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Assessing the Effects of the Mud Snail, Ilyanassa Obsoleta, on the Benthic Microalgal Community in a Pristine Saltmarsh

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ASSESSING THE EFFECTS OF THE MUD SNAIL, *ILYANASSA OBSOLETA*, ON THE BENTHIC MICROALGAL COMMUNITY IN A PRISTINE SALTMARSH

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DEDICATION

This work is dedicated to my parents, whose scientific backgrounds inspired my own passion for the field. Additionally, I would like to dedicate this work to Eric Shealy, who helped me throughout the entire process. His emotional support, assistance in the field, and constant encouragement made all of this possible.

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ABSTRACT

Saltmarshes are among the most productive ecosystems globally. At North Inlet estuary, South Carolina, about one third of the primary production comes from benthic microalgae. During the tidal cycle, mobile microalgae vertically migrate through the upper 3 mm of sediment. At low tide, algae are vulnerable to a variety of grazers, including the mud snail, *Ilyanassa obsoleta*, which is abundant in tidal creeks. Many species of intertidal snails have been shown to significantly alter the community structure and density of microalgae within the sediment. The purpose of this study was to determine how *I. obsoleta* affects the benthic microalgae community in an intertidal mudflat. This study found that *I. obsoleta* moved at an average speed of 3.3 ± 1.4 cm min^{-1} and that it could cause a significant decrease in the concentration of total chlorophyll *a* when grazing an area with low snail density, but not in areas with high snail density. Areas where snails congregated were characterized by significantly higher moisture than low density snail areas. In the laboratory, snails were introduced to petri dishes with both grazed and ungrazed sediments. *I. obsoleta* spent more time on sediment that had been previously grazed by its conspecifics. When snail cues were introduced to both sides of the dish, snails showed no clear preference for location, indicating that *I. obsoleta* likely uses chemical cues to locate conspecifics and congregate towards them, despite the competition for food. Chemical cues and desiccation risk are therefore the likely driving factors for *I. obsoleta* distribution on the mudflat, rather than the availability of their benthic microalgae food source.

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LIST OF ABBREVIATIONS

BMA ..... Benthic microalgae

HPLC .....High performance liquid chromatography

CHAPTER 1

ASSESSING THE EFFECTS OF THE MUD SNAIL, *ILYANASSA OBSOLETA*, ON THE BENTHIC MICROALGAL COMMUNITY IN A PRISTINE SALTMARSH

1.1 INTRODUCTION

Estuaries are important transition zones that have diverse functions for coastal systems. These environments serve as nurseries for the juveniles of many species and play important roles in nutrient cycling. Vital ecosystem processes rely on primary productivity occurring within the marsh; estuaries on average have some of the highest primary productivity values of any ecosystem. This production comes primarily from the marsh grass, *Spartina alterniflora*, and the benthic microalgae (BMA) community, with other inputs from phytoplankton and macroalgae. North Inlet, a relatively pristine salt marsh estuary on the coast of South Carolina, has high primary productivity compared to similar systems. This estuary produces as much as 1059 g C m^{-2} yr^{-1}, whereas other, especially more northern, marshes produce as little as 201 g C m^{-2} yr^{-1} (Dame et al., 1986). Of the total, annual production, benthic microalgae account for 318 g C m^{-2} yr^{-1} at North Inlet (Dame et al., 1986). The microphytobenthos community accounts for a significant portion of the estuary's total annual production, coming in at a close second only to *S. alterniflora* (Dame et al., 1986; Pinckney and Zingmark, 1993b). The marsh system has diverse habitat types, including intertidal sandflats and mudflats, shallow subtidal, and *S. alterniflora* zones. These habitats are defined by their physical

characteristics, including light availability, sediment type, and vegetation, all of which have the potential to influence the productivity of benthic microalgae.

Benthic microalgae are important sources of fixed carbon and energy in estuarine systems. They also produce oxygen (O_2) and facilitate nutrient cycling between the sediment and water column (Armitage et al., 2009). The microphytobenthos is composed of a variety of taxonomic groups, including cyanobacteria, euglenophytes, and chlorophytes, but primarily consists of diatoms (Paterson and Hagerthey, 2001). Mobile benthic microalgae vertically migrate through the upper 3 mm of sediment during each tidal cycle (Pinckney et al., 1994). This causes a noticeable change in sediment color, from gray to brown. During this time, many types of invertebrates graze on the BMA near the sediment surface. Benthic microalgae may be responsible for as much as 50% of the carbon assimilated by these benthic grazers (Sullivan and Currin, 2000). This grazing facilitates the transfer of energy and biomass up the estuarine food web.

Mud snails (*Ilyanassa obsoleta*) are common grazers in sandflats, mudflats, and saltmarshes along the Atlantic Coast (Connor and Edgar, 1982). These snails spend most of their time on the sediment surface, where they are known to graze on the microphytobenthos, as well as on macroalgae and dead organic matter (Connor and Edgar, 1982). Gut content analyses have found that *I. obsoleta* stomach contents were enriched in chlorophyll and that mobile diatoms accounted for 40-45% of the content, whereas there was a near absence of meiofauna or animal remains (Feller, 1984; Connor and Edgar, 1982). Many feeding characterizations have been provided for *I. obsoleta*, but the current agreed upon dominant feeding mode is herbivory and detritivory; it is a facultative carrion feeder and is likely unimportant as a predator to benthic meiofauna

(Feller, 1984; Nichols and Robertson, 1979). While *I. obsoleta* individuals graze, they leave a trail behind them in the mud. A study on another intertidal snail species, *Littorina littorea,* found that the snails could create distinguishable spatial heterogeneity in the periphyton community due to their slow movement (Sommer, 1999). A study by Alvarez et al. (2013) showed that the snail, *Heleobia australis*, negatively affected the microalgal assemblages within the intertidal mudflat. The *H. australis* selectively grazed on cyanobacteria, chlorophytes, and euglenophytes, and had a significant effect on the microphytobenthos structure and concentration. Armitage et al. (2009) also found that intertidal snails (*Cerithidea californica*) could have a significant effect on the benthic microalgal assemblage in an intertidal mudflat, specifically depleting the diatoms. It is clear that intertidal snails can impact the microphytobenthos and play an important role in the path of organic C and energy into the estuarine food web.

This study assessed the effect of *I. obsoleta* grazers on the concentration of benthic microalgae in the surface sediment of the North Inlet estuary. *I. obsoleta* are common in the intertidal mudflats, and decrease in abundance as *S. alterniflora* cover increases. The mud snails show spatial heterogeneity in their distribution across the mudflat; they congregate in areas that tend to be moist, while avoiding higher, drier areas. Intertidal mudflats can be further subdivided into microhabitats based on snail density. Some areas have a very high concentration of snails, and will henceforth be referred to as "high density" mudflats, whereas regions with few snails will be referred to as "low density" mudflats. Differences in the physical and biological conditions between these two microhabitats may influence the benthic microalgae community, and therefore food availability for *I. obsoleta*.

H1: The concentration of benthic microalgal biomass will be significantly different between the “low density” and “high density” microhabitats.

Previous studies indicate that measuring the chl *a* concentration in the top 5mm of estuarine sediments are suitable estimates of the BMA biomass available to grazers, including *I. obsoleta* (Pinckney and Zingmark, 1993a). A significant grazing effect would cause a shift in the abundance of the benthic microalgal community within grazed areas.

H2: *I. obsoleta* will cause a significant decrease in the total benthic microalgal biomass within grazed pathways, compared to adjacent ungrazed areas.

Mud snails tend to congregate in large groups rather than grazing independently in areas with less competition. This grouping behavior could signal a highly productive area of the mudflat or may indicate the use of chemical cues. Studies have shown that *I. obsoleta* follow the mucus trails of conspecifics, but not those of other distantly related snail species (Ng et al., 2013). There are many reasons that snails may follow conspecific trails; these include, but are not limited to, homing, mate location and communication, nutrition, and energy conservation (Ng et al., 2013). The innate ability to detect conspecific chemical cues may drive *I. obsoleta* distribution in the mudflats.

H3: *I. obsoleta* will utilize conspecific chemical cues to choose grazing location.

Determining the impact of intertidal snail grazers in such a habitat will provide important estimates of benthic microalgal community composition for a pristine estuarine system. These findings can also help predict widespread fluctuations in the microphytobenthos throughout the marsh in response to variable rates of grazing by snails.

1.2 METHODS

1.2.1 Study site

All data were collected in the North Inlet estuary at the Belle W. Baruch Institute for Marine and Coastal Sciences in Georgetown, SC. North Inlet is a small (3200 ha) *Spartina* dominated marsh system characterized by its numerous tidal creeks, intertidal mudflats, and sandflats (Pinckney and Zingmark, 1993b). Samples were collected in the intertidal mudflat near Oyster Landing (33°20'58"N and 79°11'34"). The mudflat is a mosaic of microhabitats; moist regions tend to have dense snail cover, while areas with drier sediment typically have fewer snails. These two microhabitats were analyzed in this study and will henceforth be referred to as “high density” and “low density” mudflats, respectively.

1.2.2 Field estimates of microalgal community composition in mudflat microhabitats

The community composition of benthic microalgae (BMA) was estimated by taking sediment samples at low tide from different areas in the intertidal mudflats. Fifteen surface sediment samples were collected for both the “high density” and “low density” mudflat microhabitats to estimate the average available BMA community biomass. Sediment samples were collected by pressing the lid of a 2 ml microcentrifuge tube into

the sediment surface. This method extracted a small core from the sediment surface, 7.8 mm in diameter (47 mm^2), and 3 mm deep (143 mm^3). Excess sediment around the lid was removed so no extra volume was added to the sample. Samples were then closed into individual microcentrifuge tubes and covered to reduce light exposure. All sediment samples were analyzed for photosynthetic pigments, which can be used as an estimate for the microalgal abundance of different algal classes (Cartaxana et al., 2006). Samples were prepared for high performance liquid chromatography (HPLC) following the protocol outlined below under 'Analytical Methods'. Preliminary analysis of HPLC data indicated that the BMA community primarily consisted of diatoms, as indicated by high concentrations of fucoxanthin. Therefore, this accessory pigment was selected for further examination.

The concentration of total chlorophyll *a* (total chl *a*) and fucoxanthin per gram of sediment was tested for normality (K-S test) and homogeneity (Levene's test) in the "low density" and "high density" mudflat samples. The pigment concentrations from the two mudflat microhabitats were compared using a single factor analysis of variance (α=0.05).

1.2.3 Microhabitat sediment moisture

The moisture content of the mudflat was estimated by taking surface sediment samples at low tide. To collect the samples, a small wooden frame (interior dimensions 16.5x11.5 cm, area of 189.75 cm^2) was placed randomly in each of the microhabitats of the mudflat. In the "high density" mudflat, snails were carefully removed from the sampling area after frame placement. The top 3 mm of sediment was then collected using a spatula and placed into a pre-weighed 50mL screw-top vial to prevent moisture loss. Five samples were taken in each of the two microhabitats.

Vials were weighed with the wet sediment, prior to being freeze dried for 24 hours and weighed a final time. The percent water by weight was tested for normality and homogeneity in the "low density" and "high density" mudflat. The percent water was compared for the two microhabitats using an ANOVA to determine if there is a significant difference in sediment water content by location.

1.2.4 Field estimates of I. obsoleta grazing effects

To determine the effect of grazing snails on the BMA community, a surface sediment sample was taken in front of a grazing *I. obsoleta* and directly behind, within the grazed pathway. Fifteen grazing pairs were collected in the "high density mudflat" and 15 pairs were collected in the "low density" mudflat. Since snails were not typically present in the "low density" mudflat, a nearby active snail was selected and placed into the microhabitat. The snail was given an acclimation period to return to normal movement before any samples were obtained. Snail grazing sediment samples were collected using a small metal spatula to scoop up the thin surface layer of the sediment. Each sample was placed into its own 2 ml microcentrifuge tube, which was held with limited light exposure until returned to the lab. All samples were prepared following the methods outlined in 'Analytical Methods'. Each snail used throughout the entire experiment was collected, weighed, and measured to the nearest hundredth of a mm with calipers. All snails were returned to the field after data were collected.

The concentrations of total chlorophyll *a* and fucoxanthin were analyzed for normality and homogeneity for both mudflat microhabitats. The pigment concentrations were analyzed with a paired t-test for the samples collected in front of an individual snail

and from behind it in the “low density” mudflat. Another paired t-test compared the pigment concentrations for the snail pairs in the “high density” mudflat.

1.2.5 Field estimates of I. obsoleta grazing rate

A total of 10 snails, 5 on each of two days, were chosen from the mudflat at random and brought back to the lab for preparation. Each snail was rinsed, dried, and marked using a distinct color of acrylic paint and placed back into the field for an acclimation period of 30 sec. After this interval the snails had returned to normal movement and were filmed for 2 min. Snails were unmisted during this test, as there was sufficient moisture from the environment to elicit movement. During the analyses of the videos, each snail’s position was recorded every 10 sec to determine the distance traveled during the 2 min period. The width of the grazing path was measured to find the total area covered in the time interval. These values were used to determine the average grazing rate of each snail.

In situ grazing rates on the mudflat were also measured. On the first day of the procedure, the 5 marked snails were placed at a common starting point, marked by a stationary post in the mudflat. These individuals were left in the field for 24 h in order to determine the distance covered in that time period. The following day, as many snails as possible were located in the marsh and a tape measure was used to determine their straight-line distance from the post. At this time, the next 5 snails were placed at the same starting point and left for another 24 h. On the third day, as many as possible were located and their straight-line distances traveled were measured.

A census was taken to estimate the number of snails present in the marsh. A quadrat 0.5m x 0.5m was placed in the tidal mudflat at random and the snails within the area were counted. This census was conducted 10 times to find an average snail density.

A linear regression was performed to determine the relationship between snail weight (cm) and snail length (g). Another linear regression was performed with the independent variable snail length (cm) and the dependent variable straight-line distance traveled in 24 hours (cm). A final linear regression analyzed the relationship between snail weight and the straight-line distance traveled in 24 h.

1.2.6 Laboratory estimates of I. obsoleta grazing effects

An independent measure of snail grazing effects was conducted in the laboratory to estimate the proportion of the algal community consumed by snails in a given amount of time. Fourteen surface sediment samples were collected to a depth of approximately 3mm, the depth over which BMA can vertically migrate (Pinckney et al., 1994). Each sample was homogenized and approximately equal masses were weighed and placed into two petri dishes. One snail was added to one of the dishes and allowed to graze for 30 min, while the other matching dish remained ungrazed as a control. The plates were misted intermittently with filtered seawater to stimulate snail movement and compensate for evaporated water. After the grazing period, the snail was removed and the samples were transferred to individual 50 ml centrifuge tubes. Samples were held and prepared following the protocol prescribed in 'Analytical Methods'.

Concentrations of total chlorophyll *a* and fucoxanthin were analyzed for normality and homogeneity. Concentration of total chl *a* in the petri dish pairs was

analyzed with a paired t-test to determine if snails caused a significant decrease. A separate test compared the fucoxanthin concentration for the petri dishes.

1.2.7 Grazing location preference by I. obsoleta

To determine if snails use chemical cues to select grazing location, eight samples of the top 3mm of sediment were collected from the marsh. Each sample was homogenized, a control sample taken, and similar masses spread into two petri dishes. One petri dish was left ungrazed, while the other petri dish was stocked with 10 *I. obsoleta*. The two dishes were left for 30 min to allow for grazing to occur. They were periodically sprayed with filtered seawater to compensate for evaporation and to promote snail movement. Once 30 min had passed, the snails were removed from the dish, the sediment samples were each homogenized, and a portion was transferred to a new petri dish. This third petri dish was divided in half, with one side randomly selected to hold the grazed sediment and the other the ungrazed sediment. A new snail was placed in the center of the divided petri dish, and allowed to graze for 5 min while being filmed. The snail was then removed from the dish and the ungrazed and grazed sediments were carefully collected, so as to prevent their mixing, and placed in their own 50 ml centrifuge tubes.

To determine if snails chose to graze on areas where other *I. obsoleta* had grazed, the same procedure described above was performed. However, during the 5 min grazing period on the third petri dish, the sediment was misted periodically with filtered sea water with snail chemical cues in it. The snail cue seawater was prepared by placing 20 snails in 200 ml of filtered seawater for 30 min. A total of 8 sets of samples were collected with

the snail cue addition. Samples were prepared following the methods outlined below in 'Analytical Methods'.

Concentrations of chl *a* and fucoxanthin were independently analyzed for normality and homogeneity for each of the treatments. The concentration of total chl *a* was analyzed with an ANOVA comparing the control, the ungrazed, and the grazed samples with regular water misting during the individual snail grazing. Another ANOVA compared fucoxanthin concentrations for the petri dish sets with control water misting. The same analyses were performed for the datasets with snail cue seawater misting during the individual snail grazing periods.

The videos of snail grazing events were analyzed to determine the amount of time spent on each side of the petri dish. The times that snails spent on each side of the dish were analyzed for normality and homogeneity for both regular water misting and snail cue water misting. A paired t-test was performed on the times spent on each side of the dish with the control misting to determine if the snails spent significantly more time in the ungrazed or grazed side. Another paired t-test was performed on the time data collected from the experiment with the snail cue infused water, to determine if the snails are responding primarily to the cues of other snails.

1.2.8 Analytical methods

All samples for photopigment analysis were stored with limited light exposure in a -80^{o}C freezer until they were prepared for HPLC analysis. Sediments were freeze dried for 24 h and prepared for HPLC analysis by adding 90% acetone and 50 µl of carotenal (used as an internal standard) per 1 ml of acetone to each sample. The solvent/sediment mixture was agitated vigorously and then held in a -20^{o}C freezer for 24 h to allow for

pigment extraction to occur. After the allotted time, extracts were removed from the freezer and centrifuged at 13,400 RPM for 90 sec to decrease turbidity. A 3 ml syringe with a flat-tipped needle was used to decant the extract, which was then filtered through a 0.45 µm filter. An aliquot of the sample was placed into the HPLC vial and ammonium acetate was added (250 µl per 1 ml of sample). The samples were then analyzed by HPLC. All sediments were retained, dried, and weighed for each sample to estimate the concentration of photosynthetic pigments per gram of dry sediment in the marsh.

Filtered extracts (250 µl) were injected into a Shimadzu HPLC with a single monomeric column (Rainin Microsorb, 0.46 × 1.5 cm, 3 µm packing) and a polymeric (Vydac 201TP54, 0.46×25 cm, 5 µm packing) reverse-phase C18 column in series. A non-linear binary gradient consisting of solvent A (80% methanol : 20% 0.5 M ammonium acetate) and solvent B (80% methanol : 20% acetone) was used for the mobile phase (Richardson et al., 2006). Absorption spectra and chromatograms (440 ± 4 nm) were obtained using a Shimadzu SPD-M10av photodiode array detector and pigment peaks were identified by comparing retention times and absorption spectra with pure standards (DHI, Denmark). The synthetic carotenoid β-apo-8'-carotenal (Sigma) was used as an internal standard.

1.3 RESULTS

1.3.1 Field estimates of microalgal community composition in mudflat microhabitats

A single factor analysis of variance was used to determine if there was a significant difference between the concentration of total chl *a* in the "high density"

mudflat compared to the "low density" mudflat. The data satisfied the major assumptions for ANOVA. There was no significant difference in total chl *a* between the two microhabitats (Figure 1.1; $F_{1,13}$=1.384, p=0.249). Furthermore, a single factor ANOVA indicated that there was no significant difference in the fucoxanthin concentration of "high density" and "low density" mudflat sediments (Figure 1.2; $F_{1,13}$=2.748, p=0.109). Thus there were no differences in total microalgal biomass or diatom biomass between the areas of high and low snail density in the mudflat.

1.3.2 Microhabitat sediment moisture

An ANOVA was performed to determine if there was a significant difference in the sediment moisture by weight between the two microhabitats. There was a significant difference between the percent water in the "high density" and the "low density" mudflat ($F_{1,8}$=32.205, p=0.000468). The "high density" sediment was on average 77.26±2.39% water by weight, whereas the "low density" microhabitat was 69.64 ± 1.83% water (Figure 1.3). Thus there is a significant difference between microhabitat moisture, which may be important for snail behavior.

1.3.3 Field estimates of I. obsoleta grazing effects

Paired t-tests were used to determine if there were significant differences between the photosynthetic pigments in the sediment in front of a snail and within the grazed pathway behind it in the mudflat. Two outliers were removed to normalize the data pigment concentrations in the "high density" mudflat, leaving a total of 13 samples. There was no significant difference in the concentration of total chl *a* g^{-1} of dry sediment in the ungrazed and grazed areas in the snail trails on the high density mudflat (Figure 1.4; t=0.289, df=12, p=0.777). There was also no significant difference between the

concentration of fucoxanthin in the "high density" mudflat (Figure 1.5; t=0.388, df=12, p=0.705). This indicated that snails do not significantly affect the microalgal biomass or diatom biomass during grazing in the high density mudflat.

There was a significant difference in the concentration of total chl *a* between the grazed and ungrazed areas in the "low density" mudflat (Figure 1.4; t=2.200, df=14, p=0.045). There was significantly more chl *a* in the ungrazed areas in front of snails (166.69 ± 74.71 µg/g) than in their grazed pathways (127.89 ± 68.00 µg/g). There was no significant difference between the concentration of fucoxanthin in the grazed and ungrazed areas, however the probability only slightly exceeded the designated α level of 0.05 (Figure 1.5; t=2.058, df=14, p=0.059). Thus *I. obsoleta* can significantly decrease the microalgal biomass and substantially decrease the diatom biomass in areas with low snail density.

The percent difference between the BMA concentration in front of a grazing snail and behind it was calculated for both the "high density" mudflat and the "low density" mudflat (Figure 1.6). In the "high density" mudflat there was a minimal mean difference between the BMA in ungrazed and grazed areas. However, in the "low density" mudflat, there was a substantially higher concentration of BMA in the ungrazed area in front of a snail compared to that in the grazed area behind it. This suggests that *I. obsoleta* can significantly decrease the BMA in areas with low snail density, but not in areas of high density.

1.3.4 Field estimates of I. obsoleta grazing rate

In the intertidal mudflats of North Inlet, there are an average of 217.3 ± 130.99 snails 0.25 m^{-1}. A least-squares linear regression analysis of the dependent variable snail

length (cm) and the independent variable snail weight (g) indicated a significant linear relationship between the variables (n=9, adj r^2=0.597, p=0.009) (Figure 1.7). The equation for the relationship was:

(1) Snail weight= (1.026* snail length)-0.608

Mud snails were capable of traveling a mean distance of 3.3 ± 1.4 cm min^{-1} and covering an area of 1.05±0.48 cm^2 min^{-1}. One snail was excluded from all analyses because it failed to move a detectable distance within the 2 min time interval allotted. If these values are extrapolated out over a 24 h period, assuming constant snail movement, then the average distance traveled is 4672.5 ± 2057.1 cm and the average area covered is 1517.3 ± 696.1 cm^2. However, in a 24 h period, mud snails traveled a straight line distance of 115.8 ± 82.1 cm, covering only 38.4 ± 29.9 cm^2. After 48 h, they had traveled a straight-line distance of 211.5 ± 128.7 cm, covering an area of 77.0 ± 46.8 cm^2. These values are substantially lower than those that would be expected when estimates are extrapolated from the distance per minute values above because those include path deviations from the straight line. This indicates that snails are inactive for a substantial period of the time, which could be due to diurnal cycles or the rise and fall of the tides.

A linear regression of the relationship between snail length (cm) and the straight-line distance traveled in 24 h (cm) indicated a significant linear relationship between the two variables (n=7, adj r^2=0.682, p=0.014) (Figure 1.8). The equations for the relationship was:

(2) Distance travelled in 24 hours= (-672.697 * snail length)+1150.956

A least-squares linear regression analysis of the dependent variable of straight-line distance travelled in 24 h (cm) and the independent variable of snail weight (g)

indicated a significant linear relationship between the two variables (n=7, adj r^2=0.974, p=0.000023) (Figure 1.9). The equation for the relationship was:

(3) Distance travelled in 24 hours= (-608.762* snail weight)+706.925

1.3.5 Laboratory estimates of I. obsoleta grazing effects

A paired t-test was used to determine if there was a significant difference between the concentration of photosynthetic pigments in ungrazed petri dishes and dishes that had been grazed by a single snail for 30 min. There was no significant difference in the total chl *a* concentrations in the grazed and ungrazed petri dishes (Figure 1.10; t=0.946, p=0.361). Similarly, there was no significant difference in the concentration of fucoxanthin between the dishes (Figure 1.11; t=0.894, p=0.388). This indicates that individual mud snails cannot cause a significant decrease in the biomass of benthic microalgae or diatoms in an area of 56.7 cm^2 in 30 min.

1.3.6 Grazing location preference by I. obsoleta

Mud snails were allowed to graze in a petri dish with heavily grazed and ungrazed sediment. The time spent in each side of the dish was analyzed with a paired t-test. When the snails were misted with regular sea water, they spent more time in the grazed side of the petri dish (185.71 ± 72.41 s) than in the ungrazed side (79.43 ± 70.91 s). When one outlier was removed, this difference was nearly statistically significant (Figure 1.12; t=2.292, p=0.062). However, when the snails were misted with seawater infused with snail cues, the mud snails did not show a clear preference for the grazed side of the dish (t=0.362, p=0.730). Therefore, *I. obsoleta* seems to prefer to spend more time on sediment with conspecific cues.

An ANOVA showed that there was nearly a significant difference in the concentration of total chl *a* between the two sides of the dish and their control during the experiment with regular seawater misting (Figure 1.13; $F_{2,5}$=3.345, p=0.058). The grazed and ungrazed had similar concentrations of total chl *a* (228.95 ± 116.38 µg/g and 225.47 ±9 8.73 µg/g, respectively), whereas the control sample had a lower concentration (130.16 ± 63.89 µg/g). In a test of fucoxanthin concentrations under regular seawater misting conditions, there was a nearly significant difference between the control, grazed, and ungrazed (Figure 1.14; $F_{2,4}$=3.410, p=0.055). One outlier had to be removed from that analysis to normalize the data. Again, the control had a lower concentration of the photosynthetic pigment (48.37 ± 21.77 µg/g) compared to the grazed and ungrazed, which were similar (96.78 ± 49.15 µg/g and 100.95 ± 48.68 µg/g, respectively). This indicates that the inherent variation of BMA biomass in the sediment is greater than that caused by mud snail grazing.

The same analyses were completed for the sediments from the snail cue misting. An ANOVA showed that there was a significant difference between the concentrations of total chl *a* in the control, grazed, and ungrazed samples (Figure 1.13; $F_{2,5}$=8.485, p=0.002). An R-E-G-WF post hoc analysis showed that the grazed and ungrazed samples were a homogenous group and that they were significantly different from the control. The grazed and ungrazed samples had total chl *a* concentrations of 146.79 ± 41.21 µg/g and 222.19 ± 134.84 µg/g, respectively, whereas the control had a total chl *a* concentration of 373.24 ± 133.15 µg/g. An ANOVA was also used to determine if there was a significant difference in the concentration of fucoxanthin between the three treatments with snail cue misting. With one outlier removed, there was a significant

difference between the three treatments (Figure 1.14; $F_{2,4}$=16.842, p=0.000075). An R-E-G-WF post hoc analysis again showed that the grazed and ungrazed were a homogenous group, with fucoxanthin concentrations of 61.93 ± 15.38 µg/g and 70.83±16.85 µg/g; the control sample group was significantly different, with a mean fucoxanthin concentration of 127.63 ± 32.61 µg/g. This leads to the conclusion that the inherent variation in BMA biomass is more significant than that caused by grazing mud snails.

1.4 DISCUSSION

In this study, the mudflats of North Inlet, SC were divided into two microhabitats based on the density of *I. obsoleta* grazers. Despite substantial spatial heterogeneity in the benthic environment, as shown in previous research and here, there was no significant difference between the "high density" mudflat BMA biomass and the "low density" sediment biomass (Figure 1.1). This indicates that the areas where the snails congregate are not chosen based on an abundance of their primary food source. A previous study found that chl *a* stimulated snail activity to a small extent, and only once they were already active (Orvain and Sauriau, 2002). Future studies should examine how responsive these snails are to their different potential food sources. It is unclear why the snails are congregating in these areas of relatively average food availability. It may be that food availability is too unpredictable on small spatiotemporal scales for a mechanism of detection to have evolved. It is also possible that food may be present at relatively similar levels through time and space, so there is no strong need to detect it. The similarities in

BMA biomass across the mudflat suggests that the spatial distribution of *I. obsoleta* is not driven by food availability, but rather by some other factor(s).

Mud snail distribution could be driven by ecological factors, including the meiofaunal assemblage in the mudflat. Studies have shown that *I. obsoleta* does not prey directly on the meiofauna (Feller, 1984; Nichols and Robertson, 1979). However, exclusion experiments have found higher densities of both benthic diatoms and meiofauna in the absence of *I. obsoleta* (Nichols and Robertson, 1979). Future studies should examine how *I. obsoleta* interacts with the meiofauna and how strongly they impact meiofaunal distribution. Changes in community structure elicited by snail grazing are important for understanding the estuarine food web.

Abiotic factors may also be a driving force for mud snail distribution. Areas of *I. obsoleta* congregation tend to be slightly lower in elevation and have increased water availability. An analysis of sediment moisture by weight indicated that the "high density" areas of the mudflat were significantly wetter than "low density" areas (Figure 1.3). A study by Coffin et al. (2008) indicated that *I. obsoleta* distribution and movement were both affected by tide pools on the mudflat. Mud snails tended to congregate in pools after the tide receded, reaching a density ~20 times higher in pools versus outside of them; snails in the tide pools also spent more time moving than those outside of the pools (Coffin et al., 2008). Other experiments have also shown that sediment moisture content influences snail behaviors and that only in the presence of seawater, would snails become active (Orvain and Sauriau, 2002). Desiccation is a known dominant factor affecting behavior and habitat selection in many snails, including another intertidal snail, *Littoraria irrorata*, which spends most of its time on plant stalks and hard surfaces (Gómez-

Cornejo, 1993). *I. obsoleta* have a relatively small operculum compared to their shell apertures, and are thus subject to increased water loss (Hyman, 1967). For that reason, it seems likely that the behavior of this species is particularly driven by water availability.

The relationship between snail activity and moisture level is supported by the seawater misting used during this study. Preliminary observations indicated that snails held in the laboratory would become inactive, even when placed on relatively damp sediment. Sediments lost moisture to evaporation between collection and use, as well as during laboratory experiments, likely resulting in the decreased snail activity. Additionally, unmisted sediment samples in petri dishes lost over 2% of their water by weight in a 30 min period. This loss was primarily from the surface that the snail was in contact with, exacerbating the drying effect on snail activity. When experimental snails were misted periodically with seawater, the sediment would become moister and the snails would resume activity quickly. For this reason, snails were misted with seawater during the laboratory experiments performed in this study. It is possible that misting had some unintended effect on snail activity beyond promoting movement. Future studies could examine how seawater misting affects snail behavior compared to natural moisture inputs. *I. obsoleta* used in field experiments of this study were not misted with seawater. *In situ* experiments have a natural addition of seawater from the tidal creeks that keeps the snails and sediments moist, so misting was not necessary. Both the sediment moisture analysis and misting results indicate that the distribution and activity of *I. obsoleta* is driven by the physiological need to maintain moisture, rather than the need to find food on the mudflat.

Individual snails can have a significant effect on the BMA biomass on small spatiotemporal scales. This study found that there was significantly higher BMA biomass in front of a grazing snail compared to that behind it in the "low density" mudflat (Figure 4); however, this trend did not hold in the "high density" areas. It is unclear why there was no significant difference in the "high density" areas, but ideas have been proposed. It is possible that the BMA community composition in the "high density" areas is different than that in the "low density" microhabitat due to differences in grazing pressure or abiotic conditions. Previous studies have found that *I. obsoleta* shows grazing selectivity for the migratory diatoms found at the sediment-water interface (Connor and Edgar, 1982). These migratory diatoms may be relatively more abundant in the "low density" mudflat and thus they would experience a more significant change with grazing. Benthic microalgae also have extended time to restore the surface community in the "low density" mudflat, as grazing pressure is minimal in these areas (Alvarez et al., 2013). This recovery period could amplify the observed grazing effect of *I. obsoleta* on the BMA community, as there may be relatively more desirable food and higher grazing rates. However, microalgal recovery also means that *I. obsoleta* grazing effects are relatively short-lived, as the BMA community can replenish the surface. Another possible cause for the difference in observed grazing effects is the "high density" mudflat has greater variability in BMA density (Figure 1.1), and a greater chance of re-grazing as snails are more likely to cross pre-grazed paths due to their abundance. This variability in the BMA biomass and high chance of re-grazing may decrease the effect *I.* obsoleta has in the "high density" microhabitat. Despite the fact that *I. obsoleta* individuals are capable of causing a significant decrease in the concentration of BMA, this likely

happens rarely and on a very small scale, as the snails are typically grouped together in "high density" areas where they are unable to elicit a significant change.

Variations in abiotic factors have the potential to cause the disparity in *I. obsoleta* grazing effects between microhabitats. Imbalances in moisture between the two microhabitats may drive differences in BMA growth rate; wetter areas may have an increased BMA growth rate, leading to a faster recovery and decreased observed role of grazing *I. obsoleta*. Many grazers also have significant, indirect biochemical effects on the sediment as a result of bioturbation. The "high density" areas may have increased light availability due to bioturbation during grazing, which may enhance BMA growth. Studies have shown that intermediate grazing can stimulate BMA production by increasing the depth of the sediment photic zone by thinning the microalgal over-story and cropping away older cells (Alvarez et al., 2013). "High density" areas also have increased nutrient supply from fecal deposition and bioturbation, which could enhance BMA growth and diminish the grazer effect (Premo and Tyler, 2013). Alvarez et al. (2013) also found that grazing could stimulate BMA production by increasing nutrient availability. Future studies should examine how bioturbation and abiotic factors influence the rate of BMA growth, and how they in turn may affect grazers.

Experiments performed in the laboratory indicate that individual *I. obsoleta* are not capable of causing a significant change in BMA biomass over larger spatiotemporal periods. When individual snails were allowed to graze a petri dish of sediment approximately 56.7 cm^2 for 30 min, they were unable to cause a significant decrease in BMA biomass compared to the control, though the mean concentration of total chl *a* was lowered (Figure 1.10). When ten snails were allowed to graze a petri dish over a 30 min

period they were unable to significantly decrease the concentration of BMA significantly compared to the ungrazed dish, though they were able to reduce BMA biomass (Figure 1.13). Another lab study also found that *I. obsoleta* could suppress benthic microalgal communities in the surface sediment, but not significantly compared to the control (McLenaghan et al., 2011). Hagerthey et al. (2002) found that another species of intertidal snail, *Hydrobia ulvae,* did not cause a significant decrease in benthic diatom biomass over a large area in a laboratory setting. Therefore, it can be concluded that intertidal snails, including *I. obsoleta*, may not have a significant, large-scale impact on the BMA biomass within the mudflat when compared to the rate of BMA growth.

Mud snails are typically most active in the moist conditions of the "high density" mudflat, moving at an average speed of 3.3 ± 1.4 cm min^{-1}. Another study of *I. obsoleta* found the snails could move at an average speed of 2 mm s^{-1} (or 12 cm min^{-1}) which is much faster than the speeds observed in this study (Dimock, 1985). The intertidal snail *L. littorea* found an average speed of 3.55 cm min^{-1}, which is much closer to that of *I. obsoleta* observed here (Erlandsson and Kostylev, 1995). Snail speed may be dependent on a variety of factors, particularly seasonal cycles or environmental conditions. In other intertidal conditions, specifically around the base of *Spartina* stems, *I. obsoleta* are essentially inactive at low tide, potentially due to the increased risk of desiccation. From the grazing rate estimates in this study, it seems that *I. obsoleta* spends a substantial part of the day inactive. Snail inactivity may be driven by snail size. A linear regression showed that there was a significant relationship between snail length and the straight-line distance it traveled in 24 h (Figure 1.8), as well as the snail's weight and the 24 h distance (Figure 1.9). While larger snails may have the competitive advantage over their smaller

counterparts, they do not seem move around the mudflat as much. This may be because it is more energetically costly for larger, heavier snails to travel, or they may have the competitive advantage locally. Larger snails may be able to extract more BMA from the sediment per unit area than smaller individuals, as they may be able to bioturbate deeper into the sediment to obtain food. Since the large snails would have increased access to BMA, they would not need to move great distances to obtain more food. Cheng et al. (1983) found that *I. obsoleta* tissue dry weight is a function of shell length, with longer shells having a smaller shell to tissue ratio than their smaller counterparts. This indicates that larger snails have relatively more body tissue, likely incurring higher metabolic costs, but also potentially permitting enhanced grazing capabilities. Future studies should determine if snail size influences bioturbation depth and nutrient acquisition.

This study did not examine the daily routines of snail behavior, but periods of inactivity could occur during tidal inundation, when predatory crabs and fish are present, or at night, when snails were unobserved. However, previous studies have found wide ranges of mean distances traveled for *I. obsoleta,* which are not necessarily in line with this proposed inactivity. A study by Curtis (2005) found that the mud snails moved an average of 1.7 m per day, which is lower than the extrapolated values found in this study, but consistent with the 24 h straight-line distances. Another study found a mean daily displacement of 10.7 m for *I. obsoleta*, which far exceeds the findings here (Coffin et al., 2008). The driving factors for the variations in snail displacement are unclear, but they may be controlled by differences in habitat, changes in sediment moisture with tidal cycle, concentration of predators, sediment type, or seasonal cycles. If *I. obsoleta* does

have relatively long periods of inactivity, this may limit their potential impacts of on the mudflat BMA biomass.

In situ results from this study indicate that the mud snails are not exerting significant control over the BMA community in the intertidal mudflats. There was no significant difference in the BMA biomass between densely populated microhabitats and "low density" areas, as would be expected if the snails were having a strong grazing effect. Additionally, individual I. obsoleta were not able to cause a significant decrease in BMA biomass while grazing in the "high density" areas. However, when individual snail effects are scaled up to the larger scale, the effect of *I. obsoleta* grazers on the BMA community changes. Using the 24 h straight-line distance, individual mud snails cover an average of 38.4 ± 29.9 cm^2 d^{-1}. At the average observed density of 869.2 snails m^{-2} the snail population of one square meter can cover over 3.34 m^2 d^{-1}. This would indicate that the snails are either venturing away from their groups into "low density" areas, or they are crossing over the pathways of other snails. However, snail congregation areas were observed to be fairly constant with time, so the likely factor is re-grazing of pathways. This re-grazing would increase the overall snail effect on the BMA in a certain area, thus enhancing their impact. This study found that the concentration of BMA is approximately $3.4*10^5$ µg total chl *a* m^2 in both high and low density microhabitats. Analysis showed that mud snails can decrease BMA biomass by an average of 19.43% in "low density" areas (Figure 1.6). If the snails were able to exert this control over BMA in all areas of the mudflat, they could decrease BMA biomass by approximately 20%. This can be easily recovered by the BMA, which reproduces at a rate of approximately one doubling per day. However, it is likely that the mud snails in one square meter are re-grazing a

given area more than three times in order to cover an area of 3.34 m^2d^{-1}. If this were the case, then the *I. obsoleta* population in one square meter could decrease that area's BMA biomass by almost 60%. That rate of decrease cannot be matched by the growth rate of the BMA. Therefore, it is theoretically possible for the mud snail population to significant affect the BMA biomass in the surface mudflat. This is in contrast with the *in situ* results of this study, which show no significant difference in BMA biomass in "high density" compared to "low density" areas. If the snails did exert a significant grazing effect on the BMA, the two microhabitats would likely have significantly different BMA biomass. The homogeneity between microhabitats indicates that snails are probably inactive for a considerable period of time or that they are not grazing for a substantial part of the day. Future studies should examine the daily routines of snail behavior and grazing.

In addition to the physiological need to avoid desiccation, chemical cues seem to play an important role in determining the congregation and grazing behaviors of these mud snails. Previous studies have found that many species of snails, including *I. obsoleta*, are known to sense the chemical cues of their conspecifics, and often directly follow their mucus trails (Atema and Burd, 1974; Ng et al., 2013). There are many reasons that snails may follow conspecific trails; these include, but are not limited to, homing, mate location and communication, nutrition, and energy conservation (Ng et al., 2013). In this study, when *I. obsoleta* were misted with regular sea water, they showed a clear preference for sediment that had been previously grazed by conspecifics. However, when the snails were misted with sea water infused with conspecific cues, individuals did not show a preference for either the previously grazed or ungrazed sediments. These

findings indicate that *I. obsoleta* can sense the presence of conspecifics and exhibits a positive response to their cues. Therefore, the snails are likely choosing their grazing location based on sediment moisture content and conspecific chemical cues, rather than on food availability or chemical cues from grazed BMA.

This study found that *I. obsoleta* does not cause large-scale, significant decreases in the concentration of benthic microalgae in a pristine saltmarsh mudflat. However, these snails are theoretically capable of having a strong, negative grazing effect on the BMA community. *I. obsoleta* congregate together on the mudflat, but not in areas with significantly higher productivity, as might be expected. Rather it seems that this grouping behavior is due to favorable physical conditions in these lower, moister areas, and that this behavior is guided by chemical communication between conspecifics.

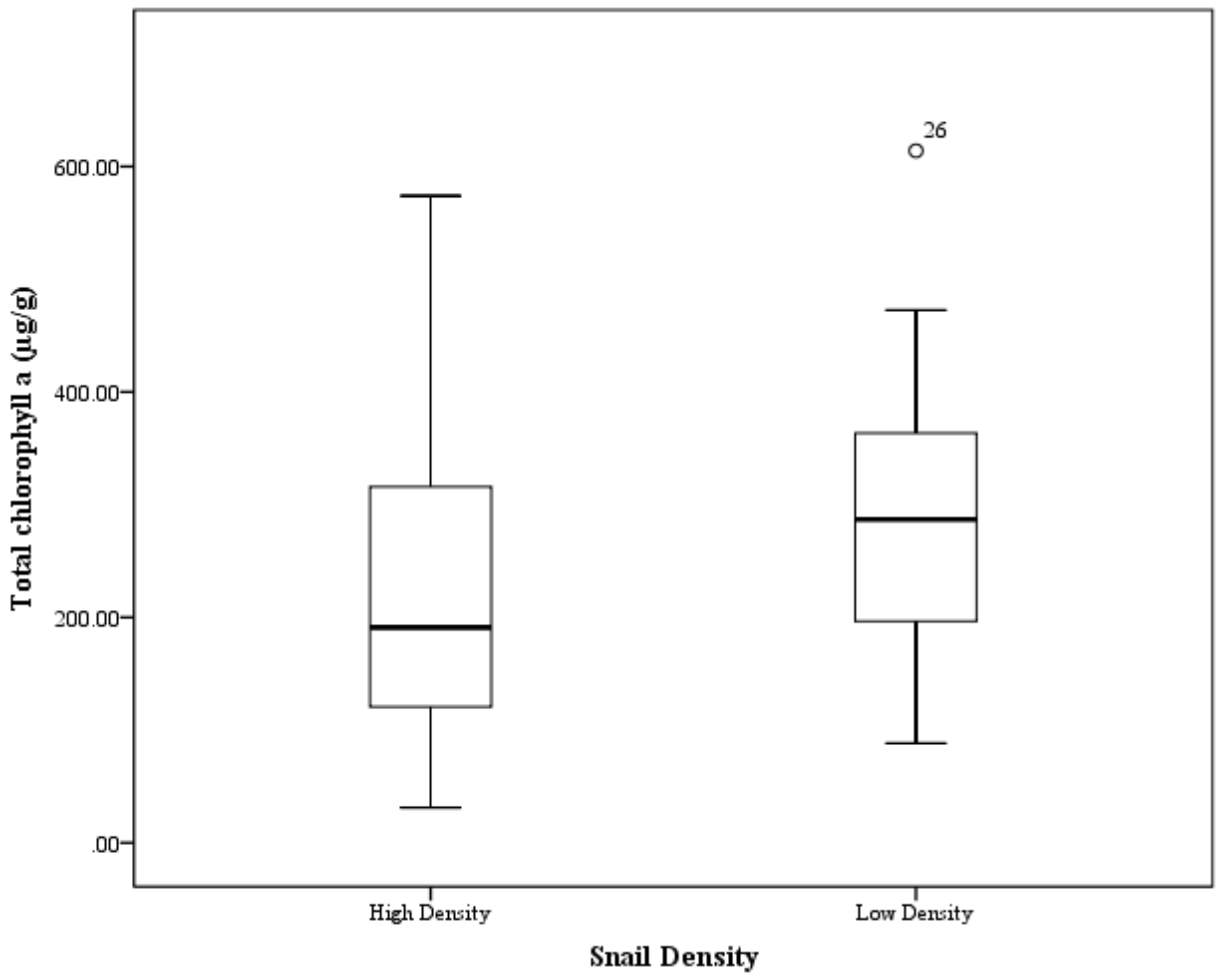

Figure 1.1 Concentration of total chl *a* in the sediment of two microhabitats. There is no significant difference between the concentration of total chl *a* in the "high density" and "low density" mudflat microhabitats (ANOVA, p=0.249).

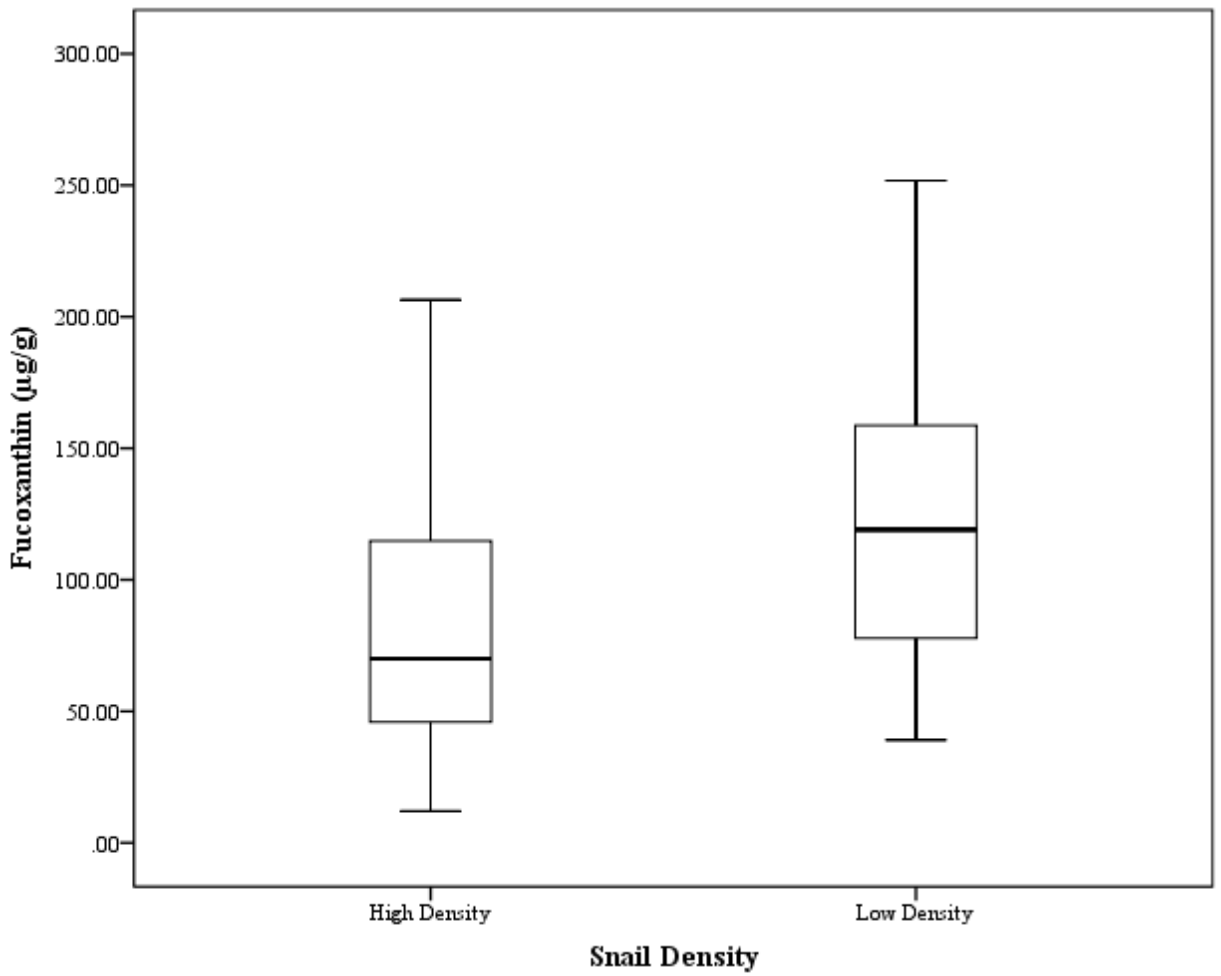

Figure 1.2 Concentration of fucoxanthin in the sediment of two microhabitats. There is no significant difference between the concentration of fucoxanthin in the high density and low density mudflat microhabitats (ANOVA, p=0.109).

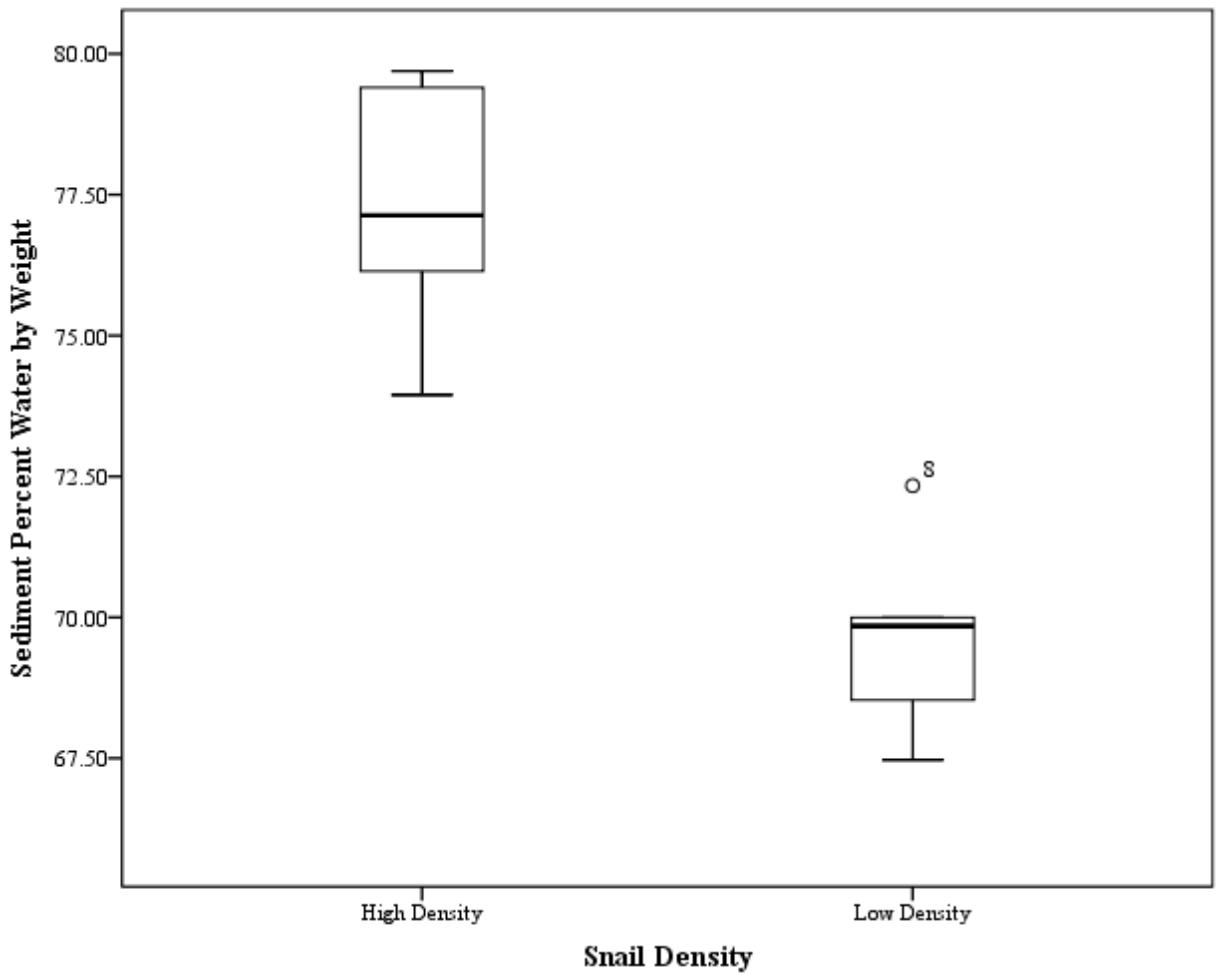

Figure 1.3 Percent water of mudflat sediment by weight. The “high density” mudflat has significantly higher water content than the “low density” mudflat (ANOVA, p=0.000468).

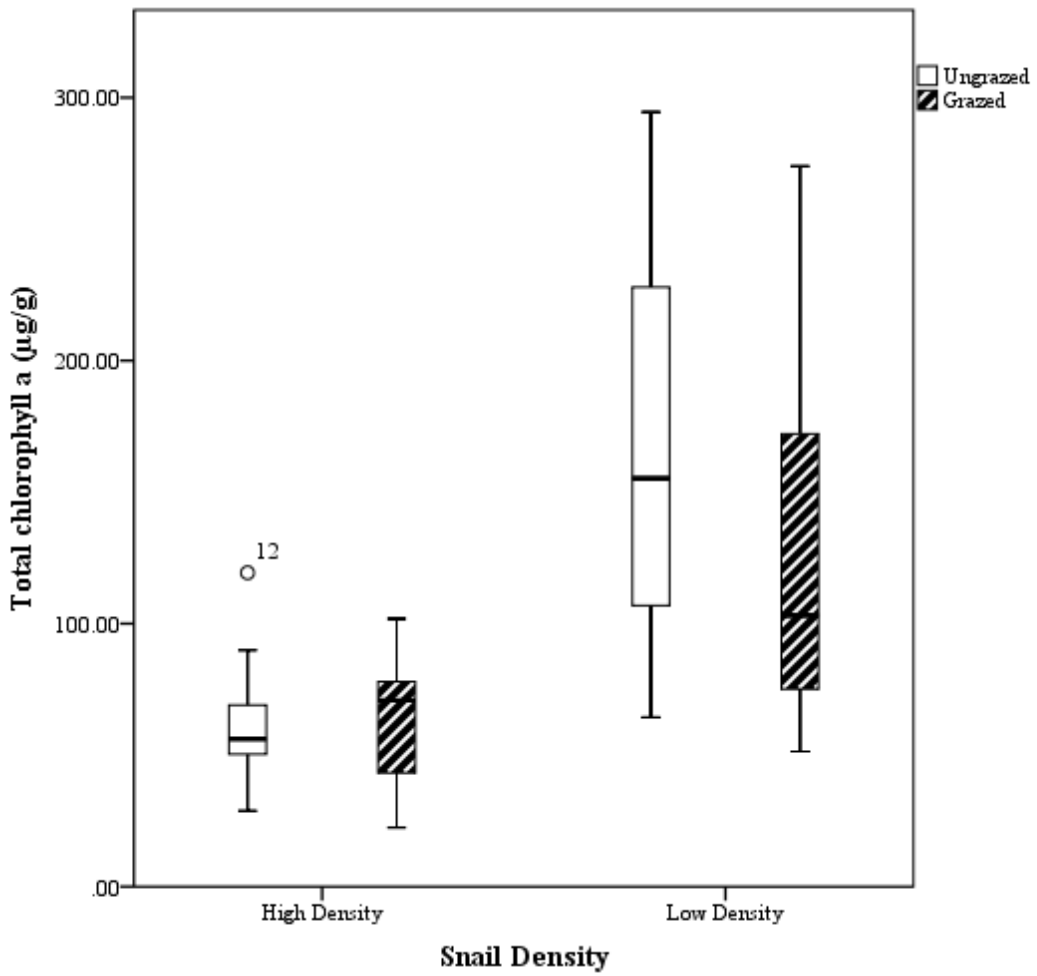

Figure 1.4 Concentration of total chl *a* in two mudflat microhabitats grazed and ungrazed sediments. There is no significant difference in the concentration of total chl *a* relative to grazing in the "high density" mudflat (paired t-test, p=0.777). There is a significant difference between total chl *a* in ungrazed and grazed areas in the "low density" mudflat (paired t-test, p=0.045).

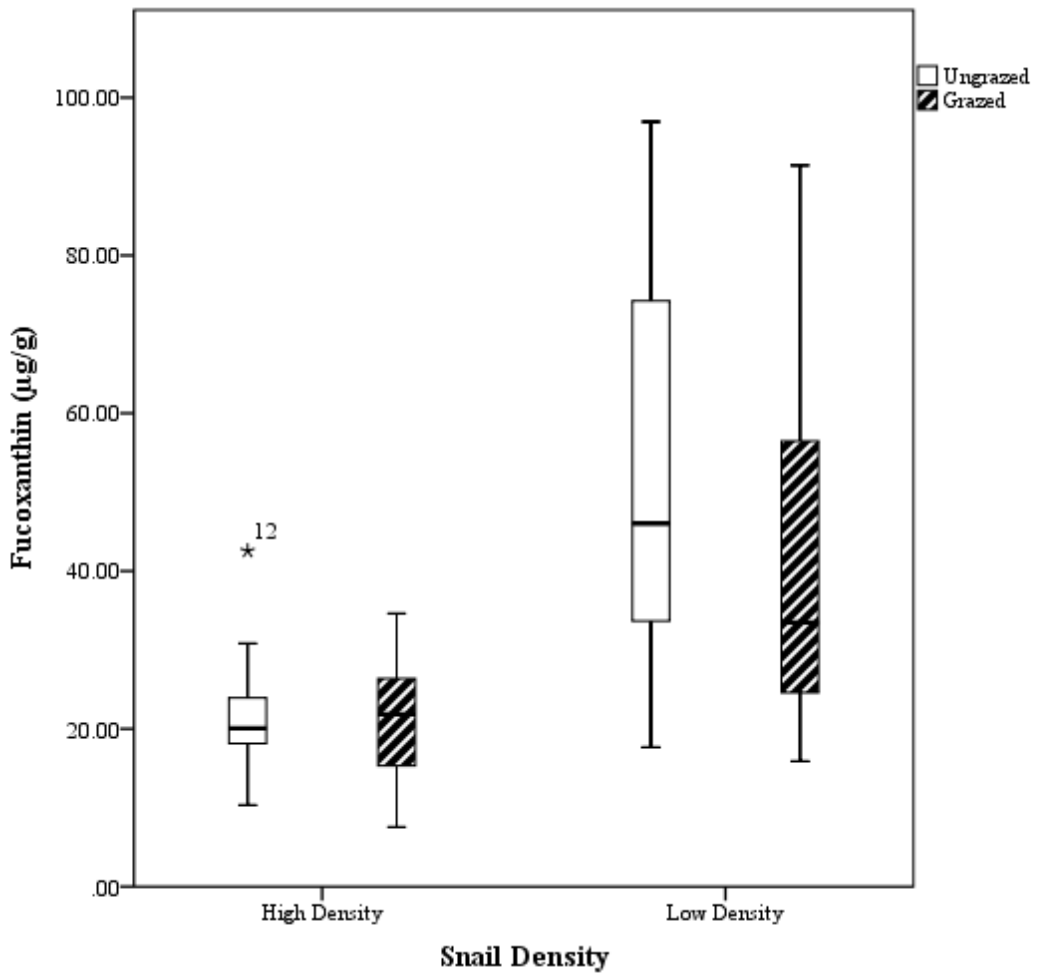

Figure 1.5 Concentration of fucoxanthin in two mudflat microhabitats, grazed and ungrazed sediments. There is no significant difference in the concentration of fucoxanthin relative to grazing in the “high density” mudflat (paired t-test, p=0.705). Data is nearly significant difference in the “low density” mudflat, relative to grazing (paired t-test, p=0.059).

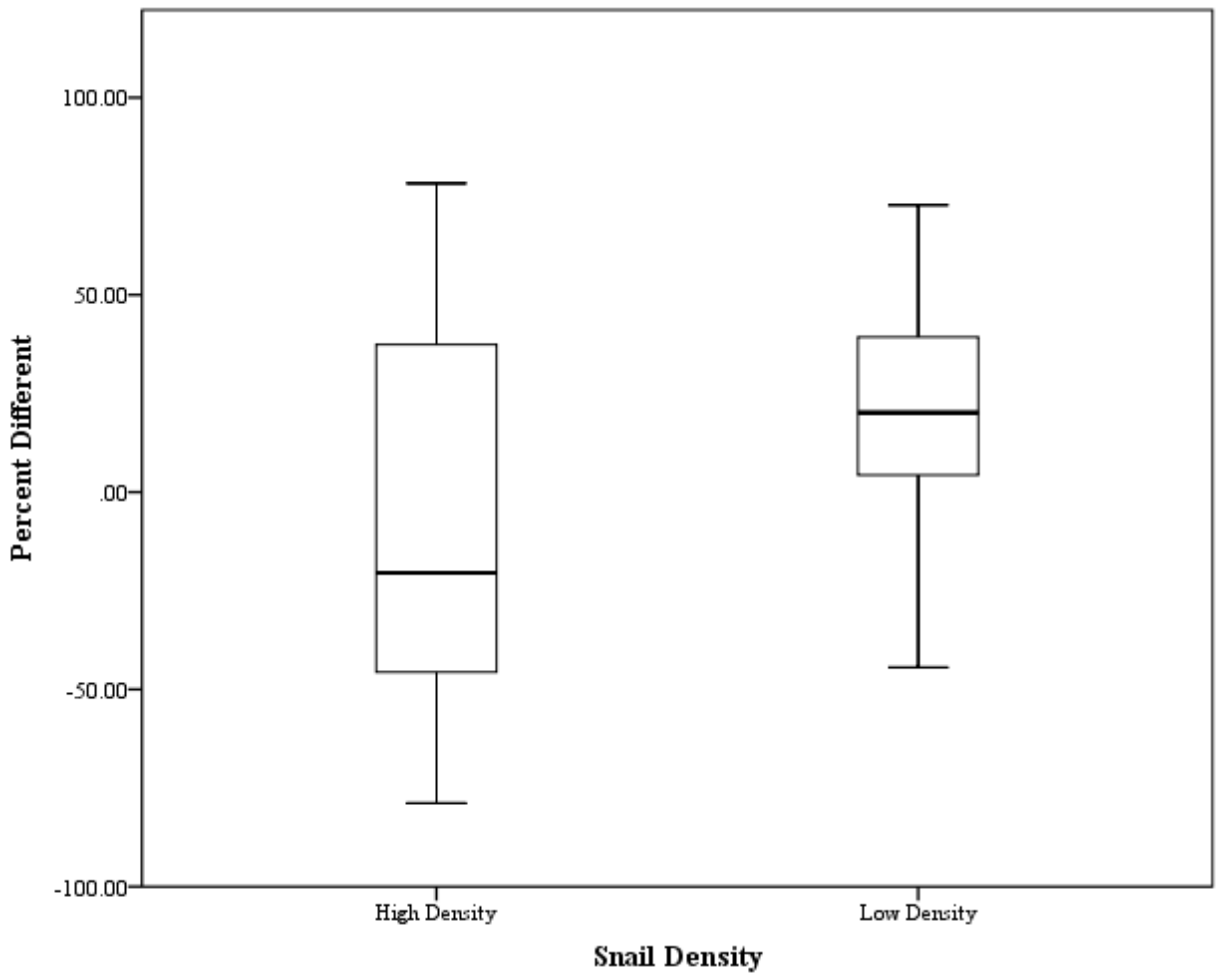

Figure 1.6 Percent difference between the concentrations of chl *a* in ungrazed and grazed sediments, normalized to the ungrazed concentration. There is a minimal mean difference in grazed and ungrazed total chl *a* in the "high density" mudflat, whereas there is significantly more total chl *a* in the ungrazed areas of the "low density" mudflat.

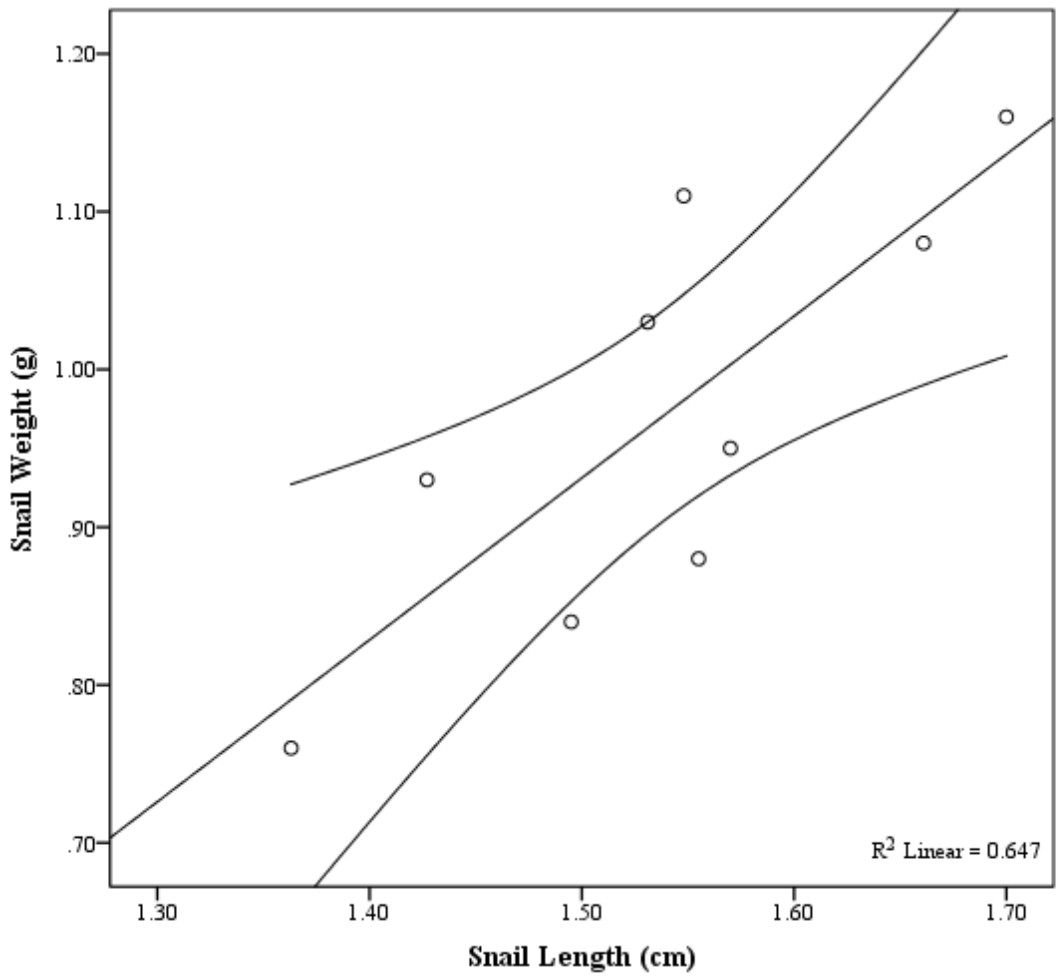

Figure 1.7 Linear relationship between snail length and weight. There is a significant, positive relationship between the two variables (p=0.009).

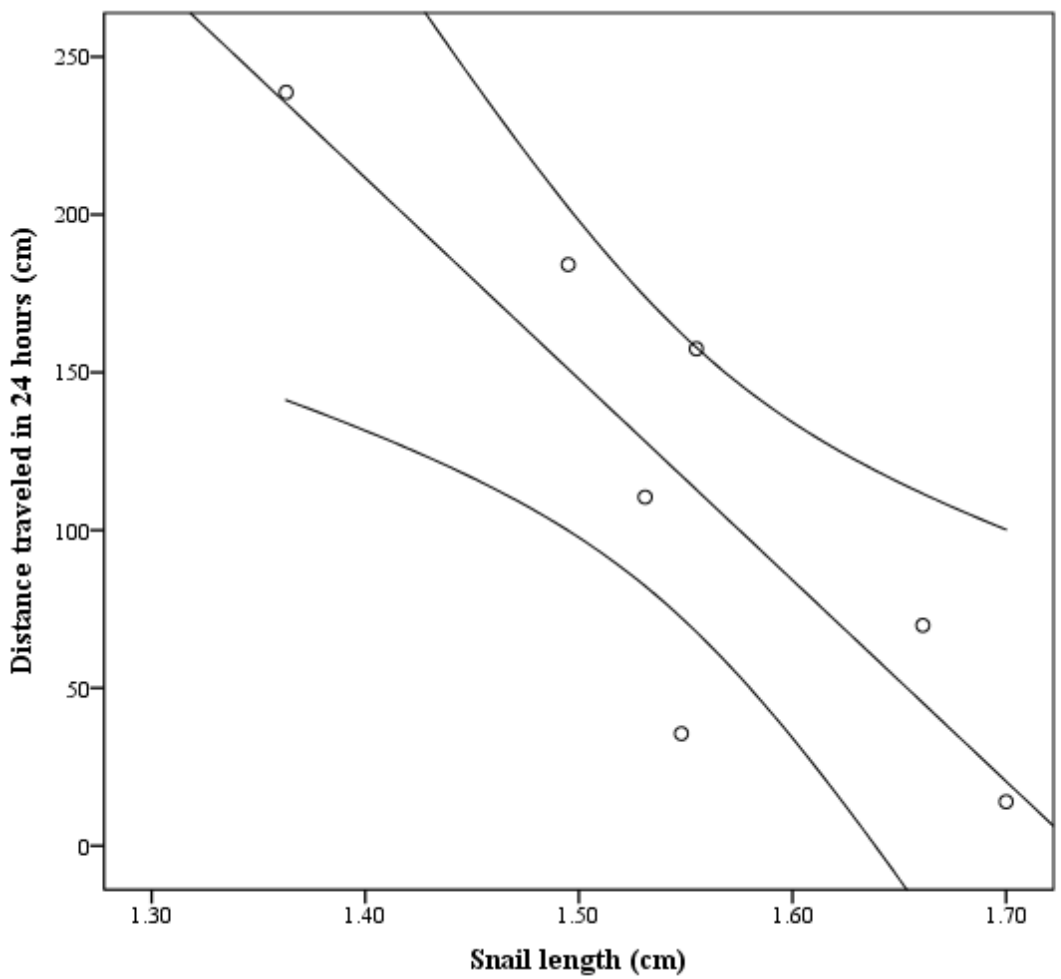

Figure 1.8 Linear relationship between snail length and 24 hour straight-line distance. There is a significant, negative relationship between snail length and distance traveled (p=0.014).

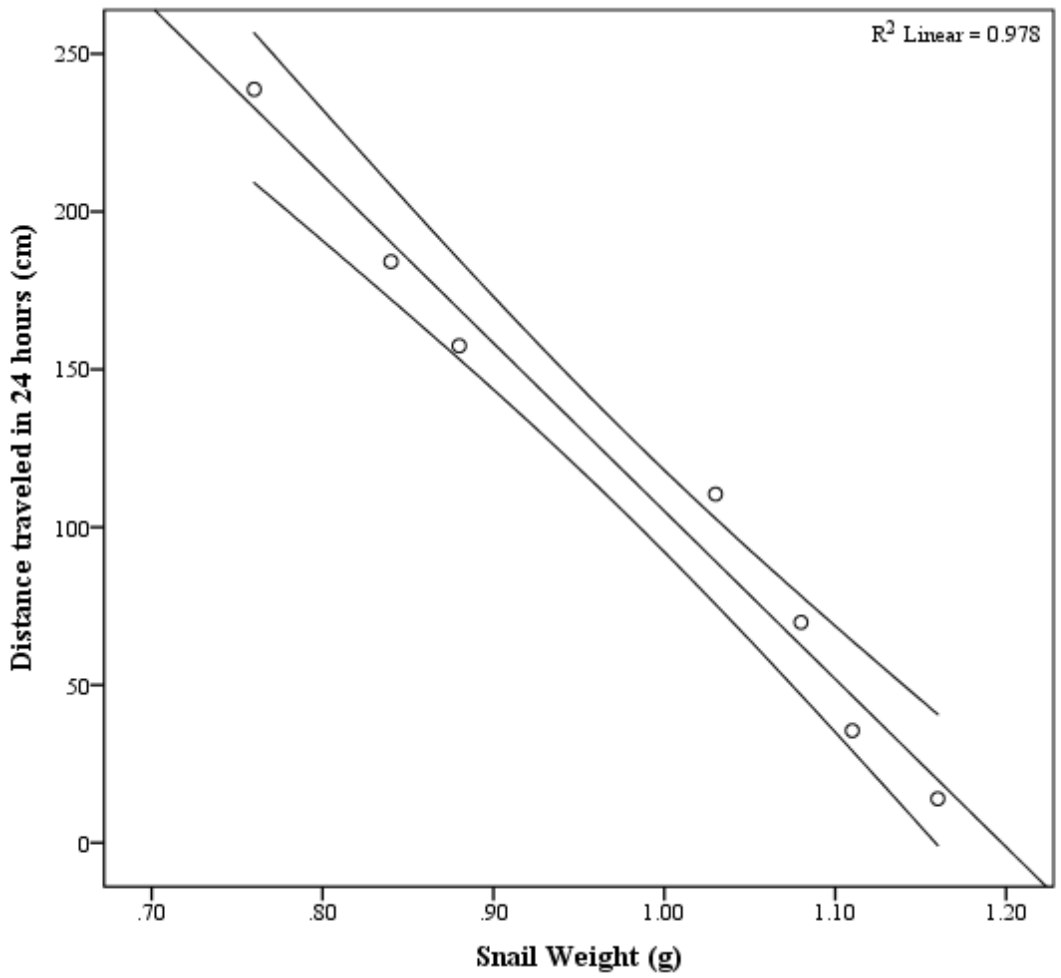

Figure 1.9 Linear relationship between snail weight and distance traveled in 24 hours. There is a significant, negative relationship between the two variables (p=0.000023).

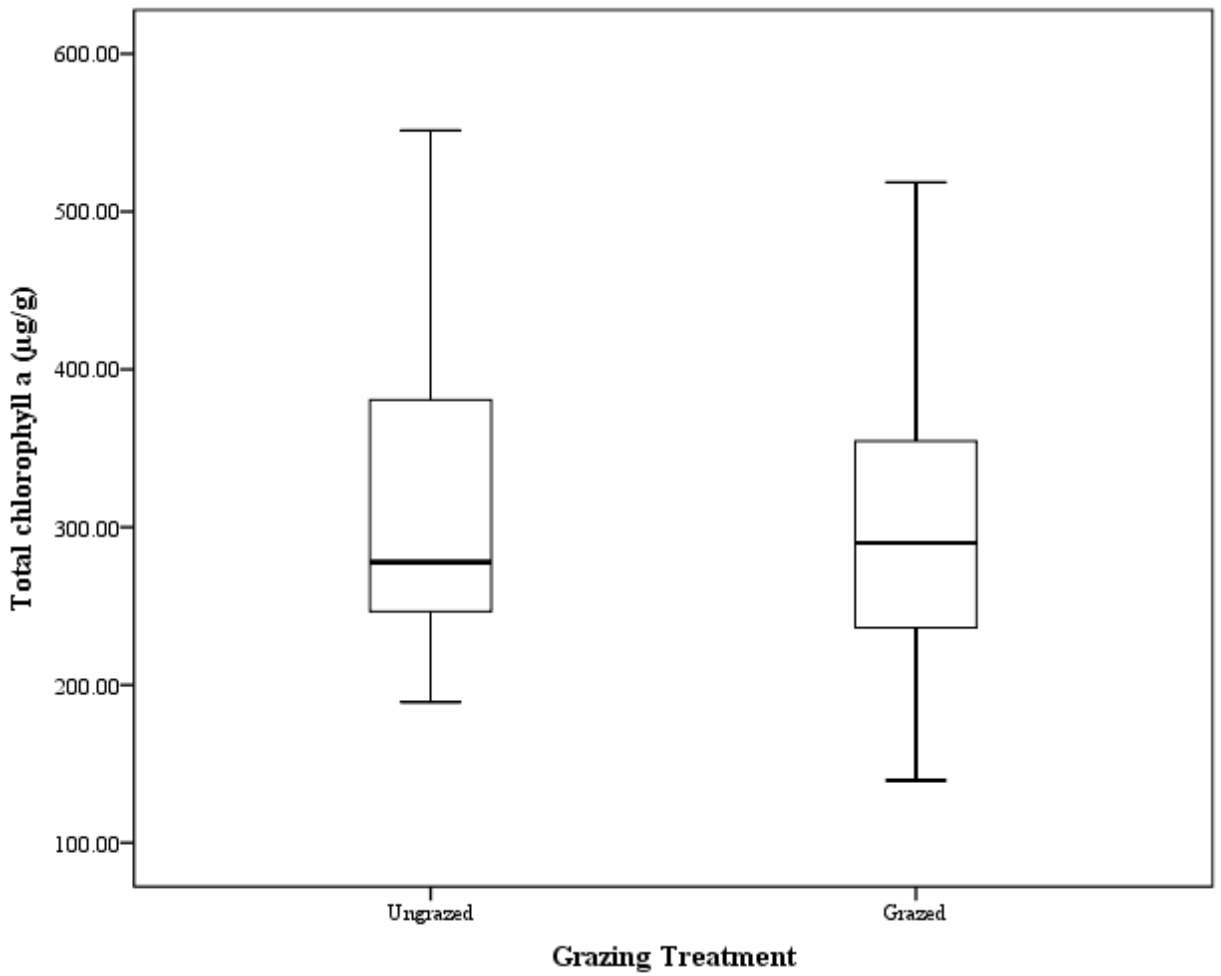

Figure 1.10 Concentration of total chl *a* in paired petri dishes. There was no significant difference between total chl *a* in the grazed and ungrazed dishes (paired t-test, p=0.361).

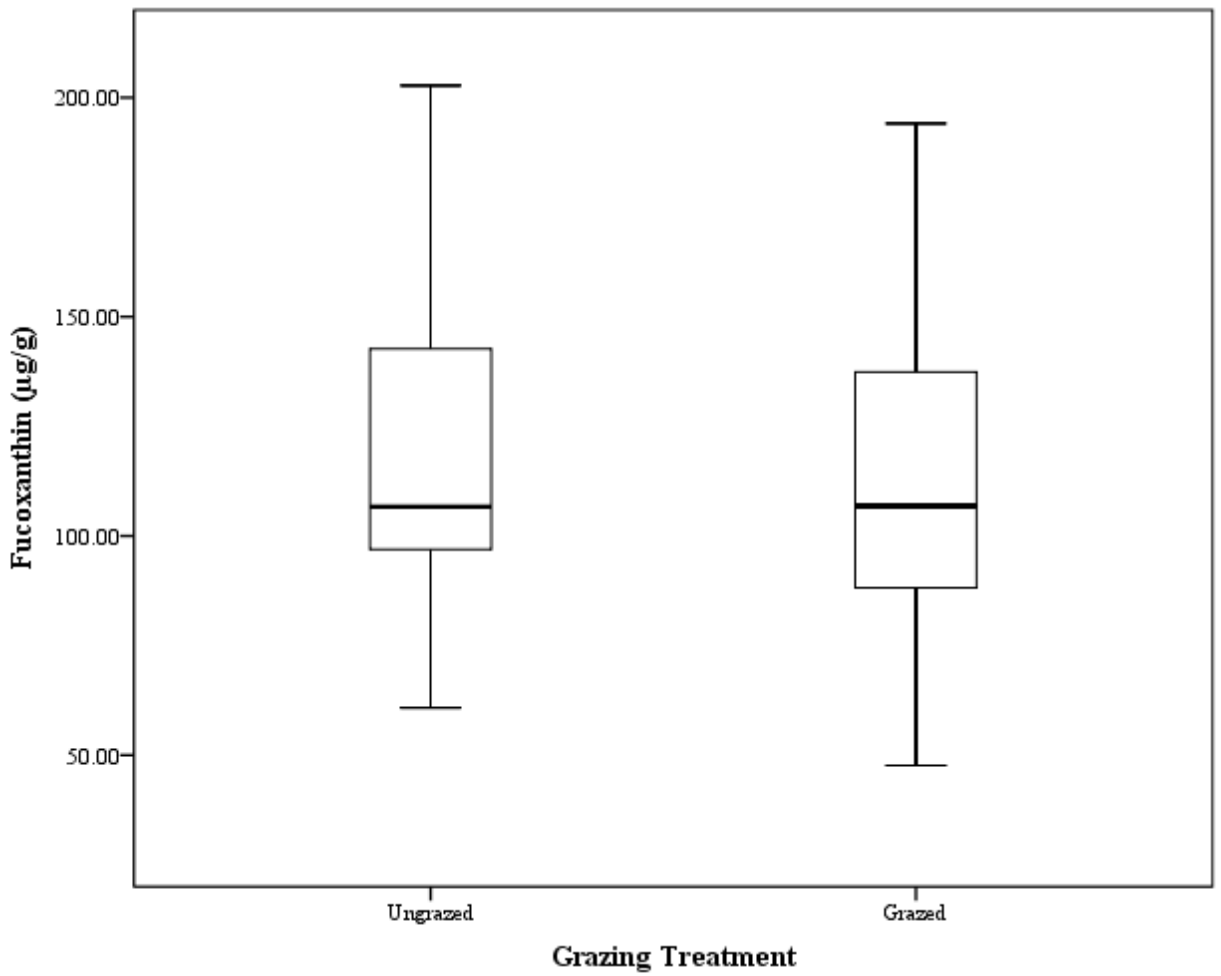

Figure 1.11 Concentration of fucoxanthin in paired petri dishes. There was no significant difference between fucoxanthin in the grazed and ungrazed dishes (paired t-test, p=0.388).

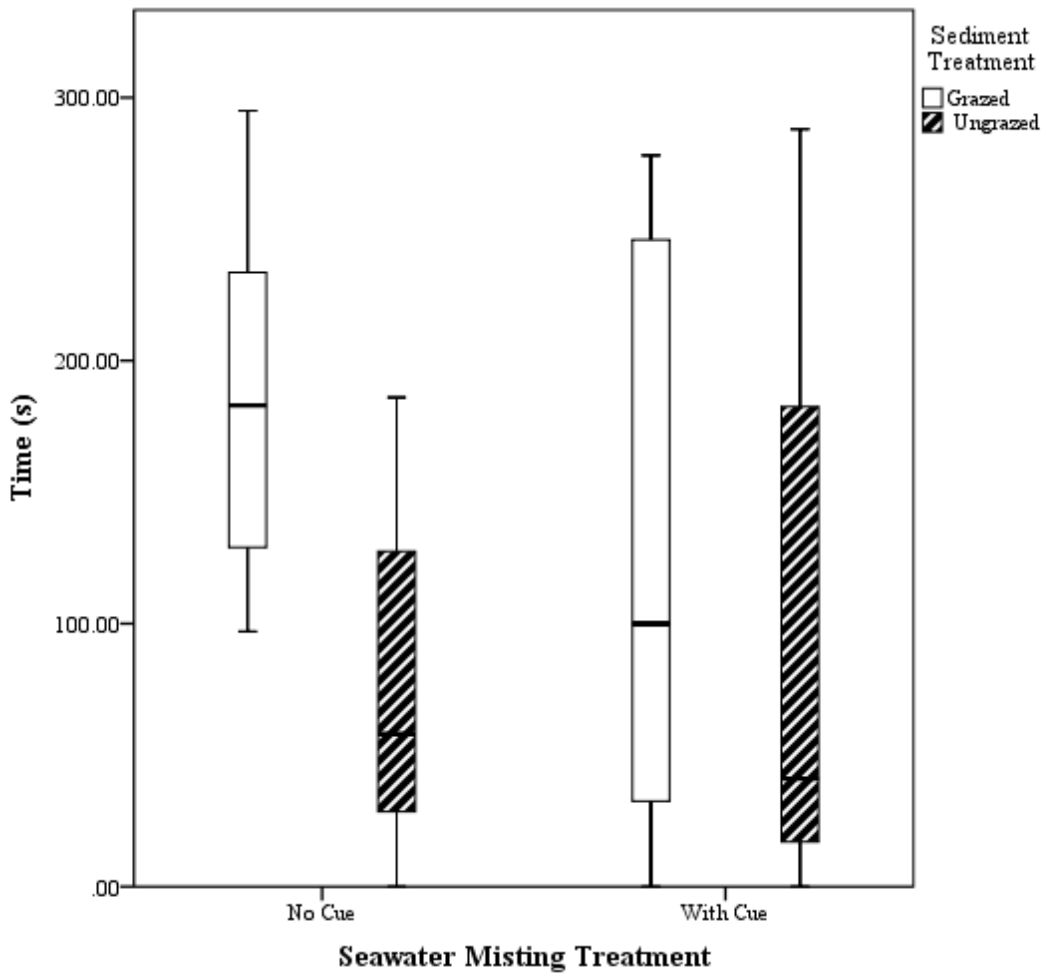

Figure 1.12 Average amount of time snails spent on different sediment treatments. Snails spent more time on sediments that had been previously grazed by conspecifics when misted with regular seawater (paired t-test, p=0.062). There was no significant difference in time spent on each side when snails were misted with snail-cue infused seawater (paired t-test, p=0.730).

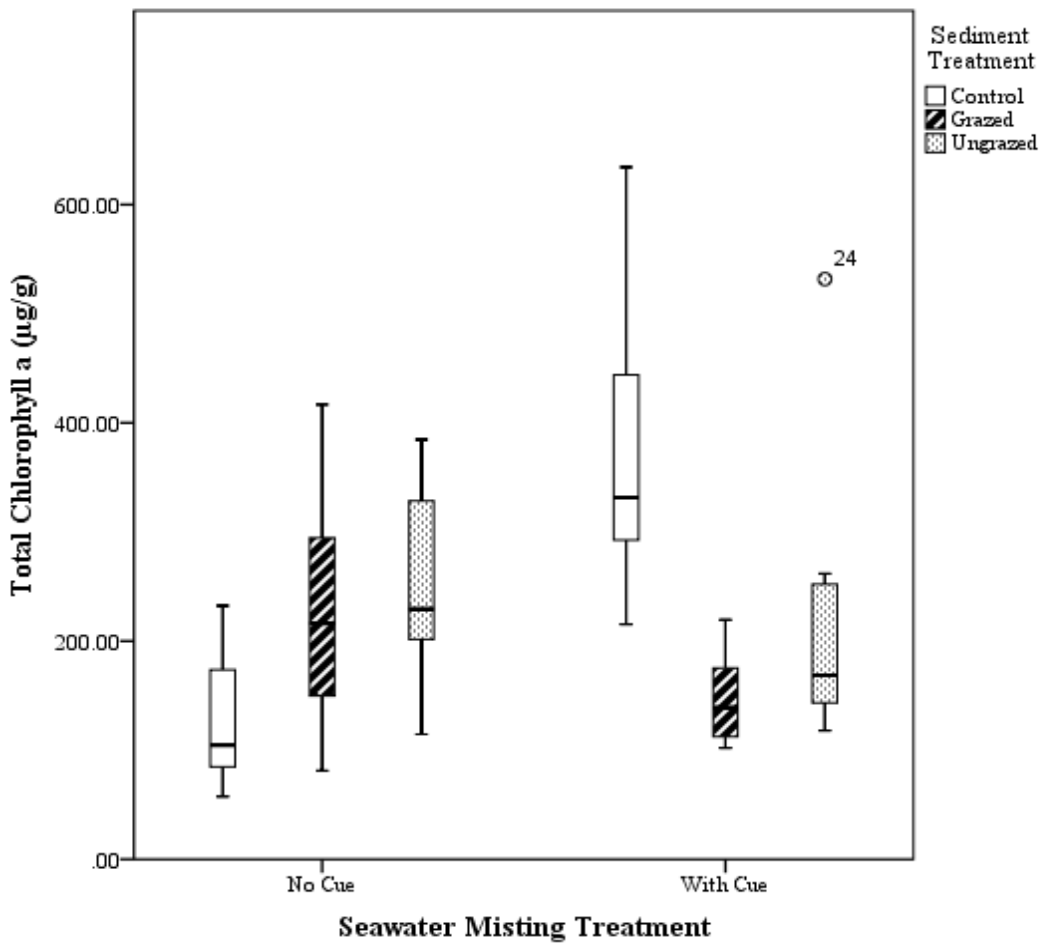

Figure 1.13 Concentrations of total chl *a* in three sediment types with misting treatments. For the No Cue misting treatment (misted with regular seawater), there was no significant difference in total chl *a* in the three sediment treatments (ANOVA, p=0.058). For the With Cue treatment (misted with snail-cue infused seawater), there are two homogenous groups: (1) the control and (2) grazed and ungrazed. There is a significant difference between groups 1 and 2 (ANOVA, p=0.002).

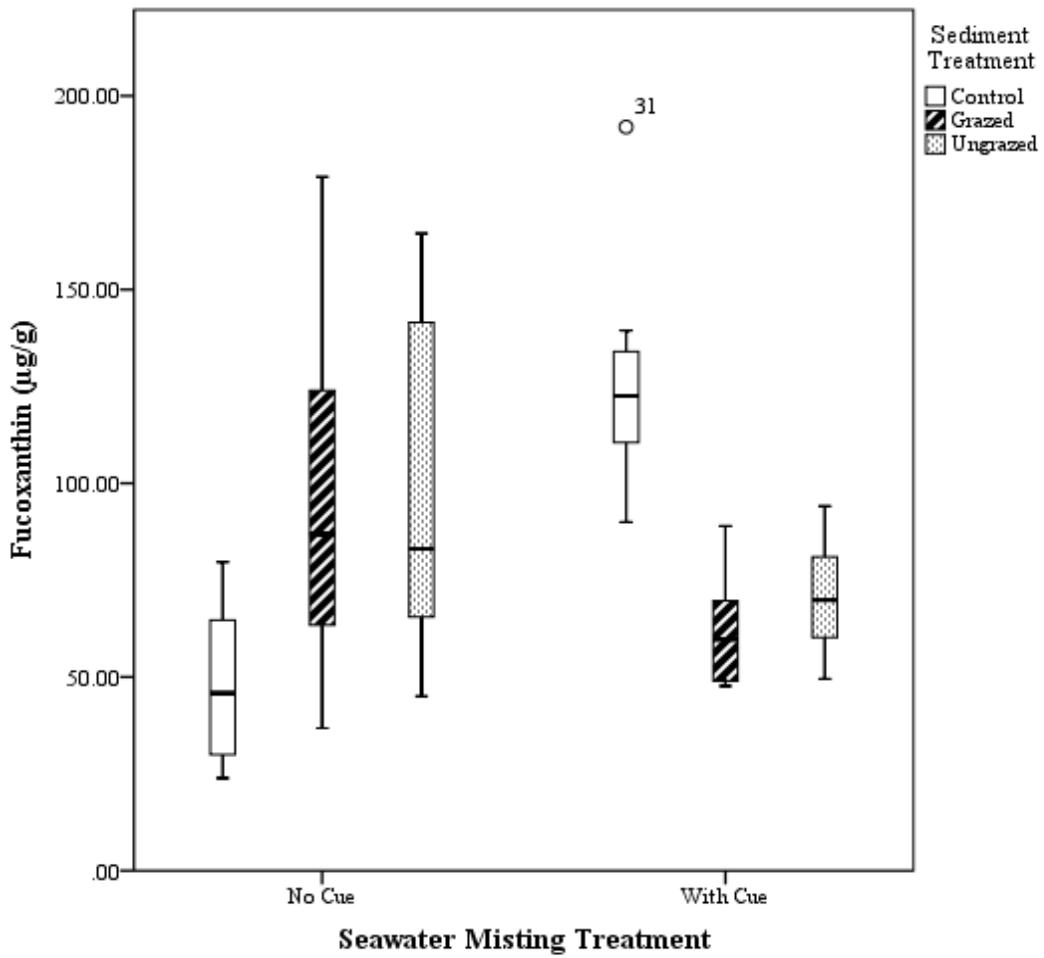

Figure 1.14 Concentrations of fucoxanthin in three sediment types with misting treatments. For the No Cue misting treatment (misted with regular seawater), there was no significant difference in fucoxanthin in the three sediment treatments (ANOVA, p=0.055). For the With Cue treatment (misted with snail-cue infused seawater), there are two homogenous groups (1) the control and (2) grazed and ungrazed. There is a significant difference between groups 1 and 2 (ANOVA, p=0.000075).

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