alteration of prey species composition (Spruzen et al. 2008). Given these scenarios, the impact of an invasive habitat modifier on shorebirds could be either negative or positive. Furthermore, the impact of an ecosystem engineer may differ among predator species, causing divergent, species-specific responses in foraging behavior and habitat selection.

Shorebirds present an optimal target for investigating the effects of invasive ecosystem engineers like *G. vermiculophylla* on predator foraging because shorebirds adjust to local conditions, feed opportunistically, and shift their prey preferences with season and local prey availability (Dierschke et al. 1999; Nettleship 2000; Jehl et al. 2001; Nebel and Cooper 2008; Hicklin and Gratto-Trevor 2010; Mathot et al. 2010; Rose and Nol 2010; Lowther et al. 2011; Poole et al. 2016). Through two observational studies and two complementary manipulative experiments at various spatial scales, we assessed how the novel physical structure of an invasive ecosystem engineer alters shorebird foraging in essential stopover habitat. We hypothesized that the presence of *G. vermiculophylla* would have significant effects on the density of shorebirds, with shorebirds preferring mudflats inhabited by the invasive engineer due to greater prey availability. However, we also hypothesized that alterations in foraging behavior would be species-specific depending on each species' niche type. Despite the potential for mixed effects, we expected net positive relationships between the invasive ecosystem engineer and shorebird density and foraging effort, given the higher epifauna abundance associated with *G. vermiculophylla*, shorebirds' opportunistic foraging during migration, and the limited physical impediment we expected the alga would present. We intended our multi-species perspective to elucidate the degree of uniformity (or lack thereof) in the behavioral responses of higher trophic levels to invasive ecosystem engineers.

## Methods

### *Large Scale Habitat Selection Survey*

To determine what habitat type (*Gracilaria*-colonized or bare mudflats) was more attractive to shorebirds, we conducted shorebird density surveys across twelve mudflats in Wassaw Sound, Savannah, Georgia (31°56’18.8”N, 80°56’53.7”W): six mudflats with *G. vermiculophylla* and six mudflats without *G. vermiculophylla* (Appendix S1: Table S1). We selected sites based on size (>1 ha), continuity of substrate, and observer access. During tides below 0 m mean lower low water (MLLW) in daylight hours, we surveyed 500 m transects parallel to the water line for shorebird abundance and species composition at each site. Along the transect, we surveyed the entire width of the intertidal mudflat from the low tide line to its highest elevation, which was visually determined based on water level predictions, time of day, and biological zonation of *Spartina* cord grass or oyster reef. The location of the 500 m transect was selected using low tide images on Google Earth, and clear landscape features were used as end points for the transect. Surveys were done by boat (and in one instance by foot due to logistical issues) ~50 m away. Care was taken to not flush the flocks, and no apparent disturbance of shorebird activity was detected across survey sites. A captain maintained a slow, consistent speed while a trained observer counted birds using 16x50 Nikon 7247 Action binoculars. A single observer identified and counted shorebirds across sites to eliminate observer differences and minimize potential error. Small sandpiper species (*Calidris spp.*) (approximately

<17 cm in length) were counted collectively as “Peeps” because the distance from the mudflats, speed of surveys, and winter plumage was not conducive to further identification. To standardize conditions between sites, we conducted surveys on days when weather was fair, i.e., with no precipitation and wind speed < 20 knots. We surveyed each mudflat twice from April 2, 2015 to May 17, 2015 (excluding two sites, House Creek and Little Tybee, that we surveyed once) for a total of 22 surveys; surveys were averaged for each site to reduce the effect of sampling date. Spring movement begins in March for these species, with their peak migrations through the southeastern U.S. occurring in April to early May (see descriptions in Warnock and Gill 1996; Nettleship 2000; Jehl et al. 2001; Nebel and Cooper 2008; Hicklin and Gratto-Trevor 2010; Lowther et al. 2011; Nol and Blanken 2014; Poole et al. 2016). We attempted to standardize bird counts by recording them at their highest levels (i.e., during peak migration). We standardized the shorebird counts as densities by estimating the total area (hectares) of each mudflat spanned by the 500 m transect using the polygon tool on low-tide images from Google Earth.

Because shorebirds are known to select foraging sites based on an array of conditions, we gathered additional data on environmental and biotic variables at each surveyed mudflat that may covary with *G. vermiculophylla* presence. Once we completed a shorebird survey, we established a 50 m transect in the middle of the 500 m transect at approximately 0 MLLW, along which we sampled *G. vermiculophylla* biomass density, epifaunal/epibenthic invertebrate density, infaunal invertebrate density, and sediment composition every 5 m for a total of 10 sampling points per site. To quantify *G. vermiculophylla* biomass and its associated epifaunal (i.e., found on seaweed) and epibenthic (i.e., found on the sediment) invertebrates, we collected all *G. vermiculophylla* in a 0.25 $m^2$ quadrat and immediately placed it in Ziploc bags for transport back to the laboratory. We also recorded large epibenthic invertebrates (~1 cm and greater) in this same quadrat found on the sediment surface. Directly adjacent to each quadrat, we collected sediment cores (10 cm diameter, 10 cm deep) for abundance of infaunal and small epibenthic invertebrates (referred to collectively as infauna hereafter) and cores for sediment type (3 cm diameter, 8 cm deep).

In the lab, we rinsed the *G. vermiculophylla* gathered from the quadrat samples, removed epifaunal and epibenthic invertebrates and preserved them in 10% formalin. We then weighed the seaweed for wet biomass (g) and dried it at 60ºC for a minimum of two days to attain dry biomass (g). For the infaunal abundance sediment cores, we sieved the cores using 250 µm mesh and filtered seawater. We immediately sorted visible infauna and preserved them in 10% formalin. We preserved the remaining sample for sorting under a dissection scope. We later transferred all preserved samples to 80% ethanol. Due to high invertebrate abundances in the sediment cores, only the first four infauna sediment cores from each site were processed. We classified invertebrates based on their lowest identifiable taxonomy. After identification, we dried the organisms in our sediment core samples at 60ºC for 3 days and weighed the organisms pooled by taxonomic unit for dry biomass (g). The ten sediment cores for sediment composition were combined by site and processed for percent composition of sand, silt, and clay by the Soil, Plant, and Water Laboratory at the University of Georgia.

To determine the relationship between shorebird density, *G. vermiculophylla* presence, and other environmental variables, we performed a linear regression using the ‘stats’ package in R 3.3.2 (R Core Team 2016). The predictor variables included algal presence (*Gracilaria* presence or absence), average infauna density (invertebrates/0.0079 $m^2$), average epibenthic invertebrate density (invertebrates/0.25 $m^2$) and the ratio of percent sand to percent silt. Sites that had an average of less than 5 g dw of *G. vermiculophylla* were categorized as absent of *G.*

*vermiculophylla*. All continuous variables were natural log-transformed to achieve normality based on visual inspection of distributions using the 'qqnorm' function in 'stats' package in R (R Core Team 2016). Independent variables were analyzed for correlation using the stepVIF function in the 'pedometrics' package in R (Samuel-Rosa 2015). Epifauna/epibenthic density was correlated with algal presence in all bird species models, and thus was excluded from the final model. We determined the relationship between algal presence and shorebird density of all species pooled as well as for individual species. In addition, we constructed all possible models and ranked them for best fit using an AICc comparison in the 'AICcmodavg' package in R (Mazerolle 2017). We also completed this analysis again, replacing average invertebrate densities with average invertebrate biomass densities (see Appendix S1 for results). We included density and biomass predictors in separate models to reduce correlation. Although biomass measurements were completed for the infaunal core data, we did not measure biomass directly for the epifaunal/epibenthic quadrat data. Thus, to estimate biomass, we calculated the average per capita biomass of different invertebrate phyla/classes in the infaunal cores and multiplied the estimated per capita biomass by the total number of individuals of the corresponding taxon in the epifaunal/epibenthic quadrat data.

To determine the effect of *G. vermiculophylla* presence on epifaunal/epibenthic and infaunal invertebrate abundances, we constructed generalized linear mixed effects models with negative binomial distributions and zero-inflation in the 'glmmADMB' package (Bolker et al. 2013) in R, with site as a random effect. We also determined the effect of *G. vermiculophylla* on epifaunal/epibenthic and infaunal invertebrate biomass densities, using linear mixed models with site, again, as a random effect. We evaluated the effect of algal presence on epifaunal/epibenthic invertebrates from the quadrat samples (total n = 120) and infaunal invertebrates from the sediment core samples (total n = 48) separately due to the different collection methods.

### *Intermediate Scale Habitat Selection - Experimental Removal and Addition of Seaweed*

Though abundance surveys provide essential information about shorebird habitat association at the large scale (>1 ha), we wanted to experimentally examine how *G. vermiculophylla* presence affects shorebirds' habitat selection and foraging. Therefore, we conducted manipulative field experiments at an intermediate scale (200 $m^2$) to determine if the birds foraged more in areas of *G. vermiculophylla* versus adjacent bare treatments. We ran the first experiment over a three-week period in April 2014, on three *G. vermiculophylla*-inhabited mudflats used in the habitat preference survey. We were unable to visit the three sites over successive days due to the need for two consecutive days of fair weather (<20 knot winds and no precipitation) and negative low tides (-0.5 to -1.0 MLLW) for each site. At each site, we haphazardly selected a location on the mudflat and established two 10 x 20 $m^2$ plots separated by 10 m. The proximity of the plots was meant to reduce variability in abiotic and biotic conditions between the plots and allowed for birds to encounter both plots in a short time period. We removed *G. vermiculophylla* from one plot and left *G. vermiculophylla* intact in the other. To remove *G. vermiculophylla* from the entire plot, while minimizing disturbance to the mud surface, we moved through the plot at low tide on flat flotation boards. We controlled for sediment disturbance by moving through the paired plots similarly and allowed the sediment to settle over a full tidal cycle before beginning our observations. Over the duration of a low tide (~3 hours), 3-minute focal bird observations were conducted for shorebirds that visited each treatment. We observed each bird for up to three minutes, or until it left the plot, recording number of pecks and overall time spent in the plot. In these experiments, peck rate is the definitive response, as it depicts true foraging effort, while time spent in a plot may be a mixture of foraging and meandering. We alternated observations between the removal versus control plots. Observations were made from the mudflat, at least 20m away from the plots behind a mobile blind, using 16x50 Nikon 7247 Action binoculars. We standardized foraging effort by each observed bird by assessing foraging rates (pecks/minute).

From mid-April to mid-May 2015, we conducted a reciprocal experiment by adding *G. vermiculophylla* to experimental plots on three mudflats where it was absent. The contrast in the effect of *G. vermiculophylla* between this addition experiment and the previous removal experiment should inform at least two factors. First, the contrast should allow us to examine whether *G. vermiculophylla*'s presence or absence at a mudflat scale (i.e., the background context) influences bird foraging decisions on the smaller, experimental-plot scale. Second, the contrast suggests whether *G. vermiculophylla* addition immediately affects shorebird habitat preference or whether its influence accrues over time (e.g., through recruitment of invertebrates). For this experiment, we left one of the paired plots as natural bare mudflat and to the other we added a standard amount of *G. vermiculophylla* (~7 kg wet biomass). This amount was the same amount of *G. vermiculophylla* that we removed from our highest density *G. vermiculophylla* mudflat in the removal experiment. Using flat flotation boards, we added *G. vermiculophylla* to the plot in a haphazard manner, attempting to create a natural spread of the seaweed across the plot. *G. vermiculophylla* was secured in place through partial burial and garden pins. Both plots were similarly disturbed and a similar number of garden pins were added to each plot. We harvested seaweed for the *G. vermiculophylla* addition treatment from a single site to ensure standard quality as well as epifauna within the *G. vermiculophylla*. We kept epifauna intact to mimic conditions on a high-density *G. vermiculophylla* mudflat. We used the same data collection methods and response variables as in the removal experiment.

We analyzed data for each experiment separately with a mixed-effects linear regression model in R, using the 'lme4' package (Bates et al. 2015). For both experiments, the response variable, pecks/minute, was natural log-transformed to attain normality and assessed as a function of treatment (*G. vermiculophylla* vs. bare mud). We also analyzed the amount of time (seconds) spent foraging as a function of treatment using a generalized linear regression analysis with a negative binomial distribution. For analyses of both response variables, we treated site as a random effect. Due to the lack of uniform presence of all shorebird species across sites, we first examined foraging rates (pecks/minute) for all shorebirds pooled (removal: n = 68; addition: n = 92). We then performed species-specific analyses on the two most common species in our experimental plots, dunlin (removal: n = 24; addition: n = 35) and semipalmated plovers (removal: n = 15; addition: n = 23). Semipalmated plovers were only present at one site for each experiment, so for their analysis, we removed the random site factor and analyzed the data using a simple linear regression model.

### *Patch Scale Foraging Behavior Study*

To determine if shorebirds utilize *G. vermiculophylla* at the smallest scale (i.e., individual clumps of seaweed attached to a *D. cuprea* worm tube, <1 $m^2$), we conducted a study that assessed individual shorebird preference for foraging directly in *G. vermiculophylla* versus the bare mud interspersed between seaweed patches. This helped ascertain whether birds at the larger scales of our study are attracted to *G. vermiculophylla* specifically for foraging quality (e.g., because high densities of invertebrates are present in *G. vermiculophylla* patches) or are just associating generally with areas invaded by *G. vermiculophylla* due to a larger scale influence the seaweed has on the environment or another correlated characteristic. Thus, studying foraging behavior at this small scale (<1 m$^2$) allowed us to determine if the shorebirds' habitat choices at the large scale (>1 ha) are related to their actual foraging preferences.

In March 2015, on five mudflats colonized by *G. vermiculophylla* (and used in the Large Scale Habitat Selection Survey), we observed up to five individual shorebirds from each of the most common shorebird species [dunlin, semipalmated plover, least sandpiper, ruddy turnstone, willet short-billed dowitcher, least sandpiper, and "peeps" (as before, remaining sandpiper species were pooled together)]. We systematically visited one flat per sampling day, over the course of 17 days. Sampling days were not successive due to the need for fair weather and negative low tides. During low tide, we observed a single focal bird for up to three minutes (or until it flew away) using 16x50 binoculars. During that time, we recorded the number of pecks in a natural *G. vermiculophylla* patch versus the surrounding bare mudflat. To compare the frequency of pecks in *G. vermiculophylla* to the seaweed's frequency on the mudflat, we quantified *G. vermiculophylla* percent cover by photographing ten 0.25 m$^2$ quadrats during the Large Scale Habitat Selection Survey. From the photographs, we estimated the ratio of *G. vermiculophylla* to bare mud at each mudflat with the image analysis software ImageJ. We estimated the average percent cover of each patch-type at each site and then averaged across all sites to obtain an overall average percent cover of *G. vermiculophylla* versus bare mud. Photographs of one site (Priest Landing) were lost and not included in this average; however, its average *G. vermiculophylla* biomass (known from the Large Scale Habitat Selection Survey) was within the range of the other sites. We used the ratio of percent cover of *G. vermiculophylla* to bare mud (0.27:0.73) to determine if the observed distribution of pecks significantly differed from the availability of patches in nature.

We analyzed the data using a hierarchical Bayesian analysis, designed specifically for ecological count data, in the R 3.3.2 package ‘bayespref’ (Fordyce et al. 2011). With this package, we estimated the strength of foraging preference for *G. vermiculophylla* patches versus bare patches for each bird and each species. The ‘bayespref’ package is preferred to other non-parametric methods because it directly estimates individual- and population-level preference, while allowing for non-normal distribution, dependent data, and uneven design (Fordyce et al. 2011). We ran models for 5,000 generations, with 10 generation burn-ins, setting prior distributions based on the expected distribution of pecks given the average proportion of percent cover of *G. vermiculophylla* patches to bare mud patches across sites. We visually assessed diagnostic plots of MCMC chain distributions to choose the most evenly mixed model. Once we generated preference strengths for each species, we compared the credible intervals (evaluated at 95%) to the proportion of *G. vermiculophylla* cover to bare mud. If the credible intervals of a species did not overlap the expected patch-type cover, the preference was considered significant.

## Results

### *Large Scale Habitat Selection Survey*

We observed a significant, positive association of *G. vermiculophylla* presence and shorebird density (birds/ha) across all sites when shorebirds were pooled ($F_{1,10} = 5.65$, $p = 0.04$, $R^2 = 0.30$) (Figure 1a). This pattern was similar when assessed for individual species. Dunlin, black-bellied plovers, willets, and short-billed dowitchers showed positive relationships with *G. vermiculophylla* ([dunlin – $F_{1,10} = 6.32$, $p = 0.03$, $R^2 = 0.33$]; [black-bellied plover – $F_{1,10} =$

11.93, $p < 0.01$, $R^2 = 0.50$]; [willet – $F_{1,10} = 15.23$, $p < 0.01$, $R^2 = 0.56$]; and [short-billed dowitcher – $F_{1,10} = 14.67$, $p < 0.01$, $R^2 = 0.55$]; Figure 1b, 1e, 1g, and 1h, respectively). Both peeps and ruddy turnstones demonstrated positive trends with *G. vermiculophylla* presence ([peeps – $F_{1,10} = 4.68$, $p = 0.06$, $R^2 = 0.25$] and [ruddy turnstone – $F_{1,10} = 4.07$, $p = 0.07$, $R^2 = 0.22$]; Figure 1c and 1d). Semipalmated plover densities did not differ significantly ($F_{1,10} = 0.14$, $p = 0.71$, $R^2 = 0.09$; Figure 1f).

Algal presence yielded the best fit model for all birds collectively and for some individual species in the AICc comparisons including ruddy turnstones, black-bellied plovers and willet (see Appendix S1: Table S2 for AICc results). Semipalmated plover density was best fit by the null (intercept-only) model. Additionally, the null model was within two $\Delta i$ for “all birds”, peeps and ruddy turnstones. However, for “all birds”, the algal presence model was over twice the Akaike weight of the null model. For ruddy turnstones and peeps, the algal presence-only model had 0.7 and 0.9 greater Akaike weights (respectively) than the null models. Other biotic and abiotic variables also accounted for variance in dunlin, short-billed dowitcher, peep and willet densities. Dunlin densities were best fit by an additive model that included ratio of percent sand to silt ($p = 0.001$) and infauna density ($p = 0.04$). Peep densities were also best fit by an additive model of algal presence ($p = 0.01$) and ratio of percent sand to silt ($p = 0.02$). For short-billed dowitchers, the best-fit model contained a significant interaction ($p < 0.01$) between infauna density ($p = 0.05$) and the ratio of percent sand to percent silt ($p = 0.01$).

Differences in the distributions of epibenthic/epifaunal and infaunal invertebrates were detected in response to *G. vermiculophylla* presence. When assessing epibenthic and epifaunal invertebrate abundances, using collection from 0.25 $m^2$ quadrats, we found that mudflats with *G. vermiculophylla* had greater epibenthos/epifauna abundances ($\chi^2_1 = 74.99$, $p < 0.001$, total n =

120; Appendix S1: Table S4) and biomass densities ($\chi^2_1 = 12.99$, $p < 0.001$; Appendix S1: Table S5). *Gammarus mucronatus* was the most abundant invertebrate within the quadrat samples (Appendix S1: Table S4), while gastropods accounted for the greatest biomass (Appendix S1: Table S5). However, when assessing infauna abundances, we found no difference between *G. vermiculophylla* mudflats and bare mudflats ($\chi^2_1 = 0.42$, $p = 0.50$, total $n = 48$; Appendix S1: Table S6). Similarly, we found no difference in infauna biomass between the two habitat types ($\chi^2_1 = 1.49$, $p = 0.22$; Appendix S1: Table S7). Marine annelids accounted for on average 63% of the infauna biomass in *G. vermiculophylla* mudflat sediment cores and 60% in bare mudflat cores (Appendix S1: Table S7). When assessing small epibenthic invertebrate biomass separately in the sediment cores, gastropods composed over 90% of the small epibenthos found in each habitat type (Appendix S1: Table S7).

### *Intermediate Scale Habitat Selection - Experimental Removal and Addition of Seaweed*

During the removal experiment, shorebirds on average foraged at a faster rate in 200 $m^2$ plots with *G. vermiculophylla* than in plots with *G. vermiculophylla* removed ($\chi^2_1 = 4.18$, $p = 0.04$, $n = 68$; Appendix S1: Figure S1a). Yet shorebirds spent similar time (seconds) in each treatment ($\chi^2_1 = 2.23$, $p = 0.14$). For the two most common birds, dunlin foraged at a significantly faster rate where G. *vermiculophylla* was intact ($\chi^2_1 = 13.87$, $p \leq 0.001$, $n = 24$), while semipalmated plovers did not forage at a significantly different rate between treatments ($F_{1,13} = 1.58$, $p = 0.23$, $n = 15$; Appendix S1: Figure S2a). We found no difference in the amount of time spent in each treatment for either species ($\chi^2_1 = 0.78$, $p = 0.38$; $\chi^2_1 = 2.58$, $p = 0.11$, respectively).

In the addition experiment, we did not detect a significant difference in foraging rate in 200 $m^2$ plots with or without *G. vermiculophylla* when all shorebirds were pooled ($\chi^2_1 = 0.009$, p

= 0.92, n = 92; Appendix S1: Figure S1b); however, pooled shorebirds tended to spend more time in *G. vermiculophylla* plots ($\chi^2_1 = 3.07$, $p = 0.08$). Dunlin showed no significant difference in foraging rate between treatments ($\chi^2_1 = 0.9$, $p = 0.34$, $n = 35$; Appendix S1: Figure S2b). Dunlin also spent similar amounts of time among treatments ($\chi^2_1 = 0.09$, $p = 0.77$). Semipalmated plovers again did not forage at a significantly different rate in bare mud versus *G. vermiculophylla* addition plots ($F_{1,21} = 2.02$, $p = 0.17$, $n = 23$; Appendix S1: Figure S2b); yet, they spent more time in *G. vermiculophylla* plots ($\chi^2_1 = 18.10$, $p < 0.001$).

### *Patch Scale Foraging Behavior Study*

At the small scale (<1 $m^2$), shorebird species differed in their foraging responses to *G. vermiculophylla* patches. Both short-billed dowitchers (n = 9) and willets (n = 6) showed no overall preference for pecking on bare mud versus in *G. vermiculophylla* patches (Figure 2a and 2g, respectively; Appendix S1: Table S8). However, individuals of both species varied substantially, with some individuals demonstrating foraging preference for bare or *G. vermiculophylla*. Least sandpipers (a species that was pooled with other peeps at the large scale, n = 15) and ruddy turnstones (n = 5) showed strong preferences for foraging in *G. vermiculophylla* patches at both the individual and population level (Figure 2c and 2e; Appendix S1: Table S8). Dunlin (n = 24) weakly preferred *G. vermiculophylla* patches at the population level, though individual preference varied (Figure 2b; Appendix S1: Table S8). In contrast, the remaining peeps (n = 7) and semipalmated plovers (n = 19) avoided *G. vermiculophylla* patches (Figure 2d and 2f; Appendix S1: Table S8).

## Discussion

Shorebirds demonstrated varied preferences for foraging habitat type across species and spatial scales, suggesting complex mixed responses to the invasive ecosystem engineer, *G. vermiculophylla*. Shorebird species were more abundant on large (>1 ha) mudflats with *G. vermiculophylla* relative to those without *G. vermiculophylla*, though the strength of this abundance effect was clearly species dependent (see Table 1 for summary). Given that introduced ecosystem engineers can have both positive and negative effects on communities (e.g. Boughton and Boughton 2014), the varied responses of these community members suggest that behavioral mechanisms may help to explain mixed effects of engineering, specifically in the context of novel habitat generation.

For ruddy turnstones, preference for mudflats with *G. vermiculophylla* at large spatial scales (>1 ha) generally reflected foraging preferences at the small, patch-level scale (<1 $m^2$) and existing literature on their tendency to forage near structure. Ruddy turnstones typically use structure to their advantage, turning over shell, stones and vegetation to reveal sheltering marine invertebrates and eggs in dense aggregations (Fleischer 1983; Sullivan 1986; Nettleship 2000). This behavior may pre-adapt the birds to readily utilize novel structure, such as that provided by *G. vermiculophylla*. In Wassaw Sound, we often observed ruddy turnstones traveling from patch to patch of *G. vermiculophylla*, flipping the invasive seaweed over with their heads or beaks and picking out epifaunal prey, including amphipods and crabs. Black-bellied plovers, a similarly sized species that also forages visually and targets large epifaunal/epibenthic prey (Poole et al. 2016), showed similar habitat selection at the large scale (>1 ha), though we could not assess their behavioral responses to the seaweed at the small scale (<1 $m^2$).

Willets, short-billed dowitchers, and dunlin had greater densities on mudflats with *G. vermiculophylla*. Indeed, algal presence was an important predictor variable within the best-fit models for these three species, though sediment composition and infaunal densities were also significant predictors. However, surprisingly, these species did not differ in foraging preference between bare mud and *G. vermiculophylla* patches at small spatial scales (<1 $m^2$), except for dunlin, which showed marginal preferences for foraging in *G. vermiculophylla* patches. These species are larger shorebirds with long beaks used for tactile foraging, often probing deep into the substrate to capture infaunal prey, though willets and dunlin also use visual detection (Stenzel et al. 1976; Rojas et al. 1999; Castillo-Guerrero et al. 2009; Novcic 2016). Given that these shorebirds forage below the sediment surface, the presence of *G. vermiculophylla* may not hinder prey detection, leading to the observed largely random foraging across patch types. This is further supported by the lack of significant difference in infaunal prey densities and biomasses between sites in the habitat preference survey (Appendix S1: Table S6, S7).

Semipalmated plovers and two peep species (western sandpipers and semipalmated sandpipers) notably avoided foraging in *G. vermiculophylla* patches at the small scale (<1 $m^2$), but did not avoid *G. vermiculophylla* at the large scale (>1 ha). Avoidance of *G. vermiculophylla* patches by semipalmated plovers emphasizes constraints of their foraging behaviors as this species relies heavily on visual detection of infaunal polychaetes and other small epifaunal invertebrates (i.e., ostracods, amphipods and small gastropods) (Rose et al. 2016). Such behavior suggests that *G. vermiculophylla* presence may impede the plovers' prey detection, although they did not avoid *G. vermiculophylla* mudflats at the large (>1 ha) or intermediate (200 $m^2$) scales. Western and semipalmated sandpipers predominantly rely on tactile detection of infaunal polychaetes and on slurping of biofilm and small planktonic invertebrates (Nebel et al. 2005;

Hicklin and Gratto-Trevor 2010; Mathot et al. 2010). Thus, their greater densities on *G. vermiculophylla* mudflats, but avoidance of small seaweed patches, may point to these birds cuing into other key factors at the large scale, such as sediment composition (Appendix S1: Table S2, S3). Notably, the third peep species, least sandpipers, strongly preferred foraging in *G. vermiculophylla* patches at the small scale – a pattern distinct from the other peeps species. Indeed, least sandpipers are known to utilize heavily vegetated microhabitats for foraging (Novcic 2016) and feed primarily on amphipods (Nebel and Cooper 2008). Thus, peeps and semipalmated plovers provide notable examples of how species identity (particularly morphology, behavior, and ecological niche) can alter the overall response of a community to an invasive ecosystem engineer.

The results of the manipulative field experiments demonstrate the complexity of *G. vermiculophylla*'s role in the observed mixed effects between the large and the small scale studies. For instance, semipalmated plovers showed no difference in peck rate between treatments in either experiment. The lack of response in foraging effort by semipalmated plovers between treatments was expected given their lack of response to algal presence in the Large Scale Habitat Selection Survey (>1 ha). Their indifference also indicates that the presence of *G. vermiculophylla* may not negatively affect semipalmated plover foraging effort, despite their avoidance of *G. vermiculophylla* patches at the small scale (<1 $m^2$). On the other hand, dunlin showed a mostly positive response to the alga. At the large scale (>1 ha), dunlin densities were greater on *G. vermiculophylla* mudflats. Additionally, at the small scale (<1 $m^2$), dunlin showed a slight preference for foraging in *G. vermiculophylla* patches, with great variability in foraging response between individuals. In the experiments, dunlin pecked at a faster rate where *G. vermiculophylla* was left intact; however, *G. vermiculophylla* had no effect on dunlin foraging in the experiments where is was newly added to mudflats. Therefore, the lower peck rate in response to *G. vermiculophylla* removal may be the result of a covarying factor (i.e., prey density or sediment characteristics) that is not expressed immediately upon the addition of *G. vermiculophylla*. Despite these contrasting results between species, it is notable that across all the experiments and observational studies conducted, only two times did a bird species demonstrate greater foraging effort or abundance in bare habitat compared to *G. vermiculophylla* (Table 1). Thus, *G. vermiculophylla* seems to be mostly neutral or positive for these birds, though the mechanism is likely different for each species examined.

Furthermore, contrasting preferences at the large scale (>1 ha) and small scale (<1 m$^2$) may indicate the importance of other habitat variables that may simultaneously affect shorebird densities and *G. vermiculophylla* presence. In fact, some of the variability in species densities, namely for dunlin, short-billed dowitchers, willet and peeps, was accounted for by sediment composition and infaunal densities in the Large Scale Habitat Selection Survey. From previous literature, we also know that *G. vermiculophylla* presence on southeastern mudflats is largely dependent on the density of native tubeworms, *Diopatra cuprea* (Byers et al. 2012; Kollars et al. 2016). *D. cuprea* density is dependent on abiotic conditions, such as salinity, sediment type, and inundation, and thus restricts *G. vermiculophylla* distributions to the lower estuary (Kollars et al. 2016). Beyond habitat characteristics, the mixed effects of *G. vermiculophylla* on shorebird habitat selection and foraging behavior across spatial scales could be partially attributable to flocking behavior, whereby birds following the cues of other species or individuals may be led to less preferred foraging habitat.

Although shorebirds nearly universally responded positively to the invasive ecosystem engineer's presence at the large scale (>1 ha), their responses at the small scale (<1 m$^2$) were mixed, despite the increased epifaunal prey availability within *G. vermiculophylla* patches (Byers et al. 2012; Wright et al. 2014). The divergent bird responses demonstrate that even polyphagous predators within the same assemblage experience effects of invasions differently. Though our methods cannot address whether the invasive engineer benefits shorebirds, and thus its impact on shorebird fitness, our results do indicate that invasive ecosystem engineers that provision additional complex habitat and boost associated prey abundance do not positively impact all native species, even those that utilize similar prey resources. Rather, predators utilize these new habitats differently across spatial scales based on inherent foraging behaviors. As invasive ecosystem engineers become more prevalent globally, the direction of their local effects may depend in part on species-specific behaviors.

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## Tables

Table 4.1. Summary of habitat preferences determined from surveys, experiments, and behavioral studies for each common shorebird species examined. Two plus signs (++) indicate a statistically significant positive relationship between *G. vermiculophylla* presence and bird density or foraging, while one (+) indicates a positive trend ($0.05 < p < 0.1$). An equal sign (=) indicates no difference between treatments. **B** indicates a significant preference for bare mudflat. The three peep species were merged for the Large Scale Habitat Selection Survey due to identification limitations; during the Patch Scale Foraging Behavior Study, least sandpipers were identifiable, while western and semipalmated sandpipers were pooled again. Dashes indicate no analysis of a species due to its absence.

| **Shorebird Species** | **Foraging Ecology** Visual, Tactile, or Mixed | **Habitat Selection Surveys** (>1 ha) | ***Gracilaria* Removal Foraging** (200 $m^2$) | ***Gracilaria* Addition Foraging** (200 $m^2$) | **Patch Foraging Preference** (<1 $m^2$) |
|---|---|---|---|---|---|
| Short-Billed Dowitcher<br>(*Limnodromus griseus*) | Tactile | + + | ---- | ---- | = |
| Dunlin<br>(*Calidris alpine*) | Mixed | + + | + + | = | + |
| Western Sandpiper<br>(*Calidris mauri*) | Mixed | + | ---- | ---- | **B** |
| Semipalmated Sandpiper<br>(*Calidris pusilla*) | Mixed | | ---- | ---- | |
| Least Sandpiper<br>(*Calidris minutilla*) | Visual | | ---- | ---- | + + |
| Ruddy Turnstone<br>(*Arenaria interpres*) | Visual | + | ---- | ---- | + + |
| Black-Bellied Plover<br>(*Pluvialis squatarola*) | Visual | + + | ---- | ---- | ---- |
| Semipalmated Plover<br>(*Charadrius semipalmatus*) | Visual | = | = | = | **B** |
| Willet<br>(*Tringa semipalmata*) | Tactile (Mixed) | + + | ---- | ---- | = |

## Figures

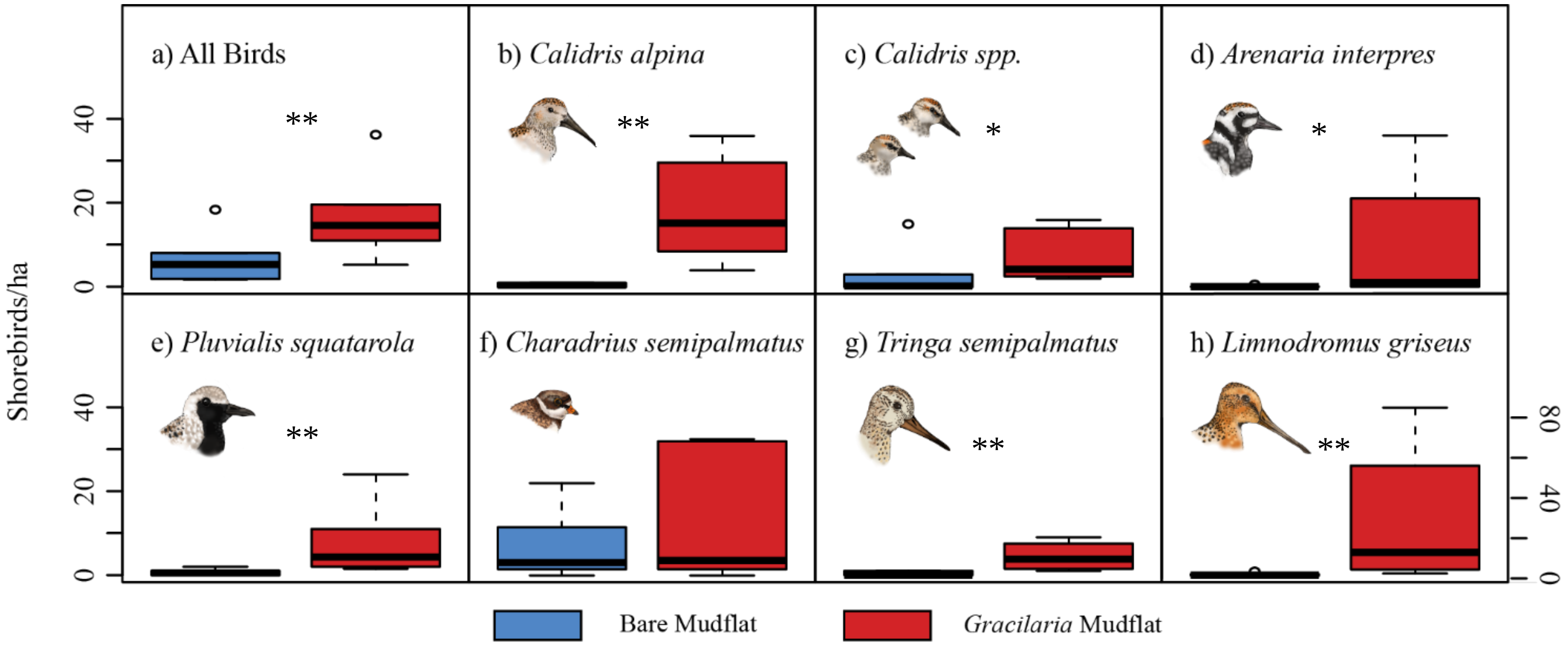

Figure 4.1. Field surveys of shorebird density (shorebirds/ha) on twelve mudflats (six with *Gracilaria* and six with no *Gracilaria*, or “bare”) for (a) all birds and (b-h) individual species. Data were analyzed after natural log-transformation using a multivariate linear regression in R (3.3.2). Figures depict the algal presence-only model. * indicates a trend (0.05 < p > 0.1) and ** indicates significant difference (p ≤ 0.05). Species are presented as follows: b) dunlin; c) peep; d) ruddy turnstone; e) black-bellied plover; f) semipalmated plover; g) willet; and h) short-billed dowitcher. Illustration credit: Rebecca Atkins

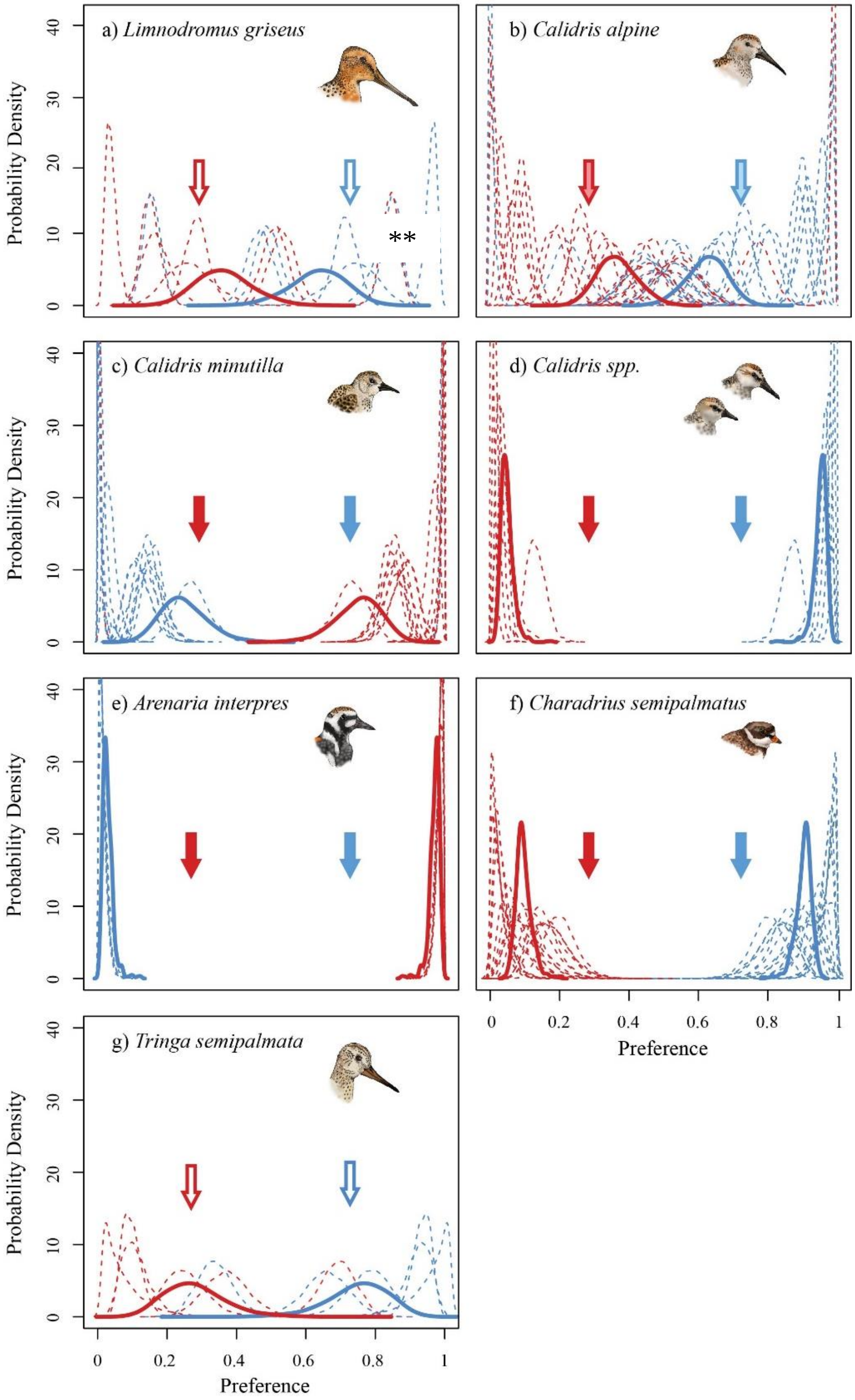
a) *Limnodromus griseus*
b) *Calidris alpine*
c) *Calidris minutilla*
d) *Calidris spp.*
e) *Arenaria interpres*
f) *Charadrius semipalmatus*
g) *Tringa semipalmata*
**
Probability Density
0
10
20
30
40
Preference
0
0.2
0.4
0.6
0.8
1

Figure 4.2. Foraging-habitat patch preferences for shorebirds during the 2015 spring migration season. We analyzed all species for individual-level (dotted lines) and population-level (solid lines) preferences using a hierarchical Bayesian analysis in R (3.3.2). Preference for *G. vermiculophylla* patches is illustrated in red and for bare mud in blue. Arrows denote where the preference curves should peak for each patch type if the birds foraged randomly, given the average percent cover of each habitat patch type. Filled arrows indicate that birds foraged differently from the random distribution, while unfilled arrows indicate that birds foraged randomly. Lightly filled arrows in panel b indicate a marginal difference from random foraging (credible intervals slightly overlap with patch-type cover). Species are presented as follows: a) short-billed dowitcher (n = 9); b) dunlin (n = 24); c) least sandpiper (n = 15); d) peep (n = 7); e) ruddy turnstone (n = 5); f) semipalmated plover (n = 19); g) willet (n = 6).

Illustration credit: Rebecca Atkins

# CHAPTER 5

# A NON-NATIVE SEAWEED DOES NOT ALTER FORAGING PREFERENCES OF EXCAVATING PREDATORS IN ESTUARINE MUDFLATS[4]

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[4] Haram, L., K. Maddox, K. Kinney, D. Dumont, E. Sotka, and J. Byers. Submitted to *Marine Ecology Progress Series*.

## Abstract

Novel species that create habitats can transform the environment of an ecosystem and influence local species interactions. The invasion of southeastern U.S. estuaries by a non-native seaweed, *Gracilaria vermiculophylla*, has altered the physical structure of previously-bare mudflats and may affect local trophic relationships. For example, the effect of the seaweed on mesopredators could be positive, due to the heightened abundance of small invertebrate prey found within it, or negative through physical interference of the seaweed on predators' excavating activity. The Atlantic blue crab, *Callinectes sapidus*, is a common generalist predator that feeds on a variety of smaller invertebrates, from crustaceans to bivalves, and acts as a trophic linkage to larger vertebrate predators. We used a combination of surveys and field and laboratory experiments to quantify the effect of this habitat-forming seaweed on the foraging behavior of *C. sapidus*. In all studies, blue crabs and other excavating mesopredators showed no foraging preference between bare versus seaweed-dominated mudflats. Thus, even though *G. vermiculophylla* has physically transformed many southeastern US estuaries, the seaweed does not seem to have affected blue crabs' distribution across habitat types or infaunal foraging strategies, at least at current seaweed densities.

## Introduction

Invasive species contribute to the alteration of biodiversity globally and can profoundly affect their recipient habitats (Vitousek et al. 1997; Wilcove et al. 1998; Gurevitch and Padilla 2004; Didham et al. 2005; Clavero et al. 2009). In particular, introduced organisms that are ecosystem engineers can generate novel habitat or can alter native habitat structure in their non-native ranges, often leading to ecosystem-level effects on abiotic conditions, such as light availability, sediment composition, and rugosity (Crooks 2002; Wallentinus and Nyberg 2007). Thus, when novel habitat structure is introduced by non-native species, it can directly and indirectly alter densities and distributions of multiple species through a variety of mechanisms (Schwindt et al. 2001; Crooks 2002, 2009; Grosholz and Ruiz 2009). Therefore, studying the effects of novel habitat on native species distributions is fundamental to understanding how communities respond to a habitat-forming invasive species.

One of the reasons that habitat structure has such a pervasive effect is that it often governs the strength of trophic relationships across ecosystem types, as the complexity of habitat structure mediates fundamental species- and community-level interactions like predation. For example, habitat structure shapes the foraging behavior of both predators and prey. For predators, despite their abilities to adapt to local conditions, foraging behavior is extrinsically constrained by prey availability and accessibility (Kramer 2001; Levi and Francour 2004). Dense habitat structure can influence predator foraging behavior by altering prey densities and thus prey availability (e.g., Schwindt et al. 2001). In many instances, increased structure leads to increased abundance and diversity of prey species that utilize the complex structure as refuge (Gotceitas and Colgan 1989; Byers et al. 2012). However, habitat structure can also shape predator foraging behavior by constraining prey detection and consumer movement with increased structural complexity, thus reducing prey accessibility (e.g. Robinson and Holmes 1984; Levi and Francour 2004). For instance, more complex habitat structure in the form of higher macrophyte density reduced encounter and capture rates of prey by bluegill sunfish, leading to lower consumption of prey and reduced fish biomass (Crowder and Cooper 1982). Thus, when introduced species create complex habitat structure, areas previously frequented by foraging predators can become zones of poor foraging quality (Levi and Francour 2004; Wallentinus and Nyberg 2007; Byers et al. 2010).

In recent decades, the habitat structure of Georgia mudflats has substantially changed with the introduction of the habitat-forming seaweed, *Gracilaria vermiculophylla* (Byers et al. 2012; Fig. 4.1). These invaded low intertidal habitats were previously composed of bare mudflats largely devoid of seagrasses and macroalgae due to high turbidity in the water column, which reduces light availability (Stephenson and Stephenson 1957; Dame et al. 2000). Additionally, the mudflats lack hard substrata to which seaweeds can attach. *G. vermiculophylla*'s attachment in these soft-bottom habitats is facilitated by a mutualistic interaction with the native polychaete, *Diopatra cuprea* (Byers et al. 2012; Kollars et al. 2016), which commonly decorates its tube with *G. vermiculophylla*, thus anchoring the seaweed to the benthos (Berke 2012). This interaction, along with the seaweed's tolerance to high stress environments, allows *G. vermiculophylla* to persist in the intertidal and shallow subtidal benthic zone (Thomsen and McGlathery 2006; Nyberg and Wallentinus 2009), forming thick mats of novel habitat in areas that once lacked macroalgae or much extensive aboveground structure (Byers et al. 2012).

The new habitat created by *G. vermiculophylla* shelters invertebrates from predation and harsh abiotic conditions (Wright et al. 2014; Bishop and Byers 2015) and may also increase their food availability (Haram, unpublished data). The seaweed's structural complexity boosts the abundance and distribution of macroinvertebrates, such as bivalves, crustacean and polychaetes, throughout its invasive range with 2- to 10-fold increases in invertebrates documented in southeastern U.S. mudflats and Denmark mudflats (Thomsen 2010; Thomsen et al. 2010; Byers et al. 2012). Given the substantial increase in primary consumers, *G. vermiculophylla* has the potential to affect multiple trophic levels simultaneously. However, *G. vermiculophylla* may also negatively affect species through physical alteration of predators' foraging grounds. In particular, excavating predators in this area are accustomed to digging on denuded sediment surfaces, and thus the presence of macroalgae may physically impede their foraging activity for infauna (Byers et al. 2010). Although many studies have determined the effects of the *G. vermiculophylla* introduction on nutrient cycling, primary productivity, and primary consumer distributions (e.g., Nyberg et al. 2009; Thomsen et al. 2009; Cacabelos et al. 2012; Gonzalez et al. 2013), few studies have investigated the effect of *G. vermiculophylla* on species interactions.

Southeastern U.S. estuaries are essential nursery and foraging habitat for many commercially and ecologically important fish and crustacean species (Dahlberg and Odum 1970; Rozas and Hackney 1983; Reichert and Van Der Veer 1991; Lehnert and Allen 2002; Ulrich et al. 2007). One predatory species that relies on these estuarine habitats, and commonly interacts with the *G. vermiculophylla*'s novel structure, is the Atlantic blue crab, *Callinectes sapidus* (Posey et al. 2005; Shervette et al. 2011). The *C. sapidus* native range in the Western Atlantic currently overlaps with the invasive range of *G. vermiculophylla*. These organisms also occupy the same habitat in the low intertidal zone, where *C. sapidus* feed upon infaunal and epibenthic invertebrates (such as bivalves, smaller crabs, shrimp, and amphipods) that proliferate in the mats formed by *G. vermiculophylla* (Laughlin 1982; Hill et al. 1989; Seitz et al. 2003a). *Callinectes* species in general are important mesopredators in mudflat ecosystems, dampening prey populations and serving as an important food-web linkage among trophic levels (Winemiller et al. 2007; Oakley et al. 2014; Byers et al. 2017). As juveniles, blue crabs benefit from the superior habitat provided by *G. vermiculophylla*, which increases protection from predation in comparison to bare mud and seagrass meadows (Johnston and Lipcius 2012). However, no research has explored how adult *C. sapidus* are affected by the seaweed. *C. sapidus* is not only ecologically important, but it is also economically important. The Atlantic blue crab fishery is Georgia's largest fishery by volume and is the second largest in economic value (Page et al. 2013). Therefore, any influence on populations of *C. sapidus* could have compounding effects on the environment and the economy of the southeastern U.S.

Thus, investigating how *G. vermiculophylla*'s complex structure affects habitat selection and foraging of *C. sapidus* helps predict the impacts of novel habitat created by an introduced species on local ecosystem structure and functioning. To quantify the effects of *G. vermiculophylla* on *C. sapidus* foraging habitat selection and predation strength, we conducted a habitat preference survey, a mesocosm foraging experiment, an *in-situ* tethering experiment, and an *in-situ* large predator exclusion experiment. We hypothesized that *Callinectes spp.* would be more abundant in areas without *G. vermiculophylla* due to the unimpeded substrate in which to dig. Additionally, we hypothesized that due to its novel, complex structure, the over-arching influence of *G. vermiculophylla* would be to interfere with *C. sapidus* foraging, and therefore *C. sapidus* would prefer to forage in areas without *G. vermiculophylla*.

## Methods

### *Habitat Preference Surveys*

**Survey Design.** To determine how frequently blue crabs interact with *G. vermiculophylla* and their preference for habitats types, we conducted surveys of crab populations on four mudflats with and without *G. vermiculophylla* cover in the Skidaway River, Wilmington River and Bull River (Appendix C: Table C1), which connect to Wassaw Sound, Georgia, USA. Surveys began on July 28, 2014 and were completed by August 20, 2014. The selected mudflats ranged in water temperature from 22 – 30.3 °C and in salinity from 28 – 30.1 psu during that time and were at least 1km apart.

At each of the eight mudflats, three sequential seine pulls were conducted parallel to the shoreline at 0 mean lower low water (MLLW) during incoming tides. The seine was 6.6 m wide (40 mm mesh) with a lead line at the bottom and floats at the top, allowing vertical coverage of the seine from the sediment to the top of the water column during pulls. We pulled each survey for 10 m before dragging the contents onshore for quantification. Each subsequent pull was started at least 10 m from the end point of the previous pull. We surveyed the contents of each seine pull for all blue crab species (*Callinectes*) native to the Southeast U.S., including *C. sapidus*, *C. similis*, and *C. ornatus*. Individuals were sexed and categorized as juveniles or adults depending on a carapace width (≤ 50 mm >, respectively). Once we finished all seine pulls at each site, we released the crabs.

To quantify *G. vermiculophylla* density on *Gracilaria* mudflats, five 0.25 $m^2$ quadrats were haphazardly placed using a blind toss method; we collected the *G. vermiculophylla* within the plots and stored it in Ziploc bags for transport to the laboratory where we rinsed the seaweed with filtered seawater, spun it for 30 seconds in a salad spinner, and then weighed it for wet biomass (g wm). We then dried the seaweed in a drying oven at 55°C until it reached a constant mass (g) (Appendix C: Table C1).

**Data Analysis.** We summed the contents of the three seine pulls at each site and analyzed the abundance of all blue crab species combined or *C. sapidus* alone as a function of habitat type (*Gracilaria* vs. bare sediment) (n = 4). Adult and juvenile crabs were analyzed separately. In R (3.3.2), we visually assessed data distributions using the 'qqnorm' function and ran a Shapiro-Wilk's test in the 'stats' package (R Core Team 2017) to determine the appropriateness of using pararmetric analyses for each of our response variables. Because our data were found to be non-normal, we analyzed relative crab abundances using non-parametric Wilcoxon Rank Sum tests.

### *Foraging Preference Assays*

**Study Species.** During June and July 2016, we collected *Callinectes sapidus* from the Wilmington River on the coast of Skidaway Island, Georgia using hook and line in shallow subtidal habitats and from otter trawls conducted by boat in the Wilmington River. The crabs used for this study (n = 10) had an average carapace width of 142.3 mm (± 8.9, SD) and were 80% female and 20% male. Prior to initiation of the experiment, we housed the crabs in an indoor flow-through seawater system at the University of Georgia (UGA) Aquarium on Skidaway Island that was continuously replenished with filtered seawater. We withheld food from the crabs for at least 48 hours prior to initiation of trials to standardize levels of predator hunger.

For the infaunal prey species for our experiment, we chose the native quahog clam, *Mercenaria mercenaria*, because this clam serves as an important food source for predatory species in the mudflat ecosystems, including *C. sapidus* (Carriker 1951; Arnold 1984; Smee and

Weissburg 2006). We obtained clams 13-15 mm in width from Sapelo Sea Farms – a local aquaculturist located ~56 km south of Skidaway Island on Sapelo Island, Georgia. We kept the clams submerged in a flow-through tank, continuously refilled with sand-filtered seawater. We allowed microalgae to naturally grow in the tanks, providing food for the clams while in the holding for up to one month.

**Experimental Design.** We conducted the foraging assays at the UGA Aquarium in a circulating seawater tank (147 cm x 99 cm x 63 cm depth) that we lined with pool-filter sand to a depth of 5 cm. By using store-bought, pool-filter sand, we ensured that the sediment was free of incidental organisms. To easily deploy organisms and extract all clams (and their fragments) at the end of each predation assay, we created two 1.27 cm-mesh baskets (~51 cm x 72 cm with 3 cm tall lips around all edges) out of hardware cloth that were buried 3 cm under the sand.

To simulate the interaction between the native *D. cuprea* tube worms that naturally anchor *G. vermiculophylla* in place on mudflats, we attached ten *D. cuprea* mimics to both baskets, representing densities of *D. cuprea* found within the Wilmington River. The worm mimics were made of twisted nylon rope (~9.5 mm diameter), anchored upright on the hardware cloth. When the baskets were buried in the sand, all but a small portion of the worm mimics were also buried, reflecting the natural positioning of *D. cuprea* tubes on the mudflat. We collected the seaweed at low tide from a nearby mudflat on Skidaway Island, Georgia along the Wilmington River (31°57'43.88"N, 81° 0'46.00"W), manually defaunated it, and kept it in an aerated flow-through system until deployment. For the bare sediment treatment, we left the tubes on one of the baskets free of seaweed. For our *Gracilaria* treatment, we attached 200 g (wet mass, hereafter referred to as wm) of live *G. vermiculophylla* to the second basket. This *G. vermiculophylla* density was representative of high densities of the seaweed found along the Wilmington River (L

Haram, unpublished data). We attached the *G. vermiculophylla* by weaving ~20 g wm of seaweed into each *D. cuprea* mimic, simulating the decorator worms' behavior of incorporating *G. vermiculophylla* into its tube. The baskets were buried on opposite sides of the tank, evenly spaced against each wall. The tops of the tube worm mimics and the *G. vermiculophylla* were exposed above the sand similarly to what occurs on the mudflat. We then haphazardly buried ten *M. mercenaria* 2 cm deep among the worm mimics, avoiding the baskets edges, in each of the treatments.

In the middle of the baskets, at their uppermost edges, we placed a small mesh cage where we acclimated the blue crab to the experimental tank conditions for one hour before initiation of each assay. We then released the blue crab and allowed it to forage freely. Assays took place under 10:14 hr light:dark cycles. After ~48 hours, we removed the blue crab and ended the assay. As we removed the hardware cloth baskets, sand sifted through the openings, allowing for easy recovery of the surviving clams and shell fragments. We recorded the proportion of clams consumed and removed any surviving clams and shell fragments. We also checked the sand bed for escaped clams or shell fragments, though we never found evidence of escape from the baskets. For each assay, we randomized which side of the tank received each treatment. We ran ten trials with ten separate blue crabs.

**Data Analysis.** We analyzed clam predation as a function of habitat using a hierarchical Bayesian analysis with the 'bayespref' package in R (Fordyce et al. 2011). The number of clams consumed was our response variable and habitat (*Gracilaria* or bare sediment) was our predictor variable. The 'bayespref' package is specifically designed for ecological count data that does not meet independence assumptions, such as in the case of choice assays (Fordyce et al. 2011). This analysis allowed us to estimate the strength of preference for both the total population of blue crabs as well as for each individual crab. We ran our models in ‘bayespref’ for 10,0000 generations with 100 generations burn-ins, based on visual inspection of scatter in diagnostic Markov chain Monte Carlo (MCMC) plots (see Fordyce et al. 2011).

### *In-Situ Tethering Experiment*

**Experimental Design.** We conducted tethering experiments on the same mudflat on Skidaway Island detailed above. The tethering experiment design mirrored the general aspects of the foraging assay design, using a total of eight paired replicates. We ran five paired replicates for seven days from June 23-30, 2016 and three additional replicates from July 25-August 1, 2016. As before, each replicate had two treatments: one plot received the bare sediment treatment while the other received 200 g of *G. vermiculophylla* for the *Gracilaria* treatment (n = 8). Again, we used 3 cm nylon rope as *D. cuprea* tube mimics and secured ten mimics to 50 cm x 75 cm hardware cloth in the same orientation as in the foraging assay. One difference from the previous assays is that, in this instance, the hardware cloth did not have lips, as instead the clams were tethered to the mesh to ensure recovery. We superglued a monofilament tether (10 lb test fishing line), 15 cm in length, to each clam. Tethered clams were placed in the flow-through seawater system for 24 hours to confirm attachment. Ten clam tethers were haphazardly chosen and tied to each hardware cloth rectangle (n = 160).

When deploying the experiment, we placed the hardware cloth rectangles 3 cm deep in the mud. The paired replicates were placed at least 1.5 m apart from adjacent pairs at 0 MLLW. We buried the clams 2 cm into the mud in an upright position to promote movement by the clams. After one week, we removed the experiment from the mudflat and counted the number of remaining clams. Any clams that were broken or missing were considered consumed. Two clams (one from each treatment) were damaged during the deployment and were removed from further analysis. Shell fragments still attached to tethers were examined to determine what type of predator consumed it. A cracked or shattered shell suggests that the clam was opened using crushing force, particularly by a blue crab's claws (Carriker 1951). Blue crabs are the primary predatory crab present on the low-intertidal mudflat that can exert the force needed to crack a clam; stone crabs, *Menippe mercenaria*, are also present, though they prefer to feed in the refuge of oyster reefs (Oakley et al. 2014; Duermit et al. 2016) and were not present in our seines. Considerable force is necessary to break a tether; therefore, we assumed all clams missing from their tethers may have been consumed by several different predator species.

**Data Analysis.** Because the tethering experiment was designed as a paired plot experiment, we again analyzed the data using a hierarchical Bayesian analysis with the 'bayespref' package in R (Fordyce et al. 2011). As in the foraging assay analysis, the number of clams consumed was our response variable and habitat (*Gracilaria* or bare sediment) was our predictor variable. We ran our models in 'bayespref' for 10,0000 generations with 1,000 generations burn-ins, as determined by visual inspection of the diagnostic MCMC plots.

### *In-Situ Predator Exclusion*

**Experimental Set-Up.** To assess the potential interactive effect of *G. vermiculophylla* cover and predation on infaunal communities, we conducted a fully factorial field experiment, crossing *G. vermiculophylla* presence and large predator exclusion. The experiment was conducted on a mudflat at the mouth of Grove's Creek in the Wilmington River in Skidaway Island, Georgia (31°58'5.15"N, 81° 0'50.04"W) at approximately 0 MLLW.

Four treatment combinations (+/- *G. vermiculophylla* and +/- predator exclusion) were replicated three times in a blocked design. Each plot was 1 x 2 m, oriented parallel to the waterline, with 2 m between each plot. The experimental blocks were at least 4 m apart. Each plot was marked by four ~1.5 m PVC poles placed in each corner. Predator exclusion treatments targeted large excavating predators, such as horseshoe crabs and stingrays, and consisted of additional ~1.5 m PVC poles placed along the perimeter of the plot every 10 cm, creating ~8 cm openings between poles (modified from Woodin 1981). All poles were pushed into the sediment to achieve a height of 1 m. The no-exclusion treatment did not receive additional poles, allowing free movement of predators. For the seaweed presence treatment, *G. vermiculophylla* was left intact in the "*Gracilaria*" treatment, while we removed *G. vermiculophylla* eight times (every one to two weeks) over the ten-week experimental duration in the "No *Gracilaria*" treatment. Continuous removal from the plots was necessary due to the decorating behavior of the present *D. cuprea* that reattach drifting seaweed within a short period of time. Care was taken during the removal process not to disturb the benthos.

After the initiation of the experiment, we counted the number of small (< 10 cm diameter) and large (≥ 10 cm diameter) predator pits. Small pits indicated foraging by mesopredators, such as blue crabs and flounder, that excavate pits in the soft sediment while feeding on infauna (Virnstein 1977; Woodin 1981). Large pits indicated foraging by larger organisms, such as stingrays and horseshoe crabs. We assessed the number of pits eight times (following the same time line as the *G. vermiculophylla* removal detailed above) over the course of the experiment to measure predation pressure on the infaunal and epifaunal communities. Pits $< 40\ \text{cm}^2$ fill within five days (Townsend and Fonseca 1998), reducing the potential to re-count pits from week to week. To determine if there are any interactive effects between *G.*

*vermiculophylla* presence and predator pressure on infaunal abundance, we collected nine evenly distributed sediment cores (10 cm diameter, 10 cm deep) within each plot to assess infauna abundance. Cores were taken to the laboratory and sieved them through 1mm mesh using filtered seawater. The samples were then preserved in 80% ethanol and were later sorted under a dissecting scope. We identified all infaunal organisms to species and enumerated them (per 0.008 $m^2$); we only included polychaetes when their heads were present. Due to the low abundance of infaunal invertebrates, we pooled all nine cores from each plot to get one density measurement from each experimental unit (infauna per 0.07 $m^2$). In addition, at the end of the experiment, we counted the number of *D. cuprea* tubes present in each plot. We enumerated *D. cuprea* tubes separately from the infauna abundance cores because *D. cuprea* are considerably larger than other infauna and can move quickly below the sediment surface within their extensive tubes.

**Data Analysis.** We analyzed the interactive effect of *G. vermiculophylla* presence and predator exclusion on the abundance of predator foraging pits using a generalized linear mixed effects model in the 'MASS' package in R. We used a negative binomial distribution for the pit densities due to overdispersion. In two separate analyses, we assessed the abundance of small and large predator pits as a function of *G. vermiculophylla* presence, predator exclusion, and their interaction. Each individual plot was included as a random effect to account for repeated measures of the pits over the ten-week duration. We also included block as a random effect; however, it did not account for a significant amount of variance, thus we did not include it in the final model.

We next analyzed the number of infauna per 0.07 $m^2$ from sediment cores and *D. cuprea* tubes per 2 $m^2$ as a function of *G. vermiculophylla* presence, predator exclusion, and their interaction using generalized linear models with negative binomial distributions (package 'MASS') in R. We separately assessed the effect of mesopredator foraging on infauna density by including the average number of small pits (detected in each plot from week two to ten) as a predictor variable. For all models, we assessed the role of block as a random effect, however it did not account for a significant amount of variance and was not included.

## Results

### *Habitat Preference Survey*

Surveys of *Callinectes spp.* distributions showed no significant difference in adult or juvenile densities between habitat types (Wilcoxon Rank Sum: W = 10, p = 0.64; W = 2, p = 0.10, respectively; Fig. 5.2a). The pattern was maintained when we isolated *C. sapidus*, as we saw no significant difference in adult or juvenile distribution between habitat types (Wilcoxon Rank Sum: W = 10, p = 0.64; W = 5.5, p = 0.51, respectively; Fig. 5.2b). *C. sapidus* accounted for 26% of the 87 juvenile blue crabs and 73% of the 22 adult blue crabs collected.

### *Foraging Preference Assays*

A total of 125 clams were consumed during the assays. On average, the crabs consumed equal numbers of deployed clams in the *Gracilaria* treatment (6.3 ± 1.1, SE) versus the bare sediment treatment (6.2 ± 1.3, SE). No significant difference in habitat preference was detected in the laboratory foraging assays. The population-level preference curves (solid lines) showed a strong overlap in habitat preference (Fig. 4.3a), indicating that blue crabs as a group showed no preference for foraging in either habitat. The bare treatment had a median preference of 0.49 (credible intervals (CI) = 0.39, 0.60), while the *Gracilaria* treatment had a median preference of

0.51 (CI = 0.39, 0.61). The response of individual replicate crabs (dashed lines in Fig 5.3a), similarly trended toward no preference, with low variance among individual crabs.

### *In-Situ Tethering Experiment*

Fifty six of the 158 clams were consumed during the field experiment. On average, equal numbers of deployed clams were consumed for both the *Gracilaria* treatment (3.5 ± 0.6, SE) and the bare sediment treatment (3.5 ± 0.7, SE). We detected no significant difference in loss of *M. mercenaria* between the habitat treatments with and without *G. vermiculophylla*, with median preference strengths of 0.51 (CI = 0.36, 0.65) and 0.49 (CI = 0.35, 0.65) respectively (Fig. 5.3b). Most of the consumed clams were cracked, and only 7% of consumed clams were completely removed from their tethers.

### *In-Situ Predator Exclusion*

The abundance of small predator pits could not be explained by the *G. vermiculophylla* treatment, the large predator exclusion, nor their interaction (Table 5.1; Fig. 5.4a). Abundance of large predator pits was marginally greater in treatments without predator exclusions, suggesting that our large predator exclusion was effective. We did not find a significant difference in large pits between the *G. vermiculophylla* presence treatments nor an interaction between seaweed and exclusion (Table 5.1; Fig. 5.4b).

*G. vermiculophylla* presence and predator exclusion did not significantly affect infaunal invertebrate density either as an interaction or as a main effect (Table 5.1; Fig. 5.5a). For *D. cuprea* tube density, we did not detect a significant interaction between the predator exclusion and the *G. vermiculophylla* treatments, or the main effect of *G. vermiculophylla* presence (Table

5.1; Fig. 5.5b). Likewise, the main effect of predator exclusion on *D. cuprea* tube abundance was not significant; however, it exhibited a positive trend, with an average of 27.5 (± 4.40, SE) tubes present in exclusion plots versus 19.3 (± 4.36, SE) tubes in no-exclusion plots (Table 5.1; Fig. 5.5b). When isolating the effect of the number of small predatory pits (per 2 $m^2$) on infaunal density (per 0.07 $m^2$), we did not find a significant relationship (GLM: $X^2_1 < 0.001$, $p = 0.89$; Fig. 5.5c).

## Discussion

*Callinectes* mesopredators showed no alteration in habitat preference or foraging behavior in response to the novel structure of *G. vermiculophylla*. The indifference of adult *C. sapidus* to *G. vermiculophylla*'s presence was consistent across habitat surveys, foraging assays, and in-situ experiments, suggesting that *G. vermiculophylla* does not deter *C. sapidus* or other excavating predators from using mudflats where the non-native seaweed is present. Our results were surprising given how substantially the invasion of *G. vermiculophylla* has transformed the historically bare soft-sediment habitat (Fig. 4.1).

Several types of benthic structure, such as seagrass and shell hash, are known to protect infauna like clams (Sponaugle and Lawton 1990; Peterson et al. 1995). The results from the present studies differed from previous research on environmental conditions that affect blue crab foraging, whereby *C. sapidus* prefer to forage on prey in less structurally complex sand and mud than in oyster shell (Arnold 1984; Sponaugle and Lawton 1990). One possible explanation for the lack of effect of *G. vermiculophylla* is that the relatively soft, flexible nature of the seaweed may not physically interfere with *C. sapidus* foraging to the same extent as other estuarine structure, especially hard, inflexible oyster shell. In fact, *C. sapidus* have been found to consume

~30% more of the mud crab *Panopeus herbistii* in *G. vermiculophylla* habitat than in oyster habitat (Bishop and Byers 2015). Furthermore, not only is oyster reef structure rigid, it is also integrated into the sediment, interfering with the ability of blue crabs to excavate infauna. Although *G. vermiculophylla* is negatively buoyant, it slightly suspends above the sediment surface during high tide. This resulting space between *G. vermiculophylla* mats and the sediment may allow for flattened mesopredators to forage freely on infauna. Additionally, water turbulence caused by increased habitat complexity on mudflats can reduce infaunal prey's detection of predators via olfactory cues (Ferner et al. 2009). Thus, the malleable yet complex structure provided by *G. vermiculophylla* may make the novel seaweed beds useful foraging grounds that counteract any effect of physical deterrence they present.

Despite the neutral response of *C. sapidus* to *G. vermiculophylla* cover, it is important to note that the *G. vermiculophylla* invasion in Georgia is relatively recent, meaning that the existing prevalence of the non-native seaweed in the ecosystem may change with time. It is possible that at the current *G. vermiculophylla* density, substantial negative effects have not yet occurred. At present, the cost of foraging within *G. vermiculophylla* may be balanced by benefits provided by the seaweed's structure, such as increased epifaunal abundance (Byers et al. 2012; Thomsen et al. 2013) or protection from higher level predators, such as bonnethead sharks (Byers et al. 2017). High densities of structural complexity in the form of seaweed mimics have been shown to function as refuge for infaunal bivalves from blue crab predation due to interference with foraging (Blundon and Kennedy 1982), suggesting that blue crab foraging habitat preference between *G. vermiculophylla* and bare sediment could shift if higher densities of the seaweed are encountered. In other areas within the seaweed's invasive range, such as the Chesapeake Bay (Thomsen et al. 2005), the *G. vermiculophylla* invasion is more pronounced, and the seaweed can form dense, high biomass mats with reduced space beneath it and the sediment surface (Thomsen et al. 2007; Thomsen et al. 2009). Not only could these high biomass mats deter mobility of predators, they could also create hypoxic conditions, which reduce blue crab foraging (Seitz et al. 2003b) and can lead to their emigration from the habitat (Bell and Eggleston 2005). Indeed, declines in benthic predator abundances have been observed in similar macrophyte invasions. For instance, red mullet, *Mullus surmuletus*, declined in Mediterranean France, following the increase in cover from the invasive *Caulerpa taxifolia* likely due to the seaweed reducing the accessibility of benthic prey (Levi and Francour 2004). Increasing density of the seaweed could also negatively affect infaunal populations living underneath it and therefore decrease the attractiveness of seaweed beds as foraging habitat for predators like blue crabs that exhibit density-dependent foraging (Seitz et al. 2001). Thus, despite our finding that current levels of *G. vermiculophylla* in Georgia mudflats have no effect on the feeding of *C. sapidus*, future studies should investigate possible biomass threshold effects on blue crab foraging.

*G. vermiculophylla* is known to increase local densities of many epifaunal invertebrates (Thomsen et al. 2013; Byers et al. 2012; Wright et al. 2014; Kollars et al. 2016) in southeastern US estuaries. Although blue crab foraging was not affected, the seaweed does positively affect blue crabs by serving as valuable nursery habitat (Johnston and Lipcius 2012). It remains uncertain, however, whether the widespread *G. vermiculophylla* affects densities and foraging behavior of the broader guild of mobile predators in southeastern US estuaries. Blue crabs represent only one of many predators that rely on mudflat habitats as foraging grounds; other non-excavating benthic-foragers, such as bonnethead sharks and red drum, could interact with the seaweed’s novel structure differently from blue crabs. Indeed, research on the same Georgia mudflats has demonstrated variability in foraging responses to the introduced seaweed among different shorebird species commonly classified as the same trophic guild (Haram, in review). Furthermore, there could be competitive interactions and indirect effects between these higher trophic level species that are mediated by the habitat structure, as has been observed on oyster reefs (e.g., Grabowski 2004). Thus, research efforts should account for species-specific differences in behavioral responses across and within trophic guilds to ensure a holistic understanding of community-level responses to *G. vermiculophylla*'s novel structure.

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## Tables

Table 5.1. Analysis of deviance table detailing the results of generalized linear regression models conducted for the *In-Situ Predator Exclusion* experiment. One model assessed the interactive effect of *Gracilaria* presence and predator exclusion on various response variables, including small and large predatory pits (per 2 $m^2$), infauna density (per 0.07 $m^2$), and *D. cuprea* density (per 2 $m^2$). The second model assessed the relationship between the average density of small predatory pits and infauna density.

| **Predictor Variable** | **Small Pits** | | **Large Pits** | | **Infauna Density** | | ***D. cuprea* Density** | |
|---|---|---|---|---|---|---|---|---|
| | $\chi^2$ | p-value | $\chi^2$ | p-value | $\chi^2$ | p-value | $\chi^2$ | p-value |
| *Gracilaria* Presence | 0.83 | 0.36 | 0.04 | 0.84 | 1.46 | 0.23 | 2.33 | 0.13 |
| Predator Exclusion | 1.96 | 0.16 | **3.69** | **0.06** | 0.01 | 0.91 | **2.98** | **0.08** |
| Interaction | 0.06 | 0.81 | 0.64 | 0.42 | 0.34 | 0.56 | 0.34 | 0.56 |
| Small Foraging Pits | --- | --- | --- | --- | < 0.01 | 0.89 | --- | --- |

## Figures

Figure 5.1. Non-native *G. vermiculophylla* structure on the sediment surface during low tide, depicting an example of the extent of *G. vermiculophylla* cover along the Wilmington River, Georgia. Photo credit: L. Haram.

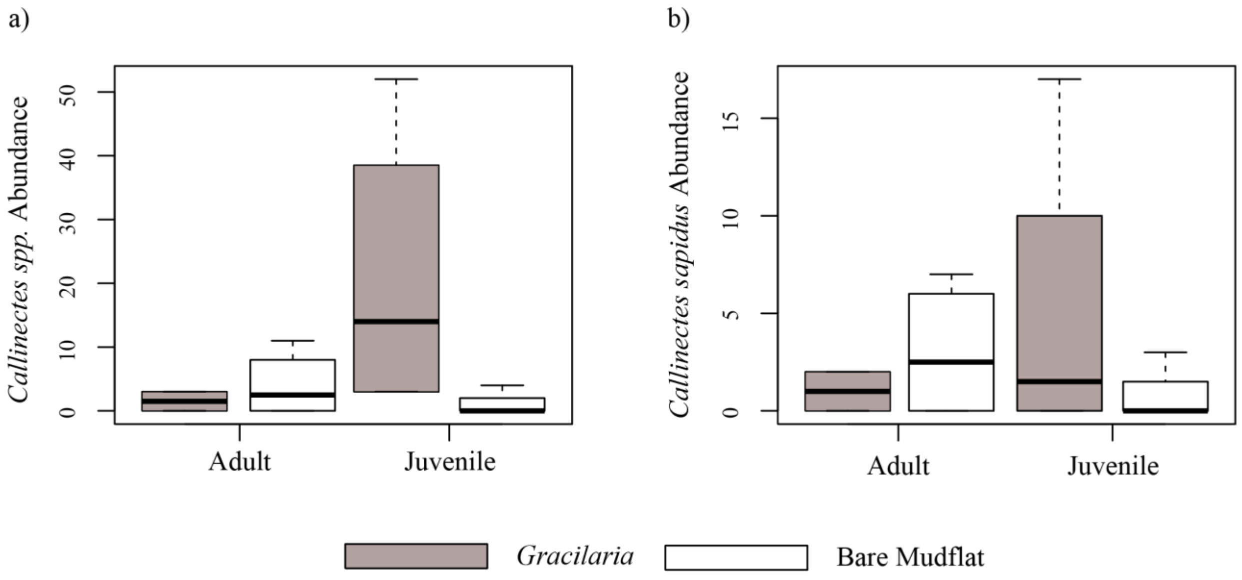

Figure 5.2. Median number of (a) all blue crab species (*Callinectes spp.*) and (b) the Atlantic blue crab (*C. sapidus*) collected on four mudflats with and without *G. vermiculophylla* cover (n = 8). *Gracilaria* mudflats are depicted in grey, while bare mudflats are depicted in white. Surveys per site consisted of three 30-meter seine pulls parallel to the shoreline over mid-intertidal mudflat during an incoming tide. *Callinectes* species included *C. sapidus*, *C. similis*, and *C. ornatus*. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

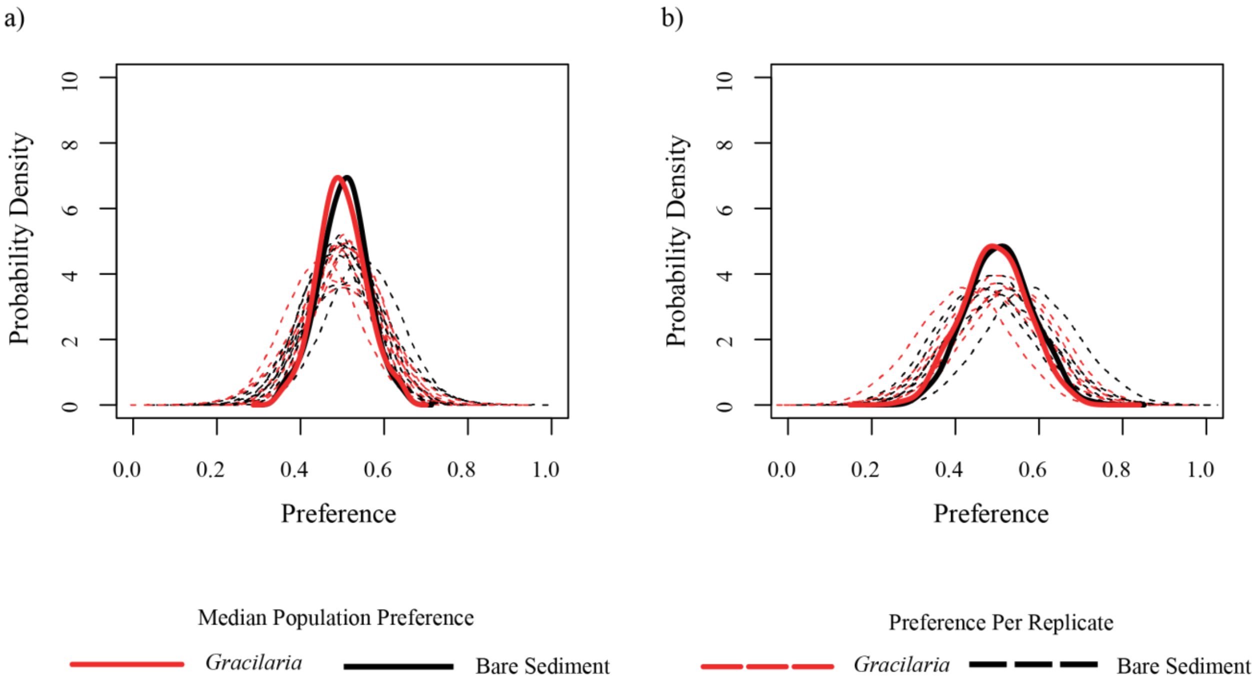

Figure 5.3. Mesopredator preference for foraging on juvenile clams, *Mercenaria mercenaria*, in habitat patches with and without *G. vermiculophylla* cover in the (a) Foraging Habitat Preference Assays (n = 10) and in the (b) In-Situ Tethering Experiment (n = 8). Red lines indicate consumption of clams in the *G. vermiculophylla* habitat, while black lines indicate foraging in the bare sand habitat. Solid lines illustrate median habitat preference across all replicates, and dotted lines illustrate in the relative loss of clams in each paired replicate between the two habitat types. No significant difference in consumption between the two habitat types was detected given the overlap of median preference between treatments for both experiments.

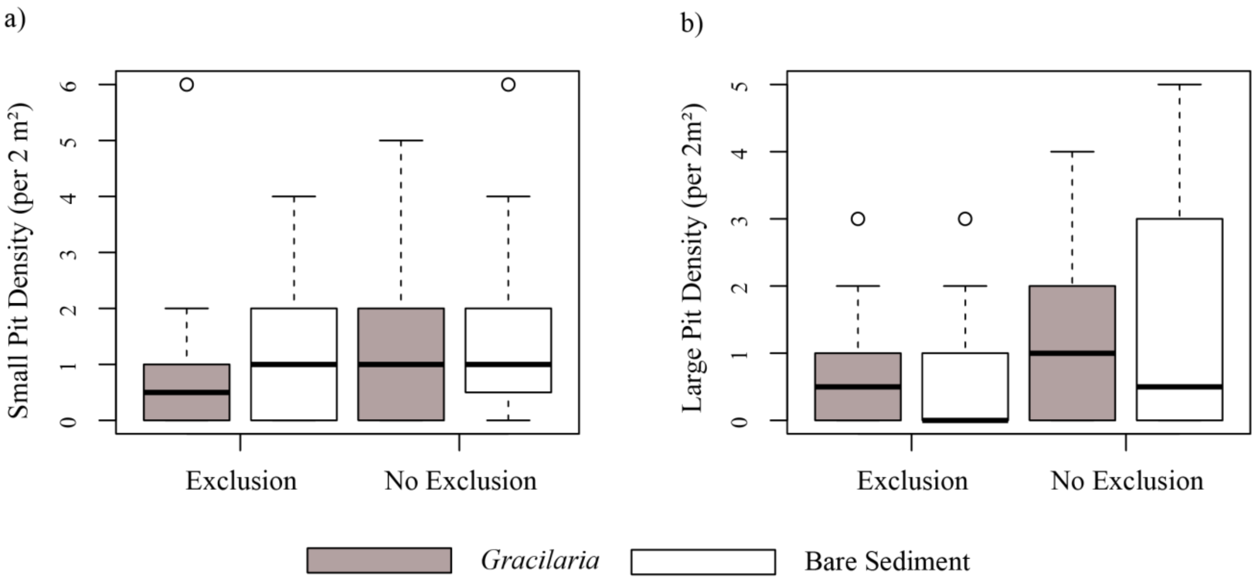

Figure 5.4. Median number of (a) small predator foraging pits (< 10 cm diameter) and (b) large predator foraging pits (≥ 10 cm diameter) over 10 weeks in 2 $m^2$ plots as a function of experimental treatments: +/– *G. vermiculophylla* and +/– large predator exclusion (n = 3 of each treatment combination). Small pits indicate foraging by blue crabs or even small fish, such as flounder. Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

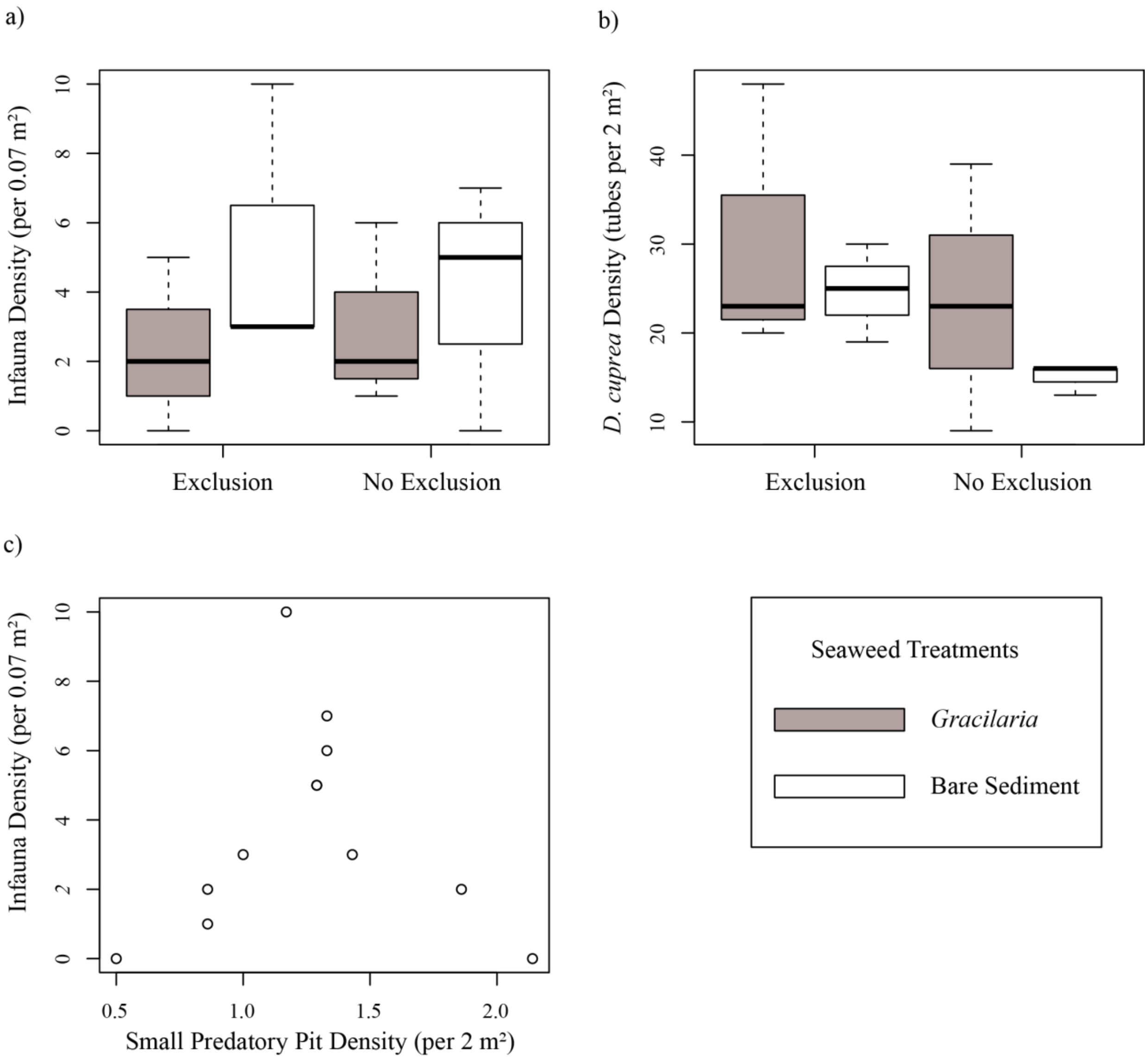

Figure 5.5. Effect of *G. vermiculophylla* and predator exclusion on invertebrate densities. (a) Median infaunal abundance per 0.07 $m^2$ among treatments crossed with *G. vermiculophylla* presence and large predator exclusion. To determine infaunal abundance, nine cores (0.008 $m^2$) were taken in each replicate plot (n = 3 of each treatment combination) at the end of the experiment and summed for a 'total infauna' metric. Cores were sieved using 1mm mesh sieve; infaunal species included polychaetes and bivalves. (b) Total infaunal abundance per 0.07 $m^2$ in response to the average number of small predatory pits (per plot). Average number of predatory

pits was determined by averaging the total number of small pits at each repeated time point. (c) Effect of *G. vermiculophylla* presence and predator exclusion on the number of tubes of the polychaete *D. cuprea*. Worm tubes were counted after 10 weeks of experimental manipulation (n = 3 of each treatment combination). Box and whisker plots represent the median and 25% and 75% quantiles; the lower and upper dashed lines illustrate the minimum and maximum values.

# CHAPTER 6

# CONCLUSION

Many invasive species are perceived as harmful to their recipient ecosystems, but they often have mixed effects on native communities depending on local community assemblages and ecosystem properties (Crooks 2002; Simberloff 2011; Simberloff et al. 2013). Despite the general acknowledgement across ecological sub-disciplines of the nuanced nature of species interactions, most research about invasive species investigates only population-level effects on native species, such as shifts in population abundances (e.g., Taylor et al. 2010), rather than alterations in behavior (e.g., Gribben et al. 2009; Byers et al. 2010). To illuminate the complexities of species introductions, we aimed to investigate how an introduced primary producer behaviorally affects trophic interactions of a native community. Thus, this dissertation highlights the direct and indirect consequences of the introduced seaweed, *G. vermiculophylla*, as a novel basal resource and physical habitat structure, on trophic interactions of native consumers in estuaries of the southeastern U.S.A.

To date, all research that investigates the role of non-native *G. vermiculophylla* as an additional basal resource in its invasive range has ignored the potential consumption by native fishes (reviewed in Hu and Juan 2014). In Chapter Two, we identified the native pinfish, *L. rhomboides*, as a potential integrator of *G. vermiculophylla* into the local trophic structure, as the fish strongly preferred *G. vermiculophylla* over the native, low-abundance seaweed, *U. lactuca*. Additionally, our research showed differences among native invertebrates in their consumption of *G. vermiculophylla* in the absence of other resources; in the presence of native *U. lactuca*, both *T. obsoleta* and *E. depressus* preferred *U. lactuca*, yet *E. depressus* consumed *G. vermiculophylla* when *U. lactuca* was not present. Thus, *G. vermiculophylla* directly affects trophic interactions in southeastern estuaries as a novel basal resource, and this work expands our knowledge of which native species consume *G. vermiculophylla*. Given that *G. vermiculophylla* is by far the dominant macroalgal resource present in these ecosystems (Byers et al. 2012), the ability of some consumers to utilize *G. vermiculophylla* as a basal resource may provide a competitive advantage over those that utilize native resources alone. More research should be conducted to identify the extent to which these species consume *G. vermiculophylla* in the field and how consumption changes seasonally.

In addition to examining which native species consume *G. vermiculophylla*, we also investigated how an abiotic factor (heightened nutrient availability) affects the integration of the non-native seaweed into the trophic structure. We found that both the pinfish *L. rhomboides* and the amphipod *A. valida* consumed similar amounts of nutrient-enriched and non-enriched *G. vermiculophylla* in the laboratory; however, when given the choice, *L. rhomboides* preferred to consume nutrient-enriched *G. vermiculophylla*. Though preference assays were not conducted in the laboratory for *A. valida*, we found that the amphipods showed varied colonization of nutrient-enriched versus non-enriched seaweed in the field depending on season, with more *A. valida* colonizing enriched *G. vermiculophylla* in the fall and similar densities of *A. valida* colonizing enriched and non-enriched *G. vermiculophylla* in the summer. Therefore, *G. vermiculophylla*'s nutrient content plays a key role in attracting consumers, which could have important consequences for patterns of consumption and species distributions across an estuary. Additional research should aim to investigate how a continuum of nutrient contents affects consumption and how it relates to the production of defensive secondary metabolites that may alter palatability for certain native consumers.

Chapters Three and Four highlight that the indirect effects of *G. vermiculophylla*'s physical structure on trophic interactions is not uniform within and across trophic guilds. In Chapter Three, we found that habitat preference and foraging behavior of shorebirds differed across species and spatial scales. Given our results, shorebirds preferred to forage in areas colonized by *G. vermiculophylla* at the large spatial scale of entire mudflats; however, the strength of this relationship depended on shorebird species. Furthermore, habitat preference at the mudflat scale did not suggest that shorebirds actively foraged in *G. vermiculophylla*, as some species, namely semipalmated plovers and the conglomerate of western and semipalmated sandpipers, avoided *G. vermiculophylla*'s structure at the small, patch scale ($<1$ m$^2$). On the other hand, some shorebirds directly foraged within the seaweed's structure at the patch scale, indicating that the heightened epifaunal abundance harbored by *G. vermiculophylla* structure may benefit some shorebird species. Additional manipulative experiments shed light on the importance of *G. vermiculophylla* structure in determining foraging effort, as shorebirds expressed higher foraging rates on *G. vermiculophylla*-colonized mudflats where *G. vermiculophylla* is left intact versus removed; yet, they showed no difference in foraging on bare mudflats where *G. vermiculophylla* was added versus left bare. These results provide evidence that shorebirds may not respond to *G. vermiculophylla*'s structure directly at the large, mudflat scale, but perhaps an unknown legacy effect of *G. vermiculophylla* colonization alters invertebrate communities. In summary, our results suggest that *G. vermiculophylla* indirectly affects the foraging behavior of shorebirds differently based on spatial scale and species identity.

Future research should investigate how *G. vermiculophylla*'s structure impacts the foraging success and site fidelity of shorebirds.

Although we found differences in behavioral responses of shorebird predators to *G. vermiculophylla* structure in Chapter Three, we were unable to determine the effect of the seaweed on predator foraging success. Thus, in Chapter Four, we aimed to more explicitly determine how *G. vermiculophylla* affects predator foraging in the laboratory and field using excavating predators, namely blue crab *Callinectes sapidus*. For *Callinectes sapidus*, we found no differences in the distribution of crabs among bare mudflats and those colonized by *G. vermiculophylla*. Additionally, we determined that *G. vermiculophylla* structure does not inhibit foraging by *C. sapidus* and other excavating predators on infaunal prey in either the laboratory or field. Thus, we determined that *G. vermiculophylla* structure does not alter behavioral foraging preferences of excavating predators. However, future studies should investigate potential threshold effects of *G. vermiculopohylla* biomass on these indirect trophic interactions, as the presence of free-floating macroalgal mats that are abundant in other areas of *G. vermiculophylla*'s invasive range (Weinberger et al. 2008; Nyberg et al. 2009; L. Haram, personal observation) may have a very different effect on native predator foraging than the patchy assemblage of seaweed observed along the Georgia coast.

Overall, we examined the direct and indirect consequences of the *G. vermiculophylla* invasion on trophic interactions in recipient communities. Our finding that *G. vermiculophylla* affects native species differently based on species identity and other external factors, such as spatial scale, season, and nutrient availability, demonstrate the complexity of species invasions. That introduced organisms can have varied effects on native species, even within the same trophic guild, highlights the importance of taking a community-level approach when investigating invasive species effects. Additionally, understanding the behavioral responses of native species is essential to predicting effects of invasive species, especially given that alterations in native species densities may not reflect important ecological consequences of invasion, such as altered quality of foraging habitat or basal resources. Because invasive species are a major source of global change and will continue to increase in number and expanse with increased globalization, future research should aim to further elucidate the nuanced nature of native-invasive species interactions to more holistically portray the state of invaded ecosystems and to better predict the outcomes of future species introductions.

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# APPENDIX A

## CHAPTER 3

### Nitrogen-Enrichment Analysis Methods

Half way through the enrichment process, we took five *G. vermiculophylla* tissue samples (~1 g) from five different thalli within each treatment bin (nutrient-enriched and non-enriched). We pooled the samples per treatment, rinsed them with deionized water, and dried them at 55°C in a drying oven for 48 hours. Once dried, we ground each pooled sample using a mortar and pestle and placed it in a glass scintillation vial. We dried the ground samples again at 55°C in a drying oven over night to remove moisture that may have accumulated during the grinding process. The samples were homogenized and ~25 mg of each sample was placed in individual foil packets. The samples were analyzed at the Skidaway Institute of Oceanography for percent nitrogen content and C:N.

# APPENDIX B

## CHAPTER 4

### Appendix S1

Table B1. Description of sites surveyed during the Large Scale Habitat Selection Survey. All sites were within the Wassaw Sound watershed in Savannah, Georgia, USA. Area (ha) refers to the size of the mudflats assessed, within which shorebird density was enumerated along 500 m transects. Sediment composition characteristics (here represented as the ratio of % sand to % silt) were attained from 10 sediment cores taken at each site along a 50 m transect that were then pooled and analyzed for percent composition. Water temperature and salinity were measured during a single point at low tide.

| **Study Site** | **Treatment** | **Latitude** | **Longitude** | **Site Area (ha)** | **Sand: Silt** | ***Gracilaria* Biomass (± SE) (g dw m$^{-2}$)** | **Water Temperature (ºC)** | **Salinity (psu)** |
|---|---|---|---|---|---|---|---|---|
| Bull River | Bare | 31°58'21.11"N | 80°55'23.95"W | 2.0 | 16.8 | 0.04 (0.04) | 22.3 | 25.7 |
| Grimball Point | Bare | 31°59'13.45"N | 81° 2'18.61"W | 1.6 | 2.5 | 0 (0) | 23.6 | 24.0 |
| Sheraton | Bare | 32° 0'7.87"N | 81° 0'19.75"W | 1.2 | 1.4 | 2.10 (2.10) | 20.8 | 22.5 |
| Little Tybee | Bare | 31°56'32.15"N | 80°55'48.99"W | 6.1 | 18.4 | 4.34 (1.83) | 25.5 | 29.7 |
| Tybee Creek | Bare | 31°57'43.94"N | 80°58'49.25"W | 1.2 | 2.1 | 0 (0) | 22.8 | 27.5 |
| Wassaw Sandbar | Bare | 31°56'17.05"N | 80°58'2.98"W | 7.6 | 95.9 | 0 (0) | 20.2 | 29.9 |
| Cabbage Mudflat | *Gracilaria* | 31°56'33.43"N | 80°58'17.80"W | 3.2 | 17.3 | 6.08 (2.16) | 27.6 | 21.1 |
| House Creek | *Gracilaria* | 31°57'26.50"N | 80°55'55.48"W | 10.0 | 32.7 | 17.44 (8.96) | 27.4 | 30.3 |
| Priest Mudflat | *Gracilaria* | 31°58'6.46"N | 81° 0'48.61"W | 5.6 | 10.7 | 22.24 (5.49) | 20.3 | 24.1 |
| Romerly Marsh | *Gracilaria* | 31°55'44.98"N | 80°59'13.79"W | 4.5 | 11.8 | 32.93 (8.34) | 21.2 | 28.4 |
| Tybee Cut | *Gracilaria* | 31°57'3.17"N | 80°59'3.14"W | 5.1 | 16.5 | 40.01 (7.31) | 26.0 | 19.1 |
| Wassaw Mudflat | *Gracilaria* | 31°54'46.75"N | 80°57'5.10"W | 5.9 | 30.5 | 12.05 (6.15) | 22.6 | 29.0 |

Table B2. Best fit models from the linear regression analysis of the Large Scale Habitat Selection Survey, including invertebrate abundances, determined by AICc and Akaike weight analysis in R 3.3.2. All models within 2 Δ*i* are presented. Models denoted as a function of 1 (e.g., log(Total Bird Density) ~1) are intercept-only null models.

| **Species** | **Candidate Model** | ***k*** | **Δ*i*** | **Akaike Weight** | **df** | **Adjusted $R^2$** |
|---|---|---|---|---|---|---|
| All Birds | log(Total Bird Density) ~ Algal presence | 3 | 0 | 0.51 | 1, 10 | 0.30 |
| | log(Total Bird Density) ~ 1 | 2 | 1.71 | 0.22 | 11 | --- |
| Dunlin | log(Dunlin) ~ log(Sand:Silt) + log(Infauna) | 4 | 0 | 0.45 | 2, 9 | 0.65 |
| | log(Dunlin) ~ log(Sand:Silt) | 3 | 1.51 | 0.21 | 1, 10 | 0.47 |
| | log(Dunlin) ~ Algal presence + log(Sand:Silt) | 4 | 1.68 | 0.19 | 2, 9 | 0.60 |
| Peep | log(Peep) ~ log(Sand:Silt) + log(Infauna) | 4 | 0 | 0.26 | 2, 9 | 0.44 |
| | log(Peep) ~ Algal presence | 3 | 0.14 | 0.24 | 1, 10 | 0.25 |
| | log(Peep) ~ log(Sand:Silt) | 3 | 0.63 | 0.19 | 1, 10 | 0.22 |
| | log(Peep) ~ 1 | 2 | 1.07 | 0.15 | 11 | --- |
| Ruddy Turnstone | log(Ruddy Turnstone) ~ Algal presence | 3 | 0 | 0.37 | 1, 10 | 0.22 |
| | log(Ruddy Turnstone) ~ 1 | 2 | 0.43 | 0.30 | 11 | --- |
| Black-bellied Plover | log(Black-bellied Plover) ~ Algal presence | 3 | 0 | 0.72 | 1, 10 | 0.5 |
| Semipalmated Plover | log(Semipalmated Plover) ~ 1 | 2 | 0 | 0.63 | 11 | --- |
| Willet | log(Willet) ~ Algal presence | 3 | 0 | 0.34 | 1, 10 | 0.56 |
| | log(Willet) ~ Algal presence x log(Sand:Silt) | 5 | 0.19 | 0.31 | 3, 8 | 0.77 |
| | log(Willet) ~ Algal presence + log(Sand:Silt) | 4 | 0.89 | 0.22 | 2, 9 | 0.65 |
| Short-billed Dowitcher | log(Dowitcher) ~ Algal presence x log(Sand:Silt) | 5 | 0 | 0.94 | 3, 8 | 0.92 |

Table B3. Best fit models from the linear regression analysis of the Large Scale Habitat Selection Survey, including invertebrate biomass (g dw), determined by AICc and Akaike weight analysis in R 3.3.2. All models within 2 Δ*i* are presented. Models denoted as a function of 1 (e.g., log(Total Bird Density) ~1) are intercept-only null models.

| **Species** | **Candidate Model** | ***k*** | **Δ*i*** | **Akaike Weight** | **df** | **Adjusted $R^2$** |
|---|---|---|---|---|---|---|
| All Birds | log(Total Bird Density) ~ Algal presence | 3 | 0 | 0.34 | 1, 10 | 0.30 |
| | log(Total Bird Density) ~ log(Epifauna biomass) | 3 | 0.53 | 0.26 | 1,10 | 0.27 |
| | log(Total Bird Density) ~ 1 | 2 | 1.71 | 0.14 | 11 | --- |
| Dunlin | log(Dunlin) ~ log(Sand:Silt) | 3 | 0 | 0.32 | 1, 10 | 0.47 |
| | log(Dunlin) ~ Algal presence + log(Sand:Silt) | 4 | 0.17 | 0.29 | 2, 9 | 0.60 |
| Peep | log(Peep) ~ Algal presence | 3 | 0 | 0.24 | 1, 10 | 0.25 |
| | log(Peep) ~ log(Sand:Silt) | 3 | 0.50 | 0.18 | 1, 10 | 0.22 |
| | log(Peep) ~ 1 | 2 | 0.94 | 0.15 | 11 | -- |
| | log(Peep) ~ log(Epifauna biomass) | 2 | 1.72 | 0.10 | 1, 10 | 0.14 |
| Ruddy Turnstone | log(Ruddy Turnstone) ~ Algal presence | 3 | 0 | 0.29 | 1, 10 | 0.22 |
| | log(Ruddy Turnstone) ~ 1 | 2 | 0.43 | 0.23 | 11 | --- |
| | log(Ruddy Turnstone) ~ log(Epifauna biomass) | 3 | 1.20 | 0.16 | 1, 10 | 0.14 |
| Black-bellied Plover | log(Black-bellied Plover) ~ Algal presence | 3 | 0 | 0.39 | 1, 10 | 0.5 |
| Semipalmated Plover | log(Semipalmated Plover) ~ 1 | 2 | 0 | 0.49 | 11 | --- |
| Willet | log(Willet) ~ log(Sand:Silt) + log(Infauna biomass) | 4 | 0 | 0.22 | 2, 9 | 0.68 |
| | log(Willet) ~ Algal presence | 3 | 0.18 | 0.21 | 1, 10 | 0.56 |
| | log(Willet) ~ Algal presence x log(Sand:Silt) | 5 | 0.37 | 0.19 | 3, 8 | 0.77 |
| | log(Willet) ~ Algal presence + log(Sand:Silt) | 4 | 1.07 | 0.13 | 2, 9 | 0.65 |
| Short-billed Dowitcher | log(Dowitcher) ~ Algal presence x log(Sand:Silt) | 5 | 0 | 0.99 | 3, 8 | 0.92 |

Table B4. Mean and standard error (± SE) of invertebrate densities ($m^{-2}$). Densities were calculated from ten replicate quadrat samples (0.25 $m^2$) taken at each site during the Large Scale Habitat Selection Survey on *Gracilaria*-colonized mudflats (, n = 6, total n = 60) or bare mudflats (n = 6, total n = 60). These invertebrates were categorized as epibenthos/epifauna in our analysis given that they were found on the surface of the mud or within *G. vermiculophylla* patches. Families with less than five individuals across cores were not included in this table. All polychaetes were combined into one group due to low abundances per species, which included *Alitta succinea*, *Diopatra cuprea*, and *Arabella irricolor*.

| **Phylum** | **Class** | **Family** | **Epibenthos/ Infauna** | ***Gracilaria* Mudflat** | | **Bare Mudflat** | |
|---|---|---|---|---|---|---|---|
| | | | | **Density ($m^{-2}$)** | **SE** | **Density ($m^{-2}$)** | **SE** |
| Mollusca | Gastropoda | *Columbellidae* | E | 4.4 | 2.1 | 0 | 0 |
| | | *Nassariidae* | E | 0.8 | 0.1 | 0.1 | 0.1 |
| | | *Calyptraeidae* | E | 3.6 | 3.6 | 0 | 0 |
| | Bivalvia | *Mactridae* | I | 0.3 | 0.1 | 0 | 0 |
| | | *Arcidae* | I | 0.9 | 0.5 | 0 | 0 |
| Crustacea | Decopoda | *Paguridae* | E | 2.4 | 1.2 | 0.3 | 0.3 |
| | | *Panopeidae* | E | 0.3 | 0.3 | 0 | 0 |
| | Amphipoda | *Gammaridae* | E | 55.7 | 16.7 | 0.3 | 0.3 |
| Annelida | Polychaeta | Spp. | I | 3.6 | 0.1 | 0 | 0 |

Table B5. Mean and standard error of invertebrate biomasses (g dry mass m$^{-2}$) calculated from ten quadrat samples (0.25 m$^{2}$) taken at each site during the Large Scale Habitat Selection Survey on *Gracilaria*-colonized mudflats (n = 6, total n = 60) or bare mudflats (n = 6, total n = 60). These invertebrates were categorized as epibenthos/epifauna in our analysis given that they were found on the surface of the mud or within *G. vermiculophylla* patches. The percent community composition is also presented for each taxon. Taxa representing less than 1% of community composition are not included in the table.

| | | ***Gracilaria* Mudflat** | | | **Bare Mudflat** | | |
|---|---|---|---|---|---|---|---|
| **Taxon** | **Epibenthos/ Infauna** | **Biomass (g dw m$^{-2}$)** | **SE** | **% Community Composition** | **Biomass (g dw m$^{-2}$)** | **SE** | **% Community Composition** |
| Gastropoda | E | 0.0923 | 0.0148 | 42.1 | 0.0221 | 0.0140 | 78.8 |
| Bivalvia | I | 0.0644 | 0.0187 | 29.4 | 0.0000 | 0.0000 | 0 |
| Amphipoda | E | 0.0387 | 0.0122 | 17.7 | 0.0015 | 0.0015 | 5.2 |
| Decapoda | E | 0.0213 | 0.0063 | 9.7 | 0.0044 | 0.0044 | 15.7 |
| Polychaeta | I | 0.0022 | 0.0008 | 1 | 0.0000 | 0.0000 | 0 |

Table B6. Mean and standard error (± SE) of invertebrate densities ($m^{-2}$). Densities were calculated from four replicate sediment cores ($0.0079\ m^2$) taken at each site during the Large Scale Habitat Selection Survey on *Gracilaria*-colonized mudflats (n = 6, total n = 24) or bare mudflats (n = 6, total n = 24). These invertebrates were categorized as infauna in our analysis given that they were collected using infauna cores. Families with less than five individuals across cores were not included in this table.

| **Phylum** | **Class** | **Family** | **Epibenthos/ Infauna** | ***Gracilaria* Mudflat** | | **Bare Mudflat** | |
|---|---|---|---|---|---|---|---|
| | | | | **Density ($m^{-2}$)** | **SE** | **Density ($m^{-2}$)** | **SE** |
| Mollusca | Gastropoda | *Acteocinidae* | E | 400.8 | 119.7 | 295.4 | 295.4 |
| | Bivalvia | *Mactridae* | I | 316.5 | 107.2 | 42.2 | 26.7 |
| Echinodermata | Ophiuroidea | *Amphiuridae* | E | 105.5 | 50.8 | 21.1 | 21.1 |
| Crustacea | Isopoda | *Idoteidae* | E | 84.4 | 42.2 | 654.0 | 654.0 |
| | Amphipoda | *Ampeliscidae* | E | 105.5 | 50.8 | 21.1 | 21.1 |
| | | *Gammaridae* | E | 253.2 | 56.6 | 253.2 | 86.5 |
| | | *Haustoriidae* | I | 189.9 | 165.9 | 126.6 | 103.4 |
| Annelida | Polychaeta | *Nereididae* | I | 506.3 | 163.4 | 84.4 | 42.2 |
| | | *Oenonidae* | I | 822.8 | 534.8 | 696.2 | 549.6 |
| | | *Capitellidae* | I | 485.2 | 315.9 | 189.9 | 116.7 |
| | | *Oweniidae* | I | 1286.9 | 320.9 | 928.3 | 805.7 |
| | | *Pilargidae* | I | 42.2 | 26.7 | 126.6 | 126.6 |
| | | *Cirratulidae* | I | 316.5 | 133.8 | 6434.6 | 6232.9 |
| | | *Spionidae* | I | 1202.5 | 541.7 | 696.2 | 372.3 |
| | | *Glyceridae* | I | 147.7 | 60.4 | 63.3 | 63.3 |
| | | *Phyllodocidae* | I | 168.8 | 120.8 | 21.1 | 21.1 |
| | | *Nephtydiae* | I | 0.0 | 0.0 | 506.3 | 506.3 |
| | | Spp. | I | 147.7 | 60.4 | 147.7 | 82.8 |
| | Oligochaeta | Spp. | I | 611.8 | 537.5 | 105.5 | 105.5 |
| Nemertea | | Spp. | I | 105.5 | 38.9 | 84.4 | 53.4 |
| Nematoda | | Spp. | I | 105.5 | 60.4 | 337.6 | 239.4 |

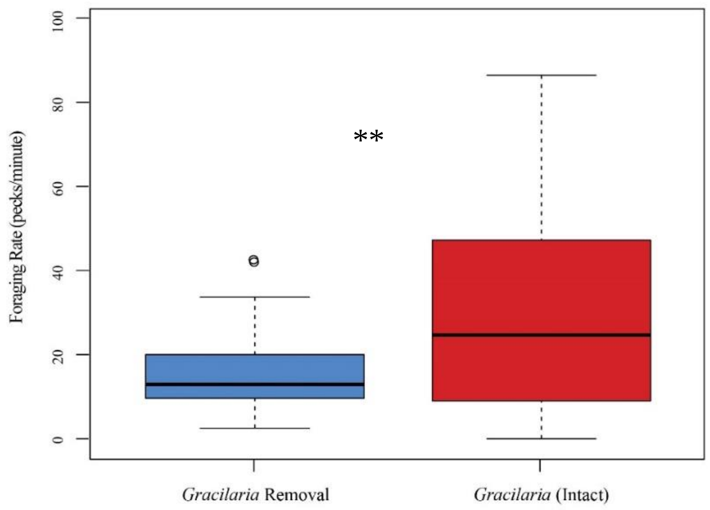
Table B7. Mean and standard error of invertebrate biomasses (g dry mass m$^{-2}$) calculated from four sediment core samples (0.0079 m$^{2}$) taken at each site during the Large-Scale Habitat Selection Survey on *Gracilaria*-colonized mudflats (n = 6, total n = 24) or bare mudflats (n = 6, total n = 24). These invertebrates were categorized as infauna in our analysis given that they were collected using infauna cores. The average percent community composition is presented for sub-group of invertebrates (i.e., 100% for both infauna and small epibenthic invertebrates). True infauna (i.e., annelids and bivalves) composed on average over 53% of the overall invertebrate community's biomass, while small epibenthic invertebrates (i.e., gastropods, isopods, and amphipods) composed over 46% of the community's biomass. Taxa representing less than 1% of community composition are not included in the table.

| | | ***Gracilaria* Mudflat** | | | **Bare Mudflat** | | |
|---|---|---|---|---|---|---|---|
| **Taxon** | **Infauna/ Epibenthos** | **Biomass (g dw m$^{-2}$)** | **SE** | **% Community Composition** | **Biomass (g dw m$^{-2}$)** | **SE** | **% Community Composition** |
| Annelida | I | 1.5 | 0.47 | 63.4 | 0.48 | 0.23 | 59.8 |
| Bivalvia | I | 0.79 | 0.53 | 33.5 | 0.32 | 0.32 | 39.4 |
| Gastropoda | E | 1.87 | 0.84 | 91.8 | 0.37 | 0.37 | 91.1 |
| Isopoda | E | <0.01 | <0.001 | 0.08 | 0.02 | 0.02 | 5.1 |
| Amphipoda | E | 0.06 | 0.04 | 3 | <0.01 | <0.01 | 1.9 |
| Total | I | 2.37 | 0.98 | 53.8 | 0.81 | 0.51 | 66.2 |
| | E | 2.04 | 0.85 | 46.2 | 0.41 | 0.37 | 33.8 |

Table B8. Patch-type preferences (and credible intervals) for shorebird species at the small scale (<1 $m^2$). Patches consisted of either naturally occurring *G. vermiculophylla* or bare mud. Preferences are presented as proportions of pecks within each patch type and were determined using a hierarchical Bayesian model in R 3.3.2 ('bayespref', Fordyce et al. 2011). Credible intervals account for 95% of the variability. If the credible intervals did not overlap the percent cover of each patch type (*Gracilaria* – 0.27; Bare – 0.73), the species were considered to demonstrate a 'significant' preference, which are represented in bold. Italics indicate a 'trending' preference.

| **Species** | **Patch Type** | **Patch Preference** | **Credible Interval (95%)** |
|---|---|---|---|
| Short-billed Dowitcher | *Gracilaria* | 0.36 | (0.21, 0.53) |
| | Bare | 0.64 | (0.47, 0.79) |
| Dunlin | *Gracilaria* | *0.37* | *(0.27, 0.48)* |
| | Bare | 0.63 | (0.52, 0.73) |
| Least Sandpiper | ***Gracilaria*** | **0.76** | **(0.62, 0.86)** |
| | Bare | 0.24 | (0.14, 0.38) |
| Peep | *Gracilaria* | 0.05 | (0.02, 0.11) |
| | **Bare** | **0.95** | **(0.89, 0.98)** |
| Ruddy Turnstone | ***Gracilaria*** | **0.97** | **(0.92, 0.99)** |
| | Bare | 0.03 | (0.01, 0.08) |
| Semipalmated Plover | *Gracilaria* | 0.09 | (0.05, 0.14) |
| | **Bare** | **0.91** | **(0.88, 0.94)** |
| Willet | *Gracilaria* | 0.26 | (0.12, 0.46) |
| | Bare | 0.74 | (0.54, 0.88) |

a) *Gracilaria* Removal Experiment



b) *Gracilaria* Addition Experiment

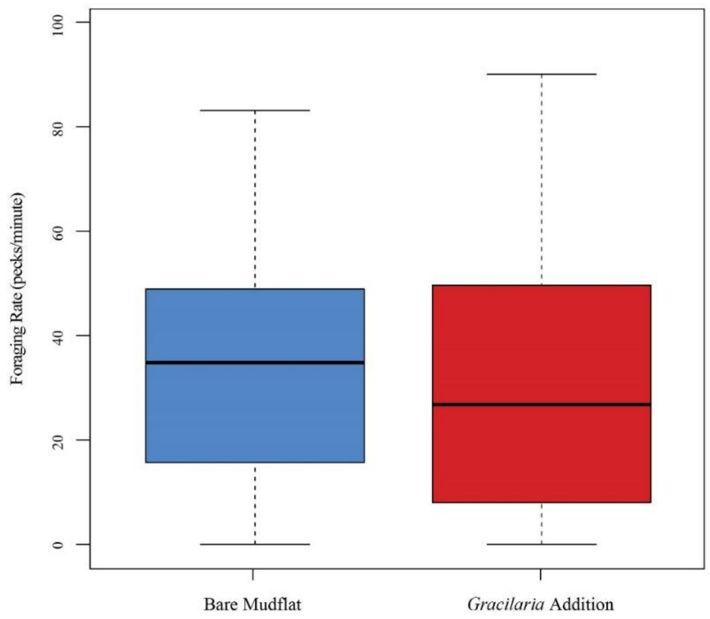
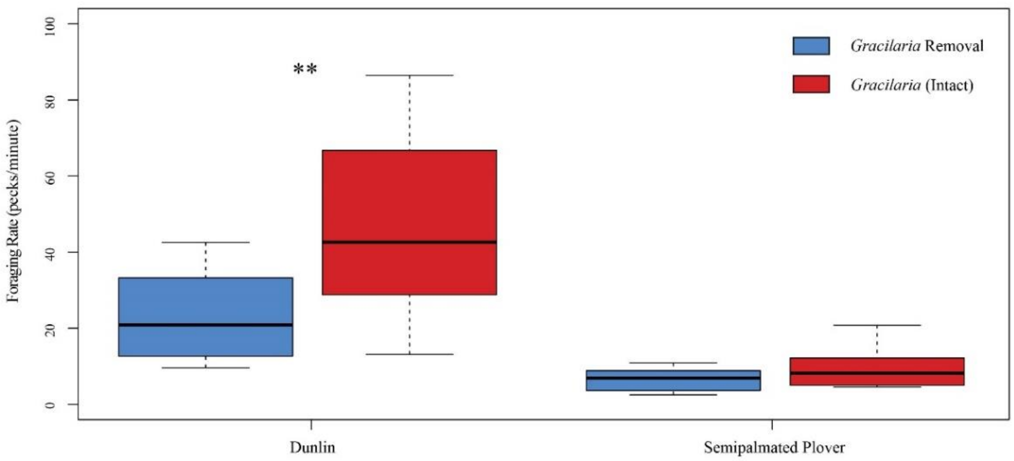


Figure B1. Average foraging rate of shorebirds in response to experimental (a) removal or (b) addition of *G. vermiculophylla* on mudflats (n = 3). Foraging rate was natural log-transformed and analyzed across all species using a generalized linear mixed model in R (3.3.2). Data were collected in spring 2014 for the removal experiment and in spring 2015 for the addition experiment. ** denotes a statistically significant difference between treatments ($p \leq 0.05$).

a) *Gracilaria* Removal Experiment



b) *Gracilaria* Addition Experiment

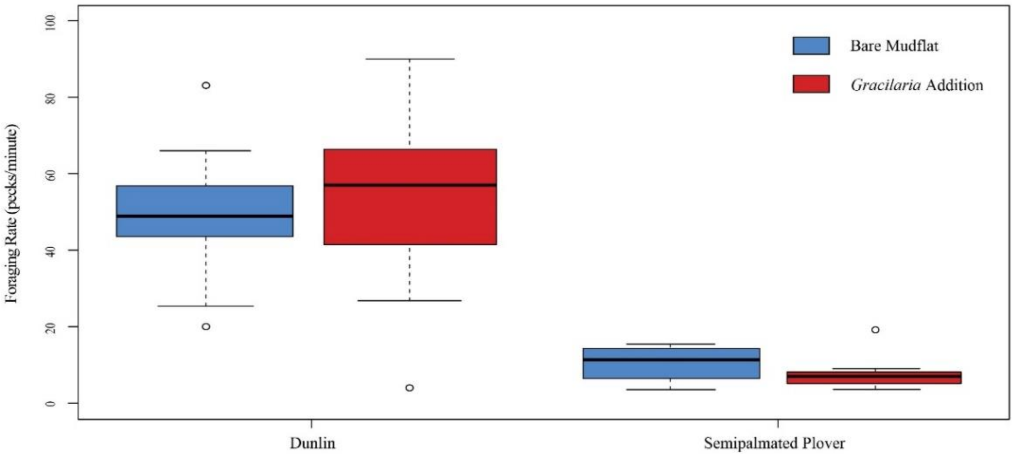


Figure B2. Average foraging rate (pecks/minute) of shorebirds in paired treatments where *G. vermiculophylla* presence was experimentally manipulated (n = 3). This figure provides species-specific detail for Figure 2. Foraging rate was natural log-transformed and analyzed separately for the two most common shorebird species (dunlin and semipalmated plover) in the (a) *G. vermiculophylla* removal experiment and the (b) *G. vermiculophylla* addition experiment. The experiments were conducted in different years (2014 vs. 2015), so the foraging rate responses should not be directly compared across experiments; however, relative patterns can be compared. ** indicates a statistically significant difference between treatments ($p \leq 0.05$).

# APPENDIX C

## CHAPTER 5

Table C1. GPS Coordinates and seaweed biomass (g) of habitat survey sites of *Callinectes spp.* in Wassaw Sound, Georgia, USA. Surveys took place on four sites with (*Gracilaria*) and without (Bare) the introduced seaweed, *Gracilaria vermiculophylla*, and consisted of three 10 m seine pulls per site. Both juvenile and adult *Callinectes spp.* were counted in each seine haul. On mudflats where *G. vermiculophylla* was present, average wet and dry biomass (± standard error) was determined from surveys that included five 0.25 $m^2$ quadrats. No *G. vermiculophylla* was present in the bare habitat types.

| Habitat Type | Latitude | Longitude | ***Gracilaria*** **Wet Biomass (g)** | ***Gracilaria*** **Dry Biomass (g)** |
|---|---|---|---|---|
| Bare | 31°59'16.98"N | 81° 1'44.04"W | --- | --- |
| | 31°57'9.29"N | 81° 3'52.10"W | --- | --- |
| | 31°54'51.39"N | 80°56'29.08"W | --- | --- |
| | 31°56'20.76"N | 80°58'6.21"W | --- | --- |
| *Gracilaria* | 31°57'43.88"N | 81° 0'46.00"W | 15.84 ± 4.24 | 1.90 ± 0.71 |
| | 31°57'2.61"N | 80°59'4.33"W | 15.08 ± 4.29 | 2.01 ± 0.75 |
| | 31°58'27.66"N | 80°55'24.61"W | 0.76 ± 0.35 | 0.10 ± 0.04 |
| | 31°56'36.37"N | 80°58'37.18"W | 11.77 ± 4.90 | 1.75 ± 0.74 |