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Assessing Feeding Preferences and Spatial Patterns of *Lagodon rhomboides* Linnaeus

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Abstract-- Mesograzers have the ability to greatly mitigate the effects of eutrophication in seagrass systems. In this study we look at pinfish (*Lagodon rhomboides* Linnaeus) as a potential epiphytic grazer and assess feeding preferences during a transitional stage in the ontogenetic diet shift exhibited by these fish. Since pinfish are abundant in seagrass meadows in the northern Gulf of Mexico, their dietary preferences have the potential to greatly impact seagrasses in this system. Twenty-four hour feeding trials were conducted to determine pinfish preference between seagrass (*Thalassia testudinum*) and algal epiphytes. St. Joseph Bay, FL was also surveyed to determine areas within this ecosystem that could be highly impacted by pinfish abundance. Significant spatial patterns were found among pinfish, as well as urchins and invertebrates, suggesting that some areas might be experiencing stronger grazing pressures. Feeding trials support previous studies showing that pinfish consume little to no *T. testudinum* and spatial patterns within St. Joseph Bay support past research showing that *S. filliforme* is a preferred seagrass for pinfish. Data regarding epiphytes as a preferred food source were inconclusive, as variation was high among treatments; further study is required.

Introduction-- The Gulf of Mexico is both a commercially and ecologically essential water body that supports numerous fisheries, diverse marine wildlife, and a profitable tourism industry (EPA 2012). There are six species of seagrass along the northern Gulf coast, although the most common are *Thalassia testudinum* König (turtle grass), *Halodule wrightii* Ascherson (shoal grass), and

Syringodium filiforme Kützing (manatee grass). Seagrasses are productive, flowering marine plants that are important to marine ecosystems, providing a number of services such as nutrient cycling (McGlathery *et al.* 2007), sediment stabilization (Orth 1977), current deflection, and dissipation of kinetic energy that provides protection from tropical storms (Fonseca *et al.* 1982). They also serve as nursery and foraging grounds for numerous vertebrates and invertebrates, many of which are commercially important (Beck *et al.* 2001, Heck *et al.* 2003). Finally, seagrasses provide globally significant carbon sequestration, with 30% of total ocean carbon storage (Duarte & Cebrian 1996). Unfortunately, the distribution of these vitally important organisms is declining at an alarming $110 \text{ km}^2 \text{ yr}^{-1}$ (Waycott *et al.* 2009).

There are many factors that contribute to seagrass decline, but the primary cause is eutrophication (Hemminga & Duarte 2000). Seagrasses grow comparably slower than other marine primary producers, but have the ability to grow in nutrient poor environments where growth of other primary producers is limited (Duarte 1995). When nutrients are added into seagrass systems, it allows the phytoplankton, epiphytic algae, and macroalgae to bloom, blocking light from seagrass leaves and causing potentially lethal anoxic sediment conditions (Hemminga & Duarte 2000). Borum *et al.* (1985) found that nutrient addition caused phytoplankton to increase 5-10 fold, while epiphytes increased 50-100 fold. Higher epiphytic biomass is also found in submerged vascular plants in estuarine ponds, and the presence of these epiphytes has been shown to cause reductions in diffusive transport of important nutrients like carbon, nitrogen, and

phosphorous, in addition to reducing available light (Twilley *et al.* 1985). Lapointe *et al.* (1994) found that land-based nutrient enrichment in the Florida Keys increased epiphytic biomass and macroalgae which, in turn, reduced dissolved oxygen in the sediment, attenuated light, and lead to an overall decline of *T. testudinum* and a gradient of habitat damage from near shore to offshore. These are powerful examples that clearly demonstrate the effects eutrophication has on seagrasses and their epiphytes.

Grazers that remove epiphytic algae could enhance the resilience of seagrasses to eutrophication (Baden *et al.* 2010, Orth and van Montfrans 1984, Tomas *et al.* 2005); however, grazers that consume both epiphytes and seagrass have negligible effects on seagrasses in eutrophic conditions (Hughes *et al.* 2004). It has been shown that many grazers prefer seagrass blades with the epiphytes still intact (Cebrian *et al.* 1996, Conacher *et al.* 1979, Lobel and Odgen 1981, Wressnig and Booth 2007). This can, in turn, have positive effects on seagrasses by facilitating the removal of older growth, which is typically the most epiphytized. Hughes *et al.* (2004) found that as water-column nutrients increased, epiphytic grazers decreased epiphytic biomass, demonstrating that immediate 'top down' forces can free seagrasses from intense competition for light, in turn protecting the grasses from other detrimental effects of eutrophication. Several studies have shown the capabilities of epiphytic grazers to reduce epiphytic biomass, mitigating the effects of eutrophication on seagrass (Hootsman and Vermaat 1985, Howard 1986, Neckles *et al.* 1992, Whalen *et al.* 2013, Williams and Ruckelshaus 1993). Because grazer preference can mediate

competition between seagrasses and epiphytes, it is important to understand which organisms grazers are actually consuming as well as the rates of consumption.

Pinfish (*Lagodon rhomboides* Linnaeus) are omnivorous fish, belonging to the Sparidae family, and inhabit Gulf of Mexico seagrass beds in the spring and summer months (Hansen 1969). Pinfish are believed to be important seagrass grazers, yet data regarding the primary components of their diet conflicts. Little is known about the life history of these fish. We know that they are omnivorous, with an ontogenetic shift in their diet from invertebrate prey in their adolescence to a mostly herbaceous diet in adulthood (Hansen 1969, Stoner & Livingston 1984). Specific pinfish size classes have been categorized into different trophic stages based on the composition of pinfish gut contents (Stoner & Livingston 1984). The literature suggests that there are 3 major trophic stages a pinfish transitions through as they age: (1) a carnivorous stage in which pinfish consume mostly small invertebrates, (2) an omnivorous feeding stage during which pinfish are transitioning from invertebrates to a more herbaceous diet, (3) an herbivorous trophic stage in which pinfish purportedly consume strictly plant material, usually dominated by seagrass. The middle, transitional, trophic stage includes pinfish that are seven to twelve cm standard length (SL). The ambiguity in this particular feeding period raises questions about pinfish's potential impacts on the seagrass beds they are found in, as pinfish diet choice could be helping or harming seagrasses. Finally, we know that pinfish are migratory, leaving estuaries in the early fall and returning in the spring (Hansen 1969), but we do

not know the specifics (such as timing) of this migratory pattern. If pinfish are important grazers, this could have a seasonal effect on the food web, since the presence or absence of mesograzers is known to have significant effects on epiphytic algae (Alcoverro *et al.* 1997, Baden *et al.* 2010, Whalen *et al.* 2013).

Gut content analyses suggested that pinfish, in later stages of their development, consume both epiphytic algae and seagrass (Hansen 1969, Stoner & Livingston 1984, Luczkovich & Stellwag 1993, Heck *et al.* 2015) and that seagrass was the major component of their diets at these stages (Hansen 1969, Stoner & Livingston 1984). Some studies have even suggested that pinfish prefer specific species of seagrass, such as *H. wrightii* or *S. filiforme* over *T. testudinum* (Prado & Heck 2011). Montgomery and Targett (1991) examined the pH of pinfish stomachs and determined that the pH was low enough to lyse plant cell walls, although digestion of the cell wall was not observed. Further investigation of the pinfish gut has revealed that there are carboxymethylcellulase (CMCase)-producing bacteria present in the intestines of pinfish (Luczkovich & Stellwag 1993). The same study also reports that CMCase-producing bacteria are most dense when pinfish are consuming a high number of invertebrate grazers, and that the bacteria taper off as the fish age to adulthood, when they become more herbivorous. The bacteria, therefore, were independent of the amount of plant material in the diet, leading to differing explanations as to how and why the bacteria are present in the guts of pinfish. One hypothesis is that the bacteria were consumed with detrital matter; other hypotheses include the possibility of the bacteria being present in the guts of the invertebrate prey consumed in

adolescence (Luczkovich & Stellwag 1993). These studies indicate the possibility of pinfish being able to digest plant material, but the ability to do so has not been definitively established.

Pinfish consume significantly less seagrass and have much longer evacuation times than well-documented seagrass grazers like parrotfish (Heck *et al.* 2015). Furthermore, data from recent feeding trials (Prado & Heck 2011) show that very small amounts of seagrass were actually consumed by pinfish ($< 0.08 \text{ gWW ind}^{-1}, \text{ d}^{-1}$). Unfortunately these feeding trials did not include epiphytic algae as a possible food source. In addition, isotopic data, which reflect food that has actually been digested and assimilated, have suggested that pinfish consume algae, not seagrass (Mutchler 2005). Heck *et al.* (2006) attributed the reduction of small crustaceans, epiphytes, and seagrass biomass in a nutrient-elevated treatment to pinfish, implying that as they grew during the five-month experiment, their change in diet allowed them to have an effect on different trophic levels. Thus, we still do not understand the preferences of this important grazer as it's diet shifts, nor do we understand the potential impacts these preferences could be having on the food web in this system. Because of their abundance, pinfish may be a key component in the food web structure of seagrass beds and critically important in system response to nutrient pollution if they are grazing significantly on epiphytes at any stage in their lifecycle.

Previous research has not sufficiently identified the potential role of epiphytes in the pinfish diet, nor has it determined the potential impacts of pinfish's ontogenetic dietary shift on the system in question. The goal of this

study was to determine any preference pinfish might have in their transitional, omnivorous trophic stage in hopes of assessing their impact on seagrass beds in the Gulf of Mexico. We hypothesize that pinfish within this second trophic stage prefer algal epiphytes to seagrass and that they are more likely to consume seagrass that still has the epibiome intact. We also aimed to assess any spatial patterns within St. Joseph Bay, FL to further understand the relationship between pinfish and the seagrasses they inhabit. If pinfish are important grazers, we expect to see distinct relationships between pinfish abundance and primary producers in the field. We would also expect to see negative relationships between pinfish and other grazers, such as urchins or gastropods, that might be potential competitors.

Materials and Methods-- Field Collection—All samples were collected in St. Joseph Bay, FL during the summer months of 2014 at seven sites along Cape San Blas (Figure 1). Sites were chosen to be representative of the seagrass beds along the entire North-South axis of the western edge of the bay. This allowed us to determine any gradients in abundance that might be associated with biological, physical, or chemical characteristics of the bay. At each site, 3 “zones” were identified at varying distances from the shore, amounting to a total of 21 sampling locations. Zone A was closer to shore and typically occurred in large dense seagrass beds in depths ranging from 0.06 m to 0.63 m (Table 2). Zone B was typically located just before a sandbar that occurred further offshore. Finally, zone C was our further offshore zone, located

on the bay side of the sandbar. At each zone we assessed pinfish abundance, invertebrate abundance and diversity, as well as percent seagrass cover along a 50 meter transect. Along the same transect, an additional urchin count was done to gain an understanding of the relative pinfish to urchin ratio within a standardized area. Pinfish and urchin abundance data were collected by snorkeling 50 x 2 meter belt transects at each zone; all pinfish and urchins within the transect were counted and recorded. A second observer snorkeled the transect to record the number of urchins in the transect. Care was taken to ensure that visibility was always suitable for identification of fish to species and so the edges of the transect were easily visible. Researchers snorkeled at a consistent rate along the transect without making any stops.

Quarter meter-squared quadrats were used to assess invertebrate abundance and diversity, as well as percent seagrass cover. Every 10 meters along the transect, collectors would sweep through the quadrat 5 times with a dip-net, counting and identifying invertebrates after each sweep (see Table 1 for taxonomic levels of classification). Pilot tests showed that few, if any, invertebrates were present after five sweeps. After invertebrates were counted, percent cover of seagrass was visually estimated. The same individual determined percent cover at every quadrat for consistency. This was done for all three zones at all seven sites along the cape.

Three *T. testudinum* shoots were collected from representative locations within each zone at each site and brought back to the lab at Kennesaw State University for further processing. In the lab, each blade from every shoot was

manually scraped with a razor blade and the epiphyte matter was collected onto pre-dried filters and placed into a drier at 60° C. Length and width of each blade was also recorded. Once filtered matter was dried, all filters were weighed to determine epiphyte mass per unit surface area of seagrass leaves.

Feeding trials-- Twenty-four hour feeding trials were used to determine feeding preferences of *L. rhomboides*. Each feeding trial included one fish and three food options: a shoot of *T. testudinum* that had been cleansed of epiphytes, a shoot of *T. testudinum* with epiphytes intact, and two strips of nitex “blades” with epiphytic colonization. These food items were never completely depleted. Fish were placed in 10-gallon aquaria 24 hours prior to the experiment to in order to acclimate. After 24 hours of fasting, the treatments were placed on each side of the tank in random positions, where they were left for 24 hours (Figure 2). After the trial, each seagrass shoot was then weighed and photographed for later image analysis using Image J 1.48v.

Pinfish were collected in St. Joseph Bay during July and August of 2014 and May of 2015 using seine nets. Fish were then transported back to Kennesaw State University via an aerated cooler. All fish were housed in aquaria with a salinity maintained around 32 ppm. Fish were fed a diet of frozen brine shrimp and a frozen marine omnivore mix of shrimps, krill, plankton, lettuce, spirulina, and spinach. Epiphytes were colonized on nitex mesh that was placed in *T. testudinum* beds in the St. Joseph Bay for several weeks until there was a thick assemblage of algae on the mesh. The nitex was kept in aquaria under grow lamps after collection. All *T. testudinum* were collected from St. Joseph Bay the

week prior to feeding trials. All trials were conducted in 10-gallon aquaria, which were divided in half with a mesh screen, allowing water to flow freely in the tank. The divider effectively created a control side and experimental side of the tank. We randomized the location of all food options, as well as which side would be the control (Figure 2). Prior to the experiment, wet weights were recorded for each seagrass shoot. All shoots were then photographed for image analysis to determine the area of each blade. Scraped seagrass shoots were scraped of epiphytes before being weighed; all epiphyte matter was filtered and dried for further analysis to determine epiphyte cover. Change in biomass was calculated as weight of the shoot before start of the trial minus the weight at the conclusion of the trial, therefore positive values indicate net loss of biomass.

Change in chlorophyll was used to assess epiphyte grazing off the nitex mesh. When the nitex strips were removed for feeding trials, one strip (or “blade”) was set aside to serve as the “before” measurement of total chlorophyll per area, since true “before” measurements could not be taken due to the destructive nature of the method used for chlorophyll analysis. After trials were complete, the total chlorophyll contents of all blades were analyzed spectrophotometrically in an acetone extraction using the equation:

$$\text{Total chlorophyll} = 11.0(\text{Abs}_{665} - \text{Abs}_{750})v/A\rho$$

Where Abs_{665} and Abs_{750} are the absorbances measured at 665 and 750 nm respectively, v is the volume of solvent into which the chlorophyll was extracted, A is the area of the nitex strip, and ρ is the path-length of the cuvette. Photos were then taken of all nitex blades to determine area via image analysis. Change

in area was calculated as before area minus after area, therefore positive values indicate net loss of area.

SAS was used to conduct two-factor ANOVA to assess the effects of site and zone on the following response variables: abundance of pinfish, percent cover of *T. testudinum*, total seagrass cover, total invertebrate abundance, abundance of urchins, total grazer abundance, and depth. Tukey's post hoc comparisons of least-squared means was used to examine differences among sites and zones when main and interactive effects were significant. Microsoft Excel was used to perform t-tests and correlations analyses for feeding trials, specifically to analyze consumption of food items between controls and experimental treatments. Data were considered significant at an alpha level of 0.05.

Results-- Field Collection: Significant zone differences were found for pinfish ($p= 0.034$), urchins ($p= 0.016$), and total invertebrates ($p= 0.034$; Table 3). Urchins and pinfish exhibited similar spatial patterns, with significantly higher abundances at the C zones (pinfish mean \pm se= 110.0 ± 16.5 , urchins= 200.0 ± 63.25) than the A zones (pinfish= 52.0 ± 17.6 , urchins= 0 ± 0), showing a trend of elevated densities at the sites further from shore (Figures 5 & 4). Total invertebrate abundance was significantly higher at the A zones (41.0 ± 11.0) than the C zones (12.0 ± 1.9 ; Figure 3). Significant site differences were found in percent cover of *T. testudinum* ($p= 0.006$) showing that there was significantly less *T. testudinum* at site one than any other site (Figure 8). Data also indicated

that there was significantly more *S. filiforme* at site one than any other site (Figure 8). Site one also had significantly more pinfish than any other site ($p=0.0189$; Figure 9). Percent cover data indicate that *T. testudinum* was the most prolific seagrass species in St. Joseph Bay and dominated all sites except for site one (Figure 8).

Significantly negative correlations were found between *T. testudinum* and pinfish, and a significantly positive correlation was found between *T. testudinum* and Total invertebrates (Table 4). There were no significant correlations between pinfish and other potential grazers in the system (i.e. snails and urchins; Table 4).

No significant differences were found in epiphyte biomass across sites ($p=0.329$; Figure 10) or zones ($p=0.487$; Figure 7), and there were no significant correlations found between pinfish and epiphyte coverage (Table 4). Two-way ANOVA analysis found no significant interaction between site and zone differences in percent cover of total seagrass (Table 3; Figure 11).

Feeding Trials-- There were no significant changes in biomass or chlorophyll found in any of the food items given to the pinfish after the 24-hour feeding period. Changes in wet weights for scraped seagrass were essentially zero, with the mean change in wet weight being -0.024 ± 0.058 g (Figure 13). In many cases the weight was higher after the trial, suggesting that the only difference was the amount of water on the blades when wet weights were determined. With unscraped seagrass, a slight change in weight was detected (mean change in weight = 0.253 ± 0.077 g). Change in biomass of unscraped seagrass was $0.047 \pm .107$ g. There was no significant difference between the

control and experimental treatments for either seagrass options (Scraped: $t=2.23$, $df= 10$, $p=0.838$; Unscraped: $t= 2.23$, $df= 10$, $p=0.606$; Figures 13 & 14), further indicating a lack of grazing on *T. testudinum* altogether. However, image analyses showed a significant change in area for the unscraped seagrass blades ($t= 2.08$, $df= 20$, $p= 0.002$), but not for the scraped seagrass blades ($t= 2.1$, $df= 18$, $p= 0.0799$; Figures 15 & 16). Pinfish SL ranged from 7.2 to 12.5 cm and there were no significant correlations found between fish size (SL and weight) and amount of seagrass consumption within or across treatments (Tables 5 & 6).

No significant differences in chlorophyll were detected during feeding trials. Variation was high across nitex strips, including the strips that were supposed to serve as before measurements of chlorophyll (Figure 12).

Discussion-- We found that pinfish and urchins demonstrate similar spatial patterns in St. Joseph Bay, FL, with high abundances in the C zones. Considering the emphasis put on urchin-seagrass relationships (Heck & Valentine 1995, Klumpp *et al.* 1993, Nojima & Mukai 1990), it is interesting that we observed significant zone patterns with urchins but not seagrasses. This could indicate that urchin distribution is being driven by something other than bottom-up effects. Adult pinfish diets have been reportedly dominated by seagrass, however, we see a negative correlation between pinfish and *T. testudinum* distribution (Table 4). There were no significant zone differences in *T. testudinum* and our data, in addition to past studies, indicate that *T. testudinum* is not a viable food option for pinfish (Prado & Heck 2011, Figures 13 & 14),

suggesting that the significant relationship observed is not a trophic one.

Furthermore, there were no significant correlations between total seagrass and pinfish (Table 4).

We did not see any significant differences in epiphyte cover across sites or zones (Figures 7 & 10) nor did we see any significant correlations between pinfish and epiphytes (Table 4). There are many factors that could be contributing to these patterns. While juvenile pinfish, reportedly, move very little and have relatively small home ranges (Potthoff & Allen 2003), this behavior is still relatively understudied and it is possible that the motility of this potential grazer is reducing any patterns we might observe across sites or zones for epiphytes. Movement decouples pinfish density from the effects of grazing on epiphyte biomass. Therefore, instantaneous associations between pinfish density and epiphyte biomass may not reflect grazing activity in the recent past. It is also possible that the assemblage of algal epiphytes is different across zones even though we did not detect a difference in biomass. We did not examine differences in community structure when estimating epiphyte biomass. Grazing by pinfish may alter epiphyte community structure without impacting overall biomass. Filamentous algae produce hair-like strands while calcareous algae is more rigid with hard thalli. Calcareous algae has been found to be herbivory resistant and unpalatable to some fishes (Tsuda & Bryan 1973, Littler *et al.* 1983), and could also be difficult for pinfish to physically remove from seagrass blades. This potential difference in relative abundance could mask any relationship between pinfish and epiphyte biomass. The influence of other,

invertebrate grazers could also be attributed to a lack in significant spatial patterns of epiphytes, as invertebrate densities were high in the A zones (Figure 3) and some of those invertebrates are probably grazing on epiphytes.

All pinfish used in feeding trials were between 7.2 and 12.5 cm, falling into the range of pinfish that purportedly consume mixed seagrasses and epiphytes as well as some invertebrates; this is the omnivorous stage in their ontogenetic diet shift (Stoner 1980, Stoner and Livingston 1984). Stoner and Livingston (1984) cited that pinfish > 10 cm have a diet made up of more than 90% plant material. With that in mind, we expected to see pinfish in our feeding trials consume both epiphytes and seagrass, but perhaps at different rates and in different quantities since this is, functionally, a dietary transition period for these fish. In our trials we observed little to no consumption of *T. testudinum* without epiphytes (Figure 13), and an insignificant amount of consumption was seen on *T. testudinum* blades with the epiphytes still intact (Figure 14). We did not observe any correlation in the size of pinfish (SL length or weight) and amount of consumption (Table 6). This supports findings published by Prado and Heck (2011) that showed when pinfish were presented with three different seagrasses, they didn't consume any *T. testudinum*. The same study indicated that *S. filliforme* was the preferred seagrass for *L. rhomboides*. Gut content studies show that *S. filliforme* is also abundantly present in the guts of pinfish > 10 cm from the Big Bend region of Florida (Stoner 1980, Stoner and Livingston 1984). We did see a significant correlation with *S. filliforme* and pinfish, as well as increased abundance of pinfish at site one, where we observed the most *S. filliforme* (Table

4, Figure 8). Stoner and Livingston (1984) suggest that pinfish dentition make *S. filiforme* easier to consume than *T. testudinum* due to the structural differences of these grasses. While our pinfish abundance data are consistent with a preference for *S. filiforme*, it is important to note that *T. testudinum* is the dominant species of seagrass in St. Joseph Bay, as well as the Northern Gulf of Mexico, and is the most available food source for pinfish in this region. Based on our data and according to previous studies, *T. testudinum* does not appear to be a viable food source for pinfish in St Joseph bay.

T. testudinum was used in this experiment because, while it is not the reportedly preferred seagrass of pinfish according to Prado and Heck (2011), it is the most abundant seagrass where these fish occur (Figure 8). We didn't find any site by zone differences among seagrass cover (Figure 11), indicating that there are similar amounts of seagrass available across our sites and zones. With the exception of site one, we see pinfish relatively homogenously distributed across our *Thalassia*-dominated sites. So the question remains, if these fish are abundantly present in *T. testudinum* beds, what are they eating? Unfortunately we could not determine change in chlorophyll due to a high level of variation in our epiphyte treatments (Figure 12). We did, however, see a slight change in biomass (Figure 14) and a significant change in area (Figure 16) of those seagrass blades with the epiphytes still intact, indicating that there might at least be some consumption occurring among these treatments. Past studies have shown preference, in other fishes, for seagrass blades with epiphytes, indicating consumption of older blades is occurring (Wressnig & Booth 2007). It is also

possible that there is simply incidental seagrass consumption occurring while fishes are grazing on epiphytes. This could explain the presence of seagrass in gut contents, as seagrasses are more difficult to digest, especially as they age (Bjorndal 1980). More trials are necessary to determine if epiphytes are a preferred food source during this dietary stage in the pinfish lifecycle. It will be necessary to reduce blade-to-blade variation among the nitex treatments in order to determine any changes in chlorophyll.

It is important to note that conclusions drawn from the significant decrease in leaf area in the feeding trials must be considered with caution. The methods for determining the area of seagrass leaves could be improved, as variation was relatively high in before and after estimates of leaf area. This variation appears to be largely due to errors in the image analysis process as after estimates of leaf area sometimes exceeded those from before measurements made on the same leaves. The differences in measurements, even in control treatments, were substantial enough to suggest that effects of measurement error during image analysis were greater in magnitude than treatment effects of pinfish grazing. Therefore, further assessment of the method is necessary to evaluate the robustness of our result.

We wanted to determine the feeding preferences of *Lagodon rhomboides* in hopes of gaining a better understanding of the services they could potentially be providing their habitat. Their ontogenetic diet shift makes them a rather unique grazer, one which has the ability to remove other potentially important grazers (invertebrates), the potential to remove harmful algae (epiphytes), and the

potential to consume seagrasses. The literature suggests that all of these organisms are being consumed at some point during the pinfish lifecycle (Heck *et al.* 2000, Prado & Heck 2011, Stoner 1980, Stoner and Livingston 1984). It is possible then, that these fish have the potential to significantly affect a number of organisms and in turn, significantly affect the food web as a whole. Pinfish's abundance in the Gulf of Mexico only increases the importance of determining their feeding preferences and, as the literature has suggested, this is no simple task.

We would like to continue to further explore this question by modifying our experimental methods in hopes of determining if pinfish are successful and important epiphyte grazers. The nitex mesh was very effective for the colonization of marine epiphytes, although the assemblage of algae on the nitex was not as homogenous as we had anticipated. Furthermore, the community structure on the nitex was slightly different than that of what we see on the *T. testudinum* blades. Qualitatively, there appear to be more stalked and tube-dwelling diatoms present on seagrass blades than we observed on the nitex, although these diatoms are still present on the nitex. We observed more filamentous blue-green algae on the *T. testudinum* blades than the nitex as well, while the nitex strips had a higher density of calcareous red algae. These algal differences could be due to a lack of nutrients on the nitex, as it is possible that epiphytes on seagrass blades are receiving nutrients from the blades themselves (Penhale & Thayer 1980, McRoy & Goering 1974). Community structure differences could also be affected by the sediments that were present in

seagrass tanks, but not in epiphyte tanks. It is likely that the sediments would contribute nutrients to the epiphytes growing in the seagrass tanks. There is a possibility that these differences in community structure could contribute to any preferences pinfish may or may not have for one treatment over another, although we did not detect any significant preferences in this study. As previously stated, it is likely that pinfish would not be interested in calcareous algae and might be more attracted to filamentous algae, as it is typically more palatable and easier to physically acquire (Tsuda & Bryan 1973, Littler *et al.* 1983).

Our feeding trials suggest that either pinfish aren't eating any plant material (because there is not evidence of *T. testudinum* consumption), or they are eating algae. Since the literature suggests that plant material is abundantly present during this trophic stage (Stoner 1980, Stoner and Livingston 1984), it would stand to reason that if pinfish aren't consuming seagrasses, they must be eating algae if they are, in fact, eating plant material. We hope that our future efforts will allow us to answer this question in more depth, which will shed more light on the potential impact of *Lagodon rhomboides* in the Gulf of Mexico.

Tables and Figures--

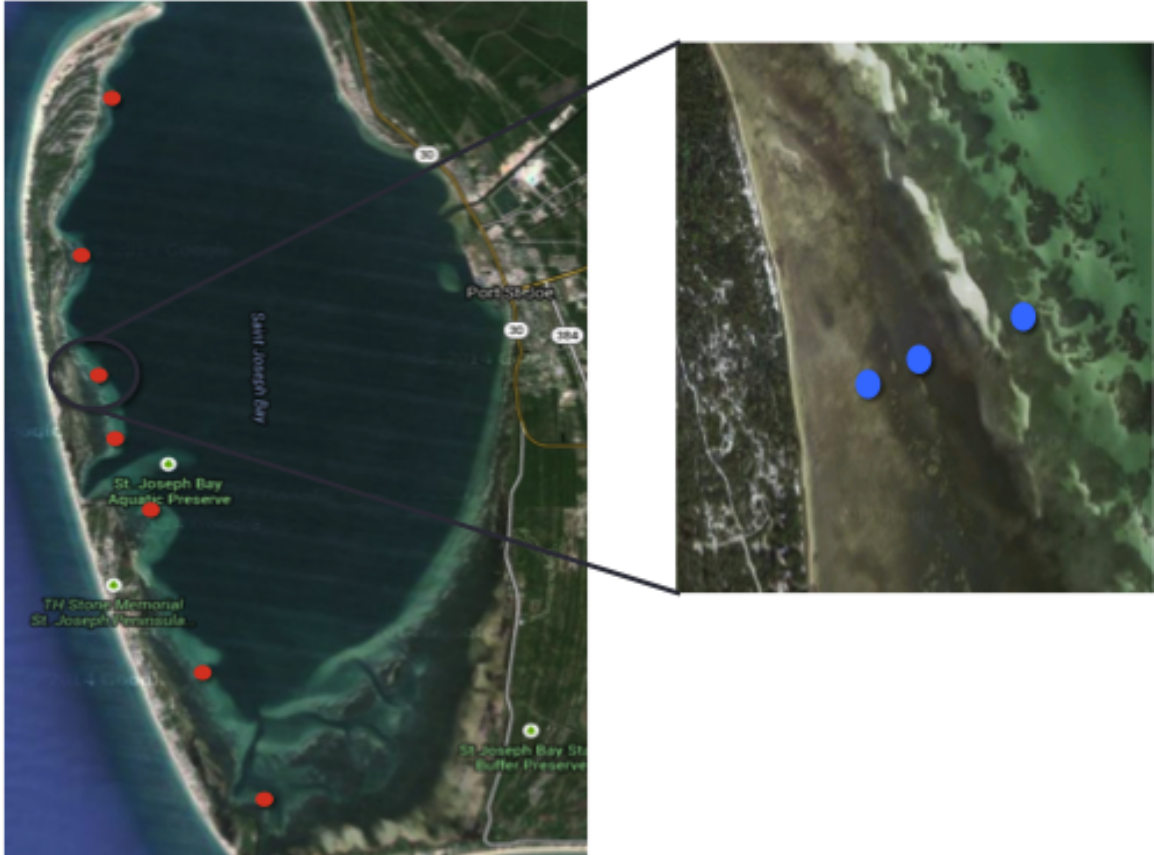


Figure 1: Left: Relative locations of all seven sites along Cape San Blas, the western border of St. Joseph Bay, in ascending order from North to South. Red dots indicate relative site locations. Right: Relative locations of zones for site 3. From left to right, blue dots show zones A, B, and C.

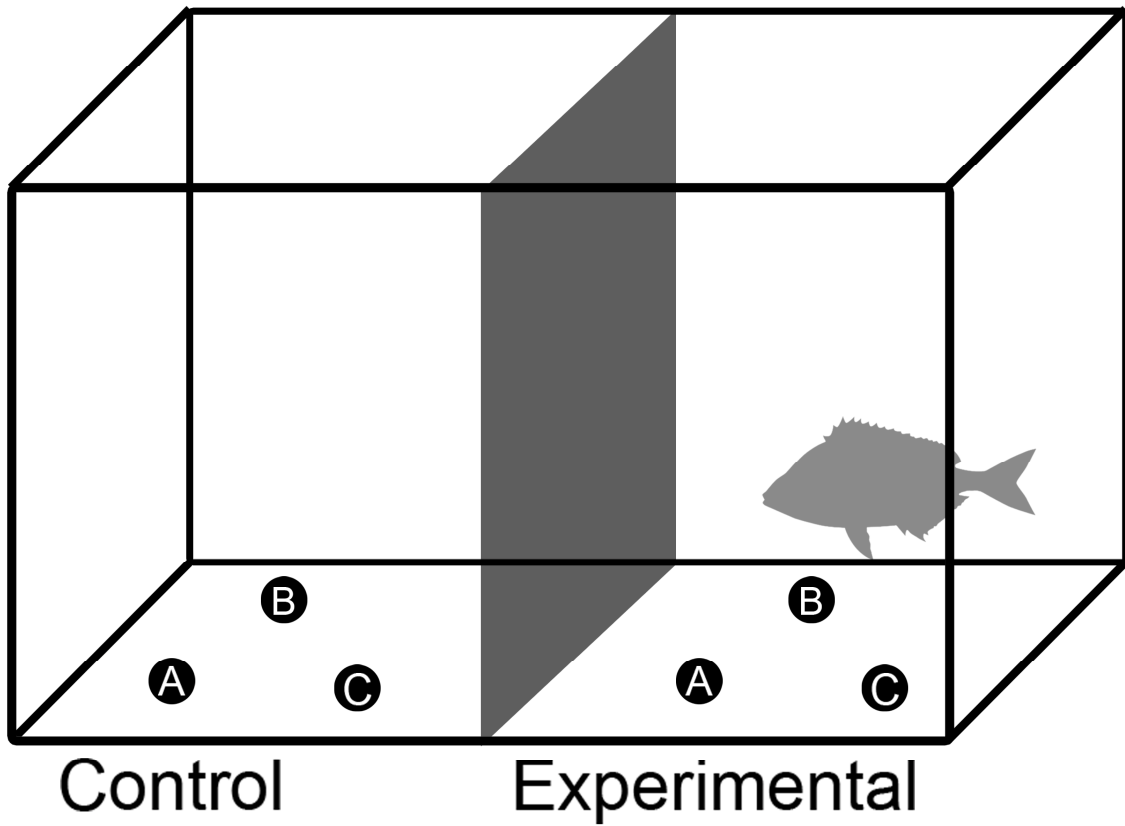


Figure 2: Schematic representation of feeding trial set-up in 10-gallon aquaria. A,B, and C represent the randomized locations for food options during feeding trials.

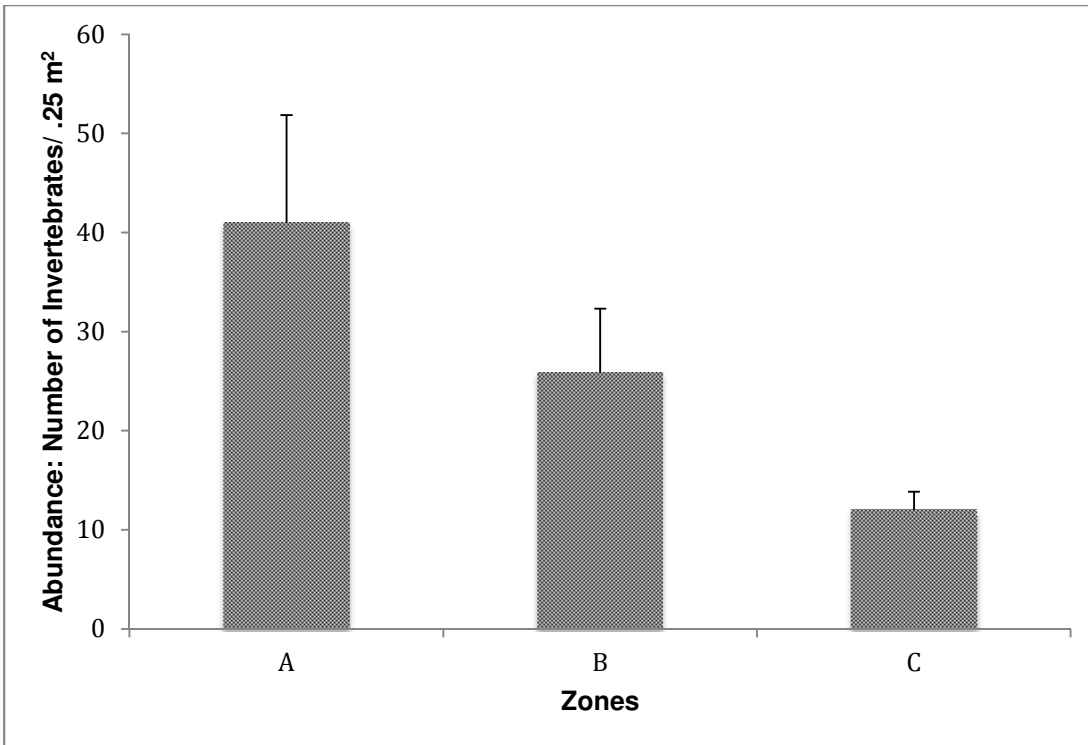


Figure 3: Total abundance of all invertebrates sampled across zones (mean±SE) N=7 for each zone.

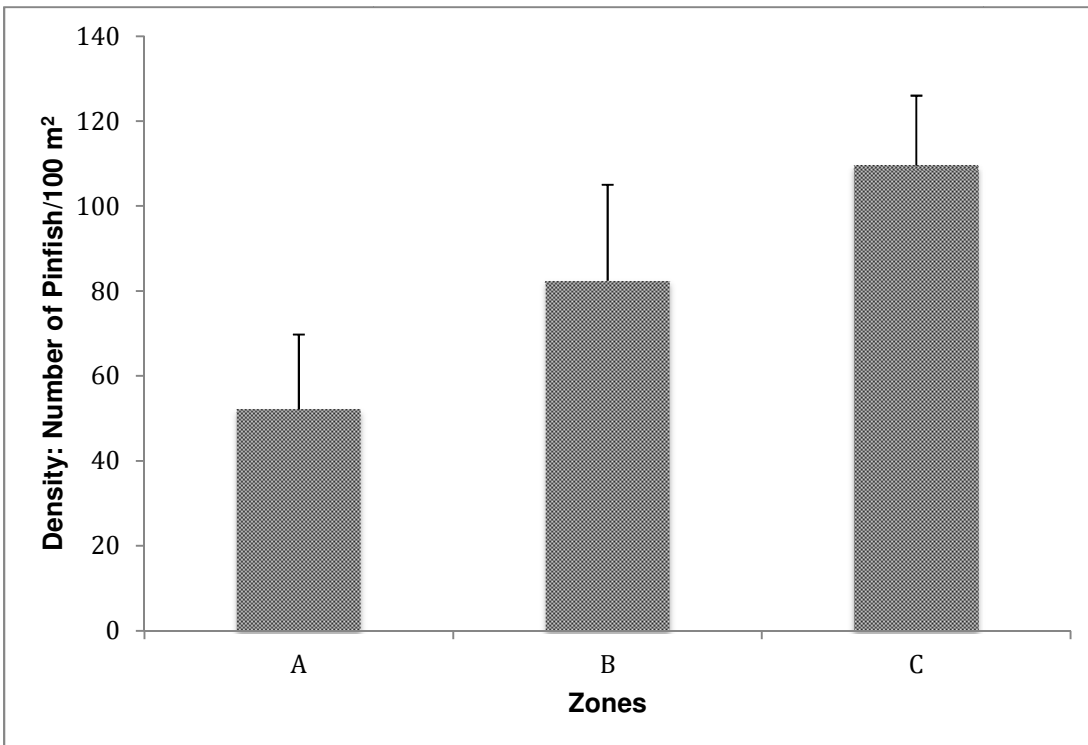


Figure 4: Densities of pinfish counts from each site across zones (mean±SE) N=7 for each zone.

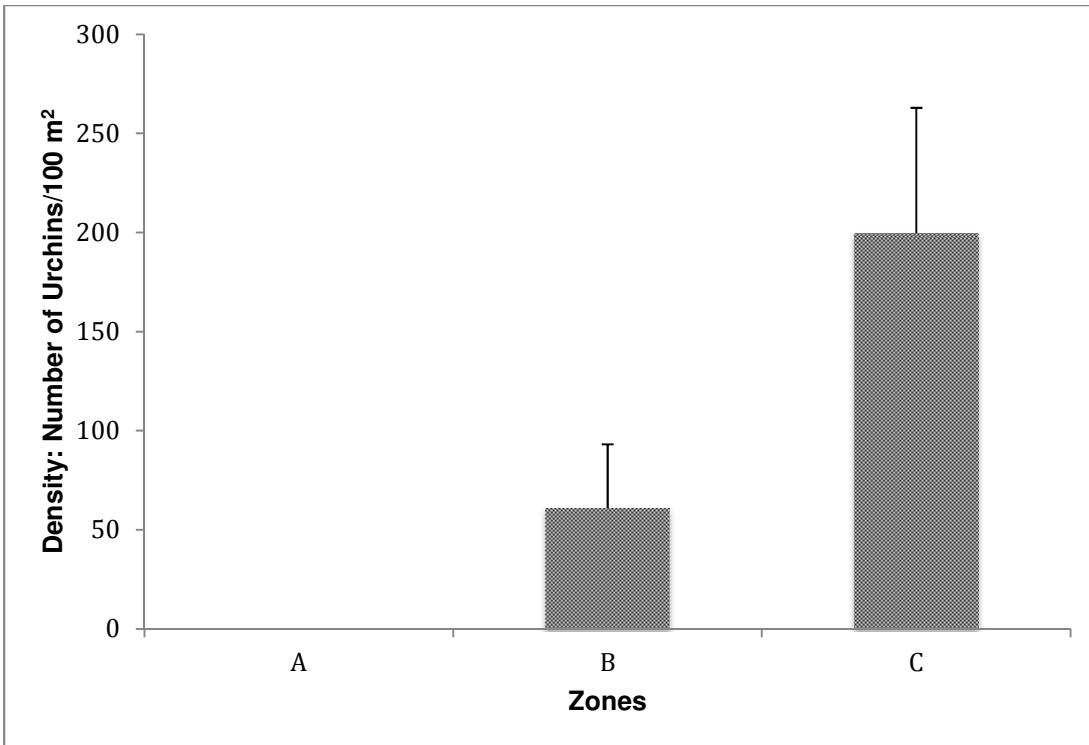


Figure 5: Densities of urchin counts from each site across zones (mean±SE) N=7 for each zone.

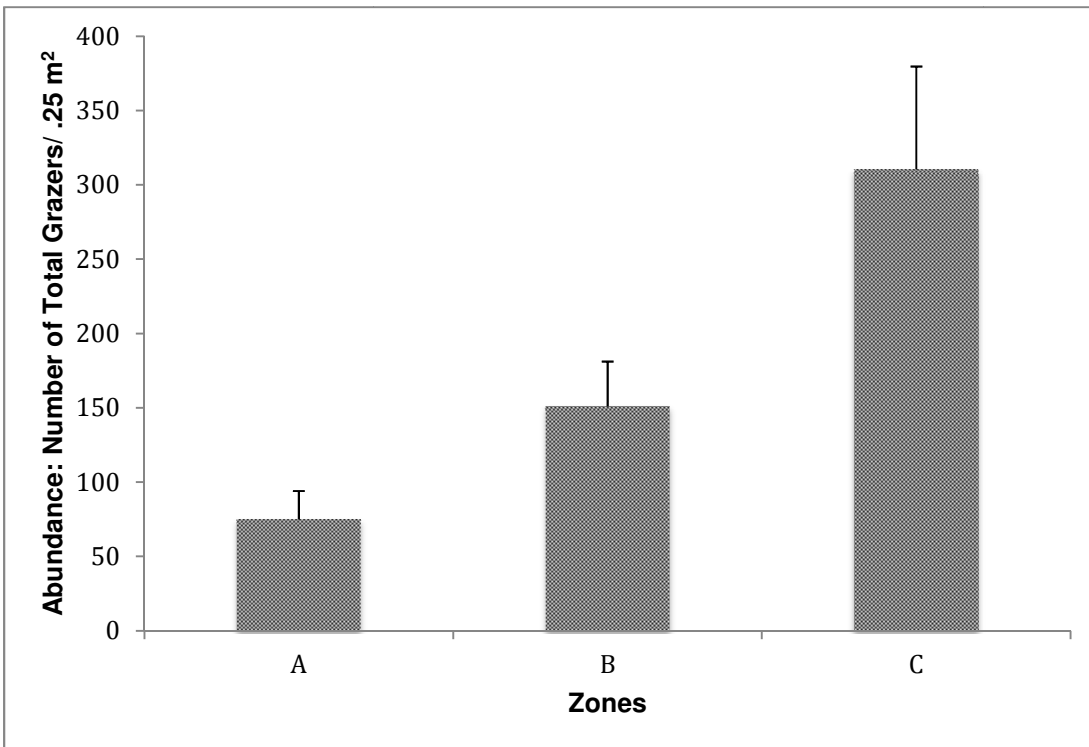


Figure 6: Abundance of total grazers (mean±SE) across zones where total grazers includes all pinfish, urchins, snails, and shrimps. N=7 for each zone.

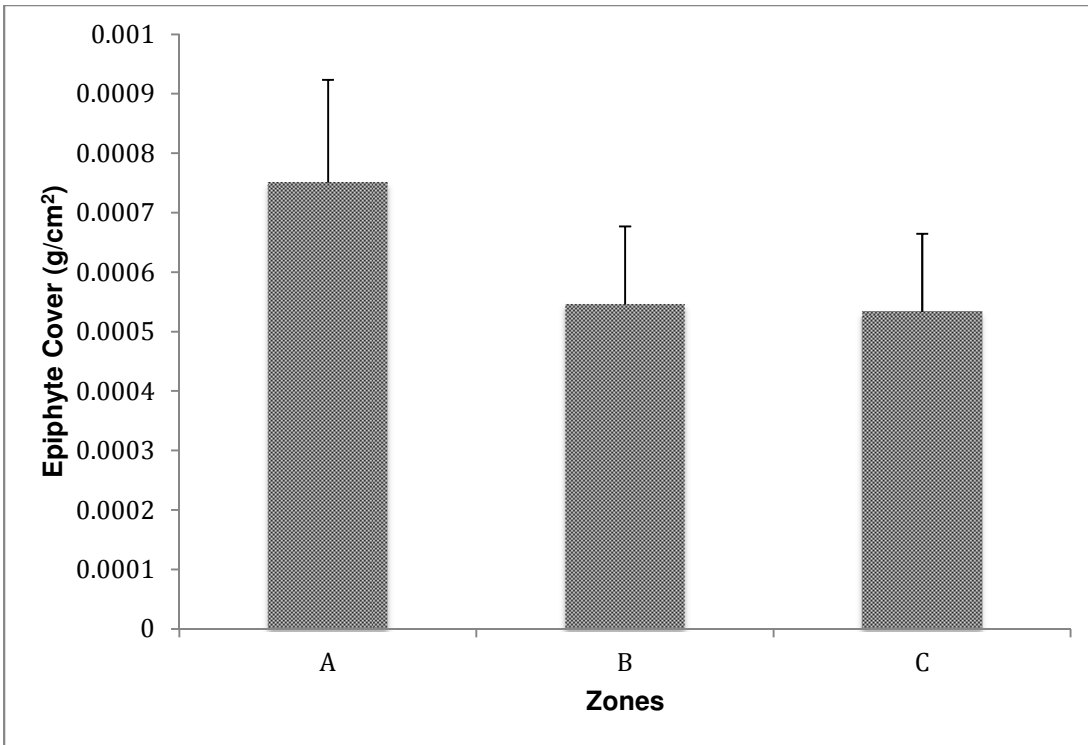


Figure 7: Total epiphyte cover (mass/area) across zones (mean±SE). N=7 for each zone.

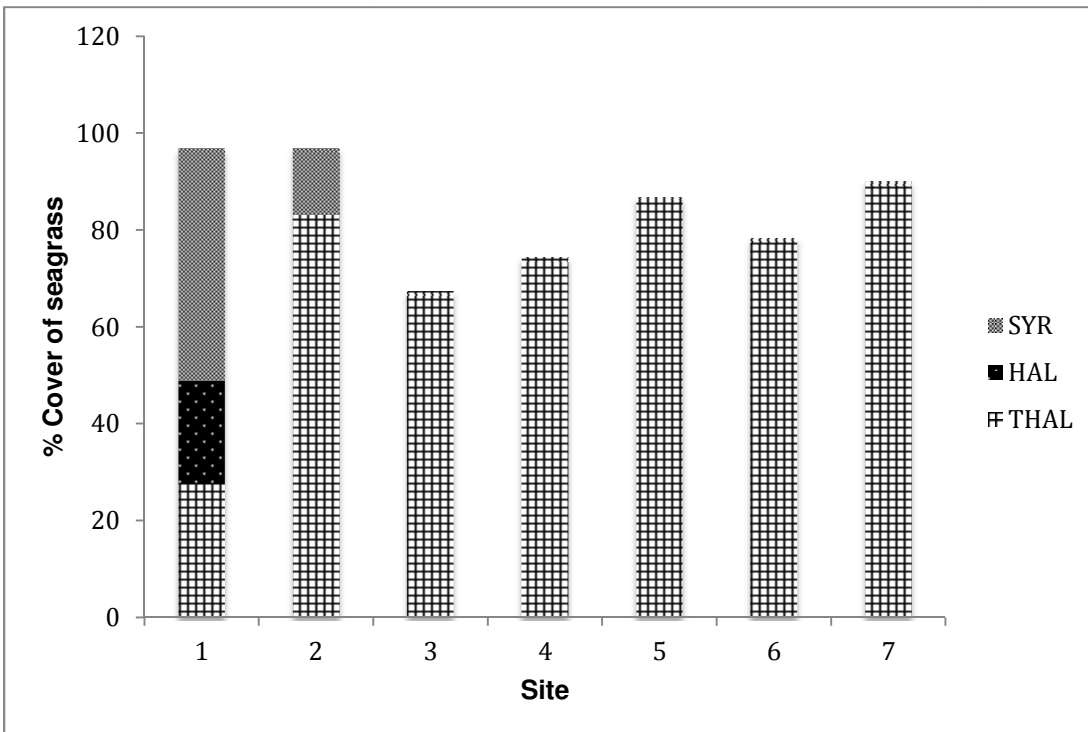


Figure 8: Distribution of seagrass across sites. SYR: *Syringodium filliforme*, HAL: *Halodule wrightii*, THAL: *Thalassia testudinum*. N= 5 at each site.

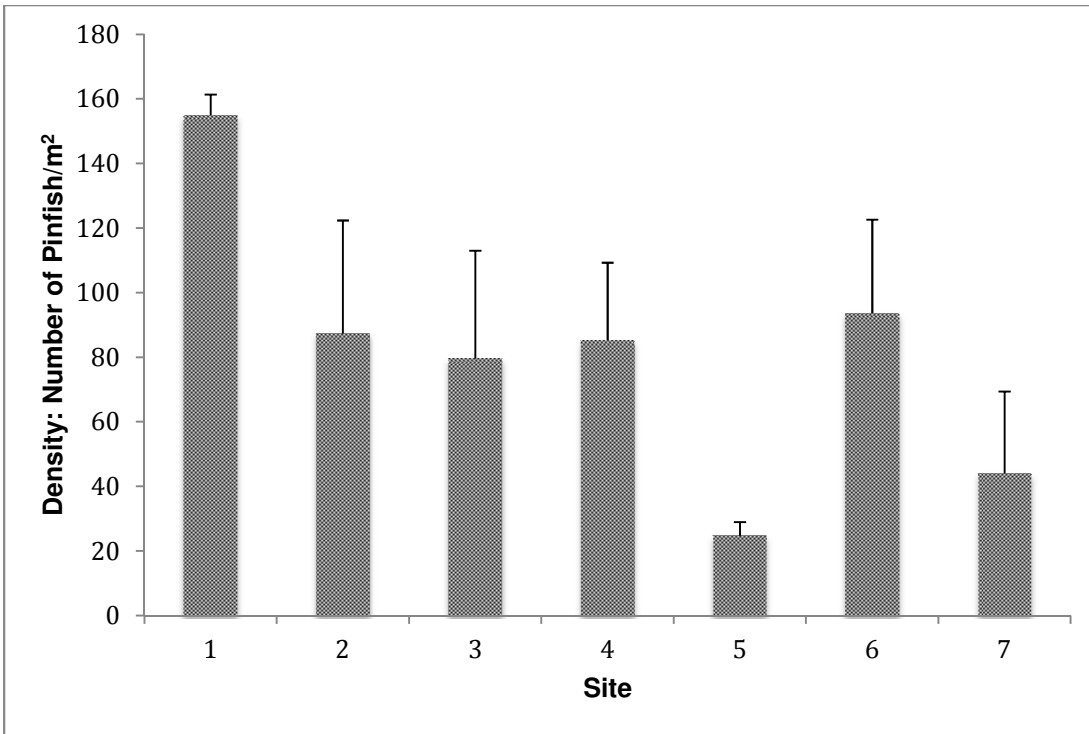


Figure 9: Densities of pinfish counts from each zone across sites (mean±SE) N=3 for each site.

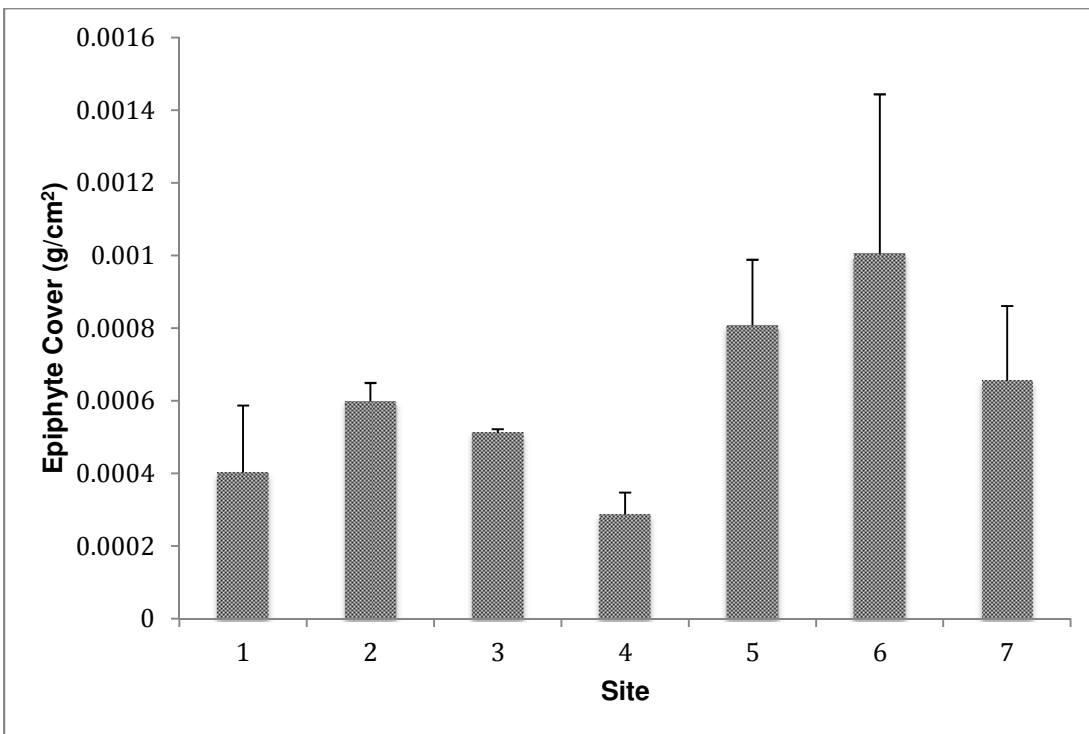


Figure 10: Epiphyte cover from each zone across sites (mean±SE) N=3 for each site.

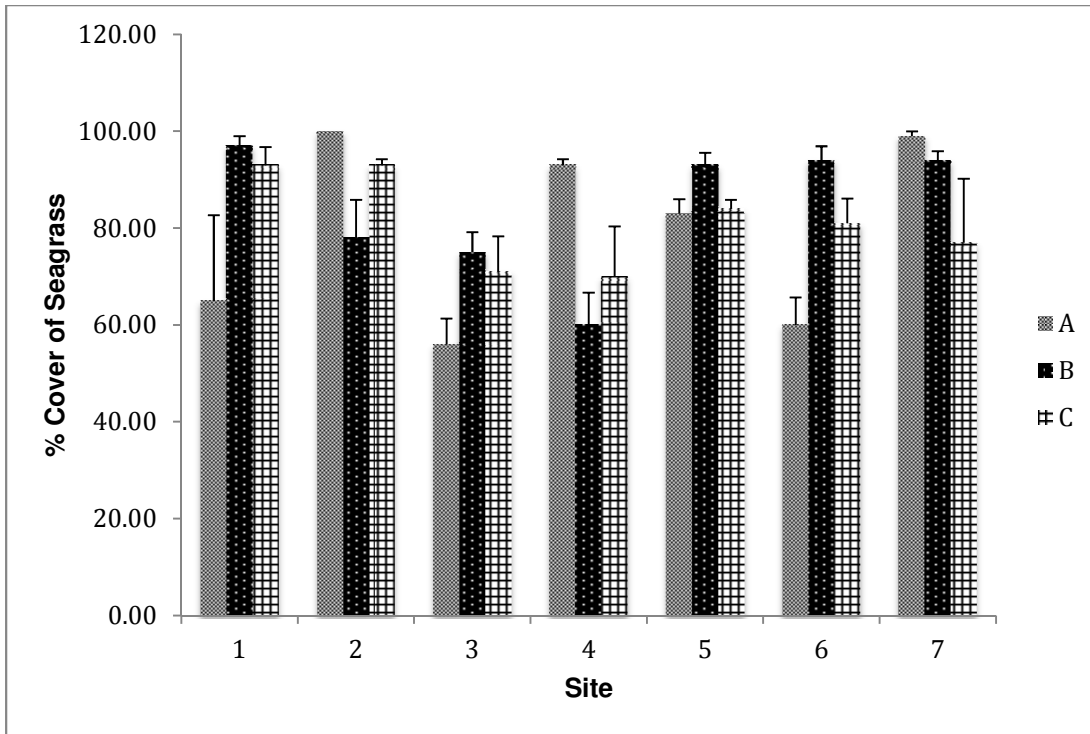


Figure 11: Site by zone distribution of total seagrass (combined *Syringodium filliforme*, *Halodule wrightii*, *Thalassia testudinum*) where A, B, and C are the zones.

Table 1: Level of taxonomic classification for all invertebrates sampled in St. Joseph Bay, FL. across sites and zones.

Invertebrate	Taxonomic Classification Level	
Shrimp	Order	Decapoda
Snails	Class	Gastropoda
Hermit Crabs	Superfamily	Paguroidea
Urchins	Class	Echinoidea
Crepidula	Class	Gastropoda
Chiton	Class	Polyplacophora
Crabs	Infraorder	Brachyura
Sponges	Phylum	Porifera
Tunicates	Subphylum	Tunicata
Starfish	Class	Asteroidea
Amphipods	Order	Amphipoda
Isopods	Order	Isopoda
Clam/ Scallops	Class	Bivalvia

Table 2: pH, salinity, dissolved oxygen, temperature, and depth for each site/zone sampled.

Site/Zone	pH	DO (PPM)	Salinity (PPT)	Temp (°C)	Depth (m)
1A	8.71	53.4	30.37	25.23	0.45
1B	8.56	133.2	31.31	28.03	0.56
1C	8.52	144.6	31.87	31.9	1.2
2A	8.73	51.7	31.59	26.84	0.06
2B	8.72	186.5	33.25	30.53	0.32
2C	8.82	103.3	32.79	29.62	0.22
3A	8.73	103.3	32.79	29.62	0.14
3B	8.69	62.9	30.89	25.46	0.3
3C	8.58	125.3	31.33	27.89	0.74
4A	8.78	137.7	31.94	31.9	0.37
4B	8.75	55.4	31.71	26.7	0.42
4C	8.77	182.6	34.24	29.28	0.53
5A	8.83	117.7	33.25	30.19	0.16
5B	8.72	67.1	34.13	26.75	0.43
5C	8.78	76.3	31.28	26	0.94
6A	8.6	138.9	31.44	27.86	0.25
6B	8.78	113.5	31.66	30.1	0.27
6C	8.57	127.3	31.15	27.32	0.62
7A	8.79	128.9	34.44	29.3	0.63
7B	8.78	129.7	34.15	30.2	0.16
7C	8.72	73.8	34.43	27.63	0.65

Table 3: Results of ANOVA testing site and zone differences in St. Joseph Bay.
*Significant p-values ($\alpha= 0.05$).

Factor	Site		Zone	
	df	p	df	p
Total Invertebrates	6	0.251	2	0.034*
Pinfish	6	0.019*	2	0.034*
Urchins	6	0.564	2	0.016*
Total Grazers	6	0.409	2	0.008*
Epiphyte Biomass	6	0.329	2	0.487
Total % Seagrass Cover	6	0.416	2	0.807
<i>T. testudinum</i>	6	0.006*	2	0.934

Table 4: Correlation results for field sampling. *Significant R-values (>0.44 <-0.44)

	R	N
<i>T. testudinum</i> vs Pinfish	-0.580*	21
<i>T. testudinum</i> vs Invertebrates	0.565*	21
Epiphytes vs Pinfish	-0.323	21
<i>S. filliforme</i> vs Pinfish	0.584*	21
Snails vs Pinfish	-0.267	21
Urchins vs Pinfish	0.266	21
Total Seagrass Cover vs Pinfish	-0.018	21

Table 5: SL and mass of all fish used in feeding trials with the change in biomass of the scraped and unscraped seagrass treatments of the feeding trials. All weights were recorded in g.

SL (cm)	Mass (g)	Scraped	Unscraped
7.2	9.46	-0.09	0
7.4	15.3	0.03	0.18
7.6	10.18	-0.01	0.44
7.9	19.98	0.02	0.13
8.5	29.58	0.25	0.79
9.3	14.04	-0.05	0.17
10	22.91	-0.09	0.14
10.3	11.04	-0.07	0.61
10.5	27.64	-0.45	0.19
11	24.51	-0.08	-0.03
12.5	17.38	0.28	0.16

Table 6: Correlation results for size of fish and change in biomass of *T. testudinum* treatments during feeding trials. *Significant R-values (>0.60 <-0.60).

	Scraped	Unscraped	N
SL (cm) vs Change in Biomass	0.002	-0.111	11
Mass (g) vs Biomass	-0.073	0.098	11

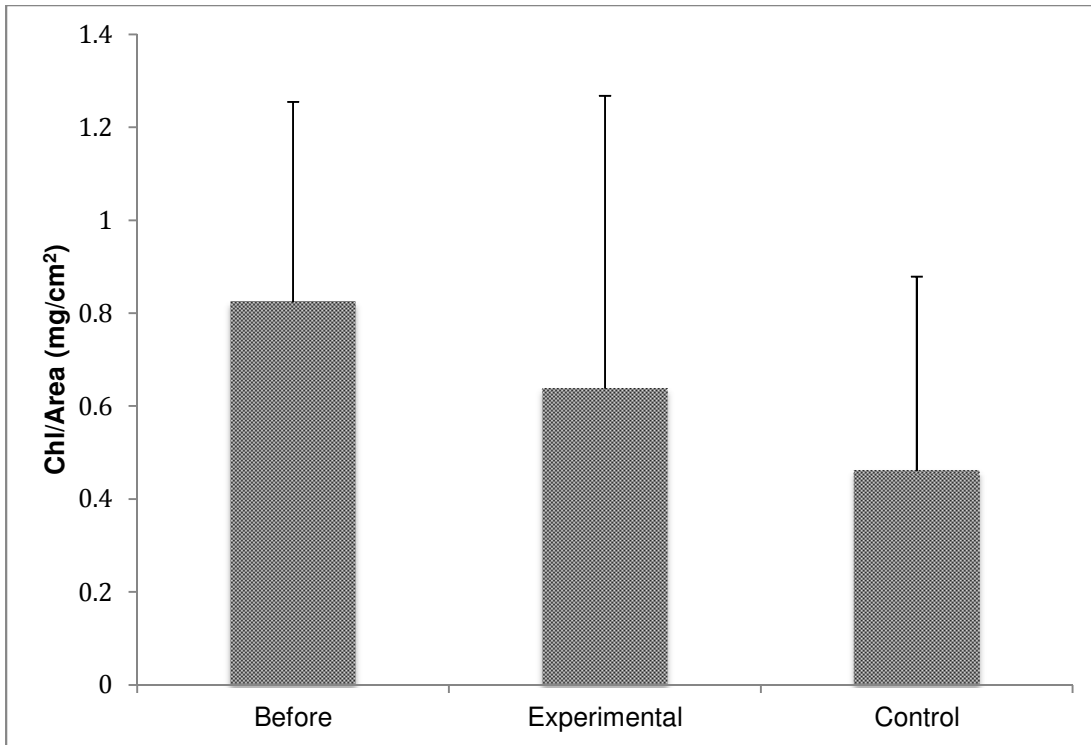


Figure 12: Total chlorophyll results from feeding trials. Error bars represent standard deviations. Before: nitex strips that were removed before experiment. Experimental: fish present. Control: no fish present. All values represent after measurements.

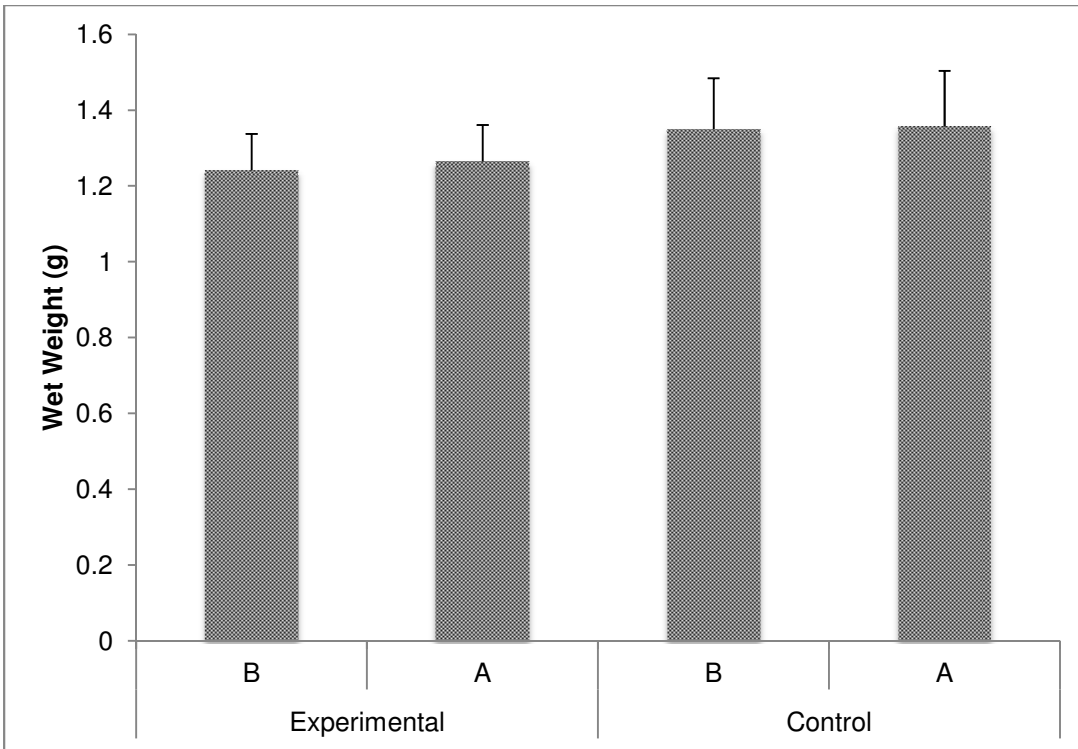


Figure 13: Change in wet weight of scraped seagrass blades (epiphytes removed) used in feeding trials (mean±SE). Experimental: Fish present. Control: No fish present. B and A represent before and after measurements. Change in biomass was calculated as before weight minus after weight, therefore positive values indicate net loss of biomass.

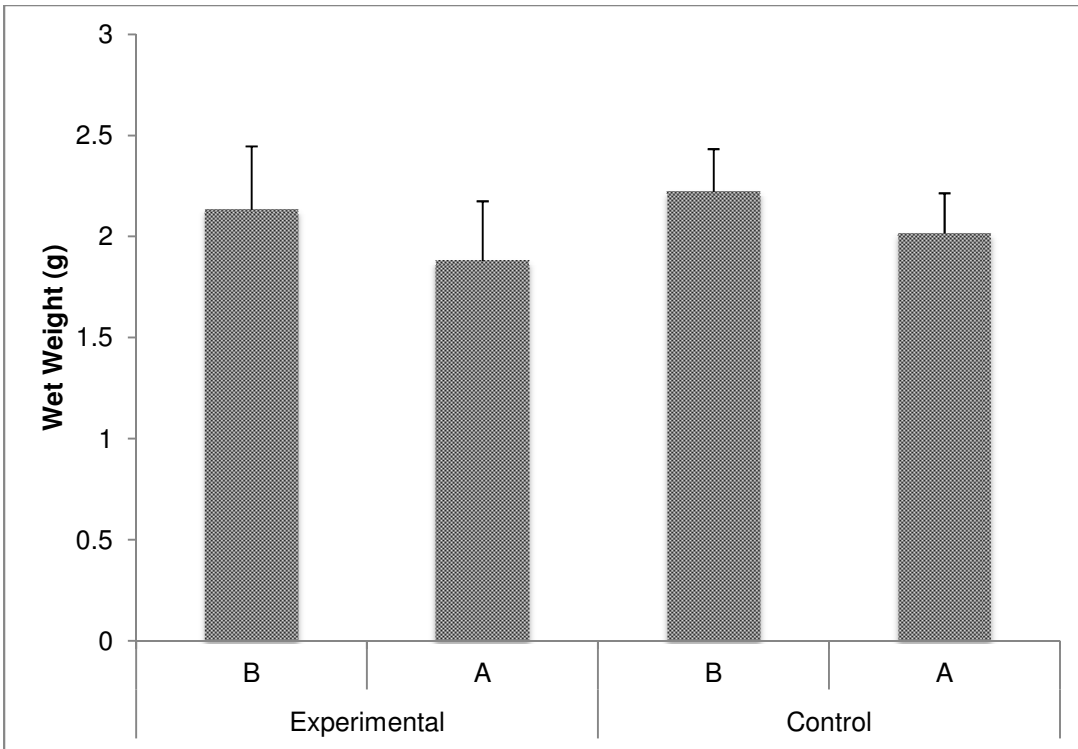


Figure 14: Change in wet weight of unscrapped seagrass blades (epiphytes intact) used in feeding trials (mean±SE). Experimental: Fish present. Control: No fish present. B and A represent before and after measurements. Change in biomass was calculated as before weight minus after weight, therefore positive values indicate net loss of biomass.

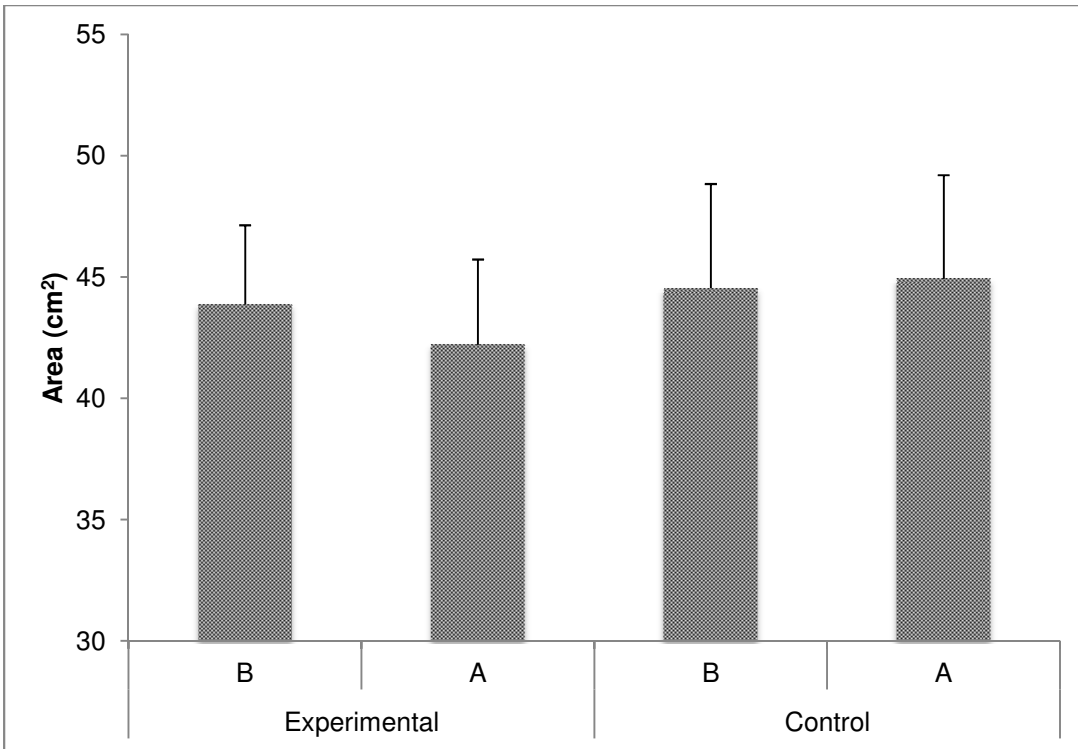


Figure 15: Change in area of scraped seagrass blades used in feeding trials (mean±SE). Experimental: Fish present. Control: No fish present. B and A represent before and after measurements.

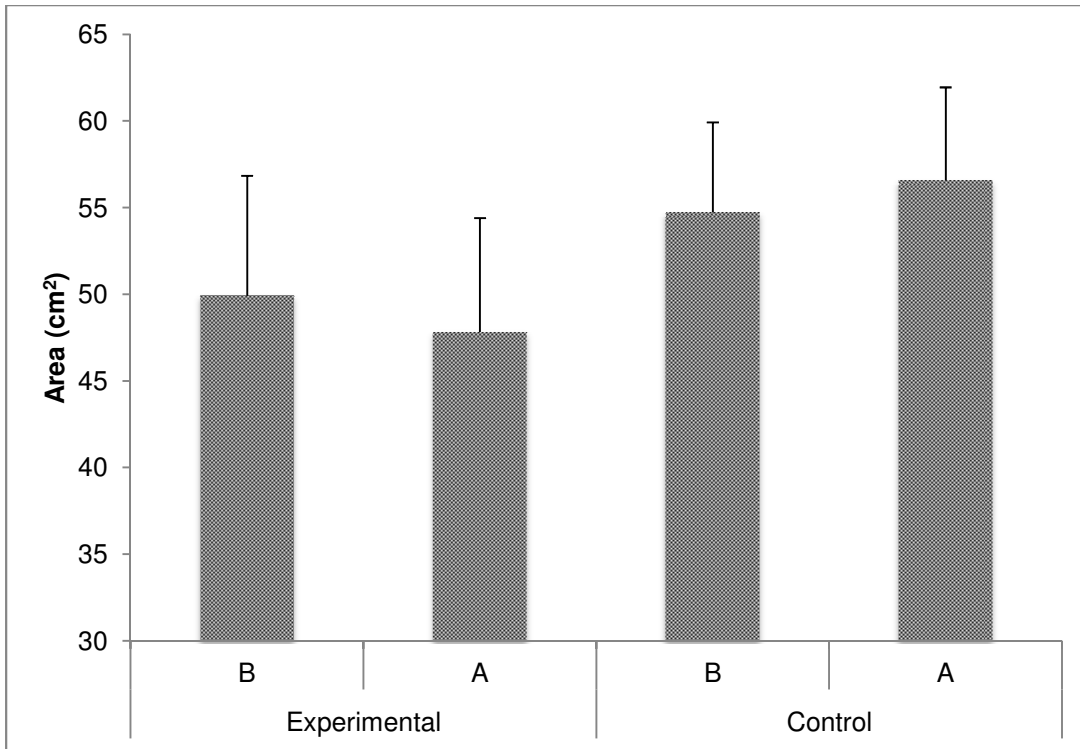


Figure 16: Change in area of unscraped seagrass blades (epiphytes intact) used in feeding trials (mean \pm SE). Experimental: Fish present. Control: No fish present. B and A represent before and after measurements.

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