ONTOGENETIC DIET SHIFTS AT MULTIPLE SPATIAL SCALES IN THE SALT MARSH PERIWINKLE, *LITTORARIA IRRORATA*

by

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(Under the Direction of CRAIG OSENBERG)

ABSTRACT

The Saltmarsh Periwinkle *(Littoraria irrorata*) is a common grazer in southeastern marshes dominated by the smooth cordgrass, *Spartina alterniflora*. I quantified *Littoraria* density, size structure, microhabitat use, and diet along elevational gradients within eight marshes ranging from Florida to Maryland. *Littoraria* density and size increased with increasing elevation within southern marshes, but size did not vary and density decreased with elevation in northern marshes. Snails in southern marshes were more likely to occur on sediments, although this habitat pattern was not reflected in their diets. Instead, stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$, showed that $\delta^{15}N$ of resources and snails increased from southern to norther marshes and that small snails shifted diet from sediments to *Spartina*-based sources as snail densities increased; diets of large snails were primarily associated with *Spartina*. Understanding *Littoraria* diet and population structure will allow us to better predict its effects on salt marsh food-web dynamics.

INDEX WORDS: stable isotope analysis, diet, microhabitat, body size, salt marsh

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CHAPTER 1

ONTOGENETIC DIET SHIFTS AT MULTIPLE SPATIAL SCALES IN THE SALT MARSH PERIWINKLE, *LITTORARIA IRRORATA*[1]

[1] Hawkins, D. G., Osenberg, C.W., and Atkins, R.L. To be submitted to *Estuaries and Coasts.*

Abstract

The Saltmarsh Periwinkle *(Littoraria irrorata*) is a common grazer in southeastern marshes dominated by the smooth cordgrass, *Spartina alterniflora*. I quantified *Littoraria* density, size structure, microhabitat use, and diet along elevational gradients within eight marshes ranging from Florida to Maryland. Snails in southern marshes were more likely to occur on sediments, although this habitat pattern was not reflected in their diets. Instead, stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$, showed that $\delta^{15}N$ of resources and snails increased from southern to norther marshes and that small snails shifted diet from sediments to *Spartina*-based sources as snail densities increased; diets of large snails were primarily associated with *Spartina*. Understanding *Littoraria* diet and population structure will allow us to better predict its effects on salt marsh food-web dynamics.

Introduction

Dietary studies provide the foundation for understanding species interactions and the structure and function of entire ecosystems (Estes 2011, Nielsen 2017). Empirical characterization of feeding interactions remains challenging, as many animals utilize diverse diets and exhibit foraging patterns that vary over space and time. This is especially true for generalist consumers whose distribution spans a large geographic range where resource availability and environmental conditions may vary tremendously (Kuo and Sanford 2009).

Traditionally, ecological studies of food web dynamics have ignored intraspecific variation in diets. Recently, this assumption has been contested, and there is increasing recognition that individual variation in diet can influence population dynamics and community structure (Bolnick et al. 2011, Tinker et al. 2012) due, for example, to its effect on a consumer's interactions with

its resources (Werner and Gilliam 1984, Zerba and Collins 1992, Bolnick et al. 2011, Griffin 2012, Singer et al. 2014, Hughes et al. 2015, Hakayawa et al. 2018).

Intraspecific variation may manifest regionally, locally, or among individuals within a local population. Intraspecific variation in body size is particularly important in explaining variation in diet as well as habitat use (Van Valen 1965, Henderson 1973, Wiens 1976, Werner and Gilliam 1984, Brown 1984, Osenberg and Mittelbach 1989, Zerba and Collins 1992, Woodword 2005, Hayakawa et al. 2018). Variation in body size and its effect on diet and habitat use can give rise to ontogenetic niche shifts (Werner and Gilliam 1984) and is central to evaluating the role of individual variation in feeding behavior and its effect on ecological and evolutionary patterns (Polis 1984, Hughes et al. 2015). For example, intraspecific resource partitioning by age (or size) classes can expand a species niche, reduce competition between age-classes, and facilitate a larger population size (Polis 1984).

Intraspecific variation in diet may be particularly dramatic for a species that inhabits a wide geographic range, in which spatial variation in resource availability can lead to variation in consumer diets (Davis et al. 2015). Regional differences in environmental factors may also act as selective forces that lead to the evolution of physiological, behavioral, or morphological differences among populations, and these differences (e.g., in energy requirements or the ability to capture and process different types of food) may bring about regional differences in feeding behavior and diet (Cabana et al 2017) even if resource availability is unaffected. Thus, diets are expected to vary across space, in addition to varying among individuals within a site. Documenting these patterns of variation is a first step to understanding variation in food web dynamics.

Traditional diet analyses such as gut and fecal content analysis are valuable tools for understanding an organism's diet, but they may not represent long-term dietary patterns. If resource distributions are patchy or vary through time, gut and fecal content analysis may only reflect the most recent resources that have been consumed. These traditional analyses are also biased towards resources that are more difficult to digest, and they don't necessarily represent what the consumer is assimilating (Silliman and Ziemen 2001). Moreover, obtaining gut and fecal contents from the same individual over multiple occasions, to expand the temporal scope of the dietary analysis, can be logistically difficult (Denton et al 2019).

More recently, stable isotope analysis (SIA) has been used to evaluate diet and its effects on food webs and trophic relationships (Currin 1995, Fry 2006, Newsome et al. 2012, Phillips et al. 2015). Stable isotope ratios of δ13C and δ15N are commonly used to identify food sources (δ13C) and trophic position (δ15N). SIA can provide a better, more robust evaluation of diet and trophic position (Newsome et al. 2012) and thus overcome the shortcomings of traditional diet analyses, especially associated with generalists or detritivores (Hentchel 1998). SIA compares isotopic composition of a consumer with its resources, and therefore helps identify resources that are being assimilated into the organism's tissue, not just what is being passed through the gut. SIA also allows us to expand the temporal scope of dietary studies by examining tissues that integrate the isotopic composition of food over a relatively prolonged time horizon (weeks to years vs. a day or less associated with traditional diet analyses) (Phillips et al 2014).

This study focuses on the omnivorous consumer, *Littoraria irrorata*, an abundant gastropod inhabiting salt marshes on the Atlantic and Gulf Coasts of the United States. *Littoraria* is particularly common in saltmarshes dominated by smooth cordgrass, *Spartina alterniflora*. *Littoraria* have a diverse diet, feeding on organic matter (detritus, diatoms, algae)

on the marsh sediment surface, live and standing dead *Spartina* tissue, and fungus that *Littoraria* "farms" by radulating live *Spartina* leaves and facilitating fungal growth in the resulting scars (Odum and Smalley 1959, Alexander 1979, Newell and Barlocher 1993, Currin et al 1995, Silliman and Ziemen 2001). In laboratory trials, *Littoraria* preferred dead *Spartina* tissue that was infected with fungi over uninfected live *Spartina* tissue and grew best when fed fungal cultures rather than live or dead *Spartina* tissue (Newell and Barlocher 1993. In a field study, *Littoraria* stomach contents contained very little live *Spartina* tissue (<4%), and instead consisted of over 95% dead *Spartina* and marsh sediments, which were difficult to distinguish in the guts (Alexander 1979, Silliman and Ziemen 2001).

Dietary patterns of *Littoraria* could be explained by *Littoraria's* use of microhabitats within salt marshes. *Littoraria* occupy the canopy of live and standing dead *Spartina*, but they also are found at the base of the *Spartina* stalk and on the marsh sediment surface. Many behavioral and physiological mechanisms have been explored to explain why *Littoraria* occupy these different microhabitats, including: predator avoidance (Hamilton 1976, Vaughn and Fisher 1988 and 1992), heat and hypoxic stress avoidance (Bingham 1972, McBride 1989, Vaughn and Fisher 1992, Hovel et al. 2001, Henry et al 2009), and desiccation avoidance (Iacarella and Helmuth 2011 and 2012). Snails are much more susceptible to predation and heat stress on the sediment surface and climb *Spartina* stalks to reduce heat stress (Henry et al. 2009) and avoid predators (Vaughn and Fisher 1988 and 1992). Thus, we would expect micro-habitat use to vary across regional populations of *Littoraria* if these populations experience differences in biotic (predation) and abiotic (heat, hypoxia, and desiccation) stressors. Further, because foraging gain and mortality risk vary across habitats (within and across marshes) and prey size (Werner and

Gilliam 1984), variation in biotic and abiotic stressors should also influence ontogenetic patterns of diet and microhabitat use.

Spatial variation in diet and habitat use might also be explained by regional differences in host plant quality. Previous latitudinal investigations in US Atlantic Coast salt marshes found that *Spartina* nitrogen content increases, and tissue toughness decreases, as latitude increases (Ho and Pennings 2013). This could influence the palatability of *Spartina* tissue or the preference of *Littoraria* for live *Spartina* vs. other resources.

This study examines the diet of two size-classes of *Littoraria*, as assessed through stable isotope analysis, at five sites across a latitudinal gradient spanning from Florida to Maryland, and across relative elevational gradients (e.g., distance from the creek) within each of those marshes. Although live *Spartina,* dead *Spartina,* and fungus have similar $\delta^{13}C$ and $\delta^{15}N$ values, making it difficult to discern their relative dietary contributions, the isotopic signature of marsh sediment is fairly unique (Haines 1976, Craft et al. 1988, Peterson et al. 1986, Currin et al. 1995), suggesting that isotopic analysis can discriminate between the contribution of plant vs sediment resources to the overall diet of *Littoraria*. This distinction can be useful because environmental stressors that influence *Littoraria* microhabitat use (predation and heat stress) are distinctly different between sediment (high predation and higher temperatures) and the canopy (low predation and lower temperatures).

When *Littoraria* biomass is high, it can have strong top-down effects on live *Spartina* aboveground biomass in Georgia salt marshes (Silliman and Ziemen, 2001; Atkins et al. 2015). The strength of this top-down affect will likely depend on the habitat use of snails – if they primarily occur on the sediment *Littoraria* would likely have smaller top-down effects on

Spartina. Understanding the spatial variation in *Littoraria* diet is therefore important to understanding spatial variation in its effects on *Spartina* and salt marsh food web dynamics.

Despite the importance of *Littoraria* to marsh ecosystems, no SIA studies have quantified diets of different size classes of *Littoraria*, nor have they compared diets across spatial gradients, such as those imposed by latitude or marsh elevation. Most past stable isotope studies of salt marshes have not primarily focused on *Littoraria,* have had small sample sizes, and have not clearly defined where (within a marsh) they collected their samples (Haines 1976, Haines and Montague 1979, Peterson and Howarth 1987, Couch 1989, Sullivan and Moncrieff 1990, Currin 1995). These studies have shown significant variation in the isotopic values of resources within marshes and within regions. Therefore, we collected samples on two spatial scales (among sites across a latitudinal gradient and within marshes across an elevational gradient) and distinguished small vs. large size classes of snails to better understand the isotopic variability of snails and their resources.

I hypothesized that (1) stable isotope values of resources and snails will vary significantly across the relative elevational gradient within a site (tested as relative distance to the creek as a proxy for elevation) – in particular, I propose that past variation in isotopic values found within sites (Haines 1976, Haines and Montague 1979, Peterson and Howarth 1987, Couch 1989, Sullivan and Moncrieff 1990, Currin 1995) may have been driven by variation in isotopic values among snails collected at different elevations; (2) stable isotope values of resources and snails will vary significantly across sites due to geographic variation in factors, such as sources of nitrogen and varying degrees of coastal protection (Haines 1976, Haines and Montague 1979, Peterson and Howarth 1987, Couch 1989, Sullivan and Moncrieff 1990, Currin 1995); (3) diet, as inferred by isotopic analysis, should be reflected by the pattern of habitat use: e.g., snails from

sites in which most snails occupy the plant canopy should have isotopic signatures that mirror the plants and not the sediments; and (4) large and small snails will have different diets, as inferred by isotopic analysis, due to ontogenetic changes in microhabitat use and diet associated with physiological stress or risk to predation.

Methods

Study Site and Design

Littoraria irrorata commonly occurs in the United States in saltmarshes from Texas to Maryland and has been found as far north as New York. In the summer of 2017, I sampled five sites from Florida to Maryland (Fig. 2.1): St. Augustine, Florida (FL) at the Guana-Tolomato National Estuarine Research Reserve; Brunswick, Georgia (GA) near St. Simon's Island; Georgetown, South Carolina (SC) near the University of South Carolina Baruch Marine Laboratory; New Point Comfort, Virginia (VA); and Deal Island, Maryland (MD). These sites span approximately eight degrees of latitude, which covers a major portion of *Littoraria's* range along the US Atlantic coast. At each site, I chose an area that had a clear elevational gradient from creek to upland without any obstructions such as tree hammocks, upland patches, or an intervening creek, and in which *Spartina* was the dominant plant and *Littoraria* was present. I was specifically interested in the saltmarsh zone where both *Littoraria* and *Spartina* were present (and not further upland) to focus on the *Littoraria*-*Spartina* interaction. I ran three transects from the tidal creek bank, corresponding to the lower distributional limit of *Spartina*, up to the upland area where *Spartina* eventually disappeared. Transects were run perpendicular to the creek and varied in length from 43m to 108m depending on marsh morphology. These transects represent a gradient from the creek bank (lowest relative elevation) to the upland (highest relative elevation). Within a site, replicate transects were placed 20m apart from one another. At five evenly spaced positions along each transect I placed a 40cm x 40cm PVC quadrat and surveyed/collected data from within the quadrat. I placed the quadrat at the same relative distance from the creek at 5 positions along each transect (0, 0.25, 0.50, 0.75, and 1.0 of the total transect length, with 0 being

nearest the creek with the lowest relative elevation, and 1 being farthest from the creek with the highest relative elevation.

In each quadrat, I counted every *Littoraria*, measured its shell width to the nearest 0.01 mm with digital calipers, and categorized its microhabitat use: a) in the plant canopy (on a *Spartina* plant and >10cm above the sediment), b) on the marsh sediment surface or on the *Spartina* stalk but close to (<10cm) the sediment surface). I pooled the snails on stalks with those on the mud since there are vertically within only a few centimeters of one another. Thus, the response was binary (in the canopy vs. on (or near) the sediments). Data on density and size-structure are reported in Appendix II. In this chapter, I focus on microhabitat use and the results for the stable isotope analyses (details are given below). All samples (snails and resources) were collected between July 14th and July 29th of 2017 within 2 hours of low tide during daylight hours.

Sample Collection and Preparation

Three types of samples from *Spartina* plants were obtained for stable isotope analysis: 1) live *Spartina*; 2) standing dead *Spartina*; and 3) fungal-infected scar tissue from live *Spartina* plants (fungus on dead *Spartina* cannot be easily distinguished visually from dead *Spartina*). I only sampled *Spartina* that had *Littoraria* feeding on them to make sure I omitted plants that were avoided by snails. I collected *Spartina* from 5 quadrats in 2 of the 3 transects, and fungal scars from 5 quadrats in 1 of the 3 transects. All samples collected for stable isotope analyses were frozen within 1 hour of collection and kept frozen until they were processed further (as described below).

Live *Spartina* samples were obtained by removing 1-5 leaves (containing no fungal-infected scars) from each of 5-10 plants and pooled together in a sample. Dead *Spartina* samples

were obtained by removing 1-5 leaves from each of 5-10 separate plants and pooled together in a sample. All live or dead *Spartina* were washed 3 times with distilled water to remove mud, salt, and any epiphytes that may have been growing on the surface. They were then dried for 48 hours via lyophilization, ground with a ball mill, and stored at 40°C until being sent for C and N analysis.

To obtain the fungal samples, I collected live *Spartina* plants that contained fungal-infected radulation scars. The fungal infected scars were dark brown or black in color, and easy to identify. All yellow or green *Spartina* tissue surrounding the infected scars was removed, leaving only the dark, infected scar tissue. Due to the small size of each scar (1-5cm in length x ~1-3mm in width), I pooled up to 40 scars per sample which were taken from up to 20 individual plants. Each scar was cut in half, with one half of each scar going into a sample for stable isotope analysis and the other halves going into a sample for fungal biomass measurement. Fungal samples for isotope analysis were then dried via lyophilization for 48 hours, ground using a ball mill, and stored at 40°C until they were sent for C and N analysis. Fungal biomass estimates were made using methods from Beni et al. (2014) at the U.S. National Poultry Research Center in Athens, Georgia, USA, by cutting the fungal scars into small pieces (~4mm^2), mixing them with potassium hydroxide in methanol, shaking them for 1 hour and sonicating them for 30 minutes in ice water. The remaining solution was then run through filter paper and combined with HPLC-grade methanol to neutralize the extract. This extract was then run through a C18 Sep-Pak filter and eluted with isobutanol. The final extract was then immediately analyzed using HPLC (Messner and Newell 2002), and the area under the HPLC curve for each sample was converted to ergosterol concentrations (nanograms ergosterol per gram of sample by dry mass) using a reference curve created with serial dilutions of an ergosterol standard (Beni et al. 2014).

Salt marsh sediment samples were obtained by collecting the top 1cm of the sediment surface from a 25 cm^2 area to collect benthic microorganisms that *Littoraria* may feed on (Couch 1989). Samples were acidified using 1N HCL to remove carbonates associated with shell material, dried via lyophilization for 48 hours, ground using a ball mill, and stored at 40^oC until being sent for C and N analysis.

Snails were collected from each quadrat, and sorted into 2 size classes (2-7mm and 9-14 mm shell width) regardless of the microhabitat they were found in. I then pooled 10 snails from each size-class into a sample (yielding a total of 140 samples: Appendix I Table 1). The samples were pooled because individual small snails did not provide enough foot tissue for a sample. The number of samples per size class and quadrat combination varied based on the number of snails available in each size class in each quadrat. Quadrats with more small snails yielded more samples of small snails, while quadrats with more large snails yielded more samples of large snails. *Littoraria* snail tissue was separated from the shell and operculum, and the foot (muscle) tissue was then dissected from the rest of the body and washed 3 times with distilled water. Each pool of 10 snails based on quadrat and size class was then dried for 48 hours via lyophilization, ground into a homogenized powder with a ball mill, and stored at 40^oC until being sent for C and N analysis.

All stable isotope analyses were performed by the University of Georgia's Center for Applied Isotope Studies in Athens, Georgia, USA. Samples were processed using an isotope ratio mass spectrometer (IRMS) to quantify $\delta^{13}C$, $\delta^{15}N$, percent carbon and percent nitrogen. The isotope ratios were compared to a standard whose isotope ratio was calibrated to international standards.

Statistical Analysis

I used linear mixed effects modeling to describe variation in the isotopic values (one model for δ^{13}C and one model for δ^{15}N) of the resources using 3 predictors: (1) Site (random effect) (FL, GA, SC, VA, or MD); (2) Relative Distance from the Creek (0, 0.25, 0.5, 0.75, or 1); and (3) Resource Type (Live *Spartina*, Dead *Spartina*, Fungus, and Sediment). I used Site as a random effect because I wasn't interested in the effect of these specific sites, but I wanted to make a statement about resource values in general. I included an interaction between Resource Type and Relative Distance to Creek:

$$Y = \beta_1(\text{Resource Type}) + \beta_2\,(\text{Relative Distance to Creek}) + \beta_3(\text{Resource Type} * \text{Relative Distance to Creek}) + (\beta_0 + b_{Site}) + \varepsilon \quad [1]$$

with fixed effects β_1, β_2, the random effect of site (b_{Site}), and normally distributed residual error ε. Models were fit using the lmer() function in R Version 3.2.4. I calculated variance inflation factors (VIFs) to test for multicollinearity; all VIFs were under 1.6 indicating low correlation between predictors.

I also used linear mixed effects modeling to describe variation in the isotopic values (one for δ^{13}C and one for δ^{15}N) of *Littoraria* using four predictors: (1) Site (random effect) (FL, GA, SC, VA, or MD); (2) Relative Distance to the Creek (0, 0.25, 0.5, 0.75, 1); (3) Size class (large vs small); and (4) Microhabitat Use (proportion of all snails of all sizes on or within 10cm of the sediments in the quadrat where the snails were collected). I only included the two-way interaction between size class and relative distance to creek because I had an a priori reason to

suspect an interaction between these two fixed effects, but not between other predictors. This resulted in:

$$Y = \beta_1(\text{Size Class}) + \beta_2\,(\text{Relative Distance to Creek}) + \beta_3(\text{Size Class} * \text{Distance to Creek}) + \beta_4\,(\text{Microhabitat Use}) + (\beta_0 + b_{Site}) + \varepsilon \quad [2]$$

All models were fit using the lmer() function in R Version 3.2.4. I calculated variance inflation factors (VIFs) for my models to test for multicollinearity; all VIFs were under 5 indicating moderate to low correlation between predictors.

Model Selection

I determined the best model for stable isotope values of *Littoraria* and their resources by creating full models (using Equation 1 for resources and Equation 2 for snails) and the random effect of site. I also fit all possible reduced models created by removing parameters. For each response variable, I ranked all models using AIC, assessed model fit using adjusted R^2 values, and drew my final inferences from the model with the lowest AIC.

Results

Ergosterol Biomass

Fungal biomass (ng of ergosterol per gram of sample) varied across sites, with fungal samples from the southern sites (FL, GA, and SC) having much higher fungal biomass than the northern sites (MD and VA) (Figure 1.2). VA and MD samples often had undetectable levels of fungal biomass.

Littoraria Microhabitat Use

Because microhabitat use of snails is a potential predictor of snail isotopic values, I briefly summarize those results here (Figure 1.3). Snail use of the sediments varied among sites: the proportion of snails on the sediments was lowest in the most southern sites (where ~20% of snails in FL and GA were found on the sediment during low tide), and greatest in the most northern sites (where ~70% of snails in VA and MD were found on the sediment during low tide). Snails at SC were on the sediment about 50% of the time. There were no demonstrable effects of snail size on microhabitat use ($p=0.13$; Figure 1.4).

Resource Isotope Values

Resource $\delta^{13}C$ varied from -23.14‰ (sediment, FL) to -12.49‰ (live *Spartina,* MD) across all samples (see Appendix I, which provides all raw isotope data). Samples from *Spartina* were relatively similar, with live *Spartina* varying from –15.19‰ to -12.49‰, standing dead *Spartina* varying from -15.42‰ to -12.89‰, and fungus varying from -15.56‰ to -13.73‰. In contrast, samples from the sediment had the lowest $\delta^{13}C$ values, ranging from -23.14‰ to -17.02‰, and these values did not overlap with other resources. Sediment showed the widest range in $\delta^{13}C$ values (range: 6.12‰), which was greater than the range across all three resource types from *Spartina* (range: 3.07‰). The lowest resource $\delta^{13}C$ values came from southern sites and the highest resource $\delta^{13}C$ values came from northern sites (Figure 1.5).

The top performing model for resource $\delta^{13}C$ included effects of site (random effect), relative distance to the creek, and resource type (Table 1.1); the zones with the largest $\delta^{13}C$ values tended to be those that were further from the creek (Appendix I Figures 1, 3, 5, 7). The most pronounced pattern was that sediment had a lower $\delta^{13}C$ value than all 3 other resources at all sites (Figure 1.5). Fungus tended to have a slightly lower $\delta^{13}C$ compared to the live and dead *Spartina*, but these two *Spartina* groups could not be easily distinguished.

$\delta^{15}N$ varied from 0.15‰ to 9.09‰ across all resource samples (Appendix I). The lowest $\delta^{15}N$ values of resources came from Florida and the highest resource $\delta^{15}N$ values came from Georgia and Virginia. All four resource types showed strong overlap in their $\delta^{15}N$ values. The top performing model for resource $\delta^{15}N$ included effects of site (random effect), and an interaction between resource type and relative distance to the creek (Table 1.1; Appendix figures 2, 4, 6, and 8). In general, $\delta^{15}N$ decreased with increasing distance, although this pattern was more pronounced for samples from *Spartina* than for those from the sediment.

Based upon these resource isotopic patterns, the contribution of live *Spartina*, standing dead *Spartina*, and fungus cannot be easily distinguished from one another, although they can be easily distinguished from samples collected from sediments based on their different $\delta^{13}C$ values.

Littoraria Isotopic Values

Snail $\delta^{13}C$ varied from -17.1‰ to -11.75‰ (Appendix I). The top performing model for *Littoraria* $\delta^{13}C$ values included effects of site (random effect), size class, and an interaction between size class and snail density (Table 1.1). Large snails had higher $\delta^{13}C$ than small snails at all sites (Figure 1.6). The $\delta^{13}C$ of small snails increased as snail density increased, but $\delta^{13}C$ for large snails was consistent across all densities (Figure 1.8). Microhabitat use was not a demonstrable predictor of $\delta^{13}C$ for the snails and was eliminated from the top model during the model selection process. In general, the $\delta^{13}C$ of large snails was indistinguishable from the values from the live and dead *Spartina* and fungal samples, but greater than the samples taken from the sediments. Small snails also had $\delta^{13}C$ more similar to *Spartina* and fungi, although sometimes had a pattern that suggested some limited use of the sediments. The exception to this general pattern were the data from Georgia, where the $\delta^{13}C$ for both small and large snails was

greater than that observed in any resource sample, suggesting the snails may have been utilizing another, yet unidentified, resource.

Snail $\delta^{15}N$ varied from 4.49‰ to 10.45‰ (Appendix I). The top performing model for snail $\delta^{15}N$ values included random effect of site and an interaction between size class and relative distance to the creek. Large and small snails $\delta^{15}N$ was 3 to 4‰ higher than all of the resources indicating, as expected, that they are one trophic level higher than these resources. Large snails generally had higher $\delta^{15}N$ than small snails at all sites (Figure 1.7). Large and small snail $\delta^{15}N$ increased from southern sites to northern sites (Figure 1.7), even though their resource $\delta^{15}N$ (with the exception of sediment) stayed relatively the same across all sites (Appendix I, Figures 2, 4, 6, and 8).

Discussion

SIA studies are an effective tool to identify trophic links in systems dominated by detrivores and other species for which gut and fecal content analysis is less effective (Hentschel 1998). Isotopic data can also be used to better understand how ontogenetic diet shifts can inform foodweb studies. In this study, I addressed several questions about the isotopic ecology of the marsh periwinkle snail, *Littoraria irrorata*, and its resources. Indeed, this is the most extensive stable isotope study of *Littoraria* -- other studies have included *Littoraria* isotope values but only as a small subset of their more extensive salt marsh stable isotope data (Haines 1976, Haines and Montague 1979, Peterson and Howarth 1987, and Currin 1995). Here, I determined: (1) there was substantial variation in the isotopic composition of snails and resources within sites (across quadrats that varied in their relative distance to creek) and across sites; (2) salt marsh surface sediments were isotopically distinct from fungus, live *Spartina*, and standing dead *Spartina*, although the fungi and plant samples could not be easily distinguished; and (3) large snails were more enriched in $\delta^{13}C$ and $\delta^{15}N$ than small snails within each site and the magnitude of this relationship varied with respect to relative distance to the creek.

Mean $\delta^{13}C$ values for snails were lower (by ~2 per mil) and mean $\delta^{15}N$ values for snails were much higher (by ~5 per mil) than reported in past studies (Currin 1995). This could be due to more extensive spatial coverage in this study than previous SIA studies of salt marshes. I collected samples across the entire gradient of the salt marsh where *Spartina* and *Littoraria* were present, and most other studies collected from one or two areas within a single marsh or two marshes within the same region (Haines 1976, Haines and Montague 1979, Peterson and Howarth 1987, and Currin 1995). Past studies may therefore have missed important sources of environmental variation driving variation in *Littoraria* isotopic patterns. However, even though

we pooled 10 snails in each sample, there was a large amount of variation between samples, implying that there is a large amount of individual variation of snail isotopic values or that there was a considerable amount of variation introduced methodologically or analytically.

The spatial differences identified in $\delta^{13}C$ values of snails among the sites could be due to different proportions of isotopically distinct food sources in their diet, but they could also be due to site differences in the isotopic values associated with a given resource. Many studies assume a single baseline value for a resource when evaluating consumer isotopic values across different sites This is likely to lead to biases (Denton et al. 2019). For example, I found that there were differences between sediment, fungus, live *Spartina*, and dead *Spartina* isotopic values among our sites (Figure 1.5). Therefore, I compared inferences drawn from the snail isotope models with those drawn from the resource isotope models to determine whether isotopic variations in the resources were driving variations in the snails (Denton et al. 2019). The $\delta^{13}C$ of different resources was greater at locations closer to the creek (i.e., at lower elevations), but snail $\delta^{13}C$ values decreased slightly at locations nearer the creek, which is opposite the pattern observed for their resources. Therefore, variation in snail $\delta^{13}C$ is not due solely to variation in $\delta^{13}C$ of their resources and is likely due to variation in some other factor such as foraging behavior.

The spatial differences in $\delta^{15}N$ values of *Littoraria* and its resources may be connected to $\delta^{15}N$-enriched anthropogenic inputs derived from human sources such as agriculture fertilizers and sewage runoff (Vizzini and Mazzola, 2006; Denton et al. 2018), rather than being connected to variation in trophic structure or foraging behavior. I found that $\delta^{15}N$ was highest closest to the creek for both snails and resources, which could be due to the longer inundation times in water derived from the estuary which is connected to rivers that bring anthropogenic nitrogen from upstream (Vizzini and Mazzola, 2006; Denton et al. 2018).

Currin (1995) provided convincing evidence using SIA that standing dead *Spartina* and benthic microalgae are important food sources for salt marsh primary consumers, such as *Littoraria* and fiddler crabs (North Carolina). Currin was able to distinguish between live and dead *Spartina* by using SIA of sulfur isotopes. Unfortunately, I was unable to do so due to financial constraints. Barlocher and Newell (1994) showed that *Littoraria* prefer to graze on standing dead *Spartina* leaves that had the highest fungal biomass. Gut content analysis of *Littoraria* indicated that most of the stomach contents of *Littoraria* was sediment and dead *Spartina* tissue (Alexander 1976; Silliman and Ziemen 2001). However, these studies did not compare gut contents of different size classes of *Littoraria*, nor did they compare gut contents across a latitudinal gradient or an elevational gradient within sites.

Obvious changes in habitat (and diet) during ontogeny will cause changes in $\delta^{13}C$ values of consumers (Hentschel 1998). However, I found no differences in microhabitat use between size classes -- small and large snails exhibited similar patterns of occurrence in the canopy and on the sediment surface (Figure 1.4). Yet, I found that large snails had consistently higher $\delta^{13}C$ than small snails (Figure 1.6), implying that large and small snails have different diets, despite their similar use of marsh microhabitats. I therefore suggest that the observed ontogenetic shift in diet, was likely due to differences in feeding ability between large and small snails.

Although *Littoraria* grow best on dead *Spartina* (Newell and Barlocher 1994), small snails have small radulas and may not be able to rasp dead *Spartina* tissue as easily as larger snails (Newell and Barlocher 1993). As a result, the diet of small snails may primarily come from fungi growing on the plants or organic matter on the sediment surface, both of which have lower $\delta^{13}C$ than dead (or live) *Spartina*. Similarly, both fungus and sediment are relatively small in particle size and easier for small snails to consume compared to *Spartina* plant tissue.

Increased consumption of fungi alone cannot explain the isotopic patterns, however, because snails had $\delta^{13}C$ values that were often lower than those found in fungus (Figure 1.5). Thus, increased feeding on sediments must be invoked to explain the observed $\delta^{13}C$ disparity between small and large snails. Of course, this does not explain why small snails would be as likely as large snails to occur in the canopy (and not the sediments), but we recorded the snails' presence in a microhabitat and not their feeding activity in the microhabitat. Perhaps the use of the canopy represents a response to another factor (e.g, predation or thermal stress). It should also be acknowledged that I collected the microhabitat data at low tide, during the hottest part of the day in the hottest time of year and only at one point in time. Therefore, this microhabitat classification may not represent where snails feed at night or during high tide as observed by Byers (2000).

Alternatively, small snails might be less enriched in $\delta^{13}C$ despite using the same food sources as large snails if they fractionate $\delta^{13}C$ less than larger snails. It has been shown that different sized animals fractionate the same food sources differently depending on their body lipid concentrations and other factors (Phillips et al 2014). Without performing feeding experiments to determine $\delta^{13}C$ isotopic fractionation of specific resources, we cannot evaluate this hypothesis.

One interesting pattern that I found was that small snails' $\delta^{13}C$ values varied much more than large snails' $\delta^{13}C$ values, and that some small snails $\delta^{13}C$ were very similar to large snails. We saw that the difference in $\delta^{13}C$ values between large and small *Littoraria* was smallest at high densities, and greatest in areas of low snail density (Figure 2.6). This suggests that within a marsh, that small snails change their foraging behavior more than do larger snails. I suggest that the reduction of small snail $\delta^{13}C$ in quadrats with low density arose because small snails are more

often eating sediment in areas of low snail density due to a lack of available fungus. This hypothesis might also explain the discrepancy between large and small snail $\delta^{13}C$ at the southern sites. Upland zones in southern sites have the highest density of large snails. Therefore, there are more snails radulating live *Spartina* thus facilitating more fungal growth. With a high availability of fungus, small snails can stay in the canopy to feed on fungus and avoid heat stress from feeding on the sediment. In areas of lower snail density (and therefore less fungus cultivation) small snails are more likely to forage on the sediment due to their inability to radulate *Spartina* leaves (Newell and Barlocher 1993). An interesting implication of this interpretation is that small snails appear to be favoring foraging gain over predation risk: i.e., they are feeding on the sediment where predation risk is high vs in the canopy where predation risk is low.

Mixing models are often used to analyze stable isotopes in order to estimate proportional contributions of resources to a consumer's diet (Phillips et al 2014). Mixing models are relatively straight-forward, but they rely on several key assumptions that can cause misinterpretation of their results. They may not be appropriate when resources have similar isotopic values, and when turnover rates or discrimination factors are unknown for the species in question (Currin 1995, Phillips et al. 2014). I determined that mixing models are not appropriate to use because resources had similar isotopic $\delta^{13}C$ and $\delta^{15}N$ values and because we don't know tissue specific turnover rates or discrimination factors for *Littoraria.* I have therefore focused on relatively crude patterns of variation in isotopic patterns to make qualitative inferences about the ecology of *Littoraria*. Follow-up studies are necessary to test these hypotheses about feeding behavior and microhabitat use.

Littoraria is an abundant and conspicuous species in Atlantic Coastal salt marshes. As salt marshes continue to experience higher stress from a changing climate (increased

temperatures, sea level rise) and increased anthropogenic effects (pollution and coastal development), it is imperative that we investigate salt marsh community structure to provide insights for more informed conservation decisions. Understanding *Littoraria* diet in different populations across its home range, and within a population across an elevational gradient gives us a better understanding of *Littoraria*'s potential effects on *Spartina* and on salt marsh food-web dynamics.

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FIGURES

Figure 1.1:

Map of sites used in this study. St. Augustine, Florida (FL) at the Guana-Tolomato National Estuarine Research Reserve; Brunswick, Georgia (GA) near St. Simon's Island; Georgetown, South Carolina (SC) at the University of South Carolina Baruch Marine Laboratory; New Point Comfort, Virginia (VA) and Deal Island, Maryland (MD).

Table 1.1: The best performing models describing the relationship between *Littoraria* $\delta^{13}C$ and $\delta^{15}N$ and Resource $\delta^{13}C$ and $\delta^{15}N$. In each case, I show the best model and the comparable model with (or without) the interaction between the included factors. Given that the *Littoraria* model had so many potential variable combinations, the top four models were included for $\delta^{13}C$ and $\delta^{15}N$. The best models were determined by lowest AIC (italicized). These were mixed effects logistic regression models where Site was a random effect and included in every model.

Model	Dependent Variable	AIC	k
Resource Type + Relative Distance to Creek	*Resource $\delta^{13}C$*	*438*	*8*
Resource Type + Relative Distance to Creek + Resource Type * Relative Distance to Creek (Full Model)	Resource $\delta^{13}C$	443	12
*Resource Type + Relative Distance to Creek + Resource Type * Relative Distance to Creek* (Full Model)	*Resource $\delta^{15}N$*	*541*	*12*
Resource Type + Relative Distance to Creek	Resource $\delta^{15}N$	548	8

Model	Dependent Variable	AIC	k
*Size Class + Relative Distance to Creek + Size Class * Relative Distance to Creek*	*Littoraria $\delta^{13}C$*	*397*	*12*
Size Class + Relative Distance to Creek+ Microhabitat Use + Size Class * Relative Distance to Creek (Full Model)	*Littoraria* $\delta^{13}C$	400	13

Size Class + Microhabitat Use	*Littoraria* δ^{13}C	401	5
Size Class + Relative Distance to Creek	*Littoraria* δ^{13}C	403	8
*Size Class + Relative Distance to Creek + Size Class * Relative Distance to Creek*	*Littoraria* δ^{15}*N*	*304*	*12*
Size Class + Relative Distance to Creek +Microhabitat +Relative Distance to the Creek* Microhabitat Use (Full Model)	*Littoraria* δ^{15}N	306	13
Size Class + Relative Distance to Creek + Microhabitat Use	*Littoraria* δ^{15}N	307	9
Size Class + Relative Distance to Creek	*Littoraria* δ^{15}N	313	8

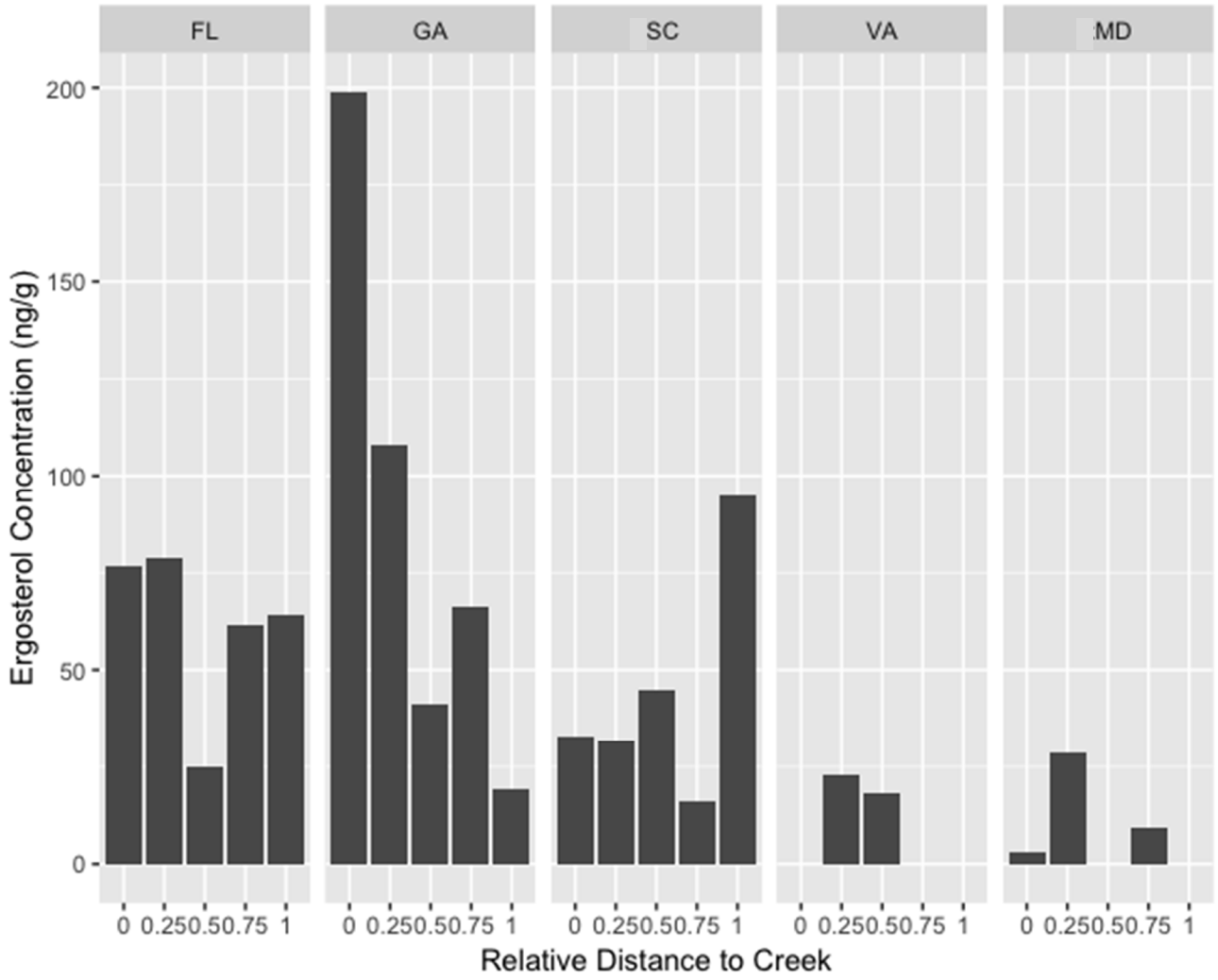


Figure 1.2: Total ergosterol concentration of fungal infected scar tissue of live *Spartina alterniflora* in each zone at each site. Zones with zero values were under the detection limit (1 ng/g).

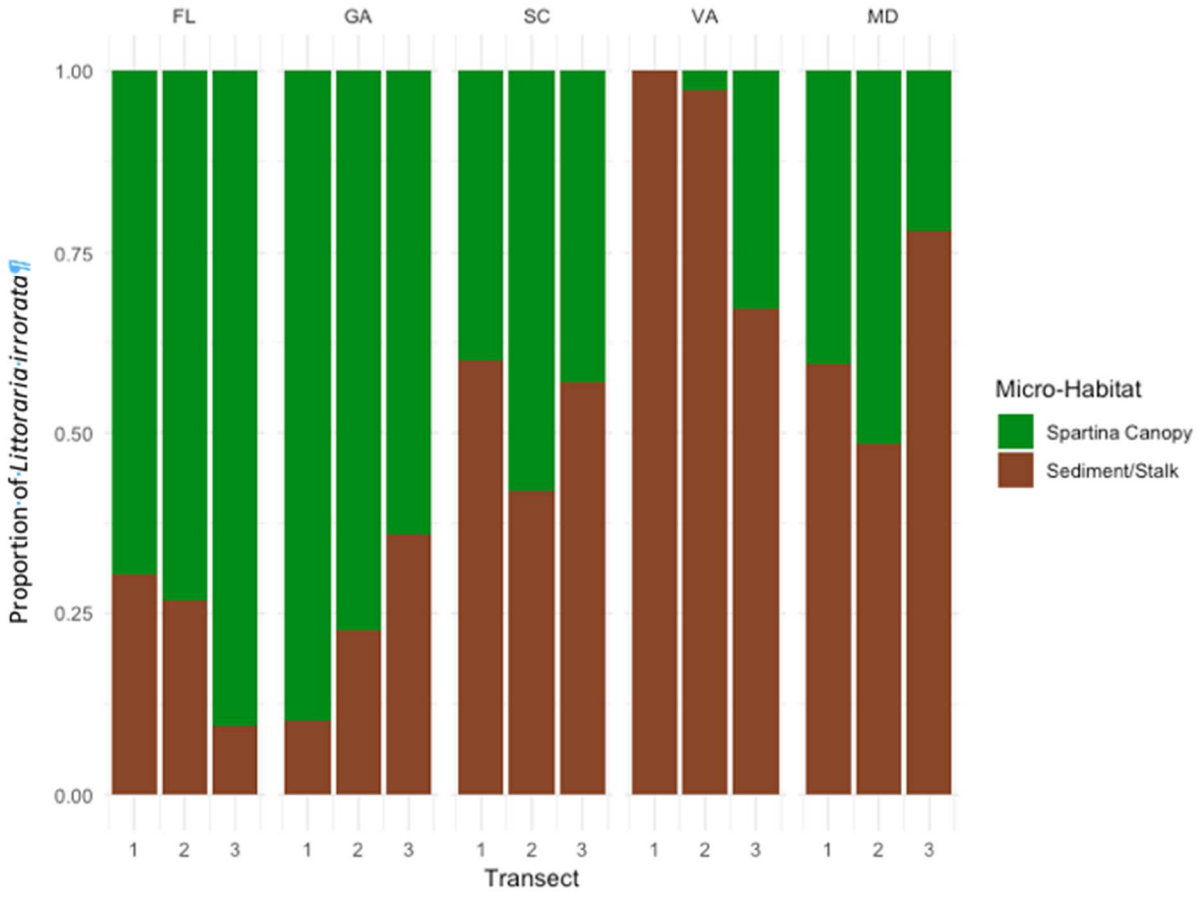


Figure 1.3: Proportion microhabitat use of *Littoraria irrorata* in each transect of each site. FL, GA, SC, VA and MD in each of the 3 transects at each site. *Spartina alterniflora* canopy (live and dead) is green, and marsh sediment/*Spartina alterniflora* basal stalk (below the 1st leaf) is brown.

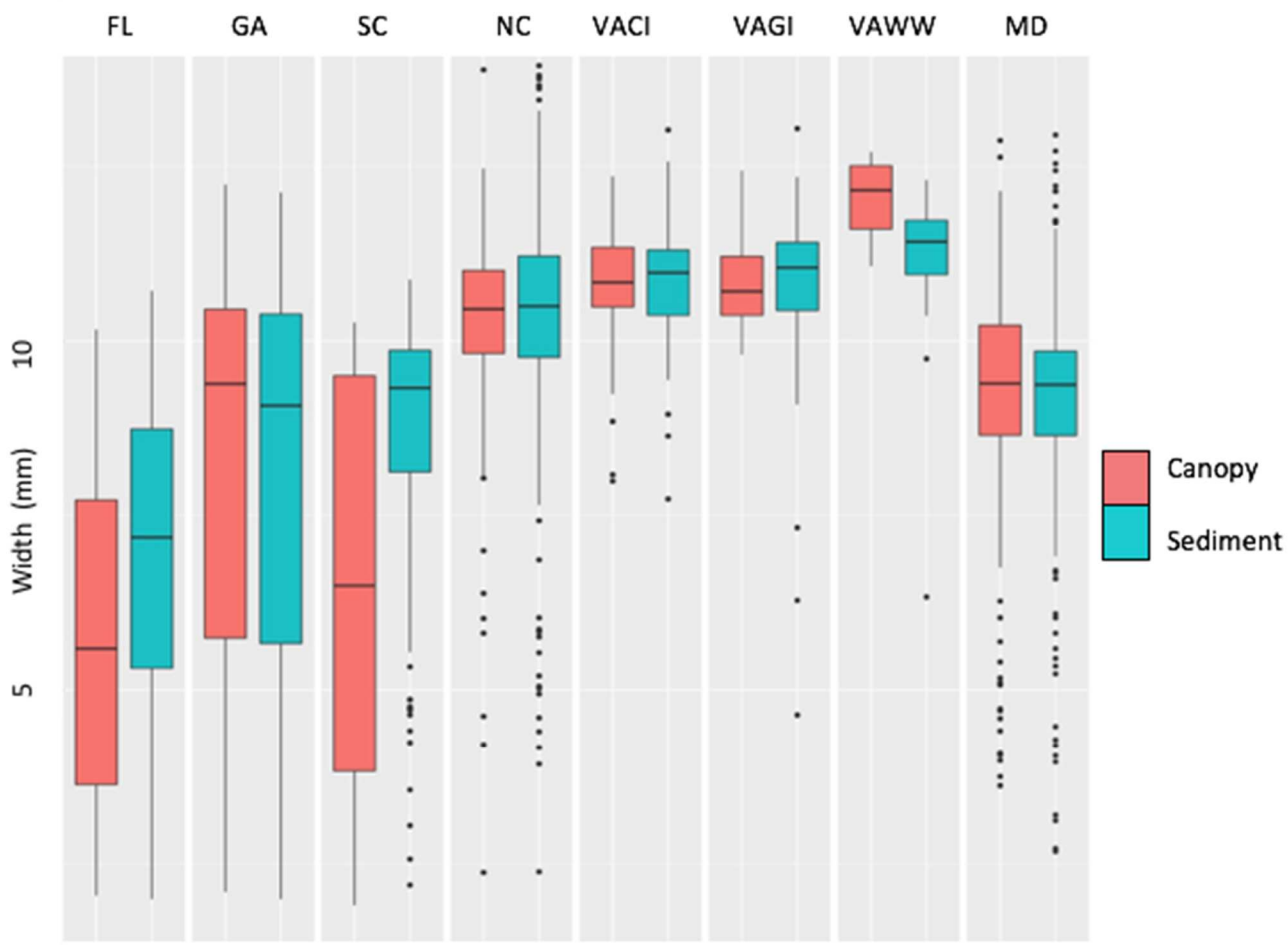


Figure 1.4: Mean body size of *Littoraria* in each microhabitat across all sites. Average body size of *Littoraria* occupying the canopy are the red boxes and average body size of *Littoraria* occupying the sediment are the blue boxes

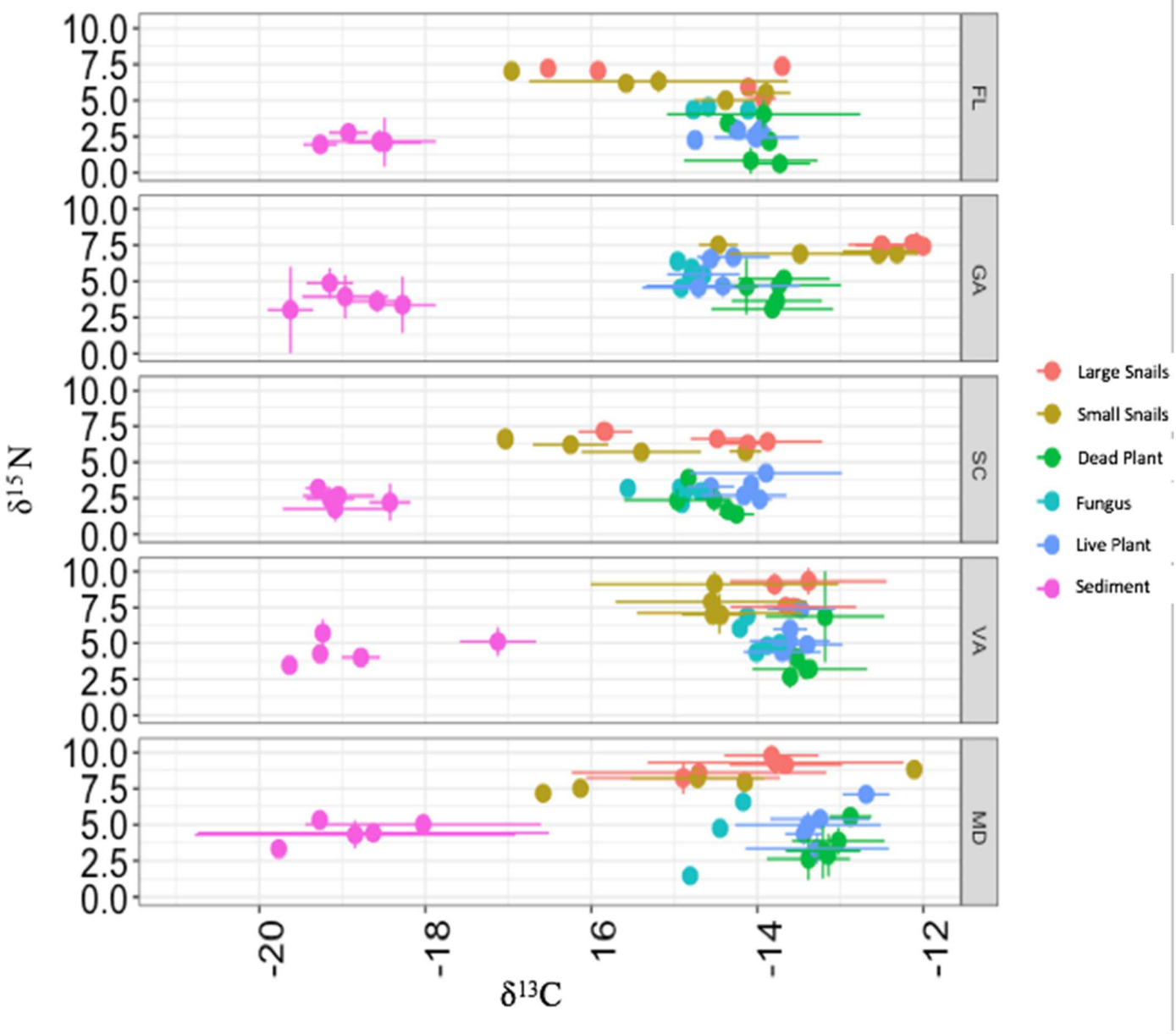


Figure 1.5: $\delta^{15}N$ and $\delta^{13}C$ isotopic values for two size classes of *Littoraria* (large *Littoraria* = red, small *Littoraria* = yellow) and four resource categories (standing dead *Spartina* = green, live *Spartina* = blue, fungus = turquoise, and sediment = pink). Averages (+/- SD) are based on n=2 samples from separate 1600 cm^2 quadrats at each relative distance from creek within each site (except for fungus which was sampled on only 1 transect). There were ten individual snails in each snail sample.

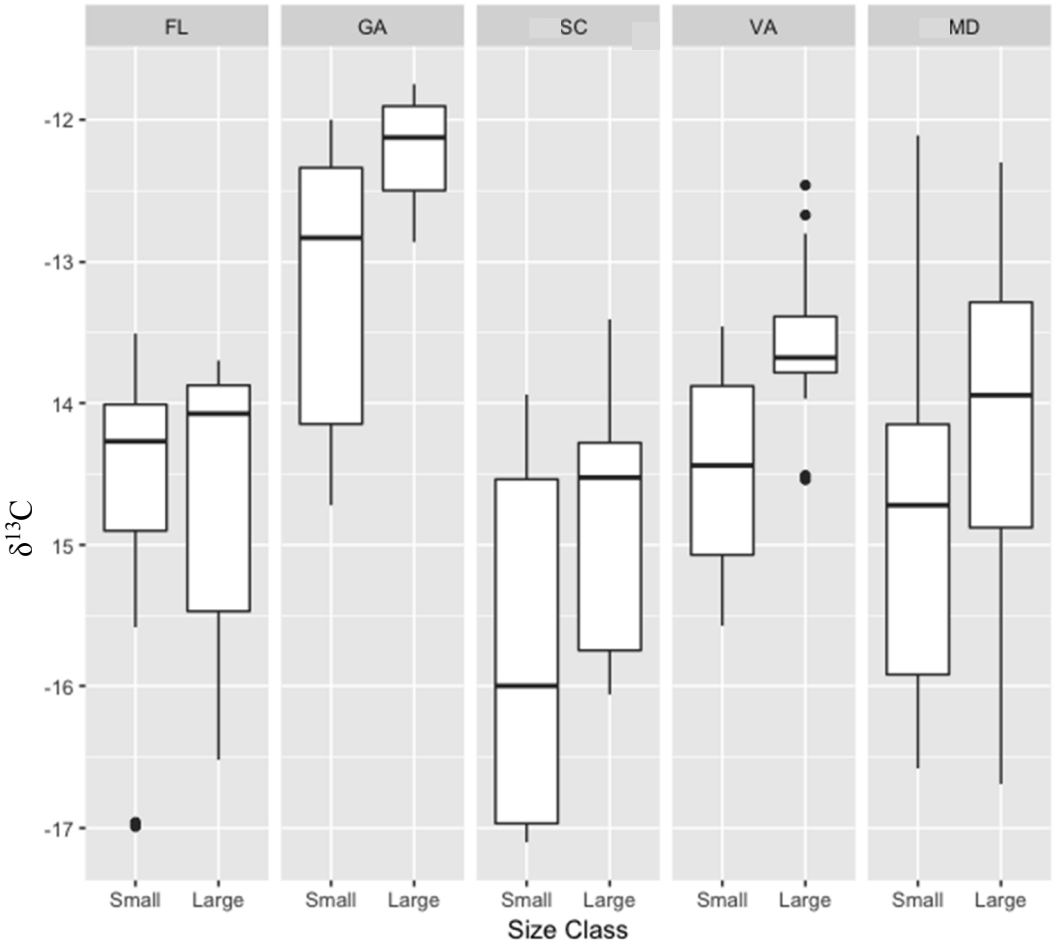


Figure 1.6: $\delta^{13}C$ values of small (left box plot) and large (right box plot) *Littoraria* at each site. Each box plot represents snails (large and small) pooled across all 1600cm^2 quadrats in a site for visual simplicity. N=15 samples per site per size class. The line represents the median, the lower and upper hinges correspond to the first and third quartiles. The whiskers extend from the hinge to the largest or smallest value no further than 1.5 times the interquartile of the hinge. Outlying points beyond the whiskers are plotted individually.

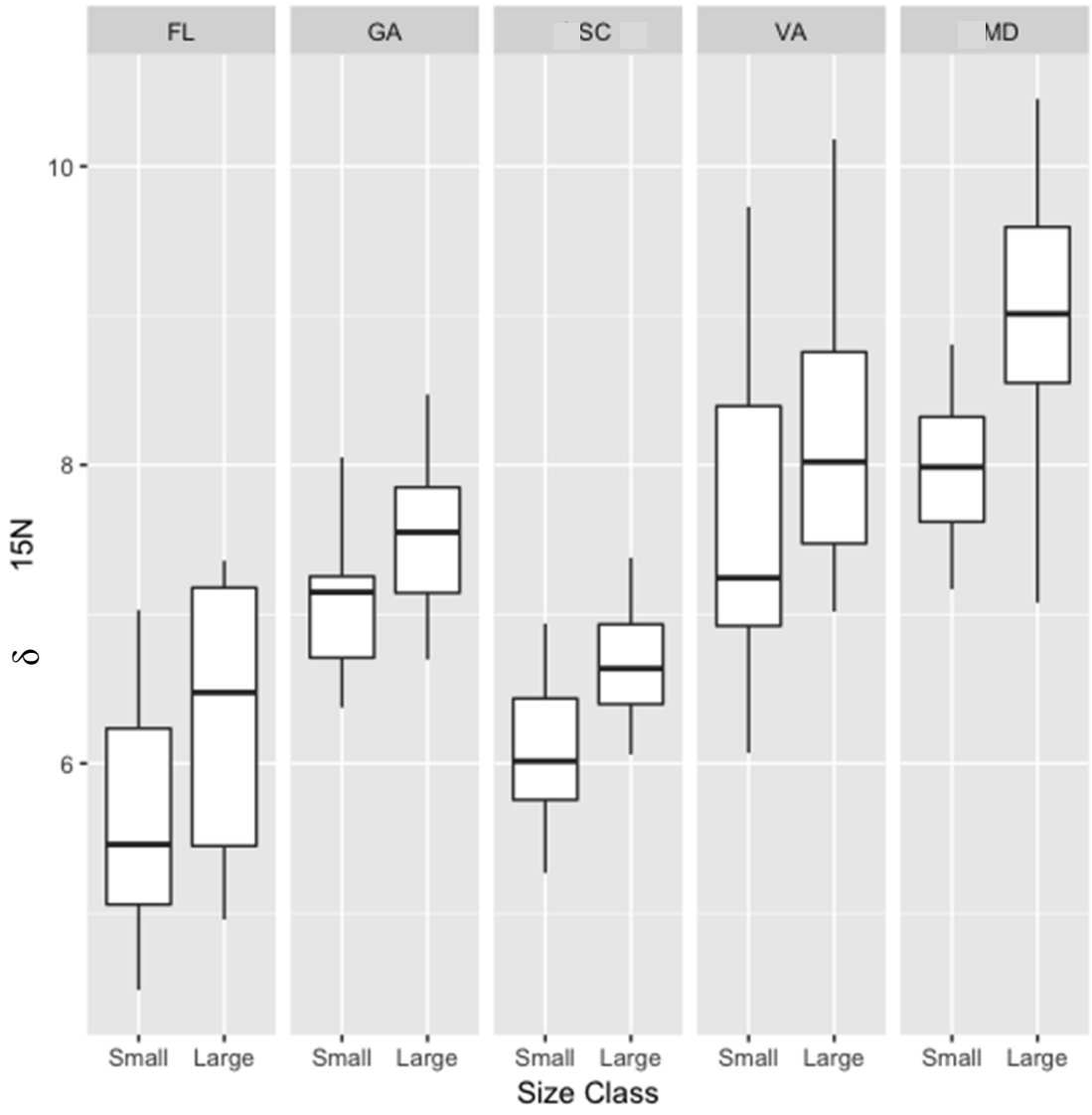


Figure 1.7: $\delta^{15}N$ values of small (left box plot) and large (right box plot) *Littoraria* at each site. Each box plot represents snails (large and small size class) pooled across all 1600cm^2 quadrats in a site for visual simplicity. The line represents the median, the lower and upper hinges correspond to the first and third quartiles. The whiskers extend from the hinge to the largest or smallest value no further than 1.5 times the interquartile of the hinge. Outlying points beyond the whiskers are plotted individually.

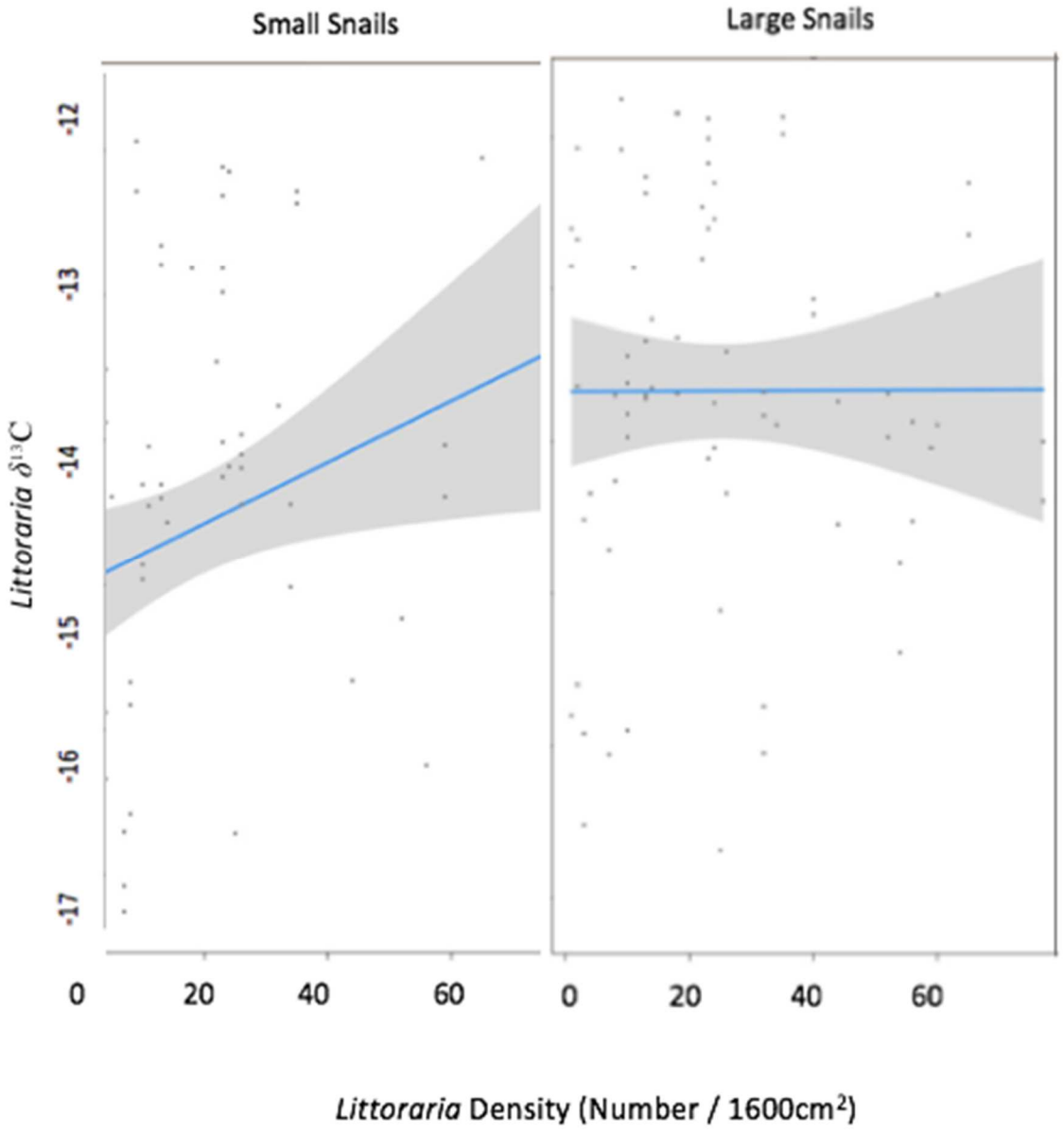


Figure 1.8: $\delta^{13}C$ of small and large *Littoraria* across a gradient of total snail densities demonstrating that the diet of small snails, but not large snails, changes with the overall density snails.

APPENDIX I

Appendix I Table 1: Stable isotope values for every individual sample of sediment, fungus, live *Spartina alterniflora*, standing dead *Spartina alterniflora*, and large and small *Littoraria irrorata* in each zone of each site. For each resource type except fungus, samples were taken from two transects. For snail samples, in which 10 snails were pooled per sample, there were often enough snails to create >1 sample from each distance from each transect.

Organism	Site	Transect	Relative Distance to Creek	δ13C	δ15N
Live *Spartina alterniflora*	FL	1	1	-14.13	2.2
Live *Spartina alterniflora*	FL	3	1	-13.93	2.85
Live *Spartina alterniflora*	FL	1	0.75	-13.65	2.2
Live *Spartina alterniflora*	FL	3	0.75	-14.37	2.66
Live *Spartina alterniflora*	FL	1	0.5	-14.06	2.41
Live *Spartina alterniflora*	FL	3	0.5	-13.87	3.6
Live *Spartina alterniflora*	FL	1	0.25	-13.91	2.24
Live *Spartina alterniflora*	FL	3	0.25	-14.8	3.27
Live *Spartina alterniflora*	FL	1	0	-14.25	2.81
Live *Spartina alterniflora*	FL	3	0	-14.65	4.43
Live *Spartina alterniflora*	GA	1	1	-14.15	6.56
Live *Spartina alterniflora*	GA	2	1	-14.48	6.54
Live *Spartina alterniflora*	GA	1	0.75	-14.96	5.81
Live *Spartina alterniflora*	GA	2	0.75	-14.34	5.18
Live *Spartina alterniflora*	GA	1	0.5	-15.19	4.05
Live *Spartina alterniflora*	GA	2	0.5	-14.23	5.05
Live *Spartina alterniflora*	GA	1	0.25	-15.07	5.21
Live *Spartina alterniflora*	GA	2	0.25	-13.76	4.13
Live *Spartina alterniflora*	GA	1	0	-14.6	6.44
Live *Spartina alterniflora*	GA	2	0	-13.98	6.92
Live *Spartina alterniflora*	SC	1	1	-14.14	3.81
Live *Spartina alterniflora*	SC	3	1	-14.01	3.09
Live *Spartina alterniflora*	SC	1	0.75	-14.07	1.97
Live *Spartina alterniflora*	SC	3	0.75	-13.87	2.89
Live *Spartina alterniflora*	SC	1	0.5	-14.36	2.88
Live *Spartina alterniflora*	SC	3	0.5	-14.76	3.69
Live *Spartina alterniflora*	SC	1	0.25	-14.52	2.69
Live *Spartina alterniflora*	SC	3	0.25	-13.8	2.76
Live *Spartina alterniflora*	SC	1	0	-14.54	3.91
Live *Spartina alterniflora*	SC	3	0	-13.25	4.58
Live *Spartina alterniflora*	VA	1	1	-13.95	6.14
Live *Spartina alterniflora*	VA	3	1	-13.27	4.14
Live *Spartina alterniflora*	VA	1	0.75	-14.03	4.37
Live *Spartina alterniflora*	VA	3	0.75	-13.37	4.35

Live *Spartina alterniflora*	VA	1	0.5	-13.7	4.93
		3			4.85
Live *Spartina alterniflora*	VA		0.5	-13.1	
Live *Spartina alterniflora*	VA	1	0.25	-13.75	6.16
Live *Spartina alterniflora*	VA	3	0.25	-13.46	5.82
Live *Spartina alterniflora*	VA	1	0	-13.18	7.57
Live *Spartina alterniflora*	VA	3	0	-13.76	7.16
Live *Spartina alterniflora*	MD	2	1	-13.89	4.04
Live *Spartina alterniflora*	MD	3	1	-12.67	2.6
Live *Spartina alterniflora*	MD	2	0.75	-13.6	4.92
Live *Spartina alterniflora*	MD	3	0.75	-13.28	3.8
Live *Spartina alterniflora*	MD	2	0.5	-14.01	5.6
Live *Spartina alterniflora*	MD	3	0.5	-12.77	4.37
Live *Spartina alterniflora*	MD	2	0.25	-13.67	5.52
Live *Spartina alterniflora*	MD	3	0.25	-12.82	5.24
Live *Spartina alterniflora*	MD	2	0	-12.89	7.21
Live *Spartina alterniflora*	MD	3	0	-12.49	6.98
Standing Dead *Spartina alterniflora*	FL	1	1	-13.47	1.12
Standing Dead *Spartina alterniflora*	FL	3	1	-13.99	0.15
Standing Dead *Spartina alterniflora*	FL	1	0.75	-13.51	1.51
Standing Dead *Spartina alterniflora*	FL	3	0.75	-14.65	0.19
Standing Dead *Spartina alterniflora*	FL	1	0.5	-13.91	2.18
Standing Dead *Spartina alterniflora*	FL	3	0.5	-14.2	1.96
Standing Dead *Spartina alterniflora*	FL	1	0.25	-14.41	3.45
Standing Dead *Spartina alterniflora*	FL	3	0.25	-13.98	0.68
Standing Dead *Spartina alterniflora*	FL	1	0	-14.75	3.2
Standing Dead *Spartina alterniflora*	FL	3	0	-13.1	4.92
Standing Dead *Spartina alterniflora*	GA	1	1	-14.07	5.45
Standing Dead *Spartina alterniflora*	GA	2	1	-13.29	4.91
Standing Dead *Spartina alterniflora*	GA	1	0.75	-14.27	5.1
Standing Dead *Spartina alterniflora*	GA	2	0.75	-13.21	4.33
Standing Dead *Spartina alterniflora*	GA	1	0.5	-14.15	3.89
Standing Dead *Spartina alterniflora*	GA	2	0.5	-13.38	3.4
Standing Dead *Spartina alterniflora*	GA	1	0.25	-14.34	3.07
Standing Dead *Spartina alterniflora*	GA	2	0.25	-13.3	3.11
Standing Dead *Spartina alterniflora*	GA	1	0	-14.03	5.99
Standing Dead *Spartina alterniflora*	GA	2	0	-14.23	3.27
Standing Dead *Spartina alterniflora*	SC	1	1	-14.4	2.2
Standing Dead *Spartina alterniflora*	SC	3	1	-14.29	1.79
Standing Dead *Spartina alterniflora*	SC	1	0.75	-14.42	1.54
Standing Dead *Spartina alterniflora*	SC	3	0.75	-14.76	2.23
Standing Dead *Spartina alterniflora*	SC	1	0.5	-14.66	2.95
Standing Dead *Spartina alterniflora*	SC	3	0.5	-14.38	1.84
Standing Dead *Spartina alterniflora*	SC	1	0.25	-14.51	2.87
Standing Dead *Spartina alterniflora*	SC	3	0.25	-15.42	1.86
Standing Dead *Spartina alterniflora*	SC	1	0	-14.77	4.15
Standing Dead *Spartina alterniflora*	SC	3	0	-14.89	3.64
Standing Dead *Spartina alterniflora*	VA	1	1	-13.38	3.2
Standing Dead *Spartina alterniflora*	VA	3	1	-13.95	3.14
Standing Dead *Spartina alterniflora*	VA	1	0.75	-13.54	3.21
Standing Dead *Spartina alterniflora*	VA	3	0.75	-14.03	2.11
Standing Dead *Spartina alterniflora*	VA	1	0.5	-13.86	3.53
Standing Dead *Spartina alterniflora*	VA	3	0.5	-13.7	2.92
Standing Dead *Spartina alterniflora*	VA	1	0.25	-13.53	4.33

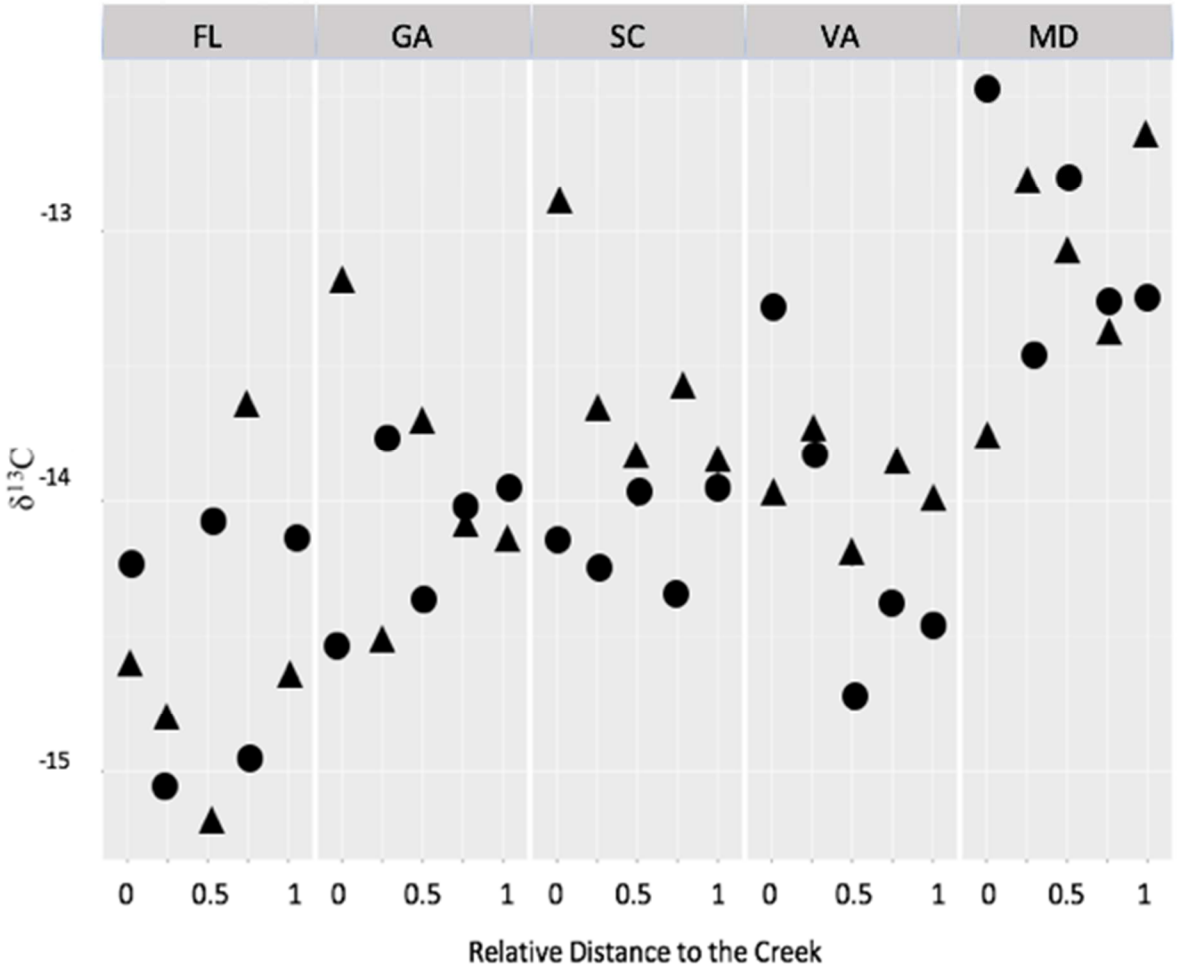
Standing Dead *Spartina alterniflora*	VA	3	0.25	-13.75	3.6
Standing Dead *Spartina alterniflora*	VA	1	0	-12.68	9.09
Standing Dead *Spartina alterniflora*	VA	3	0	-13.18	4.61
Standing Dead *Spartina alterniflora*	MD	2	1	-13.89	4.04
Standing Dead *Spartina alterniflora*	MD	3	1	-13.75	2.6
Standing Dead *Spartina alterniflora*	MD	2	0.75	-13.6	4.92
Standing Dead *Spartina alterniflora*	MD	3	0.75	-13.54	3.8
Standing Dead *Spartina alterniflora*	MD	2	0.5	-14.01	5.6
Standing Dead *Spartina alterniflora*	MD	3	0.5	-13.93	4.37
Standing Dead *Spartina alterniflora*	MD	2	0.25	-13.67	5.52
Standing Dead *Spartina alterniflora*	MD	3	0.25	-13.79	5.24
Standing Dead *Spartina alterniflora*	MD	2	0	-12.89	7.21
Standing Dead *Spartina alterniflora*	MD	3	0	-13.77	6.98
Fungus	FL	1	1	-14.26	3.02
Fungus	FL	1	0.75	-14.11	4.34
Fungus	FL	1	0.5	-14.31	4.36
Fungus	FL	1	0.25	-14.77	4.56
Fungus	FL	1	0	-14.59	4.81
Fungus	GA	1	1	-14.81	5.22
Fungus	GA	1	0.75	-14.79	5.93
Fungus	GA	1	0.5	-14.66	5.36
Fungus	GA	1	0.25	-14.92	4.53
Fungus	GA	1	0	-14.96	6.39
Fungus	SC	1	1	-15.56	3.18
Fungus	SC	1	0.75	-14.91	2.15
Fungus	SC	1	0.5	-14.86	3.05
Fungus	SC	1	0.25	-14.93	3.22
Fungus	SC	1	0	-14.68	2.95
Fungus	VA	1	1	-14.01	4.35
Fungus	VA	1	0.75	-13.88	4.82
Fungus	VA	1	0.5	-13.73	4.98
Fungus	VA	1	0.25	-14.12	6.9
Fungus	VA	1	0	-14.21	6.03
Fungus	MD	3	1	-14.81	1.47
Fungus	MD	3	0.75	-14.66	3.84
Fungus	MD	3	0.5	-14.5	4.46
Fungus	MD	3	0.25	-14.45	4.74
Fungus	MD	3	0	-14.17	6.56
Sediment	FL	1	1	-21.87	0.69
Sediment	FL	3	1	-23.14	2.36
Sediment	FL	1	0.75	-19.41	1.62
Sediment	FL	3	0.75	-19.12	2.31
Sediment	FL	1	0.5	-19.03	1.63
Sediment	FL	3	0.5	-18.07	2.73
Sediment	FL	1	0.25	-18.8	0.91
Sediment	FL	3	0.25	-18.18	3.32
Sediment	FL	1	0	-19.09	3.17
Sediment	FL	3	0	-18.76	2.36
Sediment	GA	1	1	-18.95	4.11
Sediment	GA	2	1	-19.35	5.63
Sediment	GA	1	0.75	-18.6	2.87
Sediment	GA	2	0.75	-19.33	3.89

Sediment	GA	1	0.5	-17.99	1.99
Sediment	GA	2	0.5	-18.56	4.76
Sediment	GA	1	0.25	-18.87	3.09
Sediment	GA	2	0.25	-18.29	4.15
Sediment	GA	1	0	-19.82	4.91
Sediment	GA	2	0	-19.43	5.15
Sediment	SC	1	1	-18.64	1.15
Sediment	SC	3	1	-19.53	2.41
Sediment	SC	1	0.75	-19.35	2.22
Sediment	SC	3	0.75	-18.94	2.68
Sediment	SC	1	0.5	-19.35	2.31
Sediment	SC	3	0.5	-18.74	3.02
Sediment	SC	1	0.25	-18.25	1.31
Sediment	SC	3	0.25	-18.6	3.13
Sediment	SC	1	0	-19.4	3.13
Sediment	SC	3	0	-19.18	3.24
Sediment	VA	1	1	-18.61	3.77
Sediment	VA	3	1	-18.94	4.24
Sediment	VA	1	0.75	-21.6	3.34
Sediment	VA	3	0.75	-17.67	3.6
Sediment	VA	1	0.5	-21.27	4.09
Sediment	VA	3	0.5	-17.26	4.41
Sediment	VA	1	0.25	-19.3	6.38
Sediment	VA	3	0.25	-19.17	5.03
Sediment	VA	1	0	-16.8	5.83
Sediment	VA	3	0	-17.45	4.38
Sediment	MD	2	1	-21.93	3.51
Sediment	MD	3	1	-17.6	3.12
Sediment	MD	2	0.75	-20.21	4.99
Sediment	MD	3	0.75	-17.48	3.62
Sediment	MD	2	0.5	-20.12	4.67
Sediment	MD	3	0.5	-17.13	4.19
Sediment	MD	2	0.25	-19.03	5.52
Sediment	MD	3	0.25	-17.02	4.53
Sediment	MD	2	0	-20.84	4.91
Sediment	MD	3	0	-17.7	5.72
***Littoraria irrorata* (small)**	FL	1	1	-14.01	4.96
***Littoraria irrorata* (small)**	FL	1	1	-14.9	5.06
***Littoraria irrorata* (small)**	FL	2	1	-14.27	4.69
***Littoraria irrorata* (small)**	FL	3	1	-14.35	5.34
***Littoraria irrorata* (large)**	FL	1	1	-14.04	5.3
***Littoraria irrorata* (large)**	FL	3	1	-13.82	4.96
***Littoraria irrorata* (small)**	FL	1	0.75	-13.99	5.96
***Littoraria irrorata* (small)**	FL	1	0.75	-13.86	5.31
***Littoraria irrorata* (small)**	FL	2	0.75	-13.51	4.49
***Littoraria irrorata* (small)**	FL	3	0.75	-14.22	6.24
***Littoraria irrorata* (large)**	FL	1	0.75	-14.11	5.9
***Littoraria irrorata* (small)**	FL	1	0.5	-15.58	6.18
***Littoraria irrorata* (large)**	FL	2	0.5	-13.7	7.36
***Littoraria irrorata* (small)**	FL	2	0.25	-16.99	6.82
***Littoraria irrorata* (small)**	FL	3	0.25	-14.35	5.46
***Littoraria irrorata* (small)**	FL	3	0.25	-14.23	6.69

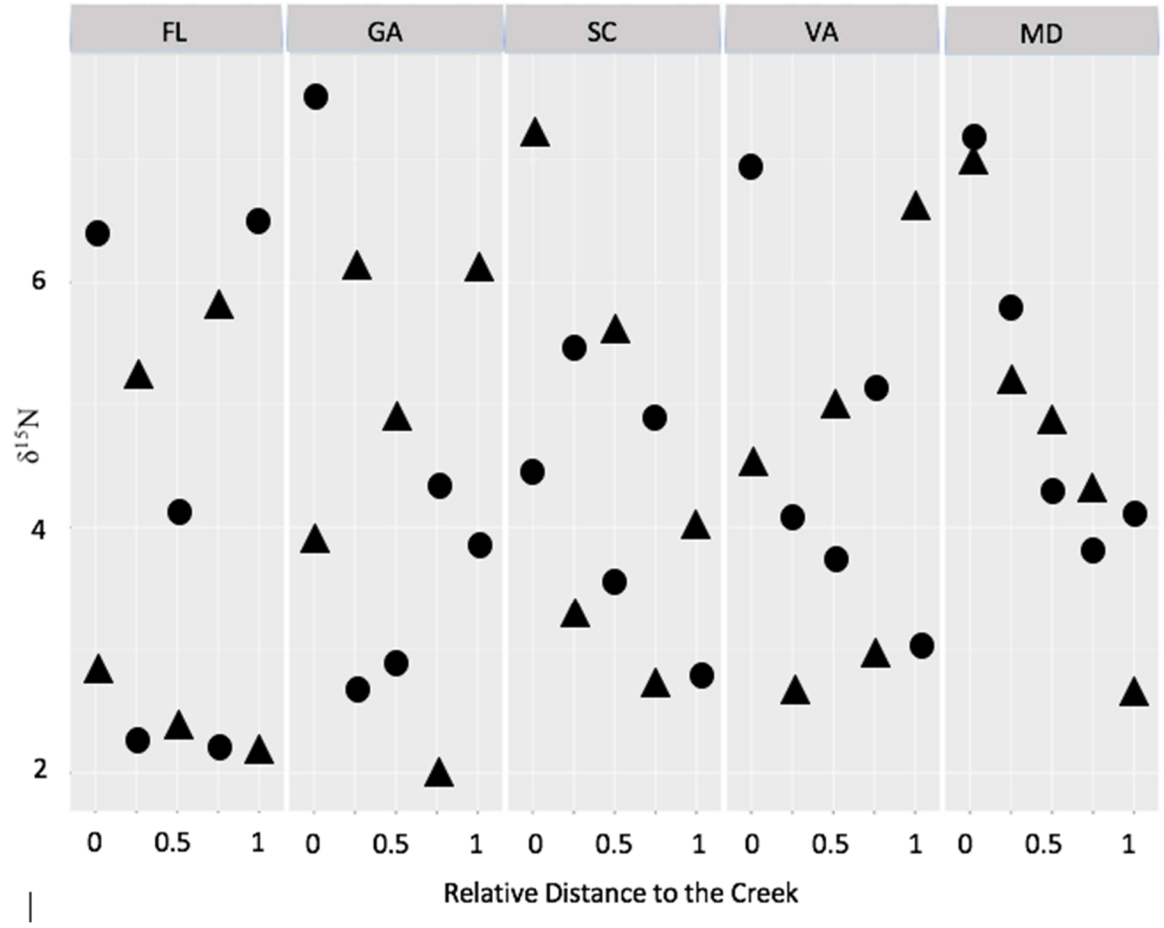
***Littoraria irrorata* (large)**	FL	3	0.25	-15.92	7.06
***Littoraria irrorata* (small)**	FL	1	0	-16.96	7.03
***Littoraria irrorata* (large)**	FL	1	0	-16.52	7.22
***Littoraria irrorata* (small)**	FL	2	0	-13.97	6.75
***Littoraria irrorata* (small)**	GA	1	1	-12.2	6.81
***Littoraria irrorata* (small)**	GA	2	1	-12.84	7.3
***Littoraria irrorata* (large)**	GA	1	1	-12.3	7.63
***Littoraria irrorata* (large)**	GA	2	1	-11.84	7.69
***Littoraria irrorata* (large)**	GA	3	1	-12.54	7.24
***Littoraria irrorata* (large)**	GA	3	1	-11.85	7.88
***Littoraria irrorata* (small)**	GA	1	0.75	-12.33	7.26
***Littoraria irrorata* (small)**	GA	2	0.75	-12.17	6.69
***Littoraria irrorata* (small)**	GA	2	0.75	-12.36	6.49
***Littoraria irrorata* (small)**	GA	3	0.75	-12.41	7.23
***Littoraria irrorata* (large)**	GA	1	0.75	-11.88	7.13
***Littoraria irrorata* (large)**	GA	2	0.75	-11.87	8.12
***Littoraria irrorata* (large)**	GA	3	0.75	-12.6	6.7
***Littoraria irrorata* (large)**	GA	3	0.75	-11.98	8.47
***Littoraria irrorata* (small)**	GA	1	0.5	-13	7.12
***Littoraria irrorata* (small)**	GA	1	0.5	-12	6.62
***Littoraria irrorata* (small)**	GA	2	0.5	-12.33	6.63
***Littoraria irrorata* (small)**	GA	3	0.5	-12.84	7.18
***Littoraria irrorata* (large)**	GA	1	0.5	-11.75	7.45
***Littoraria irrorata* (large)**	GA	2	0.5	-12.08	6.92
***Littoraria irrorata* (large)**	GA	2	0.5	-12.17	7.56
***Littoraria irrorata* (large)**	GA	3	0.5	-12.01	7.76
***Littoraria irrorata* (small)**	GA	1	0.25	-12.69	6.78
***Littoraria irrorata* (small)**	GA	2	0.25	-14.02	7.24
***Littoraria irrorata* (small)**	GA	3	0.25	-14.41	7.22
***Littoraria irrorata* (small)**	GA	3	0.25	-12.82	6.38
***Littoraria irrorata* (large)**	GA	1	0.25	-12.26	7
***Littoraria irrorata* (large)**	GA	1	0.25	-12.37	7.19
***Littoraria irrorata* (large)**	GA	2	0.25	-12.86	8.21
***Littoraria irrorata* (small)**	GA	1	0	-14.72	7.51
***Littoraria irrorata* (small)**	GA	1	0	-14.19	8.05
***Littoraria irrorata* (small)**	GA	2	0	-14.37	7.07
***Littoraria irrorata* (small)**	GA	3	0	-14.6	7.44
***Littoraria irrorata* (large)**	GA	1	0	-12.07	7.99
***Littoraria irrorata* (large)**	GA	2	0	-12.6	7.06
***Littoraria irrorata* (large)**	GA	3	0	-12.85	7.54
***Littoraria irrorata* (small)**	SC	1	1	-13.94	5.27
***Littoraria irrorata* (small)**	SC	2	1	-14.07	5.55
***Littoraria irrorata* (small)**	SC	3	1	-14.4	6.24
***Littoraria irrorata* (small)**	SC	3	1	-14.16	5.84
***Littoraria irrorata* (large)**	SC	1	1	-14.34	6.41
***Littoraria irrorata* (large)**	SC	2	1	-13.41	6.4
***Littoraria irrorata* (small)**	SC	1	0.75	-14.95	5.44
***Littoraria irrorata* (small)**	SC	2	0.75	-15.78	5.74
***Littoraria irrorata* (small)**	SC	2	0.75	-16.22	5.47
***Littoraria irrorata* (small)**	SC	3	0.75	-15.64	5.94
***Littoraria irrorata* (small)**	SC	3	0.75	-14.4	5.81
***Littoraria irrorata* (large)**	SC	1	0.75	-14.34	6.45

***Littoraria irrorata* (large)**	SC	2	0.75	-13.89	6.06
***Littoraria irrorata* (small)**	SC	1	0.5	-16.45	5.89
***Littoraria irrorata* (small)**	SC	2	0.5	-15.73	6.24
***Littoraria irrorata* (small)**	SC	3	0.5	-16.57	6.45
***Littoraria irrorata* (large)**	SC	1	0.5	-14.71	6.38
***Littoraria irrorata* (large)**	SC	3	0.5	-14.26	6.83
***Littoraria irrorata* (small)**	SC	1	0.25	-17.07	6.76
***Littoraria irrorata* (small)**	SC	2	0.25	-17.05	6.09
***Littoraria irrorata* (small)**	SC	3	0.25	-16.98	6.72
***Littoraria irrorata* (large)**	SC	1	0.25	-15.9	6.97
***Littoraria irrorata* (large)**	SC	2	0.25	-15.8	7.32
***Littoraria irrorata* (small)**	SC	1	0	-17.1	6.65
***Littoraria irrorata* (small)**	SC	2	0	-17.08	6.41
***Littoraria irrorata* (small)**	SC	3	0	-16.93	6.94
***Littoraria irrorata* (large)**	SC	1	0	-15.6	6.83
***Littoraria irrorata* (large)**	SC	2	0	-16.06	7.38
***Littoraria irrorata* (small)**	VA	1	1	-15.37	7.34
***Littoraria irrorata* (small)**	VA	2	1	-13.74	8.5
***Littoraria irrorata* (large)**	VA	1	1	-13.64	7.02
***Littoraria irrorata* (large)**	VA	2	1	-14.51	7.18
***Littoraria irrorata* (large)**	VA	3	1	-13.44	8.02
***Littoraria irrorata* (large)**	VA	3	1	-12.67	7.98
***Littoraria irrorata* (small)**	VA	1	0.75	-14.8	7.03
***Littoraria irrorata* (small)**	VA	3	0.75	-14.27	6.89
***Littoraria irrorata* (large)**	VA	1	0.75	-13.7	7.18
***Littoraria irrorata* (large)**	VA	1	0.75	-13.62	8.16
***Littoraria irrorata* (large)**	VA	2	0.75	-13.97	7.41
***Littoraria irrorata* (large)**	VA	3	0.75	-13.34	7.65
***Littoraria irrorata* (small)**	VA	1	0.5	-14.52	7.15
***Littoraria irrorata* (small)**	VA	2	0.5	-14.36	6.76
***Littoraria irrorata* (large)**	VA	1	0.5	-13.2	7.61
***Littoraria irrorata* (large)**	VA	2	0.5	-13.65	7.33
***Littoraria irrorata* (large)**	VA	3	0.5	-13.72	7.54
***Littoraria irrorata* (small)**	VA	1	0.25	-15.16	8.07
***Littoraria irrorata* (small)**	VA	3	0.25	-13.75	6.07
***Littoraria irrorata* (large)**	VA	1	0.25	-13.68	10.01
***Littoraria irrorata* (large)**	VA	2	0.25	-13.97	8.59
***Littoraria irrorata* (large)**	VA	2	0.25	-13.69	8.47
***Littoraria irrorata* (large)**	VA	3	0.25	-13.83	9.4
***Littoraria irrorata* (small)**	VA	1	0	-15.57	8.58
***Littoraria irrorata* (small)**	VA	2	0	-13.46	9.73
***Littoraria irrorata* (large)**	VA	1	0	-13.74	8.26
***Littoraria irrorata* (large)**	VA	1	0	-12.8	10
***Littoraria irrorata* (large)**	VA	2	0	-12.46	10.18
***Littoraria irrorata* (large)**	VA	3	0	-14.54	8.93
***Littoraria irrorata* (small)**	MD	3	1	-14.15	7.95
***Littoraria irrorata* (large)**	MD	1	1	-13.75	8.61
***Littoraria irrorata* (large)**	MD	1	1	-15.74	7.64
***Littoraria irrorata* (large)**	MD	2	1	-16.05	7.08
***Littoraria irrorata* (large)**	MD	3	1	-14.04	9.59
***Littoraria irrorata* (small)**	MD	3	0.75	-16.58	7.17
***Littoraria irrorata* (large)**	MD	1	0.75	-13.32	8.85

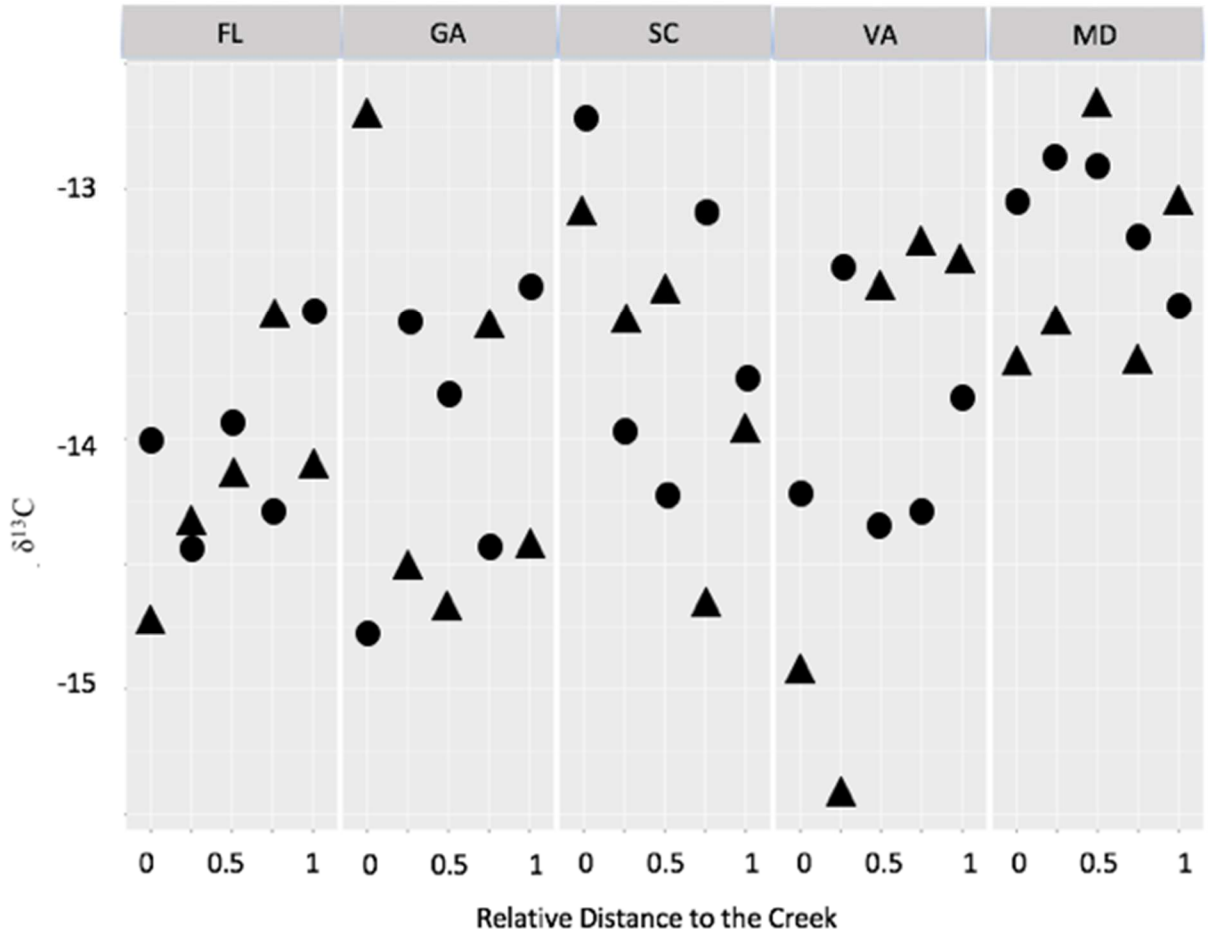
***Littoraria irrorata* (large)**	MD	2	0.75	-16.69	7.72
***Littoraria irrorata* (large)**	MD	2	0.75	-15.11	8.27
***Littoraria irrorata* (large)**	MD	3	0.75	-13.69	9.43
***Littoraria irrorata* (small)**	MD	2	0.5	-16.13	7.51
***Littoraria irrorata* (large)**	MD	1	0.5	-14.52	8.65
***Littoraria irrorata* (large)**	MD	1	0.5	-13.87	8.74
***Littoraria irrorata* (large)**	MD	2	0.5	-13.07	9.18
***Littoraria irrorata* (large)**	MD	3	0.5	-13.17	9.95
***Littoraria irrorata* (small)**	MD	2	0.25	-12.11	8.81
***Littoraria irrorata* (large)**	MD	1	0.25	-12.64	9.61
***Littoraria irrorata* (large)**	MD	2	0.25	-15.39	8.36
***Littoraria irrorata* (large)**	MD	3	0.25	-14.8	8.92
***Littoraria irrorata* (large)**	MD	3	0.25	-12.3	10.27
***Littoraria irrorata* (large)**	MD	1	0	-13.04	10.31
***Littoraria irrorata* (large)**	MD	3	0	-13.89	10.45
***Littoraria irrorata* (small)**	MD	2	0	-14.15	8.42
***Littoraria irrorata* (small)**	MD	3	0	-15.29	8.02
***Littoraria irrorata* (large)**	MD	2	0	-14	9.3
***Littoraria irrorata* (large)**	MD	2	0	-14.39	9.11



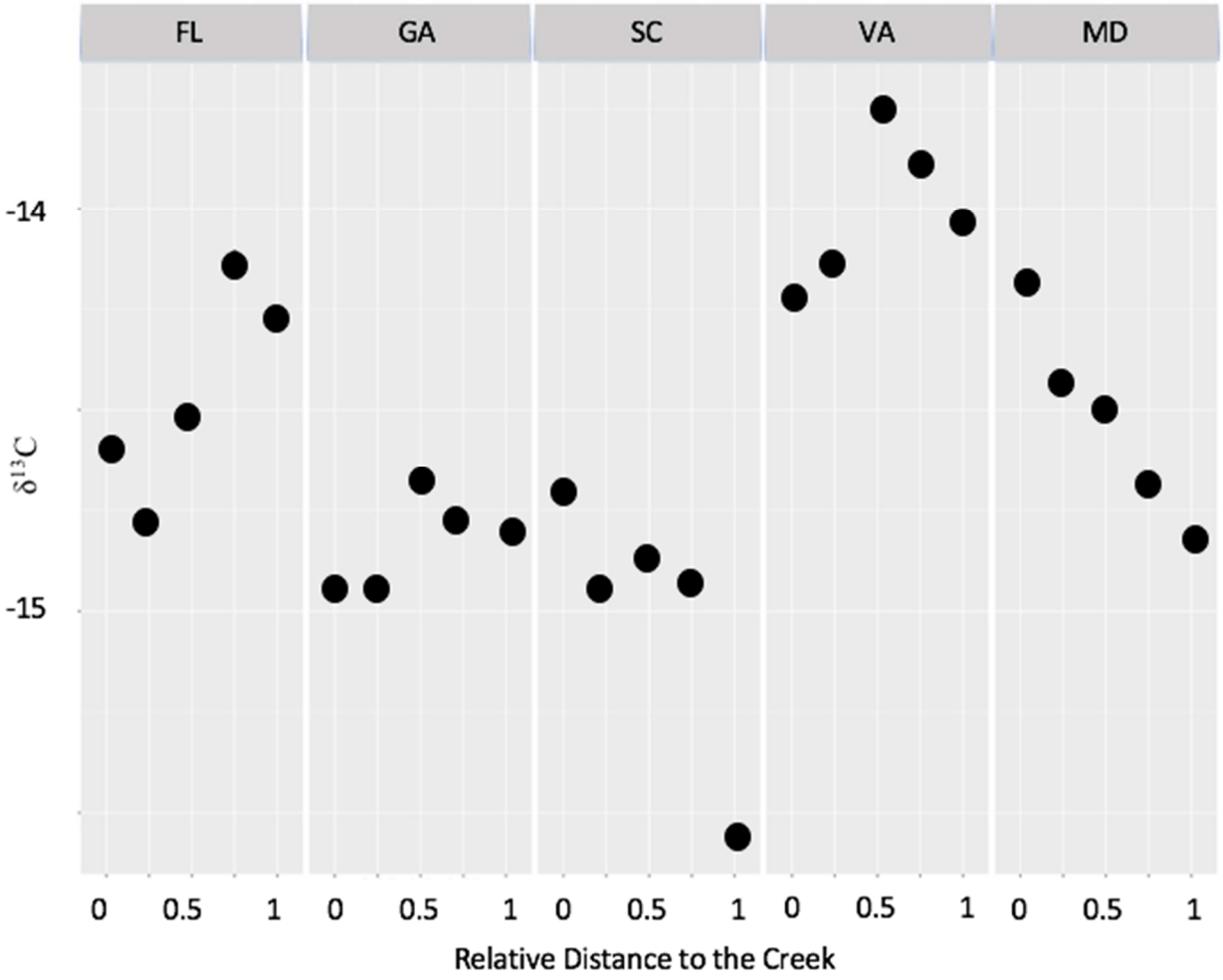
Appendix I Figure 1: $\delta^{13}C$ of live *Spartina alterniflora* in relationship to relative distance from the creek (0 to 1) in 2 transects at each site. Transect 1 is represented by circles and transect 2 is represented by triangles



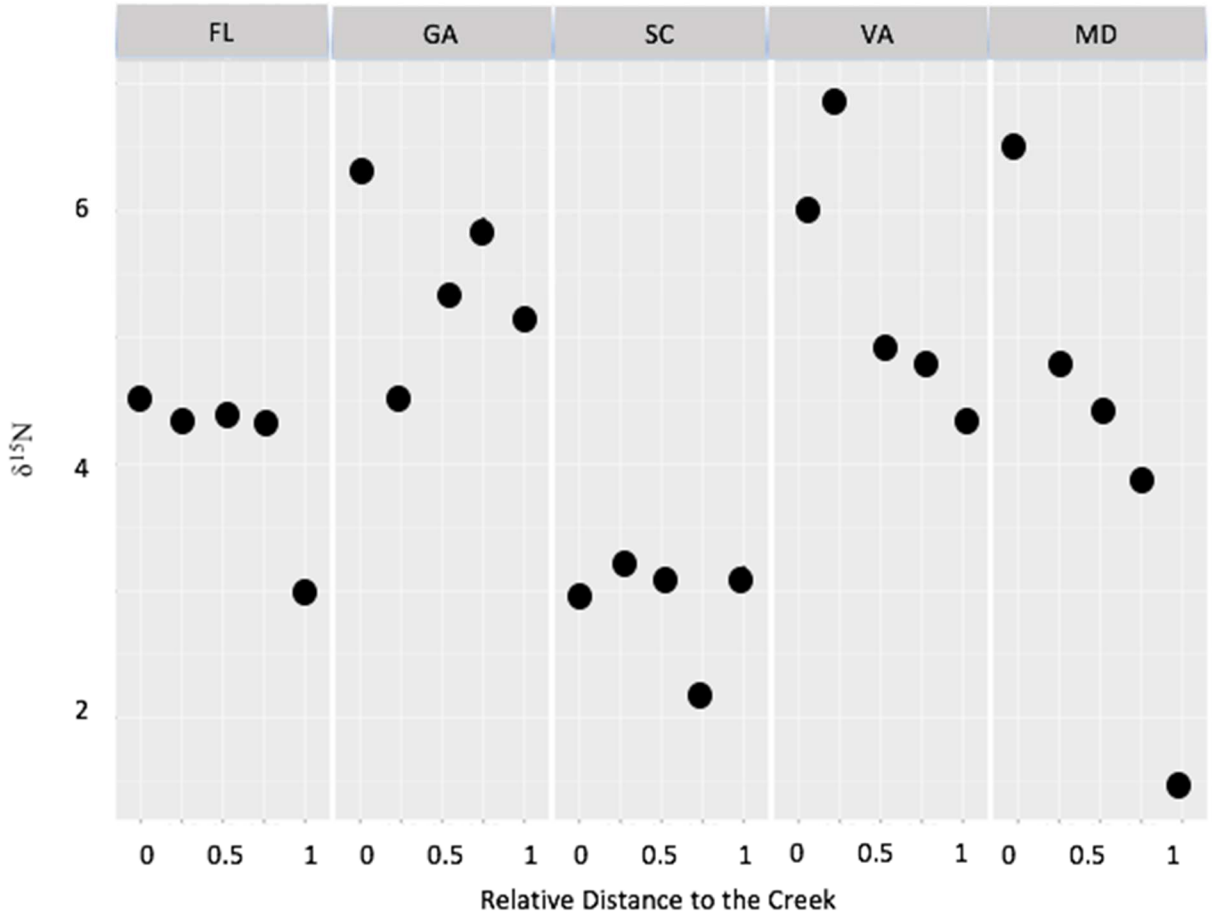
Appendix I Figure 2: $\delta^{15}N$ of live *Spartina alterniflora* in relationship to relative distance from the creek (0 to 1) at each site (n=2). Transect 1 is represented by circles and transect 2 is represented by triangles.



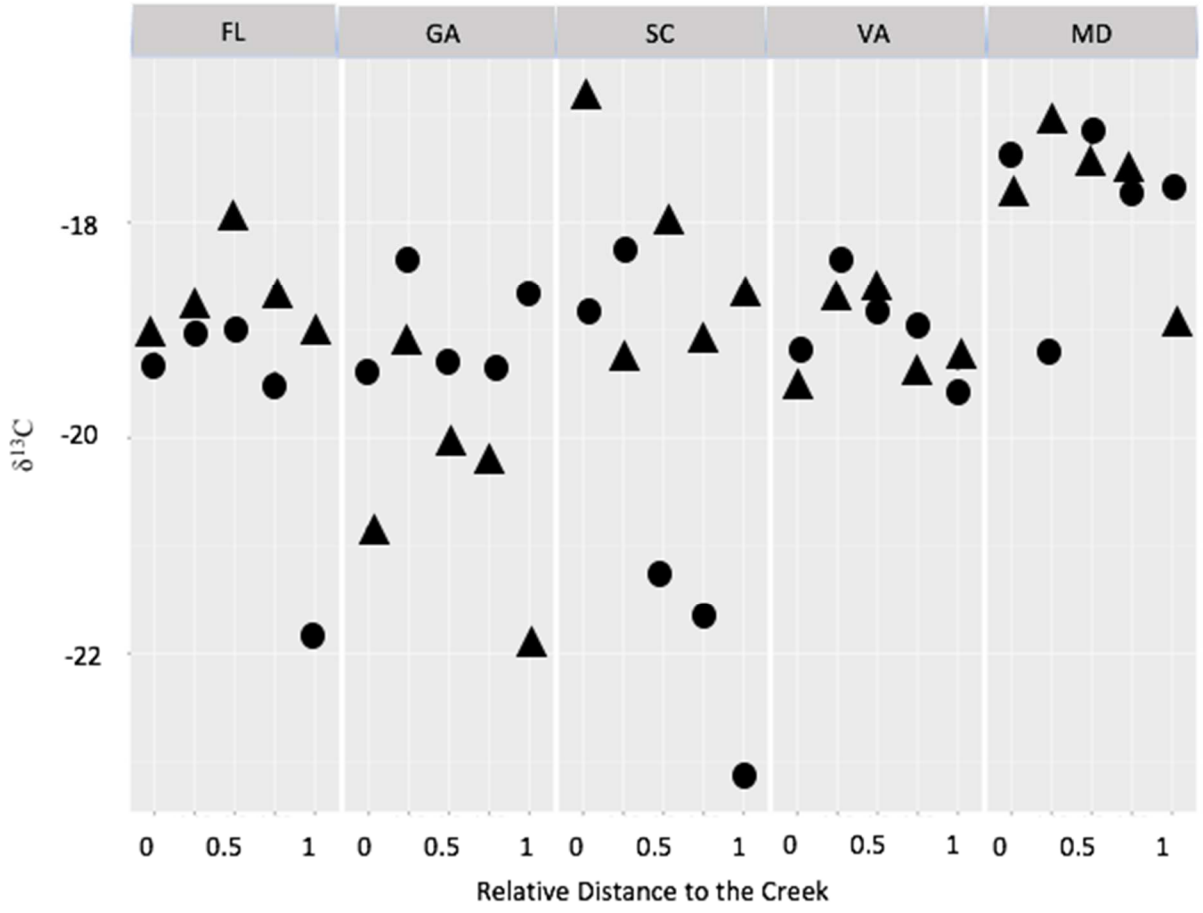
Appendix I Figure 4: $\delta^{15}N$ of standing dead *Spartina alterniflora* in relationship to relative distance from the creek (0 to 1) at each site (n=2). Transect 1 is represented by circles and transect 2 is represented by triangles.



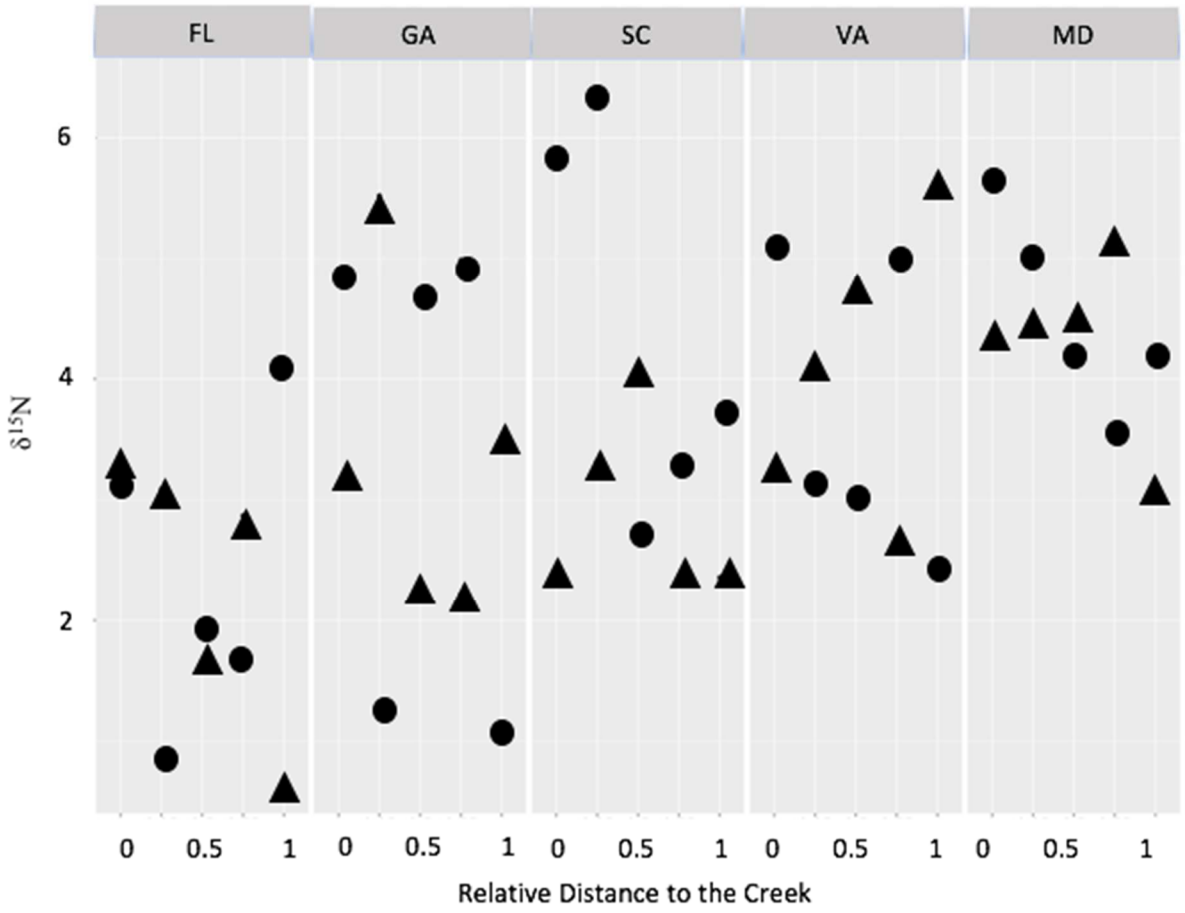
Appendix I Figure 5: $\delta^{13}C$ of fungus in relationship to relative distance from the creek (0 to 1) at each site (n=1 transect per site).



Appendix I Figure 6: $\delta^{15}N$ of fungus in relationship to relative distance from the creek (0 to 1) at each site (n=1 transect per site).



Appendix I Figure 7: $\delta^{13}C$ of sediment in relationship to relative distance from the creek (0 to 1) at each site (n=2 transects per site). Transect 1 is represented by circles and transect 2 is represented by triangles.



Appendix I Figure 8: $\delta^{15}N$ of sediment in relationship to relative distance from the creek (0 to 1) at each site (n=2 transects per site). Transect 1 is represented by circles and transect 2 is represented by triangles.

APPENDIX II

SPATIAL PATTERNS IN BODY SIZE AND DENSITY OF THE SALT MARSH PERIWINKLE, *LITTORARIA IRRORATA,* IN U.S ATLANTIC SALT MARSHES[2]

[2] Hawkins, D. G., Osenberg, C.W., and Atkins, R.L. To be submitted to *Estuaries and Coasts.*

Abstract

The Saltmarsh Periwinkle *(Littoraria irrorata*) is a common grazer in southeastern marshes dominated by the smooth cordgrass, *Spartina alterniflora*. I quantified *Littoraria* density, size structure, microhabitat use, and diet along elevational gradients within eight marshes ranging from Florida to Maryland. *Littoraria* density and size increased with increasing elevation within southern marshes, but size did not vary and density decreased with elevation in northern marshes.

Introduction

The goal of this ancillary field study was to quantify spatial patterns of body size and density at three spatial scales: among marshes along a latitudinal gradient, within marshes along a gradient of relative distance from a creek (a surrogate for elevation), and among microhabitats at a particular relative distance. Quantification of patterns at various spatial scales will establish the foundation from which more specific and mechanistic hypotheses can be addressed about the processes driving these patterns and the implications of these patterns for species interactions in the marsh ecosystem. Although this study is descriptive, I hypothesized that (1) *Littoraria* density (and biomass) will not change across sites with increasing latitude, but that periwinkle density (and biomass) will increase with increasing distance (relative elevation) from marsh creek edge due to a reduction in predation pressure by blue crab predators; (2) mean *Littoraria* size will increase with latitude (based upon field observations and previous work on the confamilial gastropod, *Littorina keenae*: Lee and Goulding, 2010); (3) mean *Littoraria* size will increase with distance from the marsh edge due to the associated reduction in size-specific predation; (4) periwinkle microhabitat-use will be similar across the latitudinal gradient (with most snails occupying the *Spartina* canopy rather

than the sediments) due to a reduction in thermal stress (lower temperatures) and predation pressures (predator refuge) in the *Spartina* canopy.

Methods

Study Sites and Design

Littoraria irrorata commonly occurs in saltmarshes from Texas to Maryland and has been found at extremely low densities as far north as New York. In the summer of 2017, I sampled eight sites from Florida to Maryland (Fig. 1.1): St. Augustine, Florida (FL) at the Guana-Tolomato National Estuarine Research Reserve; Brunswick, Georgia (GA) near St. Simon's Island; Georgetown, South Carolina (SC) at the University of South Carolina Baruch Marine Laboratory; Fort Fischer, North Carolina (NC); Chincoteague Island, Virginia (VACI); Goodwin Island, Virginia (VAGI); Willis Wharf, Virginia (VAWW); and Deal Island, Maryland (MD). These sites span approximately eight degrees of latitude and include a major portion of *Littoraria's* geographic range. Sites were chosen based on their accessibility and the presence of both *Littoraria* and *Spartina.* Within each site, I selected an area dominated by *Spartina alterniflora* (which extended from the creek edge, where it comprised a tall-form *Spartina* marsh, to upland into a short-form *Spartina* marsh). Each transect specifically began and ended where both *Littoraria* and *Spartina* occurred. At five sites (FL, GA, SC, VAWW, and MD), I sampled three transects and at three sites (NC, VACI, VAGI), due to logistical constraints, I sampled one transect. Transects were run perpendicular to the creek and varied in length from 43m to 108m depending on site-specific marsh morphology. Replicate transects were spaced at least 20m apart from one another. In Maryland where marshes were more fragmented, three separate marshes were surveyed (one transect/marsh) all located within six kilometers of each other on

Deal Island, MD. I placed a 40x40 cm^2 quadrat at five regularly spaced locations along each transect (i.e., at relative distances of 0, 0.25, 0.50, 0.75, and 1.0 of the total transect length), where relative distance of 0 refers to the quadrat at the creek's edge.

All data were collected at low tide. In each quadrat, I counted every *Littoraria*, measured their shell width (Atkins et al, 2015) to the nearest 0.01 mm with digital calipers, and recorded their microhabitat use: plant canopy (>10 cm above the sediment on living or dead *Spartina*), plant stalk (on living or dead *Spartina* but < 10 cm above the sediment) and marsh sediment surface. In general, the first blade on *Spartina* plant was located at ~10cm. For analysis, I pooled the snails on stalks with those on the mud since they are in close proximity of one another. Thus, the response was binary (in the canopy vs. on (or near) the sediments). I also counted the number of live and standing dead stems of *Spartina* in each quadrat and measured the height of 5 haphazardly chosen live and 5 haphazardly chosen dead stalks in each quadrat. I collected pore water from each quadrat in one transect per site by inserting 10cm rhizon soil moisture samplers (Rhizosphere Research Products, The Netherlands) into the top 10cm of sediment and allowing water to collect into an attached 60mL syringe. I then measured conductivity (R) and temperature (T) of the pore water using a handheld conductivity meter. I converted conductivity to salinity (S) using the Practical Salinity conversion:

$$S= a_0 + a_1T_t^{1/2} + a_2R_t + a_3R_t^{3/2} + a_4R_t^2 + a_5R_t^{5/2} \quad [1]$$

where a_0= 0.008, a_1=-0.1692, a_2=25.3851, a_3=14.0941, a_4=-7.0261, and a_5=2.7081 (Bennett 1976).

Statistical Analysis

All data analysis was performed in R version 3.3.1. Shell widths were converted to dry tissue mass (i.e. exclusive of shells, but not the operculum), using allometric equations from snails collected in the summer of 2015 at 9 locations spanning a similar latitudinal gradient. There was no significant variation in the allometric relationship among sites; therefore, I used a single, pooled allometric equation:

$$M = 6E\text{-}05W^{3.1882} \quad [2]$$

where M is dry mass (grams) and W is shell width (mm) (R. Atkins , *unpublished*).

To test for spatial differences in environmental variables, and an interaction between effects of relative distance from creek (a surrogate for elevation) and site, I fit the general linear model:

$$Y = \beta_0 + \beta_1(\text{Site}) + \beta_2(\text{Relative Distance to Creek}) + \beta_3(\text{Site} * \text{Relative Distance to Creek}) + \varepsilon \quad [3]$$

using the function lm in R, assuming error, ε, was normally distributed.

To test for spatial differences in snail population characteristics (i.e. density, biomass, and body size), I used a similar model but included the environmental variables as an additional predictor and a random effect of transect (because data were obtained from three transects at

five of the sites). Due to the hierarchical structure of the random effects, the data were fit with the function lmer in R's lme4 package:

$$Y = \beta_1\,(\text{Site}) + \beta_2(\text{Relative Distance to Creek}) + \beta_3\,(\text{Site} * \text{Relative Distance to Creek}) + \beta_4\,(\text{Environmental variable}) + (\beta_0 + b_{Transect}) + \varepsilon \quad [4]$$

When appropriate, all data were checked for normality using Shapiro-Wilks tests on the residuals, and visual examination of residual vs prediction plots.

I also ran the above models on snail density, biomass, and average shell width, but replacing the effect of site with a fixed (and linear) effect of latitude to see if variation among sites could be discerned as a latitudinal pattern. I also evaluated if absolute distance or relative distance from the creek were better predictors of snail responses. Thus, for each response variable, I ran four separate full models (including site or latitude and crossed with the inclusion of relative or absolute distance) along with the three environmental variables (stem height, stem density, salinity). For each full model, I then examined the AIC of all reduced models and selected the model with lowest AIC score. I then compared, using AIC, the four final models to assess the role of site vs. latitude and relative vs. absolute distance from the creek. Overall, the model using relative distance (vs. absolute distance) and site (vs. latitude) was best supported. I therefore emphasize the results using relative distance and site in the remainder of this appendix.

Results

Environmental Variables

Spartina stem density averaged 62.74/m^2 with a range of 10-165/m^2. Stem density varied among marshes (Appendix I Figure 9; $F_{7,78}$= 6.26, P<0.0001) and increased with increasing distance from the creek ($F_{4,78}$ = 8.40, P<0.001) (Appendix I Figure 9). GA had the lowest total stem density.

Spartina stem height averaged 42.85cm with a range of 16-93cm. Stem height varied among marshes (Appendix I Figure 10**,** $F_{7,78}$=9.29, P<0.0001) and decreased significantly with increasing distance from the creek (Appendix I Figure 10; $F_{4,78}$ = 22.61, P<0.0001). In general, stem height and stem density were negatively correlated (R^2=0.16, n=68, P=0.0007)

Pore water salinity was significantly different across marshes ($F_{7,78}$= 17.49, P<0.001), with VAGI having the lowest salinities (Appendix I Figure 11). Porewater salinity increased with increasing distance from the creek ($F_{4,78}$ > 72.84, P= <0.001) (Appendix I Figure 11).

Periwinkle Density and Biomass

I collected and measured 1768 snails from the eight sites spanning from Florida to Maryland. *Littoraria* density across all quadrats averaged 121.1 individuals/m^2 with a maximum density of 762 individuals/m^2 at NC at a relative distance of 0.5, and a minimum density of 0 individuals/m^2 at VAWW at several distances. Density varied significantly across sites (ANOVA, $F_{7,82}$ = 11.46, P<0.001), with NC having much higher densities than all other sites (Figure 1.2).

Periwinkle density varied with distance from the creek, although the pattern of change depended upon geographic location (Table 1.2). Southern sites (FL, GA, SC) had their highest snail densities in the quadrats farthest from the creek (Figure 1.3), whereas northern sites (VAGI, VACI, VAWW, MD) had their highest densities in the quadrats closest to the creek (Figure

1.3). NC showed an intermediate pattern. Snail density increased slightly with *Spartina* stem density (Figure 1.4), although this effect is largely confounded with distance from the creek.

Patterns in biomass mirrored those observed in density (Figure 1.5). Mean *Littoraria* biomass across all quadrats at all sites was 10.02 g/m^2, with a maximum biomass of 47.26 g/m^2 at NC in the quadrats that were halfway from the creek (Figure 1.6). The minimum biomass of 0 g/m^2 was observed in the zones farthest from the creek (Figure 7) in two transects in VAWW. NC had higher biomass than all other sites (Figure 1.6). As observed for density, southern sites (FL, GA, SC) all had higher snail biomass in the zones farthest from the creek (Figure 9.1) and northern sites (VAGI, VACI, VAWW, MD) had higher snail biomass in the zones closest to the creek (Figure 1.4) (Figures 1.3 and 1.4). NC had an intermediate pattern (Figure 9.1). Snail biomass increased slightly with *Spartina* stem density (Figure 1.7).

Periwinkle Size

Snail width averaged 9.65 mm, with a range of 1.91 – 13.94 mm (Appendix I Figure 7). There was a significant interaction between marsh and relative distance from the creek ($F_{39,1725}$=43.44, P= <0.001, R^2=0.48), which arose because of a divergent pattern in snail width across zones in southern vs. northern marshes. In the southern sites (FL, GA, SC), the average widths of snails increased with increasing distance from the creek, while in northern sites (NC, VACI, VAGI, VAWW, MD), snail size either decreased or showed no clear pattern (Appendix I Figure 7). Overall, snails from northern sites were larger (in terms of minimum, maximum, and mean) then were snails from southern sites (Appendix I Figure 7). Environmental variables were not included in the top models.

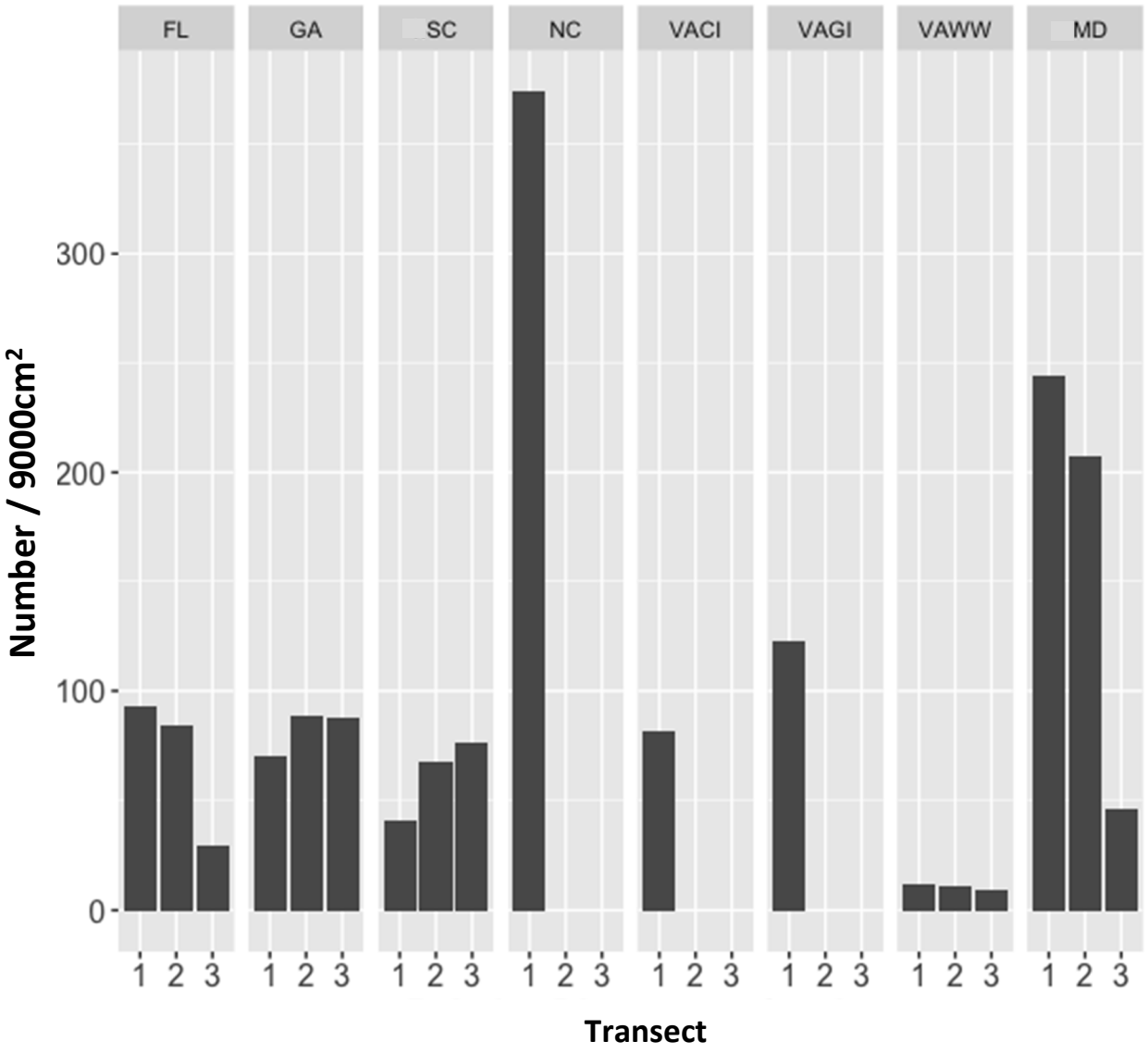
Habitat Use

Within a quadrat, snails occurred in different microhabitats and this use of microhabitats varied among sites, but not among relative distances from the creek. Snails in FL and GA sites were more likely to be found in the *Spartina* canopy (>75% of snails collected), while snails in SC, NC, VACI, VAGI, VAWW, and MD sites were more likely to occur on the sediment surface and *Spartina* stalk (>50% of snails collected), suggesting that the effect of site was largely driven by latitude.

Appendix II Figure 1: Map of sites used in this study. St. Augustine, Florida (FL) at the Guana-Tolomato National Estuarine Research Reserve; Brunswick, Georgia (GA) near St. Simon's Island; Georgetown, South Carolina (SC) at the University of South Carolina Baruch Marine Laboratory; Fort Fischer, North Carolina (NC); Chincoteague Island, Virginia (VACI); Goodwin Island, Virginia (VAGI); Willis Wharf, Virginia (VAWW); and Deal Island, Maryland (MD).

Appendix II Table 1: Latitude, longitude, stem density, mean porewater salinity, stem height, and transect length were recorded in the field. Mean high water level, and average sea surface temperature were taken from NOAA National Centers for Environmental Information.

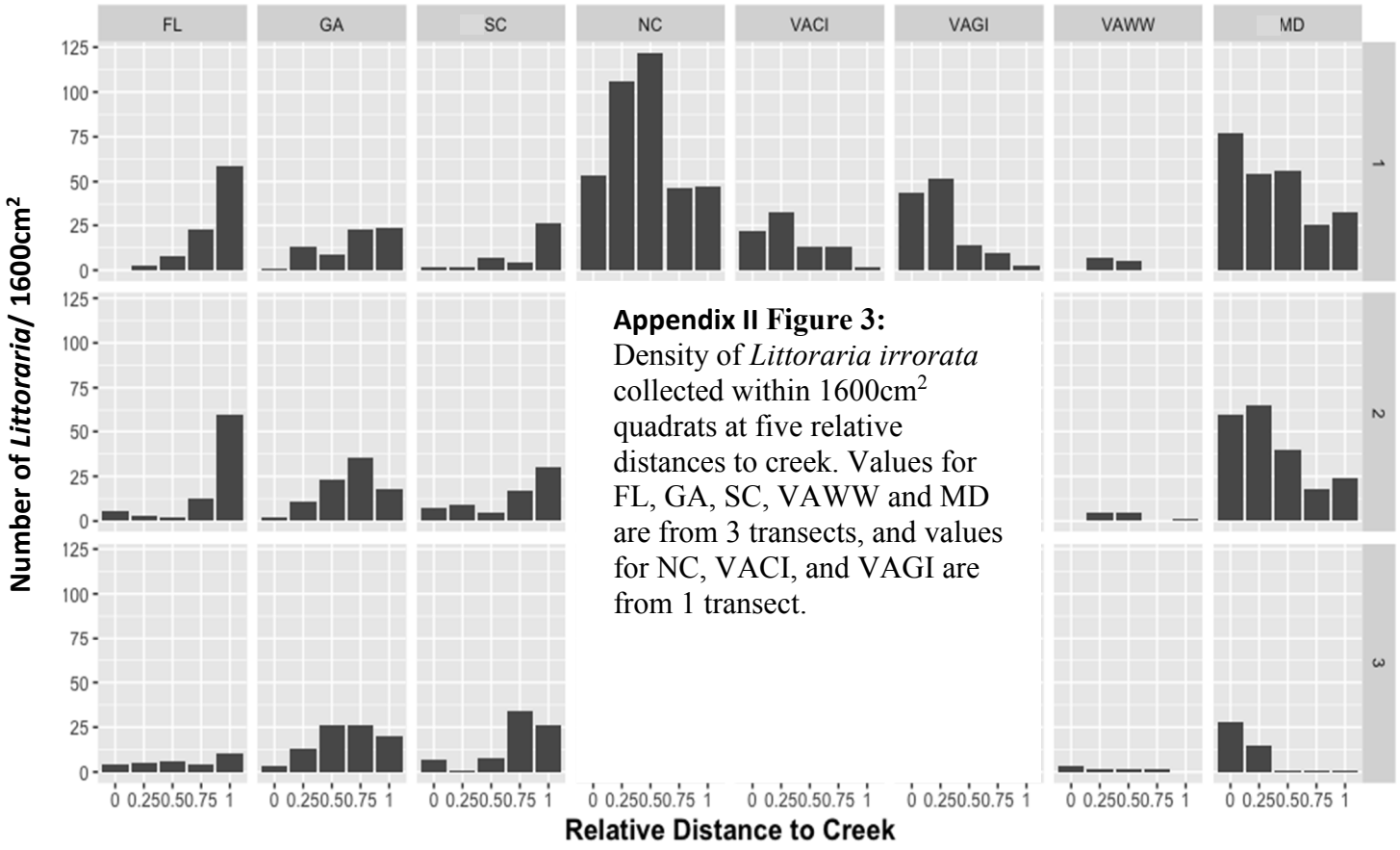
Site	Transect Number	Latitude (N)	Longitude (W)	Stem Density/ m^2	Average Pore Water Salinity (ppt)	Mean High Water Level (m)	Transect Length (m)	Average Stem Height (cm)	Average Sea Surface Temp 2017 (℃)
FL	1	30.016705	-81.344104	368 ± 124.8	41.7 ± 5.5	1.6	40	26.6	28.8
	2	30.016728	-81.344104	177.6 ± 79.5	41.7 ± 3.9	1.6	41	29.1	28.8
	3	30.016733	-81.344104	262.4 ± 31	38.48 ± 2.8	1.6	44	40.1	28.8
GA	1	31.161352	-81.468068	124.8 ± 35.2	38.7 ± 2.9	2.1	92	54.9	26.6
	2	31.161372	-81.468068	121.6 ± 41.9	43.4 ± 4.6	2.1	82	53.6	26.6
	3	31.161399	-81.468068	76.8 ± 12.8	38.75 ± 3.5	2.1	84	49.4	26.6
SC	1	33.332946	-79.198577	230.4 ± 76.2	37.1 ± 1.7	1.5	117	39.4	28.3
	2	33.332959	-79.198577	304 ± 51.3	37.1 ± 2.7	1.5	113	29.2	28.3
	3	33.332978	-79.198577	310.4 ± 58.7	35.2 ± 2.7	1.5	104	30.8	28.3
NC	1	33.995819	-77.936818	220.8 ± 56.2	32.8 ± 2.9	1.4	84	51.8	28.3
VAWW	1	37.509053	-75.808231	258.1 ± 48.3	43.4 ± 3.3	0.9	44	31.7	24.9
	2	37.509059	-75.808238	198.3 ± 22.4	38.9 ± 1.9	0.9	38	33.2	24.9
	3	37.509077	-75.808251	267.8 ±89.3	41.1 ± 2.9	0.9	44	35.7	24.9
VAGI	1	37.217288	-76.404214	327.2 ± 44.6	24.9 ± 2.2	0.8	49	47.5	24.4
VACI	1	37.891368	-75.356614	431.2 ± 70.8	35.0 ± 0.9	0.7	42	63.9	25.5
MD	1	38.137191	-75.957042	645.6 ± 107.5	40.0 ± 4.0	0.6	38	26.6	27.2
	2	38.139038	-75.949753	279.6 ± 83.1	42.7 ± 4.9	0.6	48	47.6	27.2
	3	38.178079	-75.924717	272 ± 59.9	40.3 ± 4.7	0.6	42	36.8	27.2



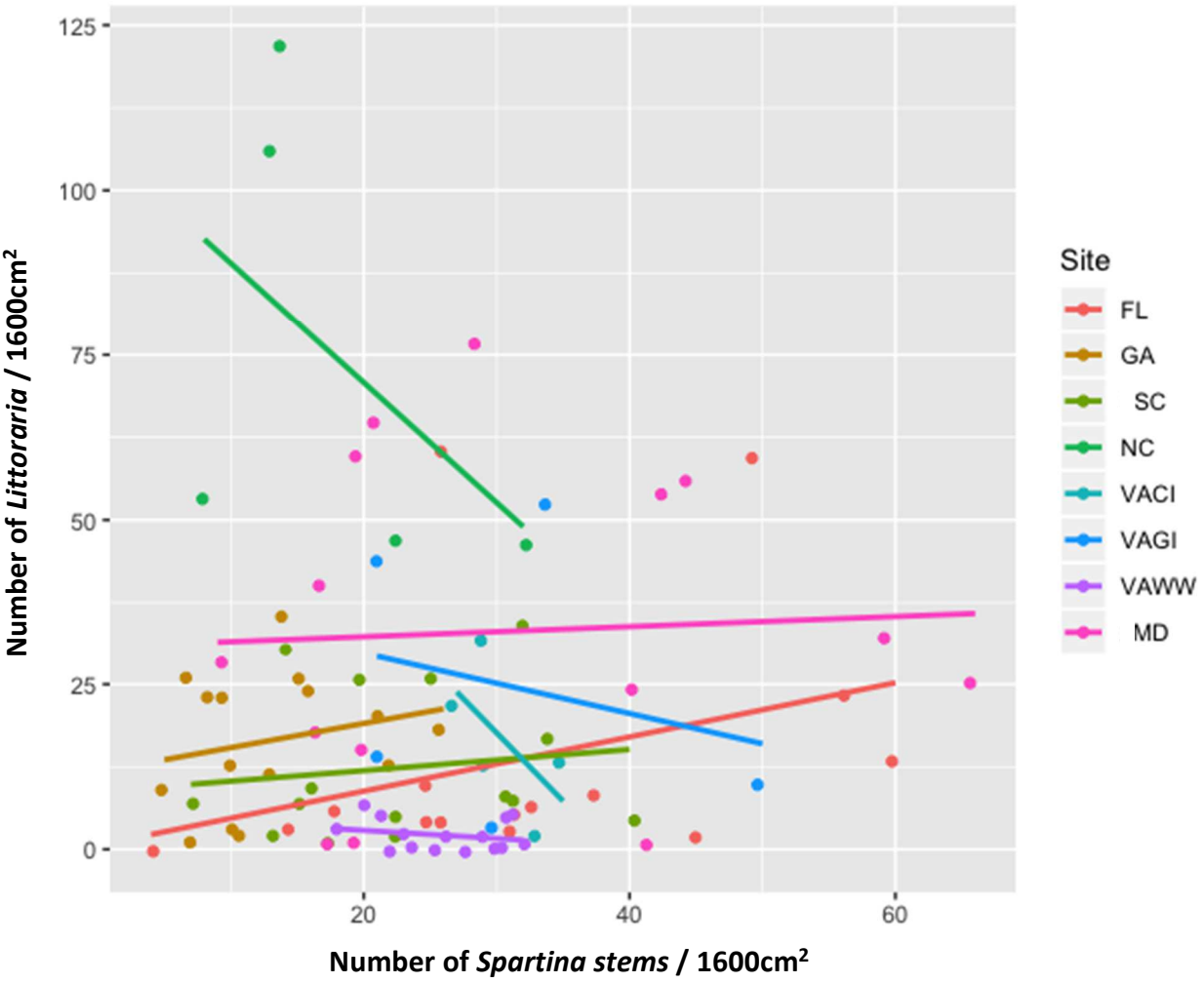
Appendix II Figure 2: Density of *Littoraria irrorata* summed across five 1600cm^2 quadrats placed along each transect (n=3 transects for each site, except NC, VAGI, and VACI which each had 1 transect: ND= no data).

Appendix II Table 2: Table illustrating the model selection process to fit the relationship between *Littoraria* density, biomass, and average width including comparisons between different classifications of spatial variables (Site vs Latitude and Relative Distance to Creek vs Actual Distance to Creek). The top model determined by lowest AIC is italicized.

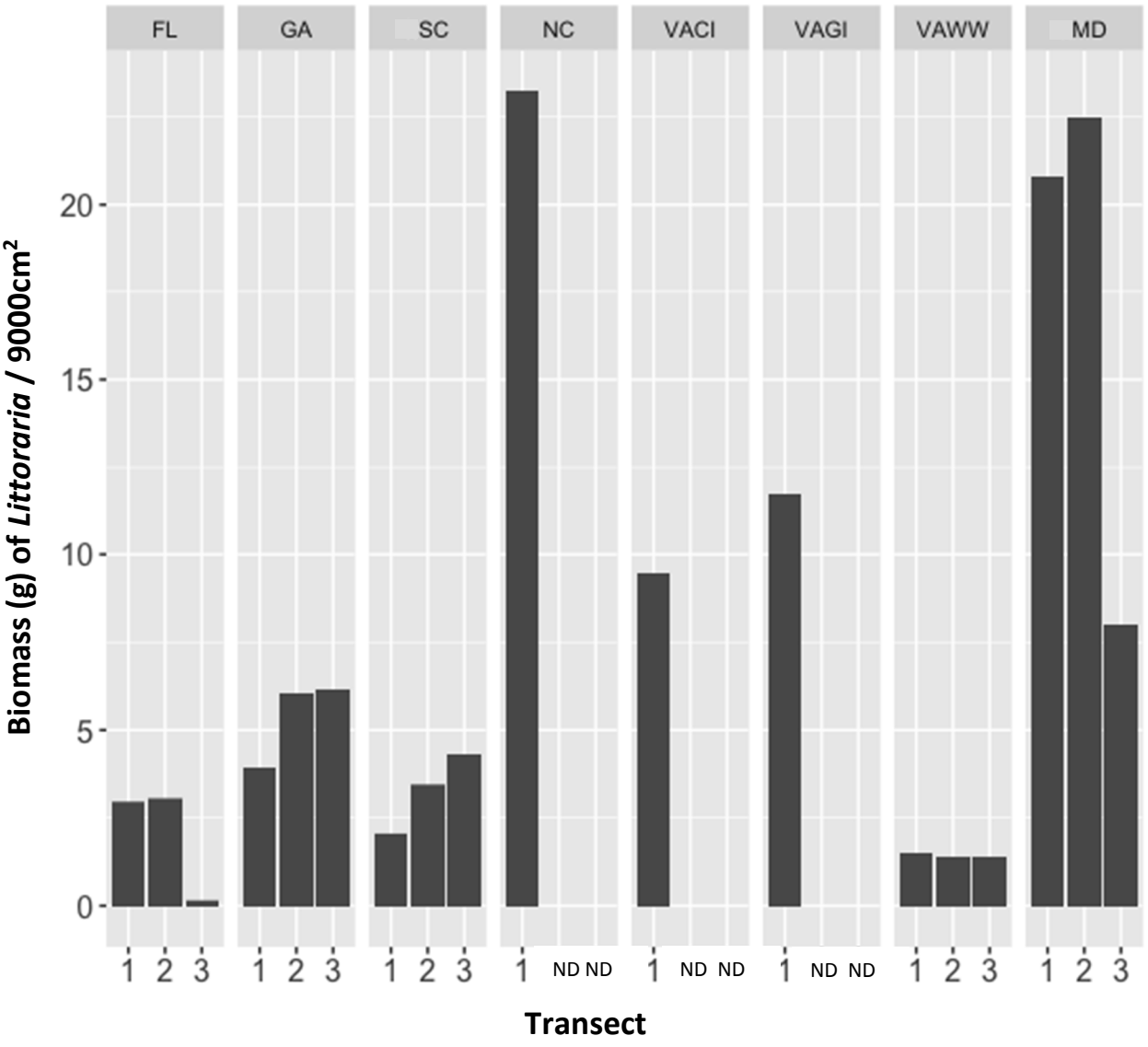
Model	Dependent Variable	AIC	logLik	Observations	k
*Site + **Relative** Distance to Creek + Stem Density + Site * **Relative** Distance to Creek*	*Density*	*496*	*-205*	*90*	*20*
Site + **Actual** Distance to Creek + Stem Density + Site * **Actual** Distance to Creek	Density	726	-344	90	19
Latitude + **Relative** + Latitude * **Relative** Distance to Creek	Density	790	-382	90	9
Latitude + **Actual** Distance to Creek + Latitude*__Actual__ Distance to Creek	Density	825	-406	90	7
Model	**Dependent Variable**	**AIC**	**logLik**	**Observations**	
Site + **Relative** Distance to Creek + Site*__Relative__ Distance to Creek	Average Width	480	-198	90	18
*Site + **Actual** Distance to Creek Site*__Actual__ Distance to Creek*	*Average Width*	*473*	*-219*	*90*	*18*
Latitude + **Relative** Distance to Creek + Latitude*__Relative__ Distance to Creek	Average Width	476	-226	90	6
Latitude + **Actual** Distance to Creek + Latitude*__Actual__ Distance to Creek	Average Width	474	-231	90	6
Model	**Dependent Variable**	**AIC**	**logLik**	**Observations**	
*Site + **Relative** Distance to Creek + Stem Density + Site * **Relative** Distance to Creek*	*Biomass*	*360*	*-138*	*90*	*20*
Site + **Actual** Distance to Creek + Stem Density + Site * **Actual** Distance to Creek	Biomass	496	-230	90	19
Latitude + **Relative** + Latitude * **Relative** Distance to Creek	Biomass	475	-225	90	9
Latitude + **Actual** Distance to Creek + Latitude*__Actual__ Distance to Creek	Biomass	490	-238	90	7



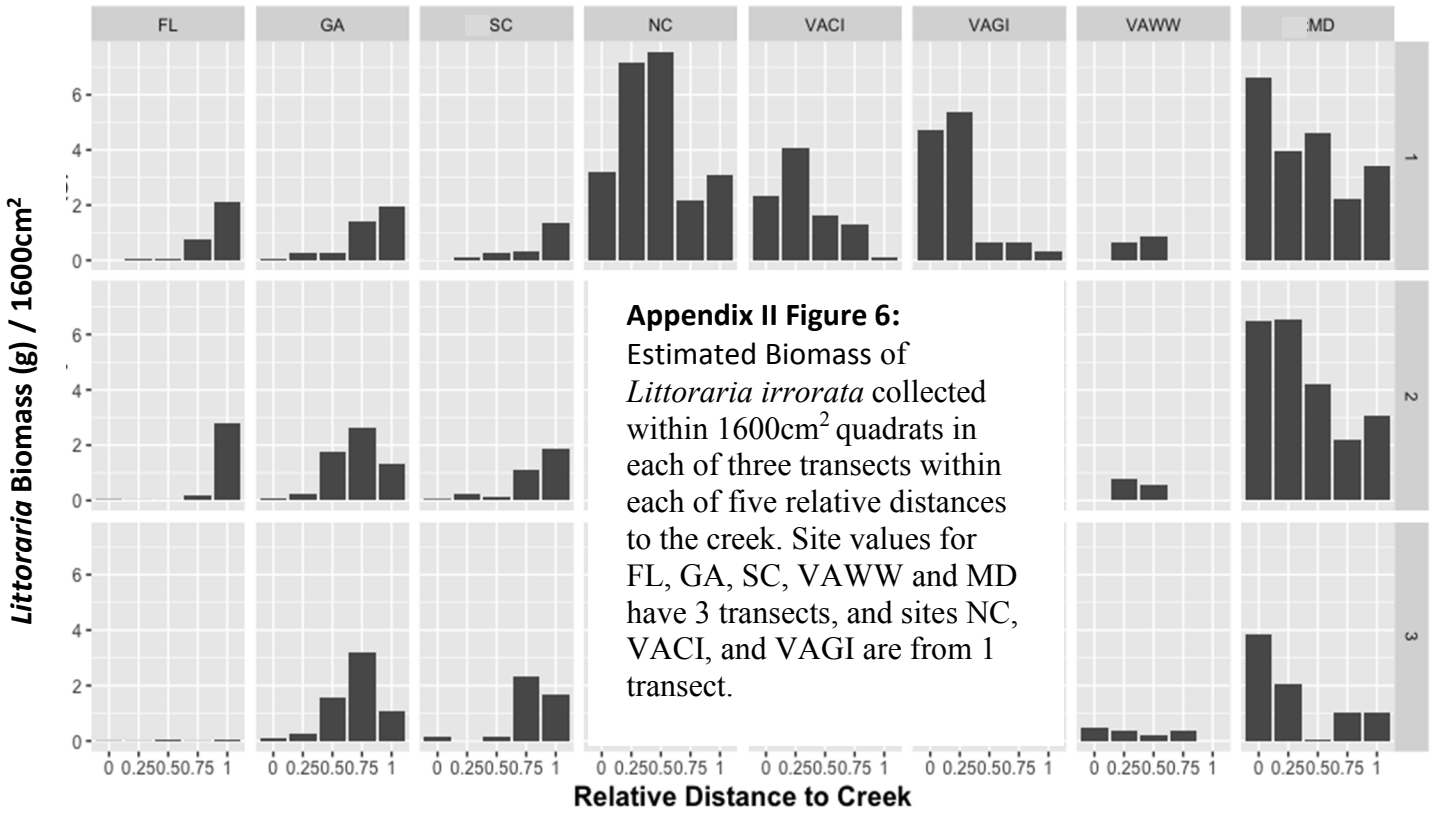
Appendix II Figure 3:
Density of *Littoraria irrorata* collected within 1600cm^2 quadrats at five relative distances to creek. Values for FL, GA, SC, VAWW and MD are from 3 transects, and values for NC, VACI, and VAGI are from 1 transect.



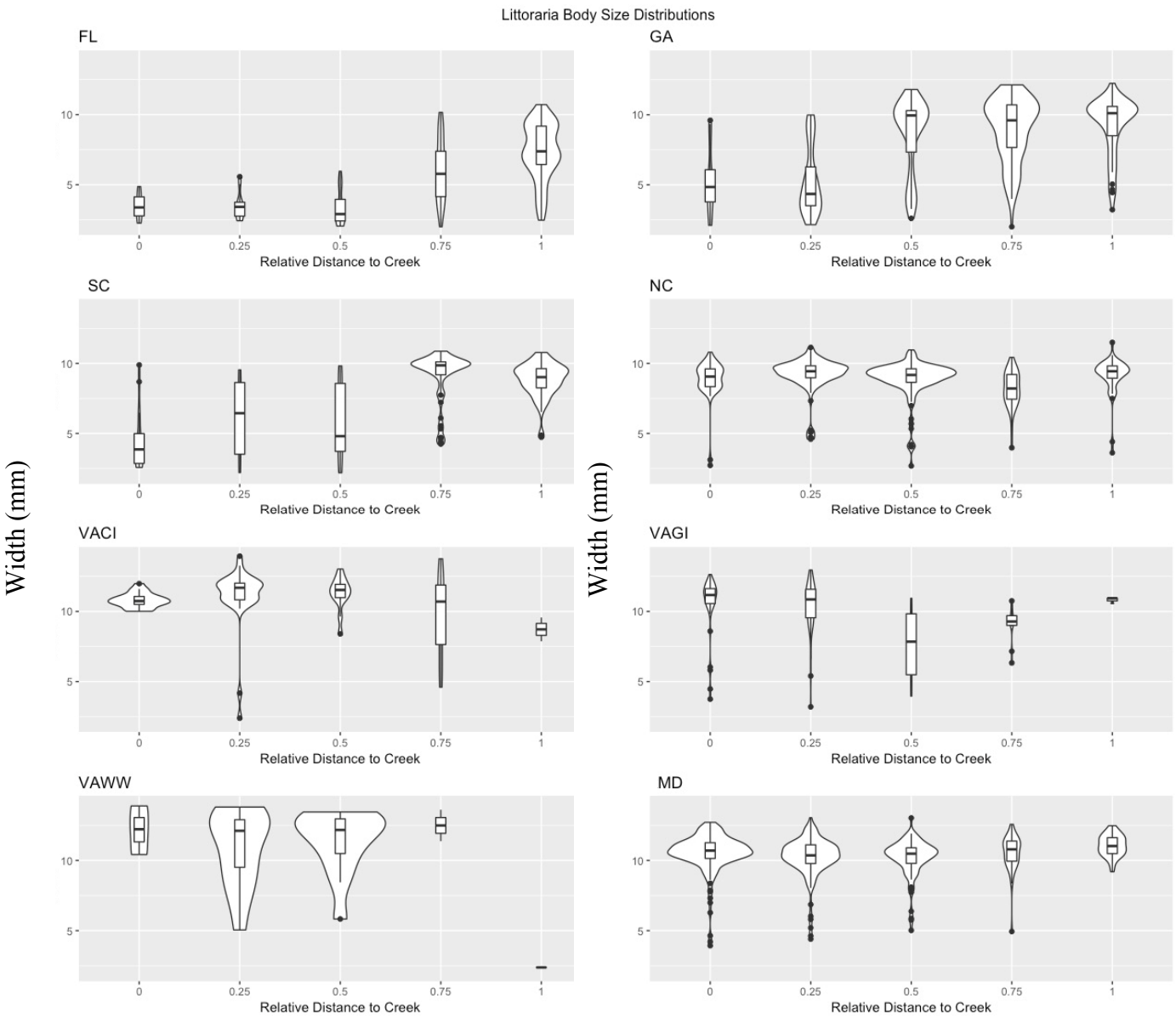
Appendix II Figure 4: The relationship between *Littoraria* density and *Spartina* stem density across all sites. FL is red, GA is orange,SC is yellow-green, NC is green, VACI is turquoise, VAGI is blue, VAWW is purple, and MD is pink. Each point represents the density of *Littoraria* and *Spartina* within a single 1600cm^2 quadrat.



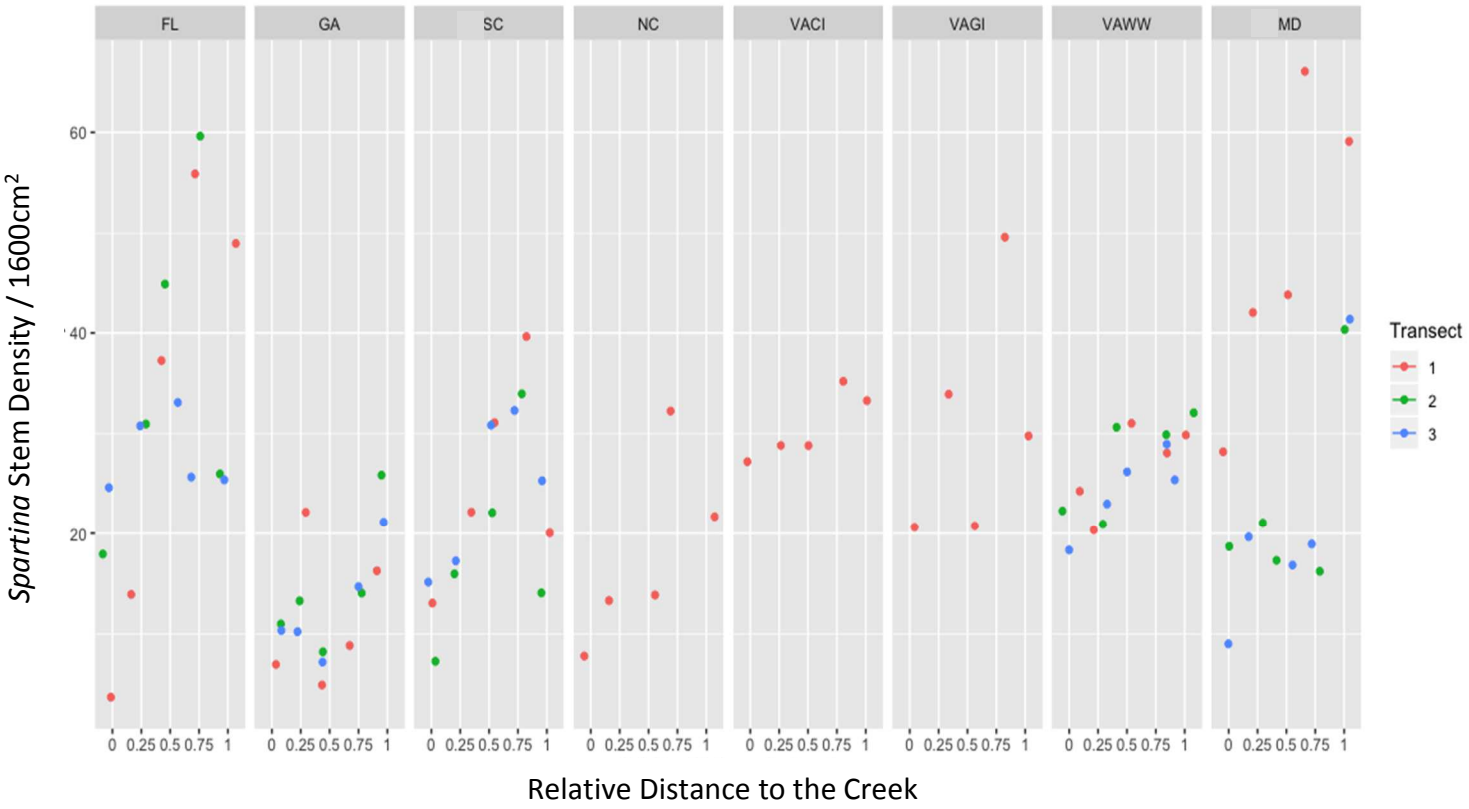
Appendix II Figure 5: Estimated biomass of *Littoraria irrorata* summed across five 1600cm^2 quadrats placed along transects (n=3 transects per site, except for sites NC, VAGI, and VACI which each had 1 transect). ND= no data.



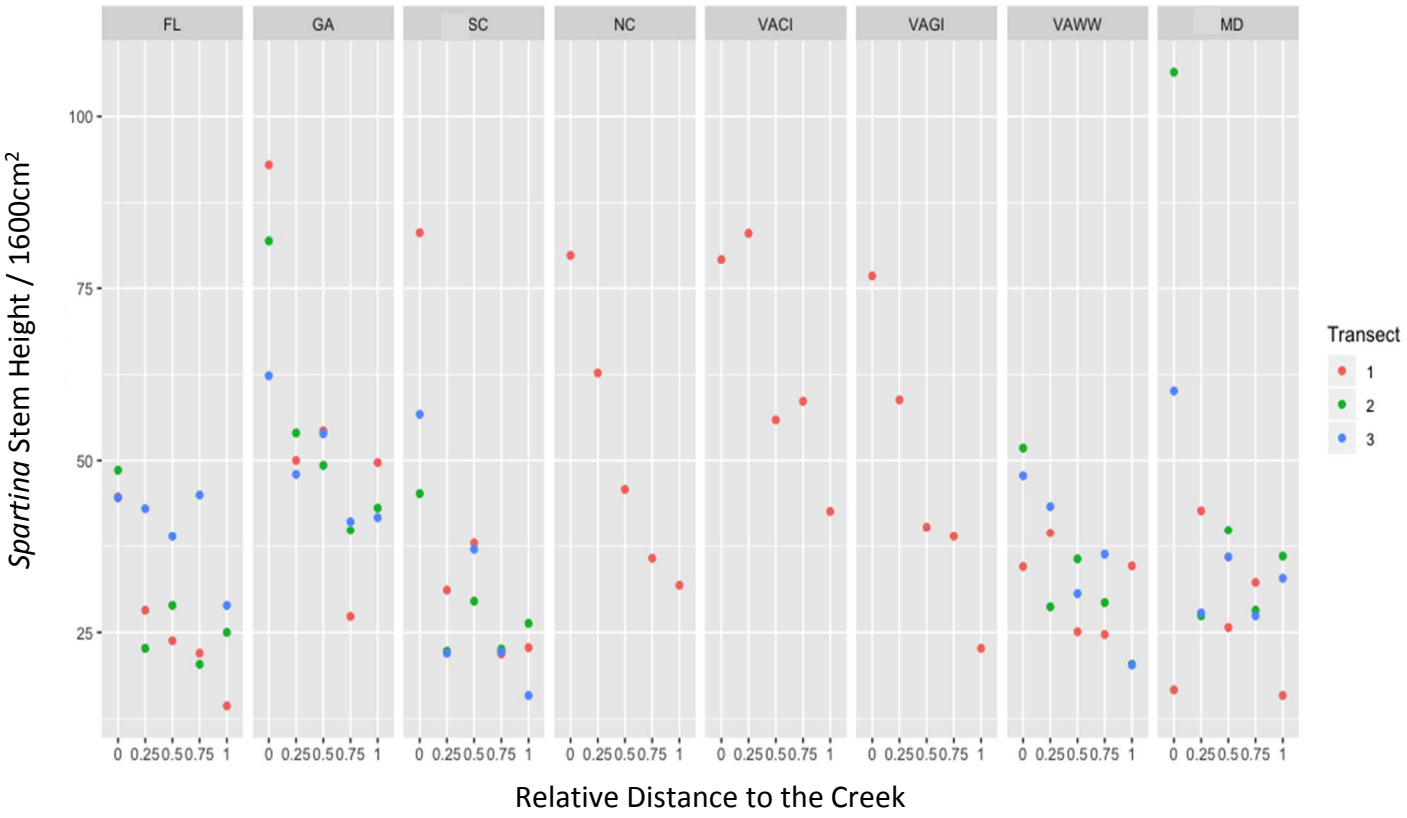
Appendix II Figure 6: Estimated Biomass of *Littoraria irrorata* collected within 1600cm^2 quadrats in each of three transects within each of five relative distances to the creek. Site values for FL, GA, SC, VAWW and MD have 3 transects, and sites NC, VACI, and VAGI are from 1 transect.



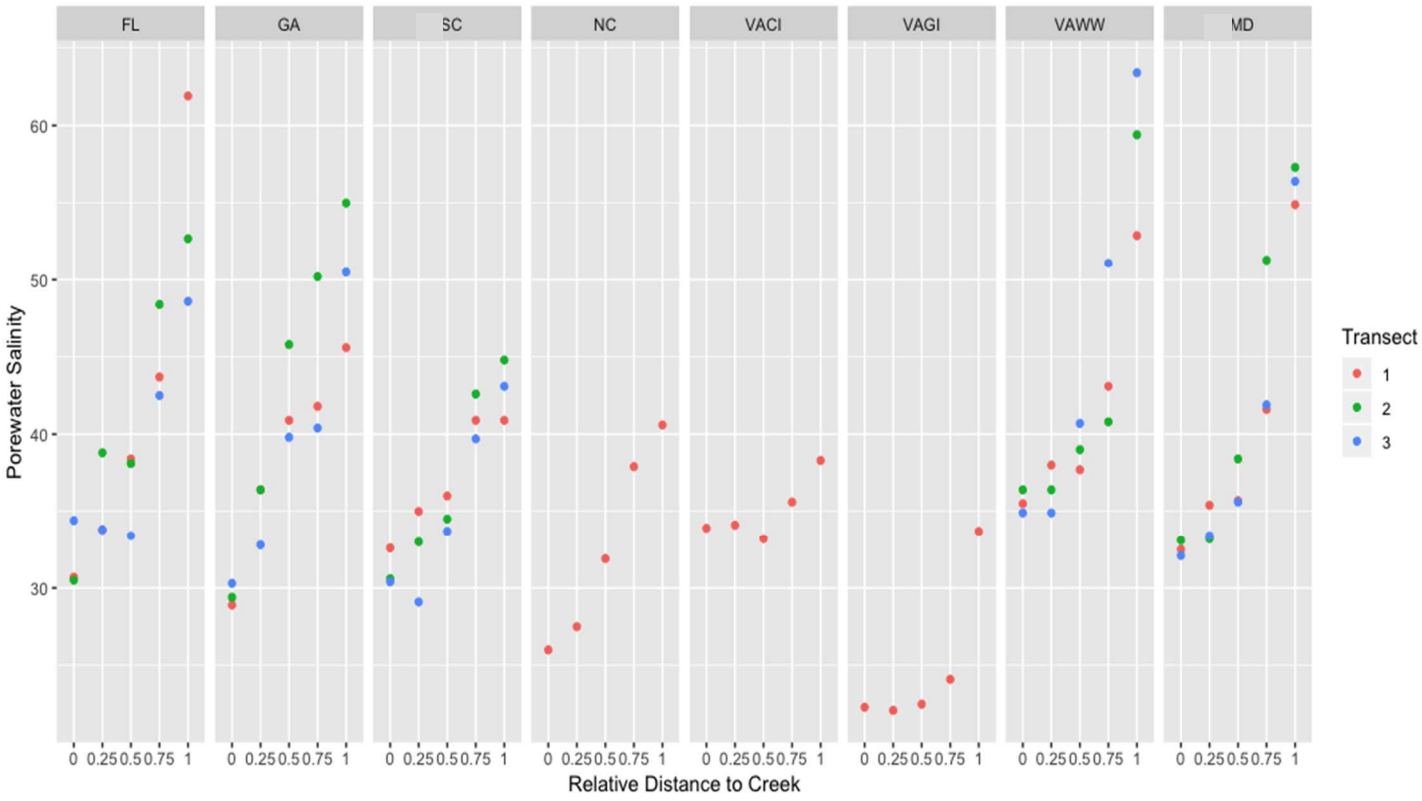
Appendix II Figure 7: Body size distributions of *Littoraria* at each site across the relative distances to the creek (0 = closest to creek and 1 = farthest from the creek). FL, GA, SC, VAWW and MD are based on pooling collections across 3 transects, and NC, VACI, and VAGI are from 1 transect. The central line gives the mean, boxes indicate ± SD, and the whiskers give the points outside of SD. The outer violin plot shows the entire smoothed distribution.



Appendix II Figure 8: *Spartina* stem density from each 1600cm^2 quadrat, across all zones in all transects at all sites. Sites NC, VACI, and VAGI were only surveyed along one transect. Red points are from transect 1, green points are from transect 2, and blue points are from transect 3.



Appendix II Figure 9: Mean *Spartina* stem height from each 1600cm^2 quadrat, across all zones in all transects at all sites. Sites NC, VACI, and VAGI were only surveyed along one transect; all other sites were surveyed along three transects.



Appendix II Figure 10: Porewater salinity across all zones, all transects at all sites. NC, VACI, and VAGI only have 1 transect; all other sites have 3 transects.