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RESTORATION OF INTERTIDAL OYSTER REEFS AFFECTED BY INTENSE
RECREATIONAL BOATING ACTIVITY IN MOSQUITO LAGOON, FLORIDA

by

ANDREA LYNN BARBER
B. S. University of North Carolina at Wilmington, 2005

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

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ABSTRACT

In recent years, intertidal reefs of *Crassostrea virginica* (eastern oyster) along central Florida's east coast have suffered extensive losses due to wakes from recreational boats. These wakes have caused extensive shell movement and sediment resuspension which results in large piles of disarticulated shells along the seaward edges of reefs. Dead margins extend up to one meter above mean high water. The creation and enforcement of "no wake" zones in the area are unlikely. Thus, there is an urgent need for an alternative restoration strategy before these oyster reefs decline any further. The goal of this project was to develop a scientifically-based restoration technique that minimized wake damage from recreational vessels on intertidal reefs in Canaveral National Seashore. To accomplish this, I tested a range of restoration measures to identify a design that best increased: 1) oyster recruitment, 2) three-dimensional structure of the intertidal reefs, and 3) biodiversity and abundances of sessile and motile species associated with reefs. As a starting substrate in all treatments, I used restoration mats, which were created by affixing 36 drilled oyster shells to 0.4 x 0.4 m pieces of black mesh (Vexar). Five mats were deployed on the fore-reef, midreef, and backreef areas of each reef. In my experiment, I manipulated two habitat conditions: 1) leveling of existing dead margins to bring the top of the dead margin below mean high water to facilitate settling of larvae, and 2) deploying artificial seagrass seaward of the mats to act as a wake buffer. All combinations of these variables and all appropriate controls were replicated on six oyster reefs each, for a total of thirty reefs. Reefs that were leveled were significantly reduced in height and this difference was maintained throughout the 1 year

study. Unleveled reefs actually increased in mean height over the 12 months. Tracking loose shells covering our restoration mats over time likewise documented that shell movement was minimal on control reefs lacking dead margins and significantly greater on reefs with dead margins. Midreef areas on reefs with dead margins were almost completely buried by loose shells.

Quarterly monitoring of the number of spat settling on all restoration mats allowed for comparisons between treatments and locations on oyster reefs. After determining that overall water flow on the fore reef areas of all treatments was similar, I tested the null hypothesis that all treatments had similar recruitment of oyster larvae. My first alternative hypothesis was that artificial seagrass would increase oyster recruitment if the grass was a successful wake buffer and minimized sediment resuspension known to be lethal to newly settled oyster or prevented disarticulated oyster shell from moving and reforming mounds. My second alternative hypothesis was that the leveling of the dead margins would increase the total reef surface area available to larval oyster recruitment and thus lead to an increase in the number of recruits and eventually 3-dimensional reef structures (when oysters grow in close proximity and affix themselves together). Statistical analyses showed the artificial seagrass did not decrease the negative impacts caused by recreational boat wakes. Hence, it is not a recommended method for reef restoration. Recruitment of oysters significantly increased over time and significantly differed on various regions of the reefs. Recruitment was always highest on the fore-reef regions and lowest on back-reef regions. Although overall recruitment did not differ among treatments, it was significantly lower on midreef regions of the impacted reefs.

This suggests that the leveling of the oyster reefs would increase the surface area available for future oyster recruitment.

To look at biodiversity, I tested the hypothesis that all treatments would have similar biodiversity on a month by month basis. Alternatively, biodiversity should always be greatest on leveled reef with artificial seagrass due to increased 3-dimensional structure nearby and longer submersion times. To enumerate biodiversity, two lift nets were placed on each reef, one contained a restoration mat and the other contained only mesh (control). In most months, the four experimental treatments were similar according to the biodiversity measures analyzed. However, biodiversity was always higher in lift nets with restoration mats when compared to lift nets with mesh only. This result again suggests that the mats as designed are important restoration tools.

Overall, my results show that placing seagrass in front of oyster reefs may not help to better restoration efforts. However, leveling dead margins on reefs and using the restoration mats is beneficial to oyster reef habitat restoration efforts. As a result of my research, restoration mats, in combination with leveling dead margins, are currently being used in a large-scale, community-based oyster reef restoration project within Canaveral National Seashore boundaries.

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CHAPTER ONE: GENERAL INTRODUCTION

Oyster restoration and strategies

Decimation of stocks of *C. virginica* throughout much of its range has prompted restoration and enhancement efforts in many states. These restoration efforts include various techniques that focus on: 1) providing substrate for settlement of oyster larvae, and 2) supplementing oyster stock with wild or reared spat. Materials that have been used as oyster substrate in restoration efforts have included oyster shells, surf clam shells (Wesson et al. 1999), limestone marl (Haywood et al. 1999; Ertel and McCall 2005), and stabilized coal combustion by-products (Andrews et al. 1997). Most often the new substrate is simply dumped on the benthos. However, some studies have used mesh bags filled with the new substrate as an alternative (Ertel and McCall 2005). The mesh bags are used to recreate the three dimensional aspect of healthy reefs (Ertel and McCall 2005). Oyster seed is sometimes used as well to help supplement the low number of recruits in ailing populations (Tweed and Jacobson 2005).

The above strategies were designed to restore overharvested or diseased oysters, especially on subtidal reefs and were not appropriate for intertidal reefs in which the primary impact is wakes from recreational boating. Thus, a restoration technique specifically designed to reduce the negative effects of boat wakes were needed.

Biology of *C. virginica*

Phylum Mollusca

Class Bivalvia

Order Pteriode

Family Ostreidae

The eastern oyster, *C. virginica*, also known as the American oyster, was first described by Gmelin in 1791. It can be found from the coasts of Brazil and Argentina northward to the Gulf of Mexico and extending to New Brunswick, Canada along the Atlantic coast (Burrell 1986; Andrews 1991; Gosling 2003). *C. virginica* is common in estuaries and coastal areas and is found both intertidally and subtidally (Gosling 2003).

General anatomy and physiology of C. virginica

The shell of the eastern oyster consists of two calcareous valves joined by a resilient hinge ligament (Gosling 2003). The shell is usually white and yellowish with purple or brown markings (Gosling 2003). *Crassostrea virginica* can grow to be 350 mm maximum length with an average length of 89 mm (Gosling 2003). The highly variable appearance of the eastern oyster is due in part to its environment. In intertidal waters, the shell is thin, irregular, and elongated. In subtidal waters, the shell is thicker and more uniform in shape (Burrell 1996). The interior of the shell is white and usually has a black or purple muscle scar (Burrell 1986; Gosling 2003).

The body is made up of the organs needed for respiration, digestion, and

reproduction of the animal (Kennedy et al. 1996). The mantle is joined at the posterior margin of the shell and forms a cap that covers the mouth and labial palps. The gills of the eastern oyster are located below the palps and consist of four demibranchs (folds) of tissue that occupy much of the ventral portions of the mantle cavity. Together with the mantle, they are the chief organs of respiration (Eble and Scro 1996; Fig. 1).

Oysters ingest planktonic material which is filtered by the gills and entrapped in mucus. This mucus is moved to the labial palps where it is sorted (Kennedy and Breisch 1980). Acceptable materials are moved from the mouth down the esophagus and into the stomach while unacceptable materials are disposed of as pseudofeces (Kennedy and Breisch 1980). The crystalline style is used to assist in the digestion process (Galtsoff 1964; Quale 1969a). After digestion, the waste products are moved from the intestines to the exhalent chamber where they are stored until disposal (Menzel 1991).

The reproductive system in bivalves is a simple pair of gonads made of branching tubules and gametes that are budded off the epithelial lining (Gosling 2003).

Crassostrea virginica is a protandric species meaning that when they first mature, all individuals are males, and later on in life, some become female (Galtsoff 1964; Burrell 1986; Mackie 1984). Sex reversals are common in adult oysters (Galtsoff 1964; Burrell 1986).

Environmental requirements

Crassostrea virginica is able to withstand a wide range of conditions including varying temperatures, salinities, water currents, and turbidity (Burrell 1986; Andrews

1979). Eastern oysters are able to survive in temperatures between 10° and 43° C, but need a temperature of at least 19.5° C for egg development, above 20° C for larval development, and between 10° and 30° C for adult growth (Galtsoff 1964; Burrell 1986). Salinity can range from 0 to 42.5 parts per thousand and still support healthy eastern oyster populations (Gosling 2003). However, optimal salinity for *C. virginica* ranges from 25 to 28 parts per thousand (Gosling 2003). Optimal water currents are between 5 and 66 cm/s (Wells 1961). Currents help to provide sufficient amounts of food, wash away biodeposits, and disperse larvae (Burrell 1996; Galtsoff 1964; Nelson et al. 2004). Fertilized eggs of *C. virginica* experience 20% mortality at silt concentrations of 0.25 g/L (Davis and Hindu 1969). At 0.75 g/L silt concentration, the growth of larvae is significantly reduced (Loosanoff and Tommers 1948; Loosanoff 1962; Burrell 1986). Adult oysters are more tolerant of turbidity and sediment resuspension. Concentrations above 1.0 g/L reduce pumping rates in adults (Davis and Hindu 1969).

Reproduction, larval dispersal, recruitment, and growth

Crassostrea virginica is a broadcast spawner. Gametogenesis and spawning are induced by environmental cues in the surrounding water, including water temperature and salinity (Burrell 1986). The presence of gametes in the water also stimulates the onset of spawning in adjacent oysters (Kennedy et al. 1996). This external fertilization results in planktotrophic larvae which develop in the water column (Mann et al. 1994; Thompson et al. 1996).

Crassostrea virginica larvae reach the trochophore stage within 12 hours of

fertilization in favorable conditions (Burrell 1986). Ninety to 95 percent of the trochophore larvae will develop into the veliger stage within 48 hours (Burrell 1986). The larvae spend the next two to three weeks moving with the currents and feeding (Bahr and Lanier 1981). After this time, the veliger develops to the pediveliger stage. Pediveligers have a well-developed foot and two eyespots, which serve as defense mechanisms by allowing them to move and to react to changes in light, and enable them to crawl on the bottom in search of suitable substrates on which to settle (Burrell 1986).

During the pediveliger stage, larvae respond to a number of stimuli that allow them to choose the most suitable habitat. Larvae respond to physical factors received through surface mechanoreceptors (Kennedy et al. 1996). They use statocysts to distinguish between horizontal and vertical surfaces and their eyes to choose areas which receive adequate sunlight (Hadfield 1978). Larvae also respond to chemical cues found on the substrate and released into the water column by adult eastern oysters (Tamburri et al. 1992). Bacterial films on the surface of oyster shells, pheromones released by live oysters, and various metabolites of oysters all act as attractants for settling larvae (Coon et al. 1985, 1990; Fitt and Coon 1992; Kennedy et al. 1996). These chemical cues evoke settlement behavior in oyster larvae and influence oyster settlement in flowing water as well as in still water (Tamburri et al. 1996).

Once a suitable habitat is found, the oyster settles, cementing itself to the substrate which is most often a conspecific oyster shell (Burrell 1986; Kennedy et al. 1996; Gosling 2003). The oyster settles on its left side making the left valve thicker and more concave to accommodate and protect the body (Kennedy et al. 1996). The right

valve is thinner and flatter (Kennedy et al. 1996). After settling, the oyster metamorphoses. It loses its velum, eyespots, and foot, and is now called a spat (Galtsoff 1964; Loosanoff 1965; Kennedy et al. 1996). A large adductor muscle, which is attached to both valves, is used to open and close the oyster's shell as well as seal it when needed (Carriker 1996). After an oyster has settled, it remains in its chosen spot for life (Burrell 1986). The oyster can no longer move away from predators or competitors, nor can it relocate if the environment becomes harsh and unsuitable for growth and survival (Zimmer-Faust and Tamburri 1994).

Growth is measured as an increase in shell or body size and typically occurs more quickly in younger oysters than in older individuals (Burrell 1986). Sexual maturity can be reached in as little as four weeks (Coke 1983). Growth and the time it takes to reach maturity are affected by tidal height, bare space around the spat, and environmental conditions (Burrell 1986; Fig. 2).

Mortality factors

Sediment loads

Oyster settlement and recruitment on oyster beds is greater where shells are abundant and silt deposits and other fouling organisms on shells are scarce (MacKenzie 1983). Many studies have demonstrated a correlation between high sediment loads and high percent silt and clay levels with decreased oyster survival (MacKenzie 1983; Kennedy et al. 1996; Boudreaux 2005; Wall et al. 2006). Mortality of larval oysters in

areas with high sediment loads may be due to continuous alteration of surface topography (Walters 1992) and abrasion of larvae by the sediment (MacKenzie 1983).

Competition

Competition for space and food plays an important role in the growth, health, and survival of *C. virginica* (Zajac et al. 1989). Organisms that compete with the eastern oyster include macroalgae, sponges, cnidarians, bryozoans, barnacles, ascidians, anemones, polychaetes, mollusks, and arthropods (Wells 1961; Gosling 2003). The main effects of competitors are: 1) prevention of settlement by coverage of available space, 2) allelochemicals that deter settlement of new recruits, and 3) overgrowth and poisoning (White and Wilson 1996). Boudreaux (2005) found *C. virginica* preferentially settled on oyster shells where no *Balanus eburneus* (ivory barnacle) or *Balanus amphitrite* (purple striped barnacle) were present. These two barnacle species were also shown to decrease juvenile oyster survival and growth (Boudreaux 2005).

Predation

Many predators can limit the growth and survivorship of *C. virginica*. When eastern oysters are in the planktonic egg and larval phases, organisms such as ctenophores, adult bivalves including adult eastern oysters, anemones, starfish, fish, and crustaceans are potential predators (Gosling 2003). After individuals settle and become spat, they are subject to predation by mud crabs, juvenile blue crabs, and flatworms (Gosling 2003; Stiner 2006). Once adults, *C. virginica* may be preyed upon by blue crabs, whelks, oyster drills, rays, and several fish species (Walters et al. 2001).

Habitat structure and predation

Oyster reef structures provide refugia along interstitial and tidal gradients for both juvenile oysters and other estuarine species (Bartol et al. 1999). This refugia protects species from environmental stressors including predation (Nichy and Menzel 1967; Menge and Lubchenco 1981; Summerson and Peterson 1984). Thus, any changes in reef architecture would be expected to influence biotic communities and subsequently biological interactions among species. Previous studies have shown changes in habitat to affect predator-prey interactions (Lipcius and Hines 1986; Seitz et al. 2001; Woodley and Peterson 2003; Grabowski 2004; Griffen and Byers 2006). Habitat complexity reduced predator foraging efficiency, increased refuge for prey species, and provided protection for intermediate predators within intraguild predation systems (Gause 1934; Huffaker 1958; Jackson et al. 2001; Byers 2002; Griffen and Byers 2006). In North Carolina, enhanced habitat complexity of oyster reefs weakened the trophic interactions between *Opsanus tau* (oyster toadfish), *Panopeus herbstii* (mud crab), and the *C. virginica* (Grabowski 2004). Vertically complex oyster reefs allowed *P. herbstii* to escape *O. tau* predation and also increased the survival of juvenile *C. virginica* (Grabowski 2004).

Stiner (2006) conducted a study in Mosquito Lagoon which focused on the interaction between juvenile oysters and three dominant predators, 1) the common mud crab *P. herbstii*, 2) *Callinectes sapidus* (blue crab), and 3) *Urosalpinx cinerea* (Atlantic oyster drill). Contrary to expectations, overall reef slope, shell configuration, and shell orientation did not significantly influence oyster mortality. However, mortality differed

between the three dominant predator species. The common mud crab *P. herbstii* was the dominant predator in Mosquito Lagoon (Stiner 2006).

Parasites and disease

The most common diseases found in adult oysters are Dermo (*Perkinsus marinus*), MSX (*Haplosporidium nelsoni*), SSO (*Haplosporidium costalis*), Bonamiasis (*Bonamia ostreae*), and *Bucephalus cuclis*. Dermo and MSX are both caused by protozoans and cause emaciation, loss of body condition, and reduced fecundity, which can lead to 100% mortality (Ford and Tripp 1996; Gosling 2003). Dermo has been reported in oysters on the Atlantic coast of the U. S. from Massachusetts down to Venezuela and into the Gulf of Mexico (Burrell 1986). MSX and SSO have only been reported north of North Carolina on the Atlantic coast (Kennedy et al. 1996; Gosling 2003). Bonamiasis is an infection of a protozoan. It causes yellow discoloration in the tissues, gill lesions, and loss of condition which can lead to 90% mortality (Gosling 2003). *Bucephalus cuclis* is a parasitic trematode that infects the gonads, gills and the digestive gland resulting in castration and 30% mortality (Ford and Tripp 1996). Of the above four diseases that affect *C. virginica*, only minimal densities of Dermo have been found in Mosquito Lagoon (Walters et al. in press).

Some bacteria, including *Vibrio* spp. and proteobacteria, occur only in oyster larvae (Ford and Tripp 1996; Gosling 2003). These bacteria cause infection that can lead to 100% and 90% mortality, respectively (Ford and Tripp 1996; Gosling 2003). These diseases were found to occur most often in hatchery reared larvae and juvenile oysters (Gosling 2003).

Study location

Research was conducted in Mosquito Lagoon, Florida within Canaveral National Seashore (CANA) boundaries. Mosquito Lagoon is the northern-most estuary in the Indian River Lagoon system (IRL). The IRL also consists of the Indian River and Banana River (Walters et al. 2001; Smithsonian 2001). Together, Mosquito Lagoon, the Indian River and the Banana River stretch more than 251 km from Ponce de Leon Inlet to Jupiter Inlet on the east coast of central Florida (Fig. 3). The IRL was designated as “an estuary of national significance” by the Environmental Protection Agency and identified as one of the most productive estuarine systems in North America, containing one of the highest species diversities of any estuary on the continent (IRL NEP 1994; Walters et al. 2001; Smithsonian 2001).

Mosquito Lagoon stretches 30 km and is located between the Atlantic coastal ridge on the west and the Atlantic beach ridge on the east (Smithsonian 2001). The average depth of the Lagoon is less than one meter, in most areas with annual salinity ranging from 25 to 45 parts per thousand (Grizzle 1990). The high levels of biodiversity found in the Lagoon are due in large part to its location along the boundary between temperate and sub-tropical climates (Walters et al. 2001).

The majority of Mosquito Lagoon is found within the boundaries of CANA. This National Park was established in 1975 to preserve and protect the outstanding natural, scenic, scientific, ecologic, and historic value of the area and to provide public recreational access to the area. The IRL generates over \$800 million dollars in revenue annually, \$300 million of which comes from fisheries, including \$13 million for

shellfisheries (IRL Comprehensive Conservation and Management Plan 1996).

Harvesting of the eastern oyster *C. virginica* is entirely confined to Mosquito Lagoon (IRL Comprehensive Conservation and Management Plan 1996). When CANA was created in 1975, there were approximately 30 oyster lease plots within the park. These leases could not be sold or transferred and had to be renewed yearly to avoid expiration. In 2001, only 14 leases remained. Commercial and recreational harvesting of the eastern oyster still occurs within park boundaries (Walters et al. 2001).

The southern-most geographic limit on the Atlantic coast for expansive intertidal reefs of the eastern oyster *C. virginica* is found within Mosquito Lagoon (Grizzle and Castagna 1995). Aerial photography and field surveys have documented uncharacteristic dead margins, found along the seaward edges of reefs, which consist of disarticulated shells mounded above the adjacent living reef. The first dead margin was noted on an oyster reef within CANA in 1943, and, in 2000, sixty reefs had dead margins, representing 9.1% of the total areal coverage by oyster reefs within CANA (Grizzle et al. 2002). The number of dead margins developing continues to increase. These uncharacteristic dead margins occupied a surface area covering approximately 10% of the reefs studied in 2000 (Grizzle et al. 2002). All of these reefs with dead margins in 2000 occurred adjacent to major boating channels (Grizzle et al. 2002).

It has been shown that recreational boat wakes increase water motion which redistributes shells and leads to high levels of sediment resuspension; both have been shown to negatively affect recruitment of *C. virginica* (Shelbourne 1957; Seliger et al. 1982; Nowell and Jumars 1984; Wall et al. 2005). The number and rate of emergence of

dead margins on oyster reefs in the IRL, especially in CANA, have been increasing at rates similar to increases in the number of boating registrations of surrounding counties. In 2001, there were over 90,000 registered boats within the counties that border the northern IRL system, and this number has increased nearly 10% annually since 1986 (Hart 1994; ANEP 2001). Many concerns with the increasing number of boaters have been well documented, the impact of rapidly increasing boat activity on important benthic organisms, including the oyster *C. virginica*, is a topic now being intensely studied (e.g. Walters et al. 2001; Wall et al. 2005; Boudreax 2005; Stiner 2006).

Importance of *C. virginica*

In the United States, no one debates the economic importance of the eastern oyster *C. virginica*. Now, a preponderance of data documents the important ecological services provided by this species. Oysters filter water and their three-dimensional reef structures provide feeding habitat and refugia from predation for juvenile and adult forms of mobile species (Sellers and Stanley 1984; Durako et al. 1988; Dame 1996; Kennedy et al. 1996; Eggleston et al. 1999), as well as substrate for sessile fauna and flora (Wells 1961; Lenihan and Micheli 2001). Mosquito Lagoon species that frequently use oyster reefs at some part of their life-cycles include blue crabs, stone crabs, shrimp, red and black drum, and spotted sea trout (Walters et al. 2001). Oyster reefs also play a significant role in stabilizing creek banks, preserving emergent shoreline vegetation, and are feeding grounds for bald eagles, wood storks, brown pelicans and numerous other species of wading birds (Walters et al. 2001).

Restoration of *C. virginica* within Canaveral National Seashore

Unfortunately, oysters and oyster reef habitat have declined dramatically along the east coast of the U.S. over the past century due to habitat degradation, overharvesting, reduced water quality, and disease. In Florida, additional anthropogenic impacts have been documented in shallow-water locations with intense recreational boating activity. Wakes produced by recreational vessels cause extensive shell movement and sediment resuspension (Walters et al. 2005). This results in large piles of disarticulated shells along the fore-reef regions of oyster reefs that extend up to one meter above mean high water (Grizzle et al. 2002). The fore-reef is the seaward side of the reef and the back reef is the side closest to land (Fig. 9).

In CANA, dead margins were not associated with increased oyster disease loads (MSX, Dermo) (Walters et al. in press). Nor was settlement (individuals <1 week old) reduced on reefs with dead margins. However, survival (>4 weeks) of juvenile oysters was significantly lower when compared to pristine reefs, potentially as a result of sediment resuspension (Wall et al. 2005). Dead margins also allow for retention and germination of both native (mangrove) and invasive (Brazilian pepper) flora, promoting changes from aquatic to terrestrial habitats (Johnson et al. 2005; Donnelly 2006). Diversity and abundances of all estuarine species on impacted versus pristine reefs was also reduced on reefs with dead margins (Stiner and Walters 2006).

Decimation of stocks of *C. virginica* throughout much of its range has prompted restoration and enhancement efforts in many states. However, strategies that were designed to restore overharvested or diseased oysters, especially on subtidal reefs, were

not appropriate for intertidal reefs in which the primary impact is boat wakes. Thus, a restoration technique designed to reduce the negative effects of boat wakes was needed. The goal of this study was to develop an optimal protocol for intertidal oyster restoration on reefs impacted by wakes associated with recreational boating activities.

During previous research conducted in Dr. Linda Walters' laboratory, a simple protocol to create miniature, mobile oyster reefs for restoration purposes was developed. These "restoration mats" were created with oyster shells attached to 0.4 x 0.4 m squares of plastic mesh (Vexar) with cable ties in the same orientation as live oysters on reefs, perpendicular to the substrate. The optimal tested shell density per mat was experimentally determined to be 36 (Wall 2004). This density maximized oyster recruitment, retention, and had the shortest time until bridges were formed by new recruits between the deployed shells. Within 1.5 years, the restoration mats were indistinguishable from the natural reef.

After many of the important questions concerning the causes of oyster degradation in Mosquito Lagoon were addressed, it was necessary to develop an optimal restoration strategy that both increased the number of oyster recruits and increased overall reef biodiversity on impacted oyster reefs within CANA boundaries in Mosquito Lagoon, Florida. I manipulated two habitat conditions on impacted oyster reefs. These habitat conditions included: 1) leveling of existing dead margins to bring the top of the reef below the water level to facilitate settling of larvae, and 2) deployment of artificial seagrass seaward of the mats to act as a wake buffer. I tested the null hypothesis that all treatments would have similar biodiversity and larval recruitment. The alternative

hypothesis for this study was that the artificial seagrass would decrease the negative impacts caused by recreational boat wakes by minimizing sediment resuspension and by preventing disarticulated oyster shells from forming new mounds. It was also hypothesized that the leveling of the oyster reefs would increase the surface area available to larval oyster recruitment; this would lead to an increase in the number of recruits and three-dimensional structure that would then lead to an increase in biodiversity.

CHAPTER TWO: METHODS

Impacted versus unimpacted oyster reefs

Four treatments were tested to determine the best design to restore oyster reefs with dead margins in CANA. Treatments included: 1) adding seagrass and leveling dead margins, 2) adding seagrass and unlevelled, 3) no seagrass and leveling, and 4) no seagrass and unlevelled. Treatments were deployed on impacted reefs, while pristine reefs were used as reference reefs (controls) (Fig. 5). Artificial seagrass was used for this experiment due to the complications of transplanting live seagrass. *Halodule wrightii* (shoal grass), the only seagrass species in northern Mosquito Lagoon, can grow to be 25 cm in length with an average range of 5-20 cm long (Littler and Littler 2000). This seagrass species is normally found surrounding oyster reefs within the study area (personal observation). Artificial seagrass was made from children's hula skirts from the Oriental Trading Company™. They were cut to 20 cm in length to simulate the maximum impact *H. wrightii* could have on an oyster reef, while still being in its normal size range (Fig. 6). On experimental reefs with seagrass, three rows of artificial seagrass were placed seaward of the reef. The row closest to the reef contained four 60 cm long pieces of artificial seagrass. The next row contained three 60 cm long pieces of artificial seagrass. The seaward row contained two 60 cm long pieces of artificial seagrass (Fig. 7). Each piece of seagrass was anchored using two circular irrigation weights attached with 120 lb. test cable ties and two garden staples. Leveling of the reefs was accomplished by raking the disarticulated shells that form the dead margins to the back of

the reef. After leveling, the top of the reef became submerged at high tide much like reference reefs (Fig. 8).

There were six replicates of each treatment and six reference reefs (no dead margins); all were randomly chosen (Fig. 9). All thirty reefs were located within CANA boundaries in Mosquito Lagoon (Fig. 9). Experimental deployment was completed on 19 February 2006. Monitoring of these sites occurred for twelve months and included: 1) comparisons of vertical reef profiles before leveling, immediately after leveling, at four months, at eight months, and at twelve months, 2) monitoring of oyster recruitment and number of bridges formed between recruited oyster shells on the restoration mats at four months, at eight months, and at twelve months, 3) percent coverage of restoration mats by displaced adult oysters and disarticulated shells at four months, at eight months, and at twelve months, and 4) monthly monitoring of biodiversity in lift nets deployed at each site for ten months.

Reef profiles

Vertical reef profiles were obtained by using a tape measure, stadia rod, compass, and Johnson 9100/40-0909 laser level secured to a tripod. Heights of all 30 reefs were measured at 20 cm intervals from the shoreline, over the highest part of the reef, to the seaward edge of the reef. By deploying metal stakes on shore and calculating compass angles, I was able to return to the exact transect locations. Note that for two of the reference reefs, the reef profiles for month 0 were discarded due to human error in data

collection. For these reefs, month 4 was used for initial height of the reef in data analysis.

Recruitment, shell coverage, and bridges formed

Fifteen restoration mats were deployed on each treatment and each reference reef in the following locations: 1) 5 mats in the fore-reef region, 2) 5 mats in the middle of the reef, and 3) 5 mats in the back reef region (Fig. 4). Restoration mats were placed approximately 0.4 m apart from each other. Each restoration mat consisted of a Vexar base which measured 0.4 x 0.4m and 36 drilled shells attached by 50 lb. test cable ties (Fig. 10). Each restoration mat was anchored using two irrigation weights at opposite corners and two garden staples at the other two corners.

Quarterly monitoring of oyster recruitment and number of bridges formed between recruited oyster shells was completed by counting the number of oyster recruits and bridges that formed. Bridges occurred when new oysters grew between and connected two shells originally deployed on the restoration mats. When restoration mats were checked for recruitment and bridges, disarticulated oyster shells that had washed up and covered the restoration mats were also observed, and a percent coverage was estimated. This information was used to compare percent coverage between locations on reefs and treatments.

Biodiversity

Two lift nets were deployed on the side of each reef to monitor sessile and mobile animals, and macroalgal biodiversity (Fig. 11). One of the lift nets had one restoration

mat with 0 shells (from hereon called Vexar only mat) and the other had one restoration mat with 36 shells (from hereon call restoration mat). Each lift net was created from a 0.6 x 0.6 m PVC frame and netting which had been treated with an anti-corrosion coating. Lift nets were anchored using four irrigation weights per net attached with 120 lb. test cable ties. Each restoration mat and Vexar mat within the lift nets was weighted with two irrigation weights that were attached using 120 lb. test cable ties.

Biodiversity in lift nets was determined by creating a list of all mobile and sessile species found in each lift net and attached to the Vexar and shells (not PVC frames, weights, or netting) and counting the number of individuals of each species. Macroalgal species attached to restoration mats and shells were identified and biomass included in my analysis of biodiversity. To quantify the biomass of each macroalgal species found, wet and dry weights were determined. Macroalgae was spun 30 times in a salad spinner, blotted dry, and weighed on a top-loading balance to obtain wet weights. The macroalgal biomass was then dried in an Econotherm laboratory oven at 70°C for 48 hours and reweighed to obtain dry weights. After organisms were removed from the lift nets each month, they were not returned to the lift nets but were released unharmed in a different location of the Lagoon. Lift nets were also cleaned of any encrusting flora and fauna and new restoration mats and Vixar only mats were placed in each lift net every month.

Relative water flow

To determine if flow rates differed between impacted and reference reefs, as well as by location on each reef, relative measures of flow rates on each of the 30 reefs were

recorded. Four 4 cm diameter spheres of plaster-of-Paris (DAP plaster-of-Paris Dry Mix Interior) were made and placed on each reef. One sphere was placed approximately two meters in front of the reef. This sphere was located seaward of the artificial seagrass on those treatment reefs that had artificial seagrass and the same distance from other reefs which did not have artificial seagrass. A second sphere was placed at the fore-reef edge. This sphere was located behind artificial seagrass on those treatment reefs that had artificial seagrass and the same distance from other reefs which did not have artificial seagrass. A third sphere was placed on the middle of each reef. A fourth sphere was placed immediately behind the back edge of each reef.

After all plaster-of-Paris spheres were made, they were dried in an Econotherm laboratory oven at 70°C for 24 hours then weighed before deployment. In the field, each sphere was anchored using one 50 lb. test cable tie and one garden staple. Twenty-four hours after deployment, the spheres were collected, dried for 24 hours and reweighed. The difference in weight was determined and provided an estimate of relative rates of flow on different locations on each of the 30 reefs.

Those reefs and locations on reefs with high flow rates had a greater difference in sphere weight before and after deployment. Those reefs and locations on reefs with low flow rates had a smaller difference in sphere weight before and after deployment. This experiment was completed five times throughout the same twelve month period that recruitment and biodiversity were monitored.

Statistical analyses

We used statistical analyses between treatments to test: 1) differences in the number of recruits, 2) differences in percent coverage of loose oysters and shells on restoration mats, 3) differences in both overall biodiversity and abundances of organisms found in the lift nets, 4) differences in reef height before leveling, immediately after leveling, and quarterly, and 5) relative water flow on all reefs. Repeated measure ANOVAs were used to analyze oyster recruitment and percent shell coverage. Oyster recruitment and percent shell coverage both violated the assumption of sphericity so Greenhouse-Geisser corrections were used (Quinn and Keough 2002; Field 2005;). Sphericity is a form of compound symmetry which assumes that the variances of the differences between data taken from the same sample unit are equal (Field 2005). Because variances were not homogeneous, Games-Howell *post hoc* comparisons were used to compare differences in reef location and treatment for recruitment and shell coverage. The number of bridges observed during the study period was not large enough for statistical analysis. Total numbers of bridges formed per treatment is presented.

Kruskal-Wallis comparisons corrected with Bonferroni were used to analyze biodiversity data from lift nets. Response variables of species richness (total number of species), density (number of individuals per lift net), macroalgal wet weights, macroalgal dry weights, number of macroalgal species, animal species evenness, and animal species diversity (Shannon-Wiener) were analyzed. Biodiversity was also analyzed using ANOVA on vectors produced from Nonmetric Multidimensional Scaling (NMS) ordinations (PC-ORD v11.5 and 15; McCune and Grace 2002) with Sorensen distance

measures. I started with random configurations and ran 200 iterations with 50 runs on real data and 30 for randomized data. I assessed dimensionality using scree plots. This procedure was repeated twice to evaluate the stability of the solutions. NMS ordinations were conducted on data of two month intervals to observe how number of individuals in each species changed from month to month in both lift nets from each reef. Prior to this, species were grouped into five categories: mollusks, crustaceans, fishes, all other invertebrates, and macroalgae. Once vectors were produced from NMS ordinations, changes in vector direction and length from month to month were analyzed using MANOVA. This allowed us to observe if treatments were significantly different from one another and if the lift net with the restoration mat was significantly different from the lift net with Vexar only with respect to number of individuals per category.

Repeated measures ANOVA was used to analyze vertical heights of reefs from month 0 (after leveling if the reef was leveled), 4, 8, and 12 months. To determine if initial leveling did significantly change the height of the reef, *t*-tests were run. All reef height analyses were performed on the mean of the maximum five heights recorded for that reef at that time period. Univariate ANOVAs were used to analyze relative water motion for each trial. A covariate of initial sphere weight was used and difference in sphere weight was log transformed.

CHAPTER THREE: RESULTS

Reef profiles

Before any manipulations were applied to reefs, reference reefs had a mean width of 1060 cm (± 182.3 cm) and an average of the five maximum heights of 13.1 cm (± 3.5 cm) (Fig. 12). The no seagrass, leveled reefs had a mean width of 1096.7 cm (± 182.3 cm) and an average of the five maximum height of 18.5 cm (± 4.2 cm) (Fig. 13). The no seagrass, unleveled reefs had a mean width of 600 cm (± 55.4 cm) and an average of the five maximum heights of 22.0 cm (± 5.7 cm) (Fig. 14). The seagrass, leveled reefs had a mean width of 543.3 cm (± 64.8 cm) and an average of the five maximum heights of 23.8 cm (± 4.8 cm) (Fig. 15). The seagrass, unleveled reefs had a mean width of 546.7 cm (± 57.4 cm) and an average of the five maximum heights of 19.0 cm (± 3.2 cm) (Fig. 16). Most reefs had, in general, a unimodal shape throughout the study. Treatment reefs that were leveled had initial heights that were significantly higher before leveling than after leveling (Table 1; Figs. 13, 15).

Between February 2006 and February 2007, the average of the 5 maximum heights of the reefs changed in a quadratic fashion through time (Tables 2, 3; Fig. 17, 18). The treatment that had the highest increase in the five maximum heights of the dead margin after 12 months was the no seagrass and unleveled treatment reefs (Table 4; Fig. 16, 17, 18). The change in the five maximum heights of the other three treatments,

including no seagrass and leveled, seagrass and leveled, and seagrass and unleveled, were not significantly different from this variable in reference reefs (Table 4; Fig. 17, 18).

Recruitment

Recruitment was significantly higher at 12 months than at 4 or 8 months (Tables 5, 6; Fig. 19). Mean recruitment for all reef types ranged from 18.96 to 51.85 for over 4 months, 24.46 to 47.68 overall after 8 months, and 12.10 to 65.80 overall after 12 months (Fig. 20). I did not find significant difference in change in recruitment among locations or treatments. However, in average across the study, recruitment was significantly different within reef locations (Table 7; Fig. 21). The fore reef region had higher recruitment on average than mid or back regions (Tables 7, 8; Fig. 22). Average recruitment on mid and back reef regions were similar (Tables 7, 8; Fig. 22).

Shell coverage

Shell coverage increased from month 4 to month 12 for treatments including no seagrass and leveled and no seagrass and unleveled (Table 9; Fig. 23). The treatment seagrass and leveled reefs decreased in shell accumulation from month 4 to month 12 (Fig. 23). Shell coverage on seagrass and unleveled reefs remained constant from month 4 to month 12 (Fig. 23). Overall in average across all the studied months, treated reefs had significantly more shell coverage than reference reefs (Tables 10, 11; Fig. 23, 24). For the treatments including no seagrass and leveled, no seagrass and unleveled, and seagrass and leveled, the middle of reefs had significantly more shell coverage than other regions (Table 12; Fig. 25, 26).

Bridges formed

The number of bridges observed during the study period was too small to perform any statistical analyses (Fig. 27). The total number of bridges formed on all reefs over the twelve month study was thirteen (Fig. 27). No bridges were observed on the middle region of any reef (Fig. 27). Seven bridges were observed on the fore reef regions (Fig. 27). The largest number of bridges found on the fore region was seen on reference reefs (Fig. 27). Five bridges were observed on the back regions of reefs (Fig. 27).

Biodiversity

Number of individuals, species richness and species evenness: effect of treatments

Thirty sessile species of invertebrates recruited to oyster shell or the Vexar mesh found in the lift nets throughout the ten month study period. Sixty one mobile species were also found in lift nets throughout the study, including twelve species of crustaceans and twenty-six species of fishes (Table 13). Twelve macroalgal species were also found attached to Vexar and shells (Table 14, 15). Measures of oyster community metrics did not exhibit any clear trends. Animal species richness in lift nets with restoration mats was not different between treatments for nine out of the ten months (Table 16). February was the only month in which treatments significantly differed from one another in species richness (Table 16). Bonferroni corrections showed reefs with the no seagrass and unlevelled treatment had significantly less species richness than reefs with the seagrass, levelled treatment (Table 17). Number of individuals in lift nets with restoration mats never differed significantly between treatments and the controls (Table 18). Wet algal

biomass, dry algal biomass, and algal species richness were only analyzed for December and January given their absence in the remaining months. Wet algal biomass in lift nets with restoration mats did not differ significantly between treatments and the controls during the two months tested (Table 19). Dry algal biomass in lift nets with complete restoration mats did not differ significantly between treatments and the controls during the two months tested (Table 20). Algal species richness in lift nets with complete restoration mats did not differ significantly between treatments and the controls during the two months tested (Table 21).

Species evenness in lift nets with restoration mats did not differ significantly between treatments or controls in any of the ten months (Table 22). The Shannon-Wiener Index in lift nets with complete restoration mats did not differ significantly between treatments or controls in any of the ten months (Table 23).

Animal species richness in lift nets with Vexar only was not significantly different between treatments or the control in nine out of the ten months (Table 16). February was the only month in which treatments significantly differed from one another in species richness (Table 16). My analysis showed reefs with the no seagrass, unlevelled treatment significantly differed in species richness from reefs with the seagrass, levelled treatment (Table 24). Numbers of individuals in lift nets with Vexar only never differed significantly between treatments and the controls (Table 18).

Wet algal biomass in lift nets with Vexar only did not differ significantly between treatments and controls in any month tested (Table 19). Dry algal biomass in lift nets with Vexar only did not differ significantly between treatments and controls in any month

tested (Table 20). Algal species richness in lift nets with Vexar only did not differ significantly between treatments and controls in any month tested (Table 21). Species evenness in lift nets with Vexar only did not differ significantly between treatments and controls for nine out of the ten months (Table 22). My analysis showed reefs with the no seagrass, unlevelled treatment significantly differed in species evenness from all other treatments including no seagrass, leveled reefs, seagrass, leveled reefs, and seagrass, unlevelled reefs in the month of August (Table 25). Shannon-Wiener Index in lift nets with Vexar only did not differ significantly between treatments or controls in any of the ten months (Table 23).

Vector analysis: effect of restoration mats

For vector analysis on number of individuals caught per category, categories included mollusks, crustaceans, fishes, all other invertebrates, and algal species. During the ten month period that biodiversity data were collected, I observed thirty-four mollusk species, twelve crustacean species, eighteen species of other invertebrate taxa, twenty-six fish species, and twelve algal species. First, NMS ordinations were conducted on May and June (Fig. 28), June and July (Fig. 29), July and August (Fig. 30), August and September (Fig. 31), September and October (Fig. 32), October and November (Fig. 33), November and December (Fig. 34), December and January (Fig. 35), and January and February (Fig. 36). Adjacent months were analyzed to observe how the number of individuals in each category changed from month to month. I obtained two dimension NMS solutions for all month to month comparisons after 30-60 iterations for most

comparisons, some month to month comparisons need 200 iterations ($P = 0.0196-0.0392$ that a similar final stress could have been obtained by chance). Out of the five categories of organisms, mollusks, other invertebrate taxa, and algae correlated most with the axes. Mollusks were always positively correlated with both axes but r^2 was highest for axis 2 in the July to August ordination ($r^2 = 0.584$). Other invertebrate taxa were always positively correlated with both axes but r^2 was highest for axis 1 in the July to August ordination ($r^2 = 0.542$). Algae was always positively correlated to both axes but the highest r^2 was found for axis 2 in the January to February ordination ($r^2 = 0.605$). In most ordinations, the lift net with the complete restoration mat clearly separated from the lift net with Vexar only (Figs. 28-36). This indicates that the lift nets containing restoration mats and lift nets containing only Vexar experienced very different species composition.

From May to June, June to July, July to August, October to November, and November to December there were significant differences between lift nets with restorations mats versus Vexar only (Table 26). From August to September, September to October, December to January, and January to February no significant differences between lift nets with the different restorations mats were observed (Table 26).

Relative water motion

Dissolution rates of plaster-of-Paris spheres were not significantly different among reefs (Table 27; Fig. 37). A two-way ANOVA showed dissolution rates were not significantly different between treatment type, but were significantly different among reef locations (Table 28; Fig. 37). Combined, these analyses show that no one reef out of the

thirty used in this study had significantly higher or lower relative water motion than the others, and thus no treatment had higher or lower relative water motion than the others. Dissolution rates were higher on exposed regions, including seaward of seagrass and seaward of reef and locations behind seagrass experienced greater relative water motion than the other two locations, including the middle and back of reefs (Table 28; Fig. 37).

When the fore reef regions of reference and impacted reefs were compared, they were found not to be significantly different (Table 29). Reference reefs tended to have lower relative water motion than the impacted reefs in this study, although it was not significantly lower (Fig. 37).

CHAPTER FOUR: DISCUSSION

Reef profiles

Significant movement of disarticulated oyster shells has been observed on intertidal oyster reefs within CANA boundaries in short-term, single pass trials associated with recreational boat wakes (Walters et al. unpublished data). The results from my study reinforce these observations that recreational boat wakes have long term impacts on intertidal oyster reefs (Grizzle et al. 2002). By comparing vertical reef profiles from the beginning and end of this twelve month study, it was shown that reference reefs, those without dead margins, did not increase or decrease in height (Fig. 12). However, the treatments reefs that were unmanipulated, the no seagrass unlevelled treatment, experienced significant increased maximum reef height when compared to reference reefs over the twelve months (Table 4; Figs. 14, 17, 18). These results suggest that leaving reefs alone will not stop dead margin growth. I did find that the other three treatments including, no seagrass and leveled, seagrass and leveled, and seagrass and unlevelled did not have significant regrowth of dead margins (Table 4; Figs. 13, 15, 16, 17, 18). Data suggests the best restoration protocol would include leveling dead margins (Fig. 18). However, if leveling dead margins is not feasible, the addition of artificial seagrass will slow the regrowth of dead margins (Fig. 18). Although some regrowth of dead margins occurred on some leveled reefs, the knowledge that leveling dead margins remains in effect for at least a year will help to restore oyster reefs in Mosquito Lagoon, Florida.

Recruitment

Recruitment was predicted to be higher on impacted reefs than on reference reefs due to Wall et al.'s (2005) observations from the same study area in 2003-2004. She observed higher water flow on the seaward edges of impacted reefs potentially due to the recreational boating activity. She also found higher recruitment on impacted reefs partially due to the increased amount of larvae being brought to those reefs by the higher water flow (Wall et al. 2005). However, they discuss the limitations of their experimental design, including the design of frames used to observe recruitment rates. The frames suspended oyster shells 15 cm above the benthos and may have allowed ample space on all shells for larvae to settle on all reefs. This may not be representative of impacted reefs because the shells on the frames did not have the same shell orientation as shells found on impacted reefs. The disarticulated shells on impacted reefs may have less surface area on which larvae can settle because they are so tightly packed and they move on a regular basis. The shells on her frames were not tightly packed and were securely attached not allowing shell movement which may damage spat (Wall et al. 2005). These differences in experimental design may have lead to the appearance of increased larval recruitment on impacted reefs, when in fact, recruitment may have actually been similar.

My experimental design showed no significant differences between treatments, in reference to recruitment of oyster larvae (Fig. 19). However, slightly higher recruitment was observed on the seagrass and leveled treatment reefs (Fig. 19). The decision to study recruitment on oyster reefs instead of survival was made for logistical reasons but could

have added to our knowledge gained from this study. Although reference and treatment reefs were exposed to the same body of water and therefore similar amounts of oyster larvae, survival of oyster larvae once they settle on the different reefs may differ between reference and treatment reefs. In fact, I observed mortality of oyster spat from 4 to 8 months on those treatments that were leveled and mortality from 8 to 12 months on reference and seagrass, unleveled treatment reefs (Fig. 19). This would be expected because of the increased sediment suspension and increased shell movement on impacted reefs (Wall 2004; Wall et al. 2005). Increased sedimentation leads to lower percent survival (MacKenzie 1977, 1983, 1996; Gunter 1979; Kennedy et al. 1996; Perret et al. 1999; Boudreaux 2005). Increased shell movement and scouring of spat also leads to decreased survival of oyster spat (MacKenzie 1983).

Differences in mean recruitment were observed between reef locations. Lower recruitment was observed on the middle and back of reefs. Recruitment on the middle of reefs was low due to the large dead margins, indicating that the top of these dead margins have been lost to recruiting oyster larvae. The dead margins extend up to one meter above mean high water making it difficult not only to recruit to the region of the reef but impossible to survive if a shell carrying a spat is pushed to the top of the dead margin by recreational boat wakes (Grizzle et al. 2002). It should be noted that recruitment on mid regions of unleveled reefs was not zero (Fig. 20). This was due to high water levels in winter months which covered dead margins on some days and allowed for minimal recruitment of oyster larvae. Recruitment on the back of reefs was lower because dead margins block normal water flow. Reduced water flow on the backs of impacted reefs

results in less recruitment in the areas because less larvae are being brought in. Again, my data suggests leveling of the dead margins would be a helpful addition to restoration protocols within CANA in Mosquito Lagoon, Florida.

Shell coverage

Shell coverage on the restoration mats was significantly higher on all impacted reefs when compared to reference reefs (repeated measures ANOVA, Figs. 22, 24). The region of impacted reefs with highest shell coverage was the middle region (Figs. 24, 25). Stiner (2006) found significantly more dead, disarticulated oyster shell on impacted reefs than on pristine reefs. These disarticulated shells are being piled up by recreational boat wakes, increasing the height of dead margins over time (Figs. 14, 15, 16, 17) and covering live oysters that recruit to impacted reefs. These observations suggest that something needs to be done with the dead, disarticulated shells to stabilize them to allow newly settling oysters time to grow and rebuild a healthy reef structure.

Bridges formed

Bridge formation occurred between 8 and 12 months. Bridges were observed on restoration mats that had between 15 and 195 spat on attached oyster shells. The average number of spat on attached oyster shells when bridge formation was observed was 79. Although the number of bridges formed during this study were too small for statistical analyses, the location of the bridges that did form was interesting. No bridges were observed on the middle region of any reef. This was likely due to lower recruitment observed on this region of impacted reefs. The most bridges were observed on the fore

regions of reefs, in particular on reference reefs that were not impacted by recreational boat wakes. Bridges also occurred on the back region of impacted reefs. The dead margins on impacted reefs may create a protected back reef area due to the dead margin acting as a buffer against wave motion, sedimentation, and the accumulation of disarticulated shells (Stiner 2006).

Biodiversity

Previous oyster reef biodiversity studies conducted within the same study area using similar lift nets found 51 mobile species and 24 sessile animal species (Boudreaux et al. 2006). In addition, 14 mobile species were found outside of lift nets for a total of 65 mobile species (Boudreaux et al. 2006). I found a similar number of mobile species, 61, and sessile species, 30, during my study. Although my species list included a few species not found in the previous study (Atlantic calico scallop *Argopecten gibbus*, *Chione elevata*, fragile sphenia *Sphenia fragilis*, crenulate tellin *Tellina squamifera*, *Maiphysa sanguinea*, polychaete worms *Sigalionidae* family, gag grouper *Mycteroperca microlepis*, pigfish *Orthopristis chrysoptera*, speckled worm eel *Myrophis punctatus*, and spotfin mojarra *Eucinostomus argenteus*), the combining of the species lists should give a complete list of species found on oyster reefs within Mosquito Lagoon, Florida. The previous study did not collect and identify macroalgal species. I found 12 species during my study.

NMS graphs for mollusks, crustaceans, fishes, other invertebrates, and macroalgae for all reefs and for the majority of month to month comparisons showed lift

nets with Vexar only grouped separately from lift nets with restoration mats. This suggests that having the 36 oyster shells attached to each restoration mat does help to attract species and increase biodiversity in the immediate vicinity of the restoration mats. It also increased the number of individuals of each species. Thus, placing restoration mats on impacted reefs may help improve biodiversity to levels like those found on reference reefs.

Although vector analyses did not indicate differences between treatments, some of the community metrics data did. During one month, February, species richness was significantly lower for lift nets with Vexar only and lift nets with the restoration mats with the no seagrass and unlevelled treatment than for the reefs with the seagrass and leveled treatment. Although this is not indicative of an improvement of reef habitat when the reef has seagrass and is leveled alone, further research may show seagrass to be a helpful addition to the restoration protocol for increasing biodiversity. In August, significantly lower species evenness was observed for those reefs with the no seagrass and unlevelled treatment than for all other treatments, reinforcing the idea that seagrass and leveling may help to improve reef habitat.

All lift nets placed on reefs with artificial seagrass were placed behind seagrass although some reefs were too small to place lift nets on shell. Those lift nets were placed on the silty benthos directly next to the oyster reef. The placement of the lift nets on the sides of the reefs was chosen so lift nets would not disturb the effects of the treatment being applied to each reef. Had we placed the lift nets in front of the reefs we may have distorted the apparent impact of the artificial seagrass. Wall et al. (2005) also noted an

increase in sediment accumulation on the seaward edge of reefs, the fore reef region, due to recreational boat wakes. This could have deterred species from entering the lift nets had they been positioned on the fore reef. If lift nets were placed in the back of the reefs, the dead margins were expected to act as a buffer to the majority of effects experienced by species living on impacted reefs (Boudreaux et al. 2005). This results in back reef usage by species to be similar between reference and impacted reefs as observed by Boudreaux (2005) and Stiner (2006). The side of the reef was chosen to avoid distorted results. However, the lift nets may have been far enough from the applied treatments to produce a conservative view of the impact of treatments in this study.

Relative water motion

A difference in relative water motion was expected between reference and impacted reefs due to results of a study conducted by Wall (2004) in the same study area. She found significantly higher water motion on fore reef regions of impacted reefs which she suggested lead to higher recruitment rates on impacted reefs (Wall 2004). During my study, relative water motion was not significantly different between reefs, treatment type, or reference versus impacted reefs when all four locations on oyster reefs were considered (Fig. 36). This observation was reflected by my recruitment rates for reference and treatment reefs (Table 4; Figs. 20, 21). Differences in results between Wall et al.'s (2005) study and mine may be attributed to the methods used for the plaster-of-Paris spheres used to obtain relative water motion. Plaster-of-Paris spheres used in Wall's study (2004) were 7.5 cm in diameter and remained in the field for one week

while mine were 4 cm in diameter and remained on oyster reefs for 20-22 hours.

Differences in results may be attributed to differences in recreational boating activity from day to day and season to season. Although our results differ, it is important to know that relative water motion is not always different on reference versus impacted oyster reefs.

CHAPTER FIVE: CONCLUSIONS FOR RESTORATION PROTOCOL

My data suggests that, in addition to utilizing the restoration mats developed prior to this study, leveling dead margins will help to restore intertidal oyster reefs in Mosquito Lagoon, Florida. Leveling the dead margins gives oyster larvae access to more area on the reef on which to settle. Leveling was also shown to remain for at least one year, which gives spat time to grow on the stabilized shells found on the restoration mats. By providing spat with shells that can not be moved by the recreational boat wakes, the newly recruited oysters can rebuild the complex three-dimensional reef structure characteristic of pristine reefs (Stiner 2006). Although artificial seagrass may have helped treatment reefs gain more “pristine” qualities in some cases, the data supporting the use of artificial seagrass was less compelling than that supporting the use of leveling dead margins and not conclusive.

It was observed that the restoration mats can and do get covered by the disarticulated shells on all impacted reefs, regardless of having been leveled or not, when they were placed approximately 0.4 m apart. Therefore, it was suggested that restoration efforts within CANA boundaries use enough restoration mats to cover all disarticulated shells on those reefs being restored. This will help to ensure that the loose shell does not move before newly settled oysters have time to recreate a healthy oyster reef.

The optimal protocol suggested by the results from this study includes the use of restoration mats and leveling of dead margins. This plan is currently being applied on

twenty reefs within CANA boundaries. In Mosquito Lagoon, recruitment of *C. virginica* can occur all year long but is lower in winter months, January to March, and peaks in spring and early summer (Grizzle 1990; Wall et al. 2005; Boudreaux et al. in review). The first phase of restoration within CANA began in May of 2007 and is intended to be completed by the end of July 2007.

Oyster reef restoration has been recognized as an important need by resource management agencies in many states along the Atlantic and Gulf of Mexico coasts of the United States (Breitburg et al. 2000). Most efforts have been directed at increasing or maintaining oyster habitat (MacKenzie 1989; Luckenbach et al. 1999; Coen and Luckenbach 2000). Results from previous studies and my study helped to develop an optimal protocol for intertidal oyster reefs affected by recreational boat wakes. Results indicated the need for: 1) appropriate substrate and substrate orientation, 2) shell density which maximizes surface area and oyster larval recruitment, 3) the removal of dead margins, and 4) stabilization of dead disarticulated shell. The restoration protocol suggested by my results and being utilized in Mosquito Lagoon, FL, may be applied in other southeastern and Gulf coast states with intertidal reefs facing similar declines.

APPENDIX A – FIGURES

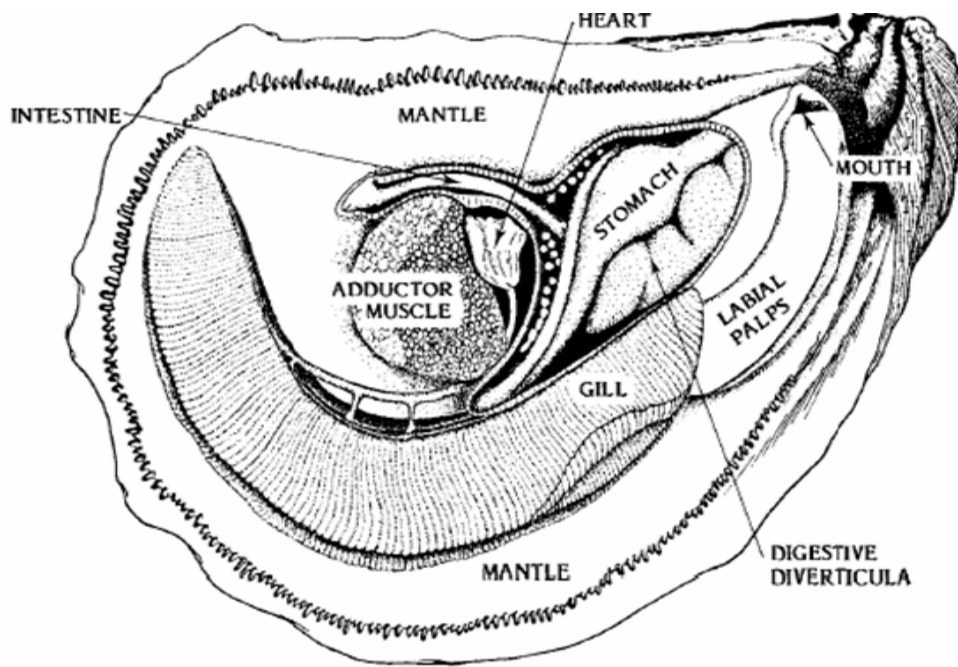


Figure 1: General anatomy of *C. virginica* (Ashbaugh 1951)

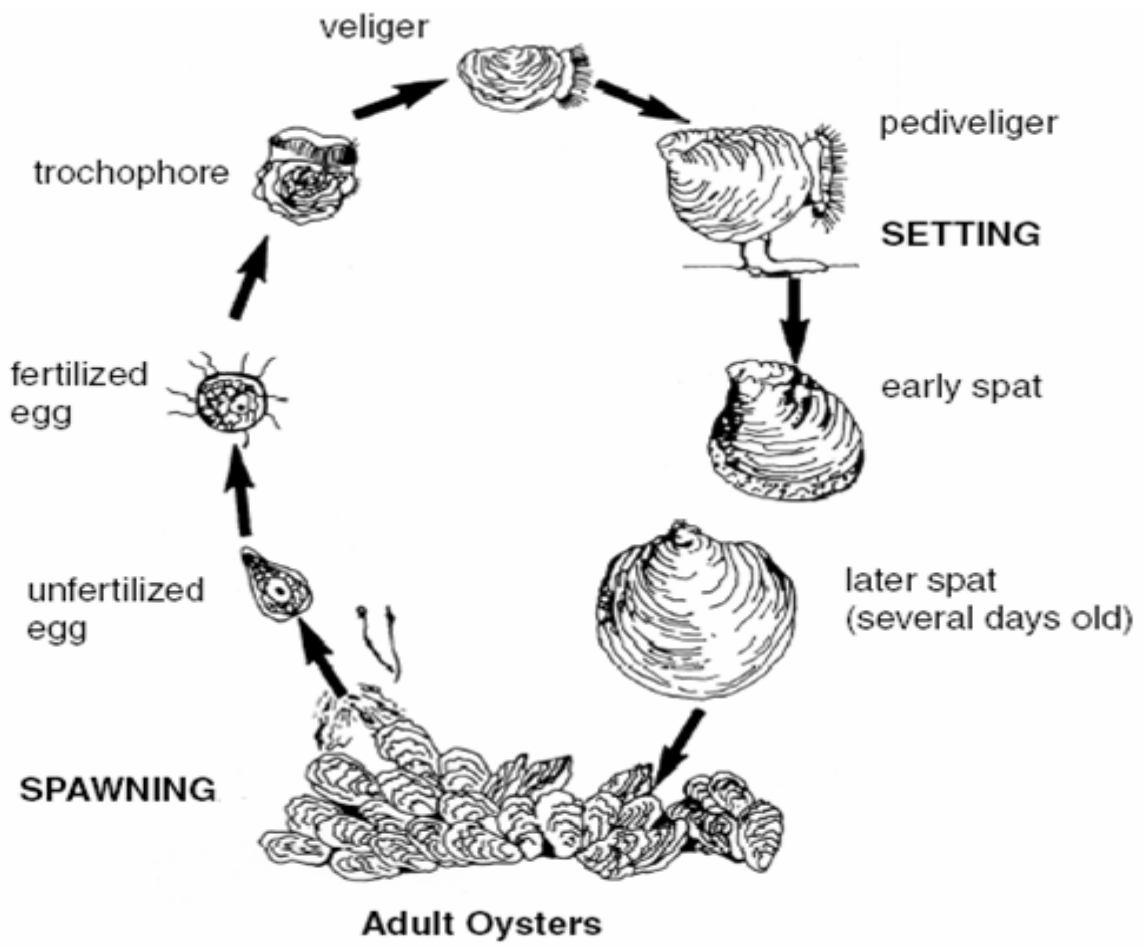


Figure 2: Life cycle of *C. virginica* (Wallace 2001)

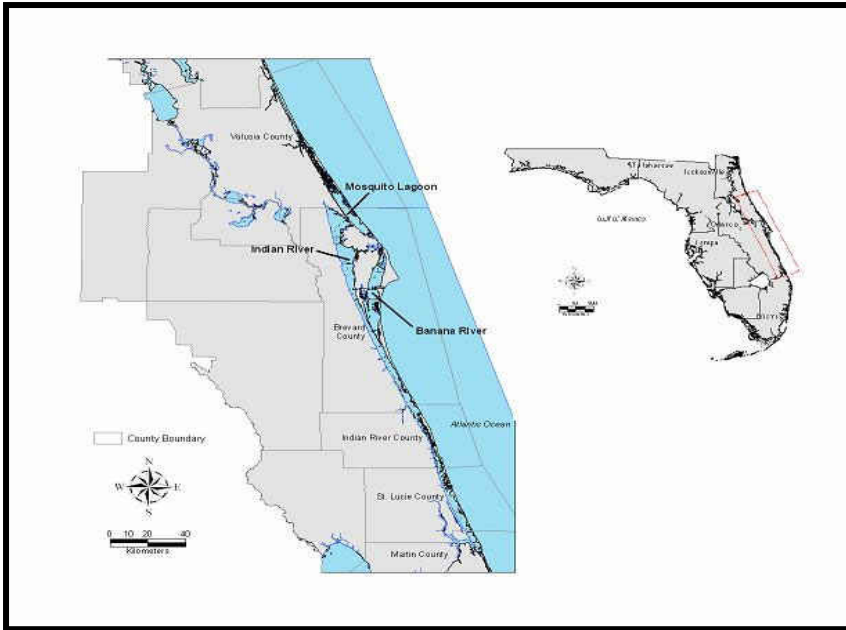


Figure 3: The Indian River Lagoon system

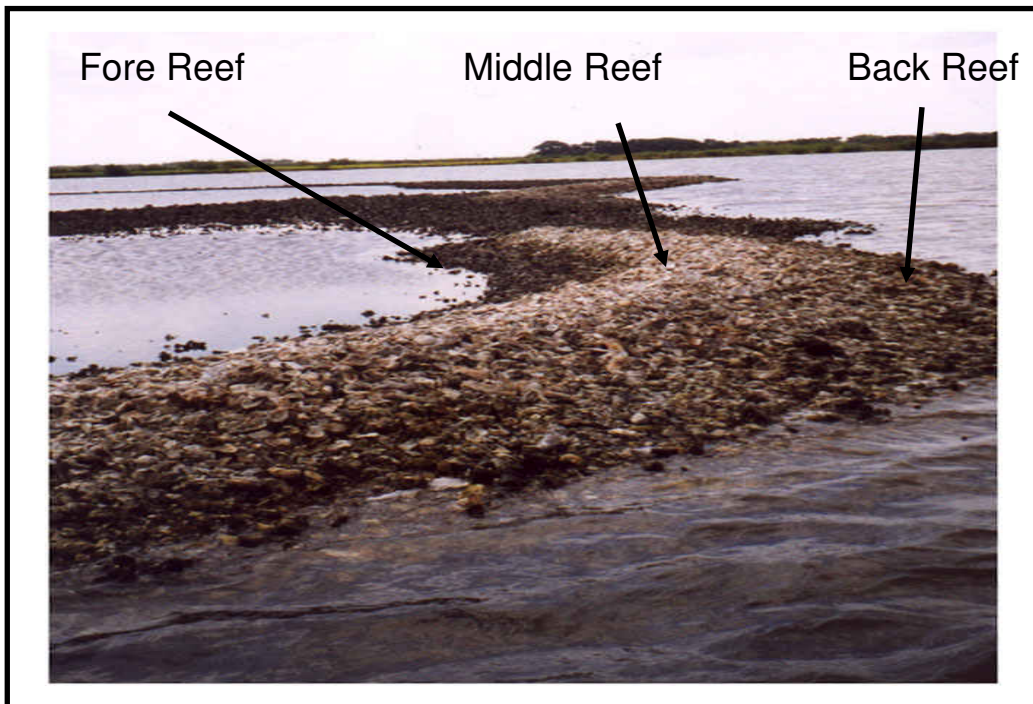


Figure 4: Fore, middle, and back regions of oyster reef in Mosquito Lagoon



Figure 5: Oyster reefs

Top: An impacted oyster reef at mean high water in Mosquito Lagoon with uncharacteristic dead margin. Bottom: A pristine (reference, unimpacted) reef in Mosquito Lagoon at mean low water.



Figure 6: Artificial seagrass used in field experiments



Figure 7: Unleveled reef with seagrass added on seaward edge



Figure 8: Leveling of a treatment reef

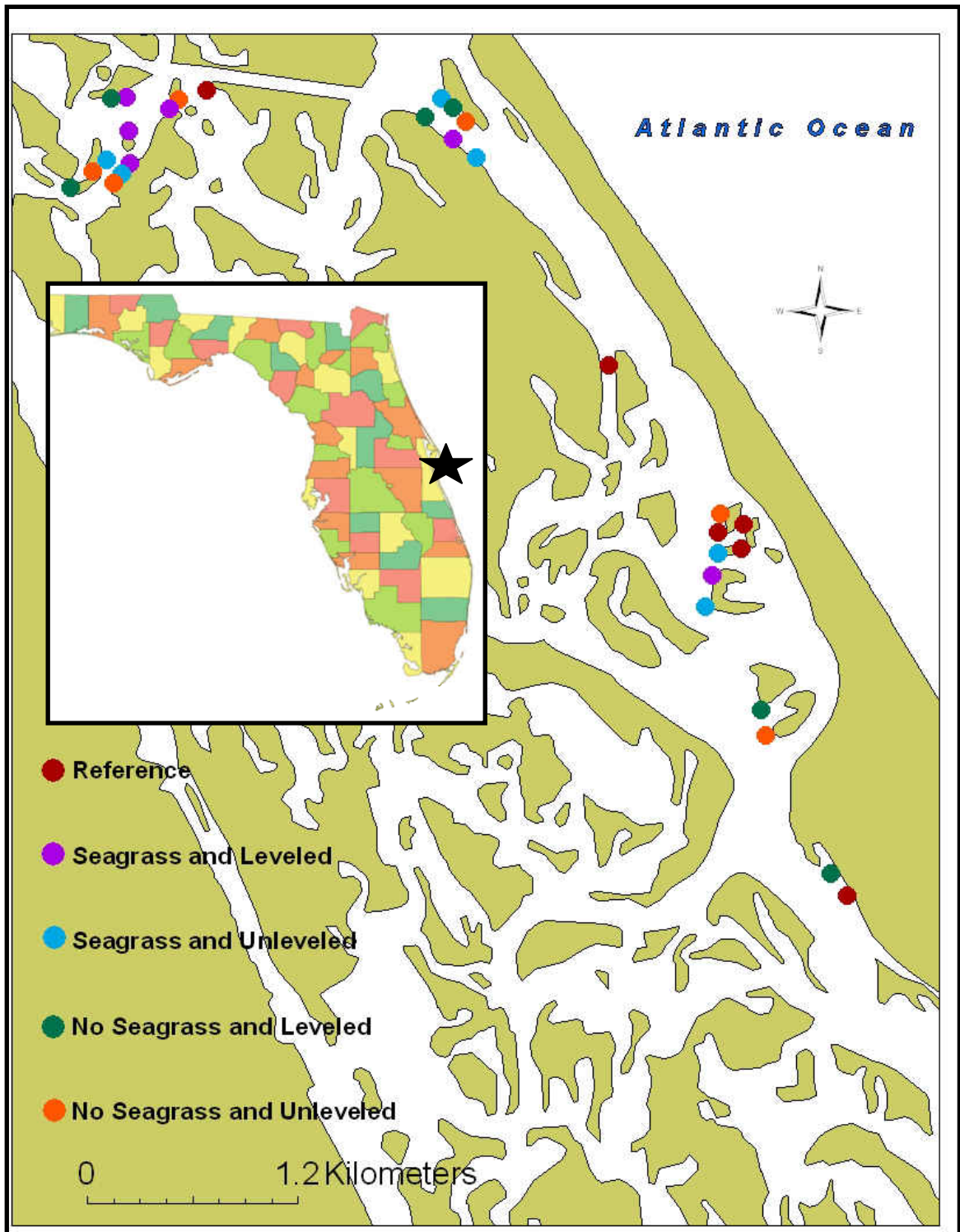


Figure 9: Study sites within Canaveral National Seashore



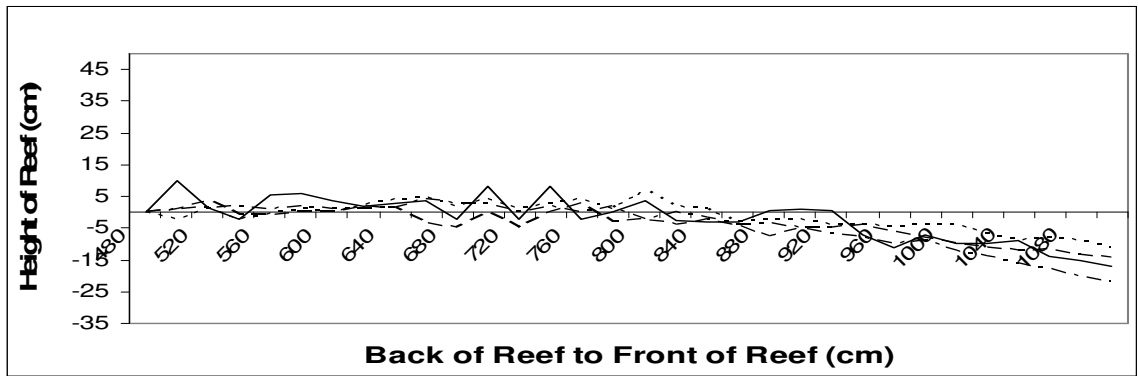
Figure 10: Example of restoration mat



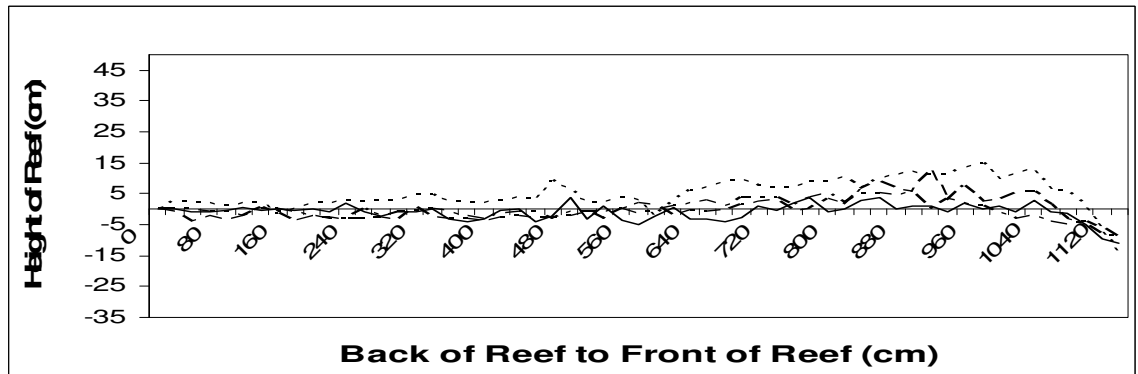
Figure 11: Submerged lift nets

Top: Lift net with Vexar only and two weights. Bottom: Lift net with restoration mat with 36 shells and two weights.

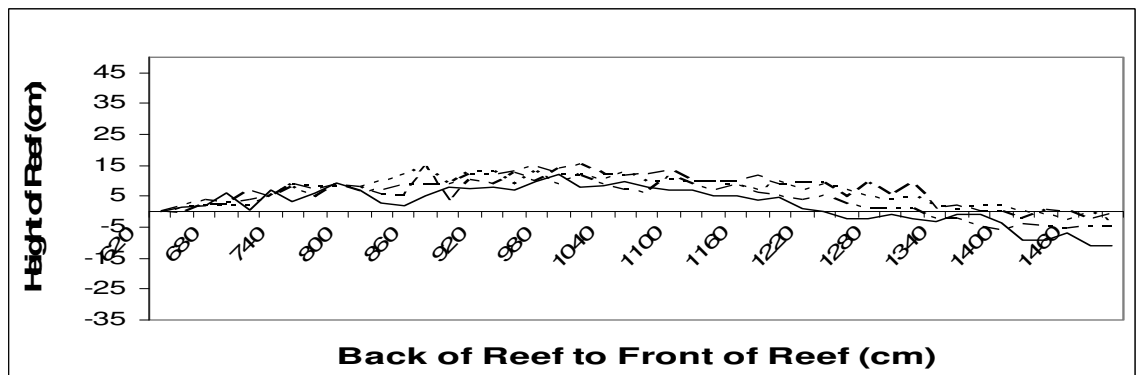
a) Reference reef 1



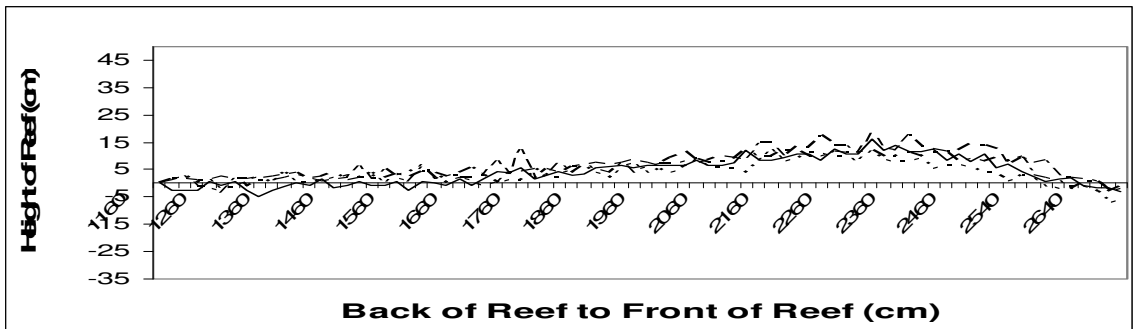
b) Reference reef 2



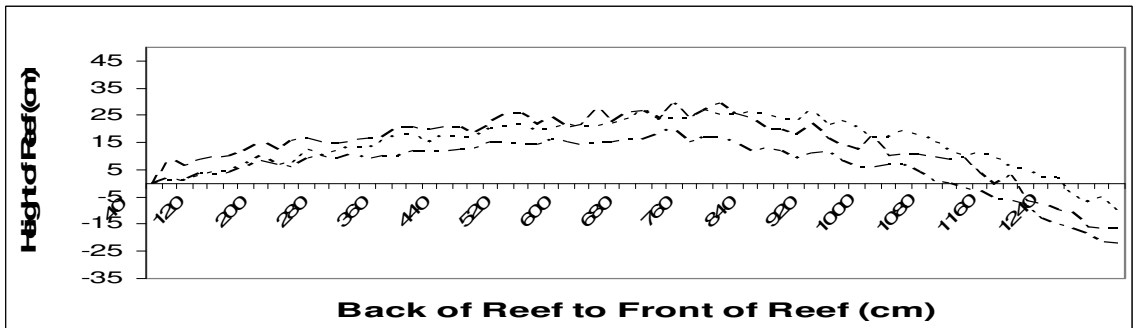
c) Reference reef 3



d) Reference reef 4



e) Reference reef 5



f) Reference reef 6

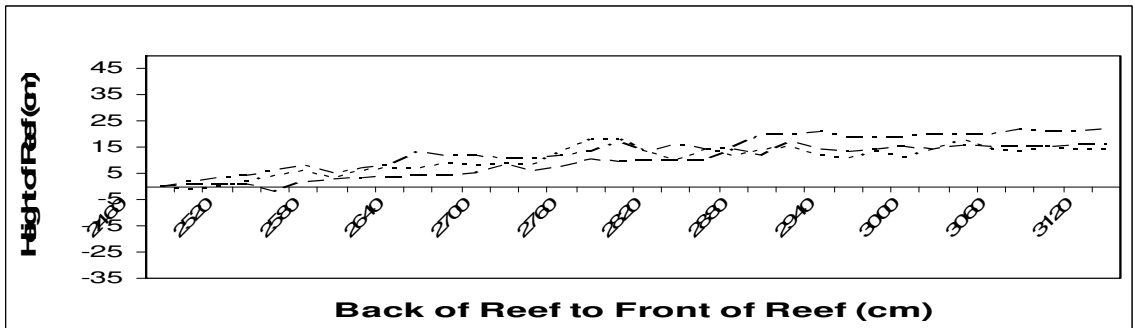
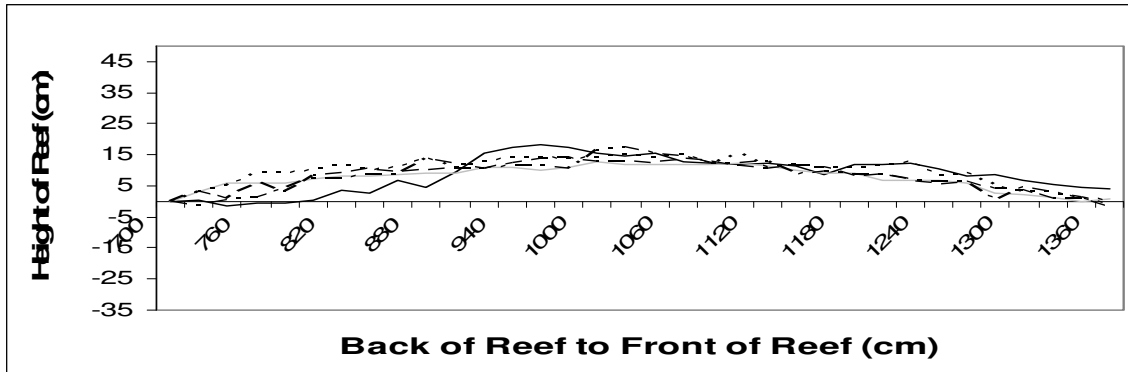


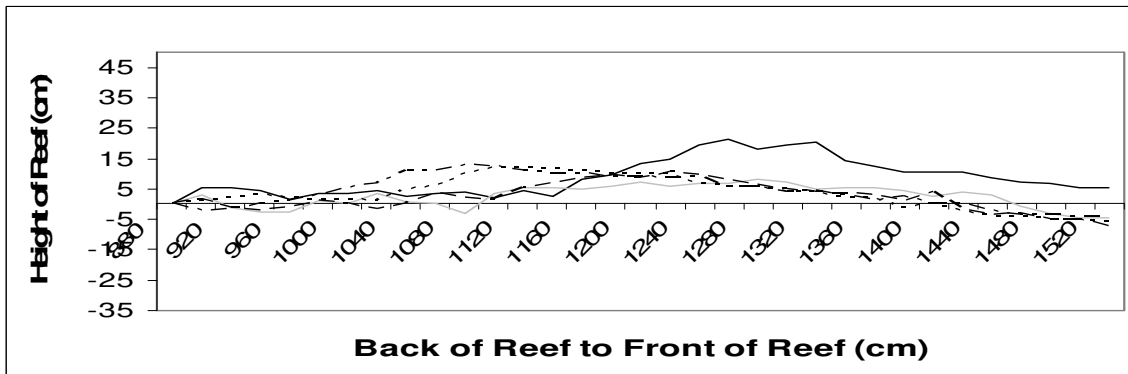
Figure 12: Reference reef profiles

— denotes profile at time zero, — — denotes profile at time 4 months, - - - denotes profile at time 8 months, — - — denotes profile at 12 months.

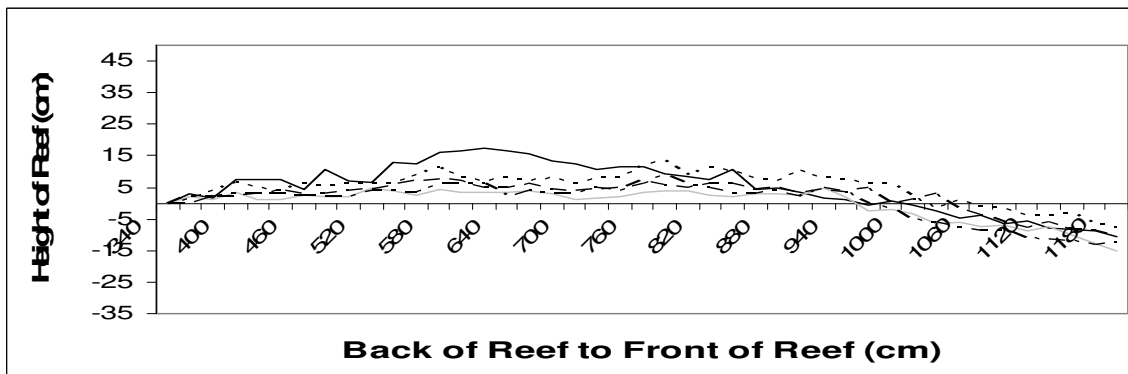
a) No seagrass and leveled reef 1



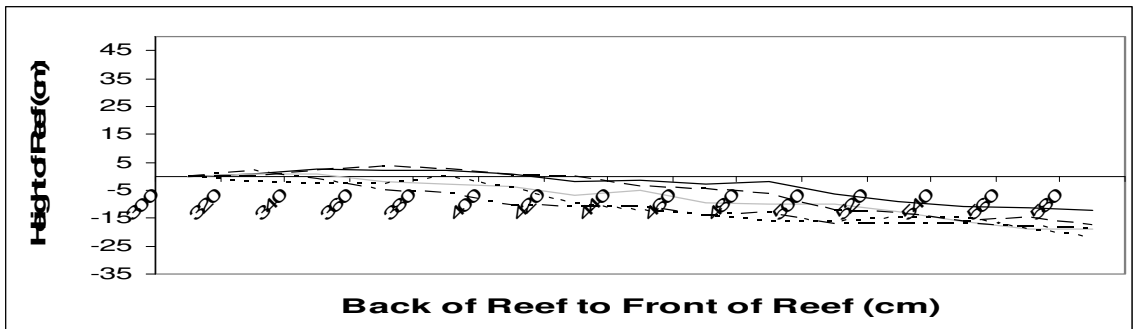
b) No seagrass and leveled reef 2



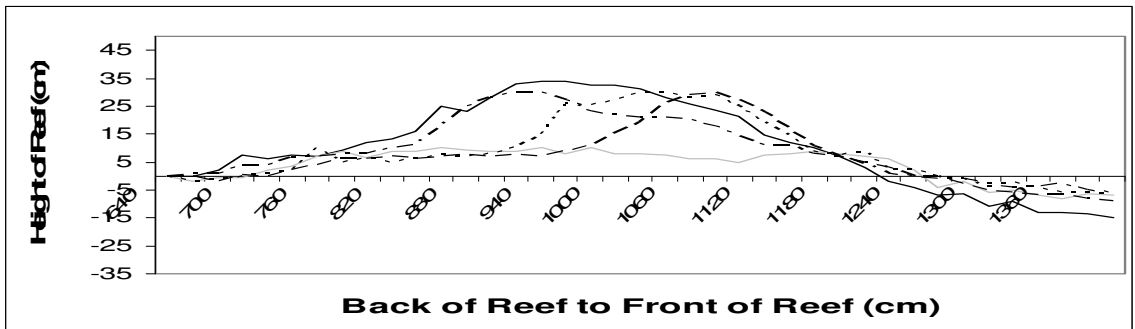
c) No seagrass and leveled reef 3



d) No seagrass and leveled reef 4



e) No seagrass and leveled reef 5



f) No seagrass and leveled reef 6

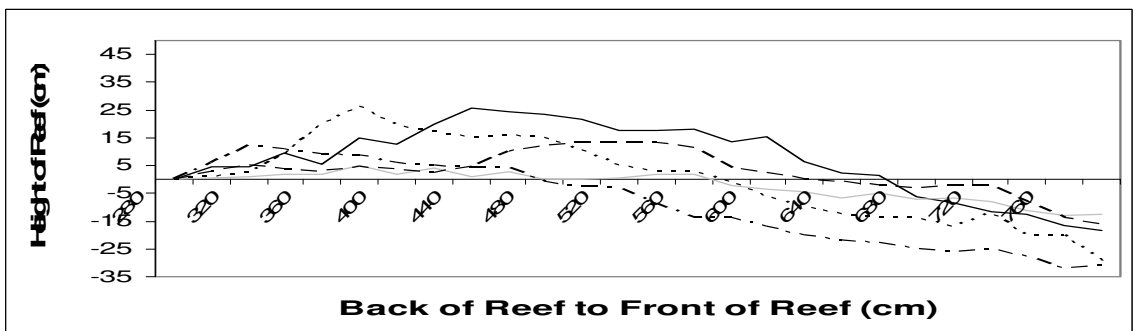
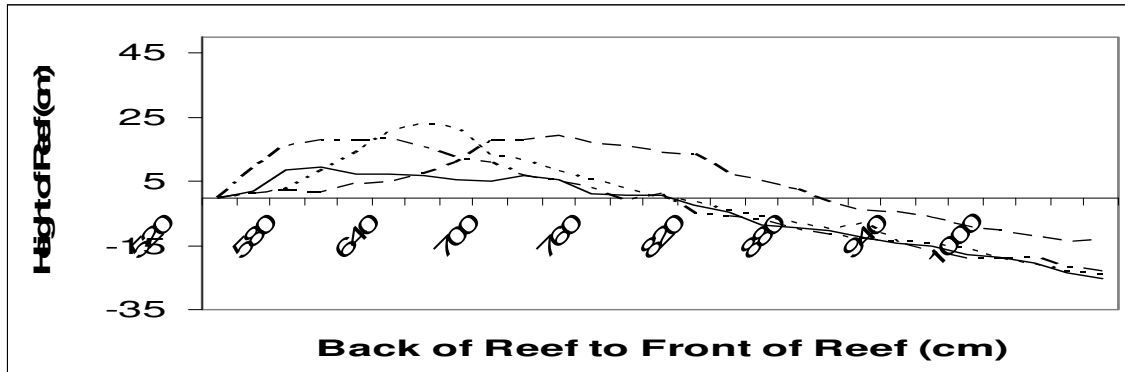


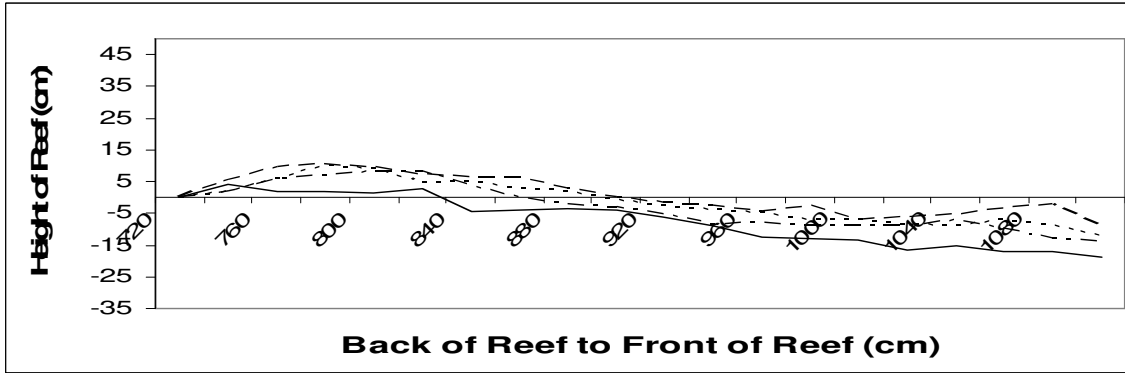
Figure 13: No seagrass and leveled reef profiles

— denotes profile at time zero, — — denotes profile after leveling, — — — denotes profile at time 4 months, - - - - denotes profile at time 8 months, — · — · — denotes profile at 12 months.

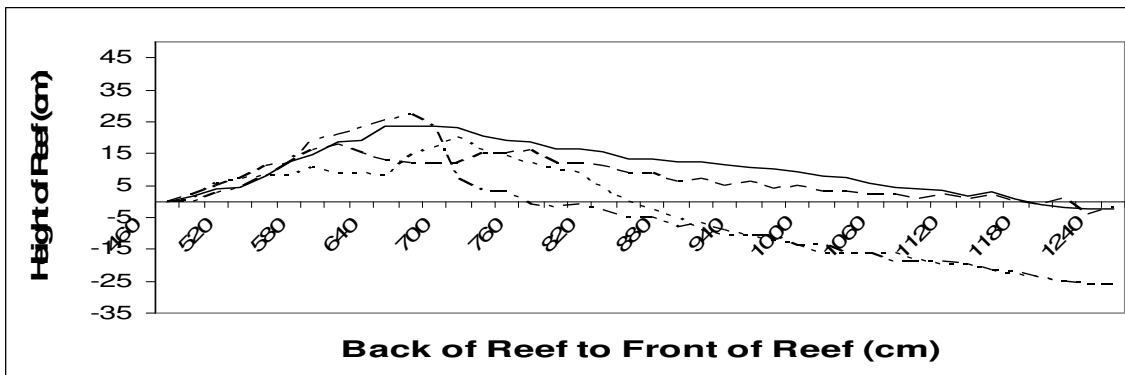
a) No seagrass and unlevelled 1



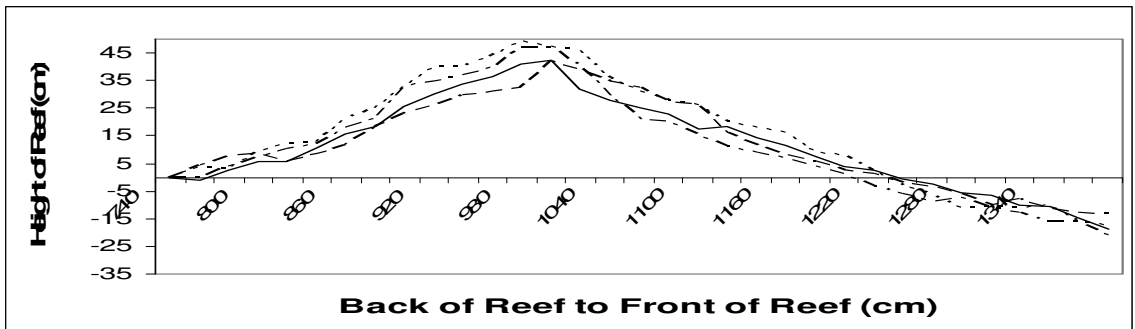
b) No seagrass and unlevelled 2



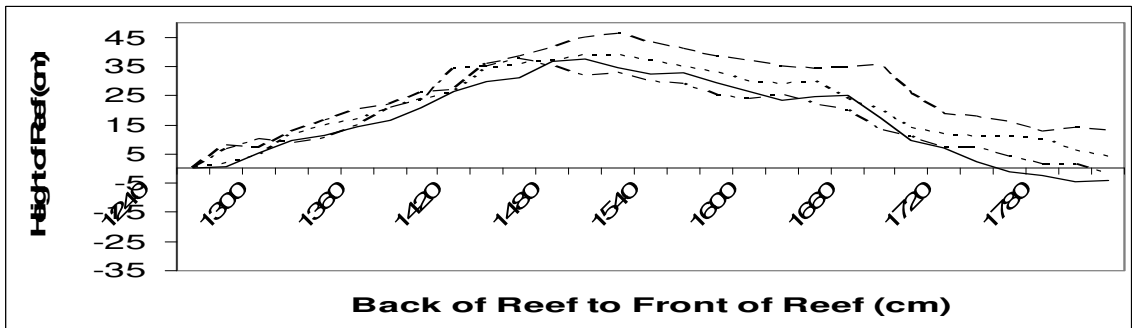
c) No seagrass and unlevelled 3



d) No seagrass and unlevelled 4



e) No seagrass and unlevelled 5



f) No seagrass and unlevelled 6

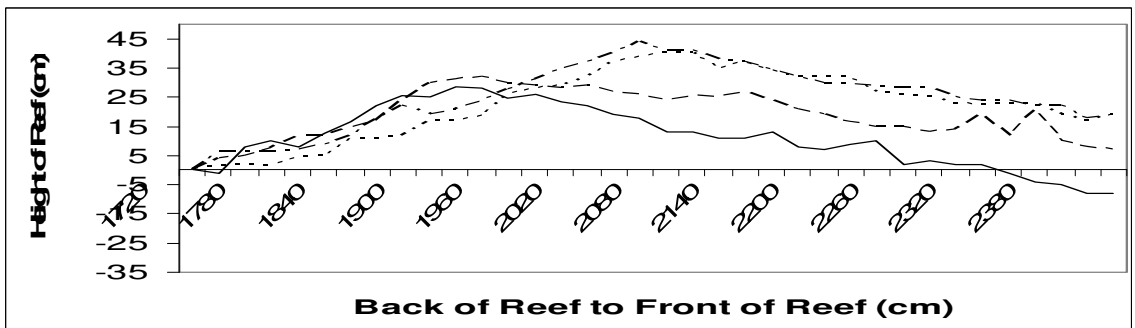
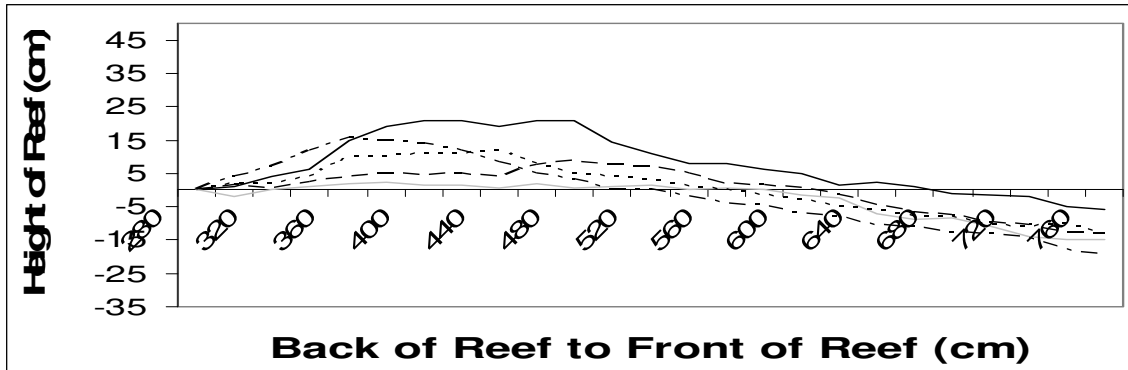


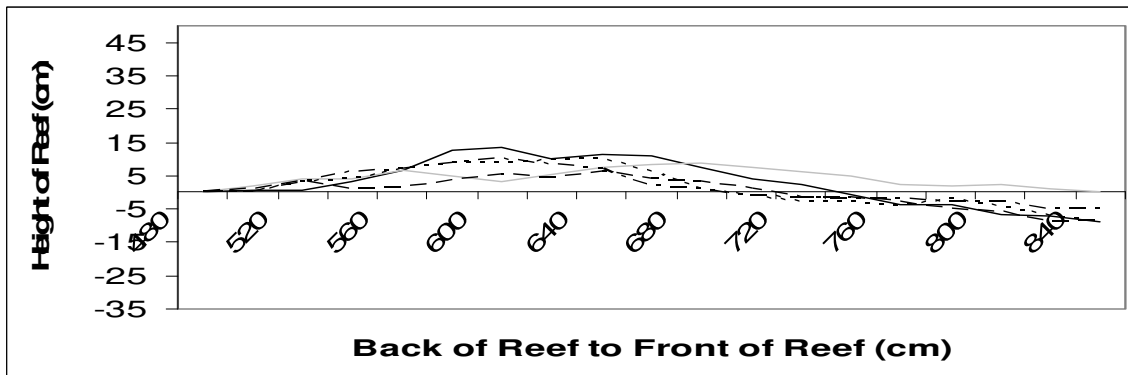
Figure 14: No seagrass and unlevelled reef profiles

— denotes profile at time zero, — — denotes profile at time 4 months, - - - - denotes profile at time 8 months, — · — · denotes profile at 12 months.

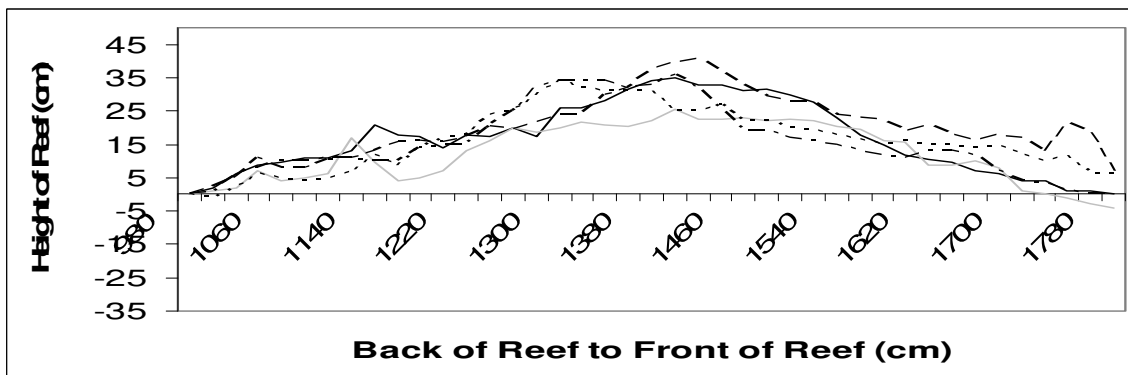
a) Seagrass and leveled 1



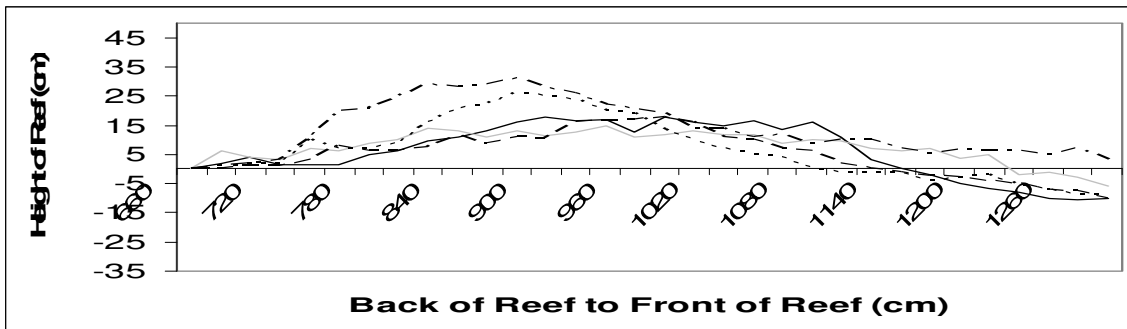
b) Seagrass and leveled 2



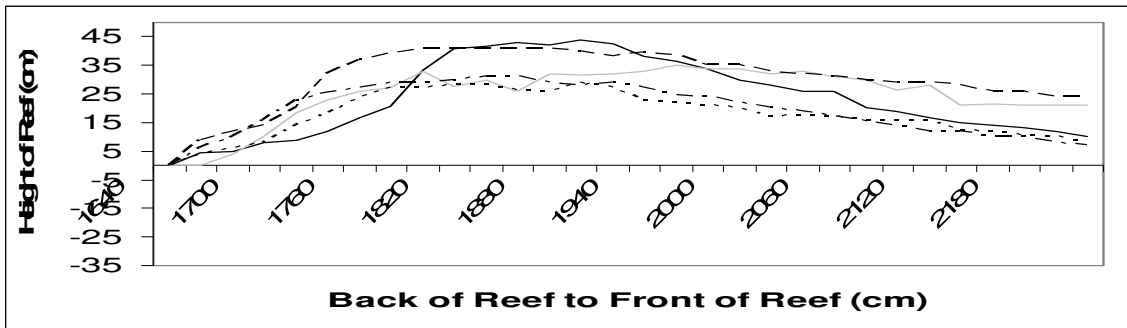
c) Seagrass and leveled 3



d) Seagrass and leveled 4



e) Seagrass and leveled 5



f) Seagrass and leveled 6

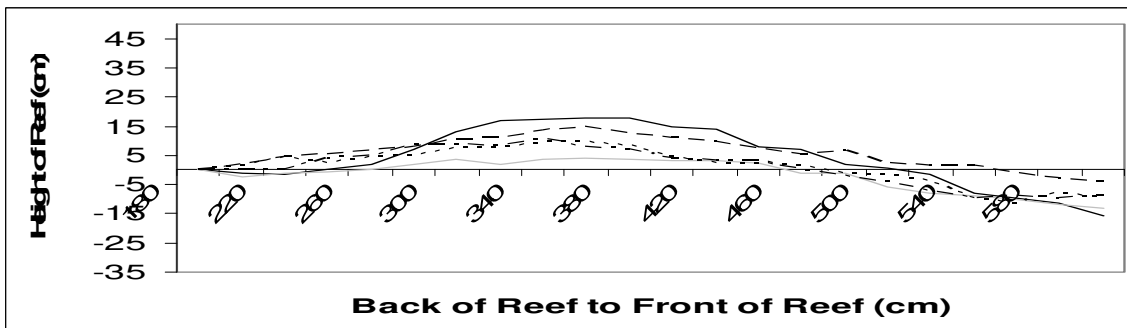
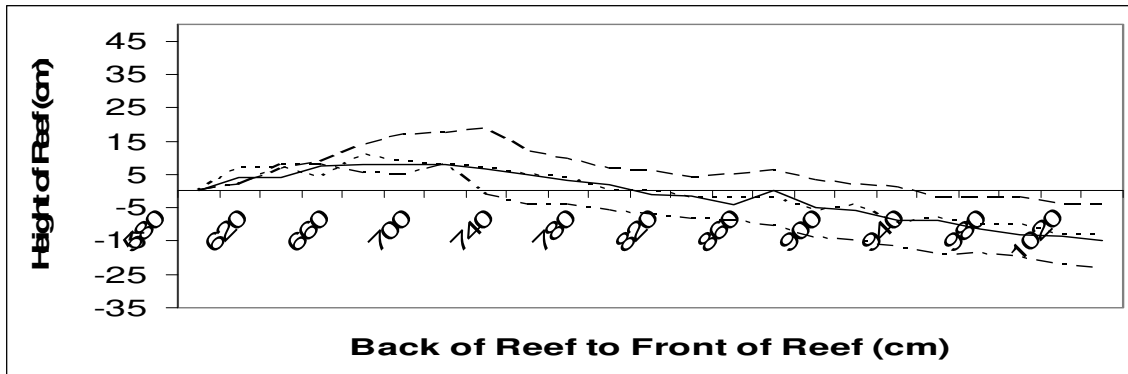


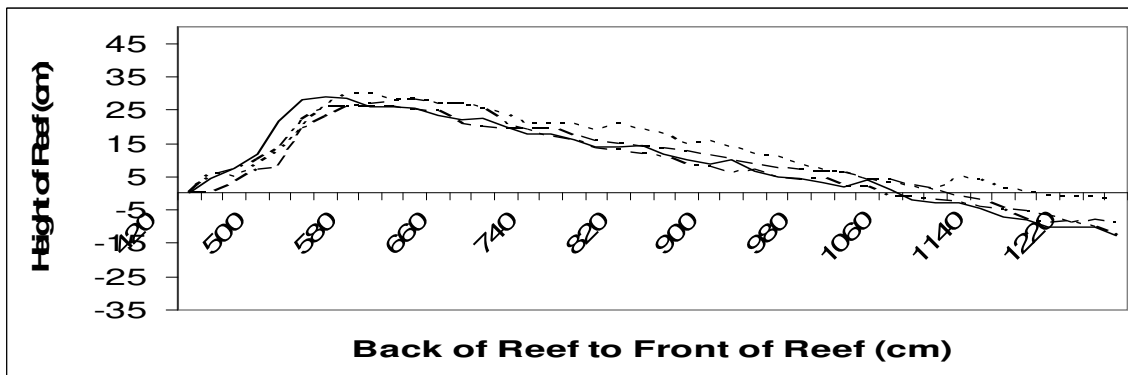
Figure 15: Seagrass and leveled reef profiles

— denotes profile at time zero, — denotes profile after leveling, — — denotes profile at time 4 months, - - - denotes profile at time 8 months, — - — - denotes profile at 12 months.

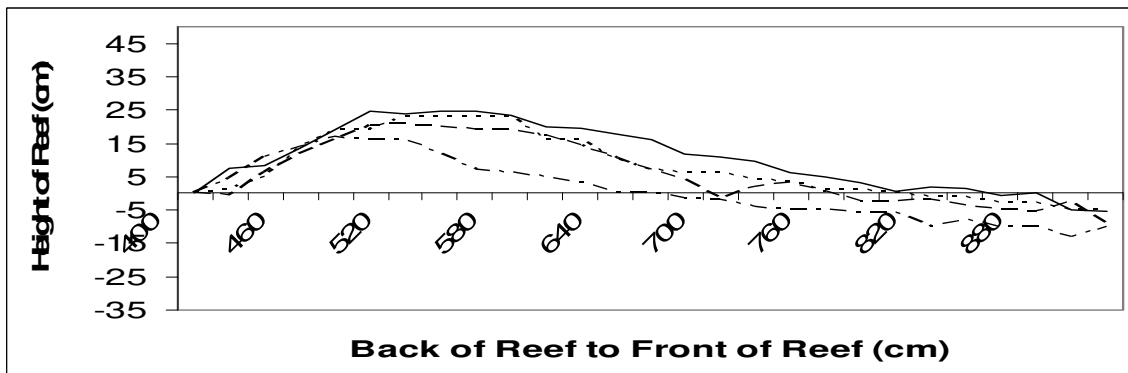
a) Seagrass and unlevelled reef 1



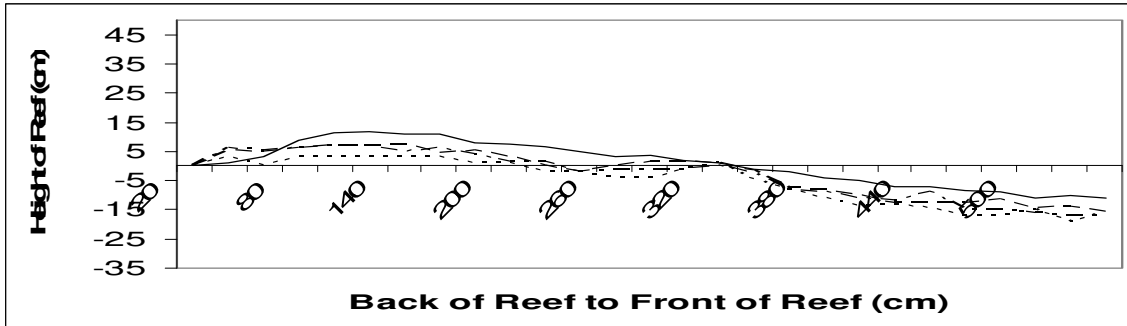
b) Seagrass and unlevelled reef 2



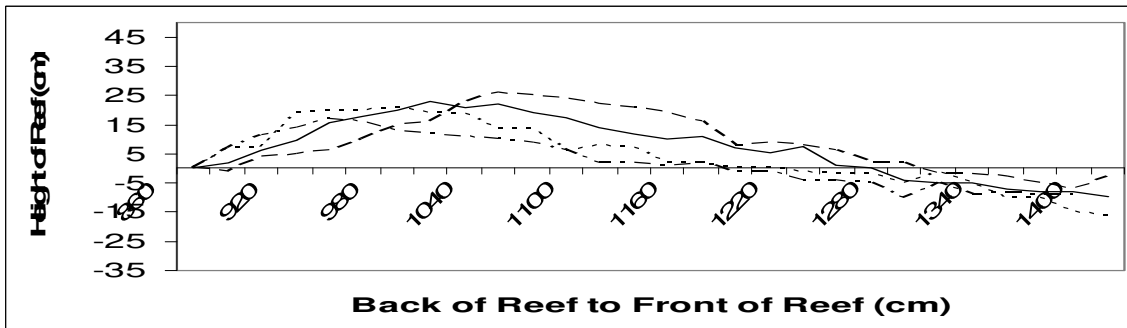
c) Seagrass and unlevelled reef 3



d) Seagrass and unlevelled reef 4



e) Seagrass and unlevelled reef 5



f) Seagrass and unlevelled reef 6

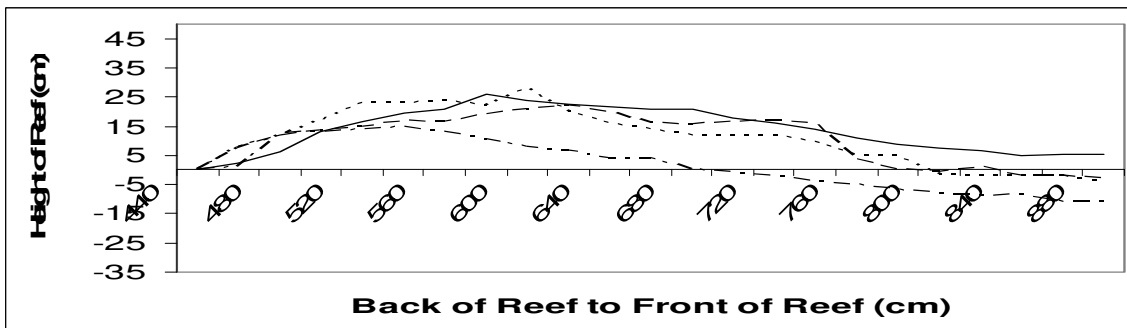


Figure 16: Seagrass and unlevelled reef profiles

— denotes profile at time zero, — — denotes profile at time 4 months, - - - - denotes profile at time 8 months, — · — · denotes profile at 12 months.

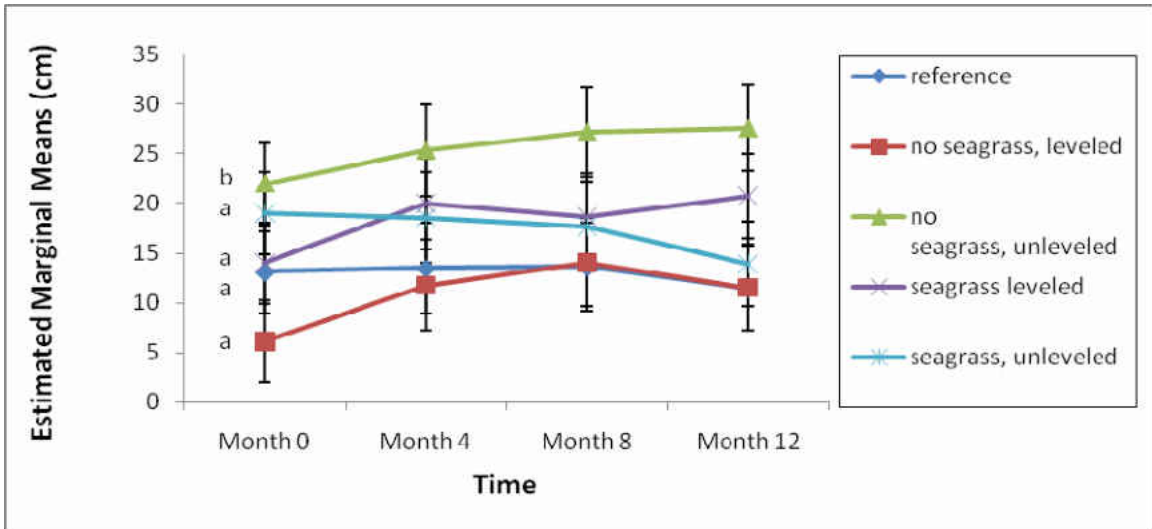


Figure 17: Change in reef heights (\pm SE) of treatments over time

Lower case letters indicate significant differences found between reference and treatment reefs as indicated by repeated measure ANOVA simple contrasts.

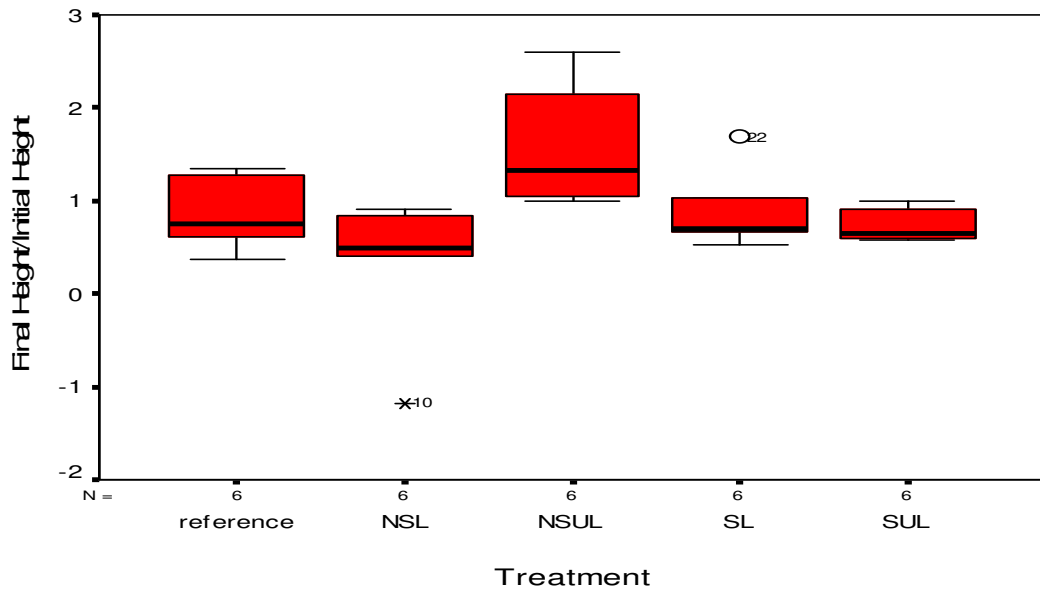


Figure 18: Box plots showing ratio of final heights to initial heights of reefs in all treatments

Treatments included no seagrass and leveled (NSL), no seagrass and unleveled (NSUL), seagrass and leveled (SL), and seagrass and unleveled (SUL).

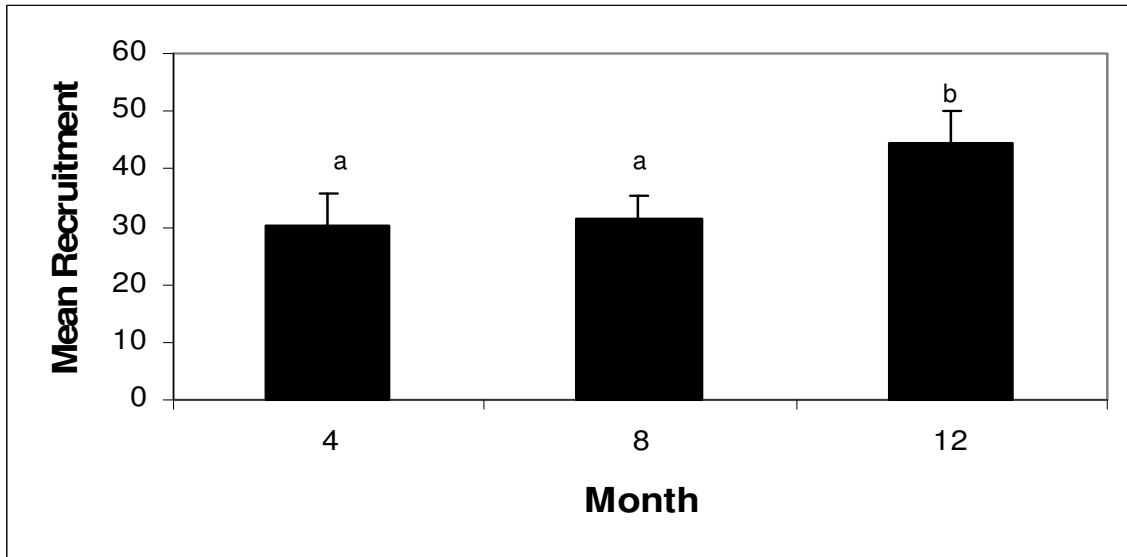


Figure 19: Mean oyster recruitment (\pm SE) at 4, 8, and 12 months overall
Lower-case letters refer to repeated measures ANOVA pairwise comparisons with Bonferroni corrections among months.

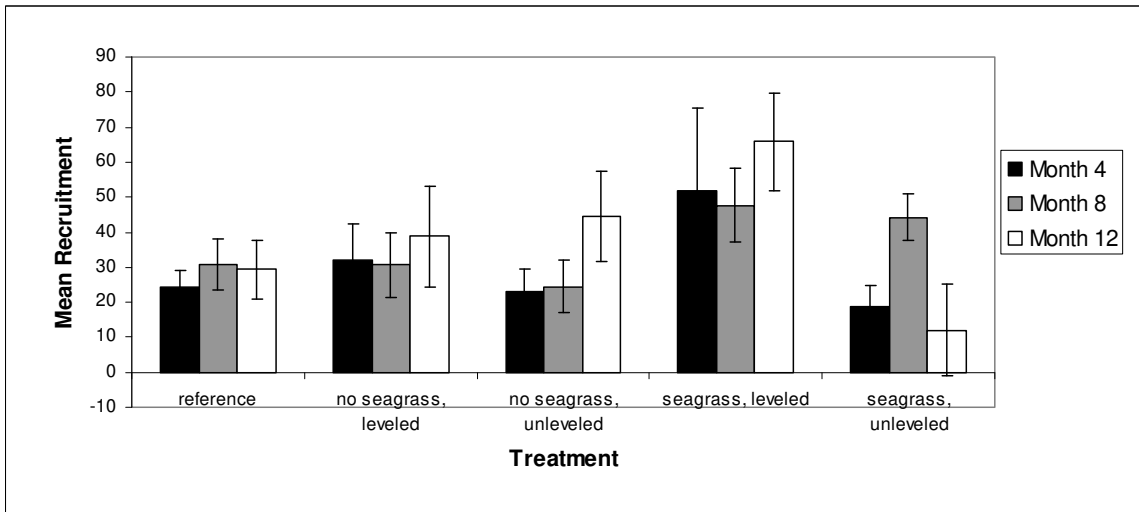


Figure 20: Mean oyster recruitment (\pm SE) at 4, 8, and 12 months for reference and treatment reefs

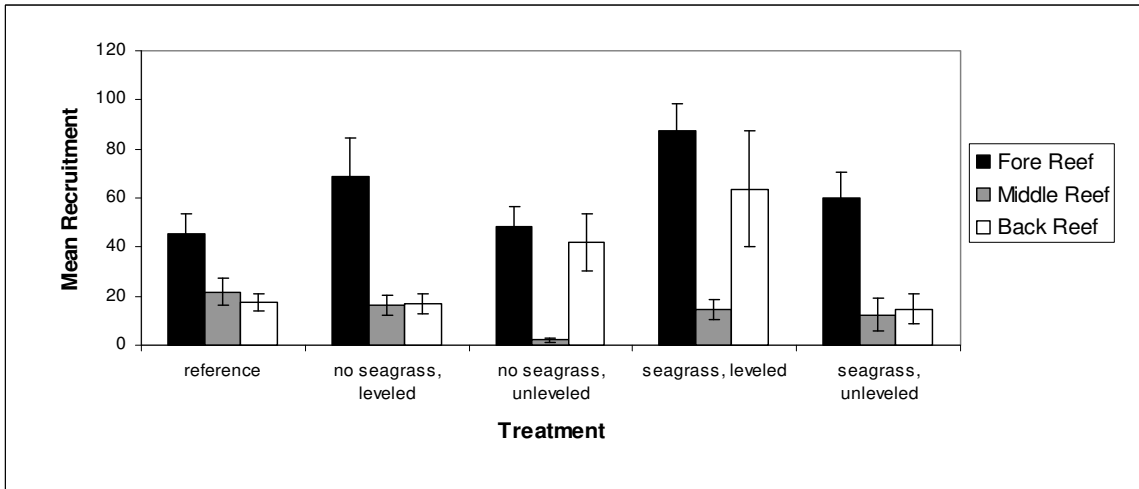


Figure 21: Mean oyster recruitment (\pm SE) for fore, middle, and back regions of reference and treatment reefs

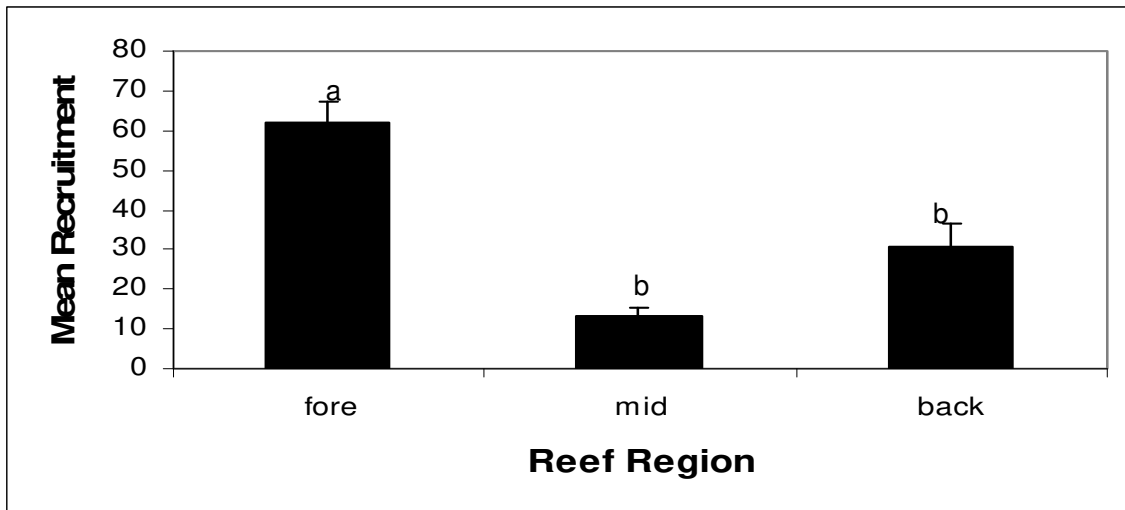


Figure 22: Mean oyster recruitment (\pm SE) for fore, mid, and back reef regions overall

Lower-case letters refer to the Games-Howell *post hoc* comparisons among locations.

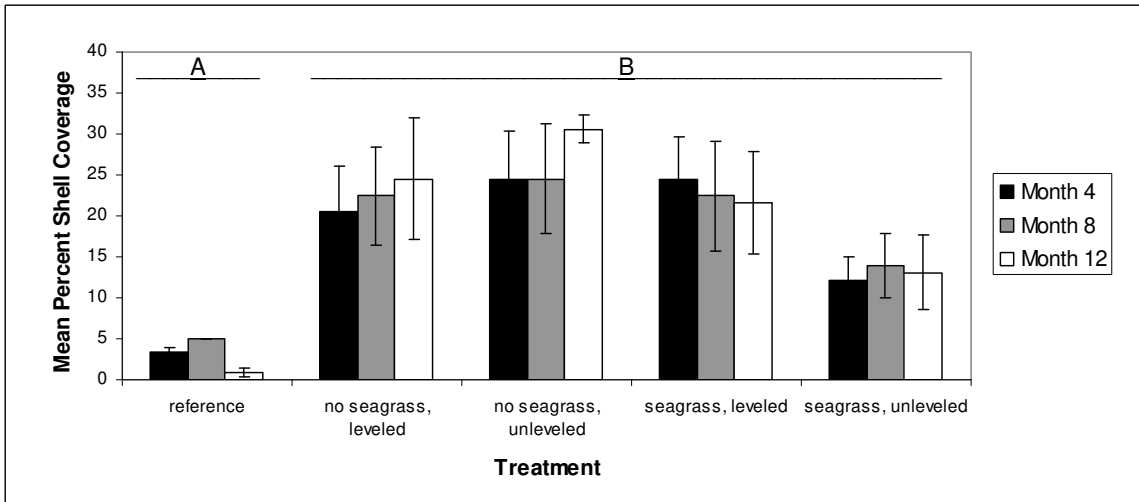


Figure 23: Mean restoration mat coverage by loose shell (\pm SE) for 4, 8, and 12 months for reference and treatment reefs

Capital letters refer to the differences among treatment reef types and the reference reefs at the $p \leq 0.05$ level according to repeated measures ANOVA comparison.

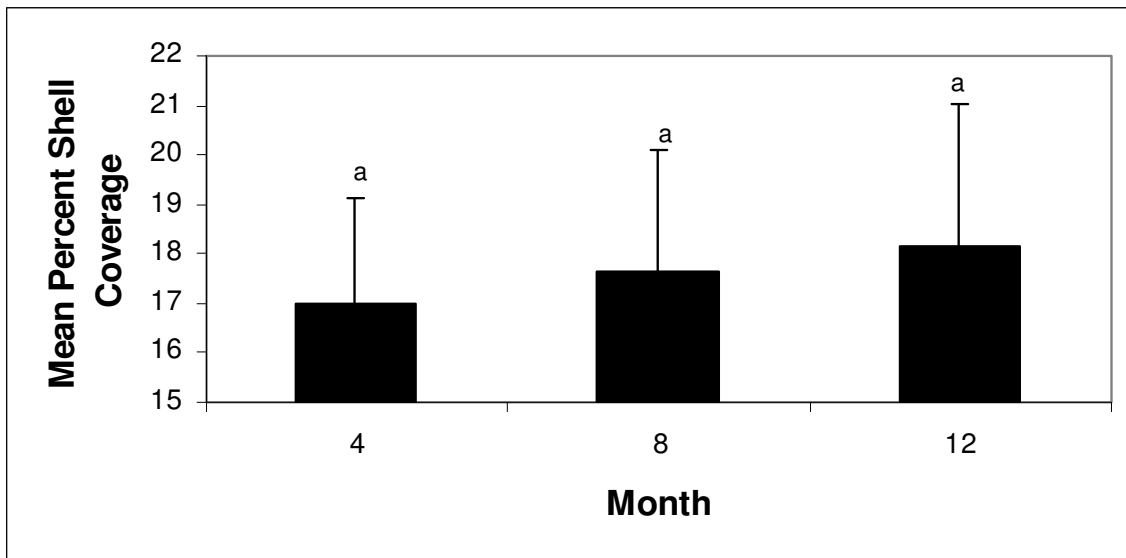


Figure 24: Mean restoration mat coverage (\pm SE) at 4, 8, and 12 months overall
Lower-case letters refer to the Greenhouse Geiser comparisons among months.

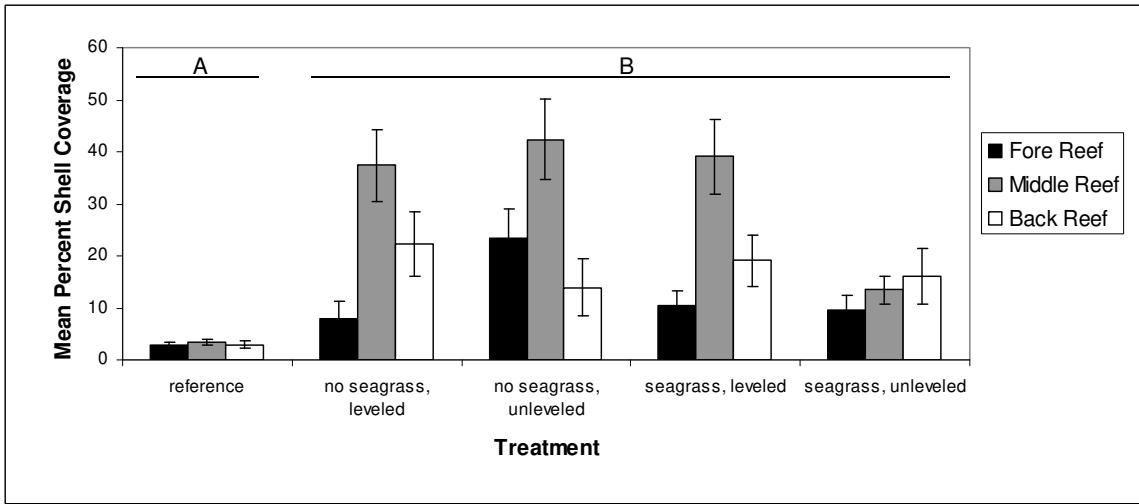


Figure 25: Mean restoration mat coverage (\pm SE) for fore, middle, and back regions of reference and treatment reefs

Capital letters refer to the differences among treatment reef types and the reference reefs at the $p \leq 0.05$ level according to repeated measures ANOVA comparison.

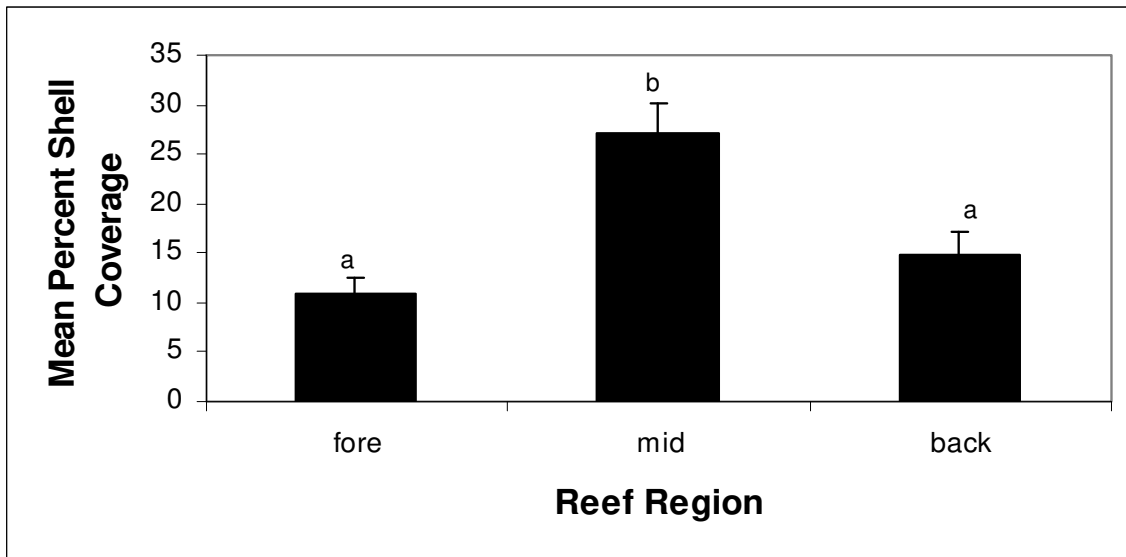


Figure 26: Mean shell coverage (\pm SE) for fore, mid, and back reef regions overall
Lower-case letters refer to the Games-Howell *post hoc* comparisons among months.

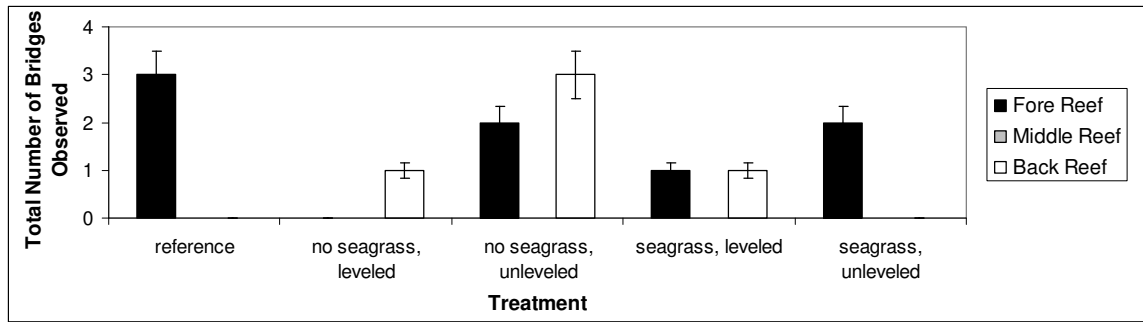


Figure 27: Total number of bridges (\pm SE) formed in the front, middle, and back of reefs for reference reefs and the four treatment types.

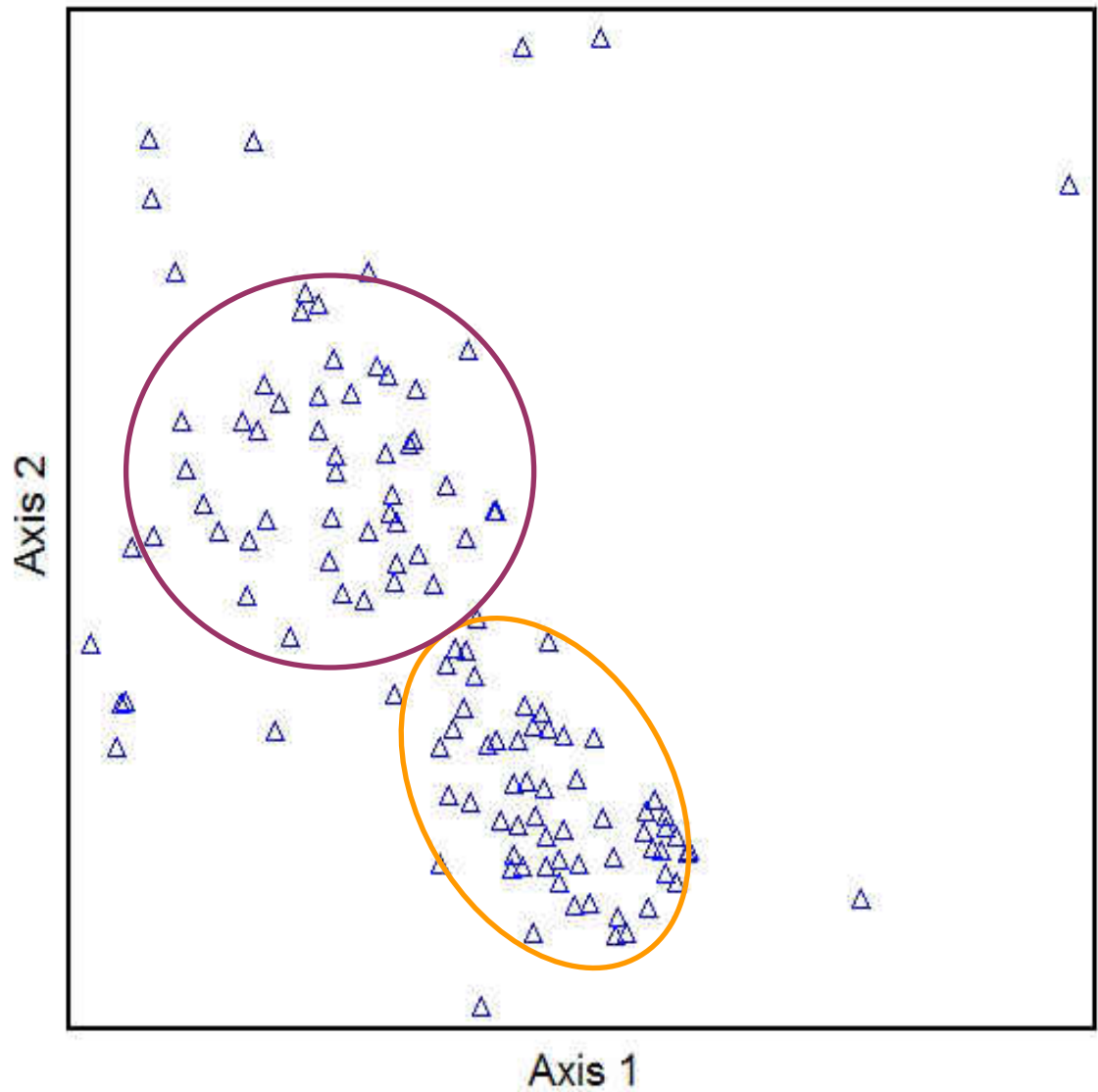


Figure 28: NMS ordination graph for change in biodiversity from May to June
The orange oval contains the majority of lift nets which had restoration mats placed in them. The purple circle contains the majority of lift nets which had Vexar only placed in them. Here, biodiversity in lift nets were significantly different.

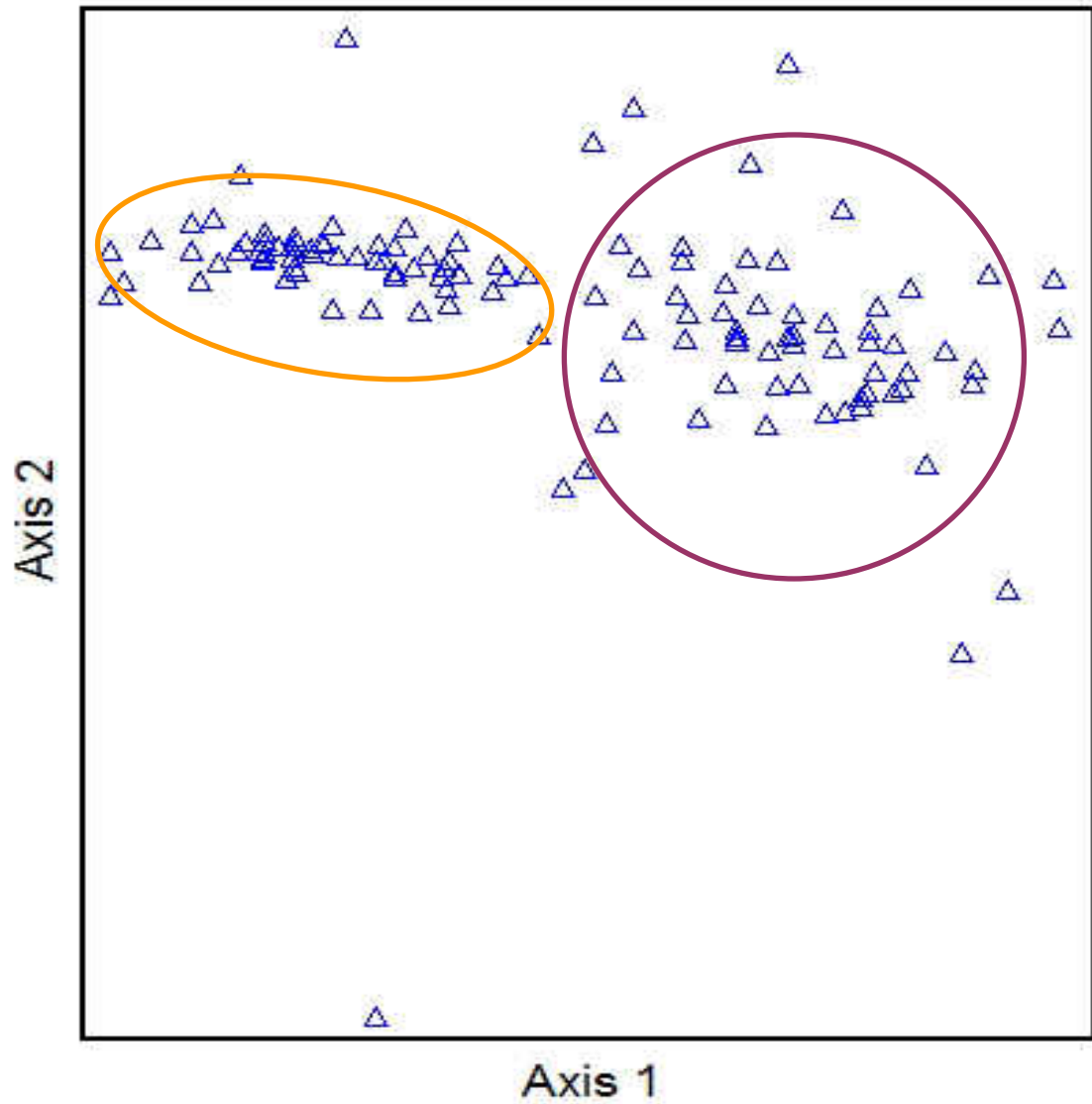


Figure 29: NMS ordination graph for change in biodiversity from June to July
The orange oval contains the majority of lift nets which had restoration mats placed in them. The purple circle contains the majority of lift nets which had Vexar only placed in them. Here, biodiversity in lift nets were significantly different.

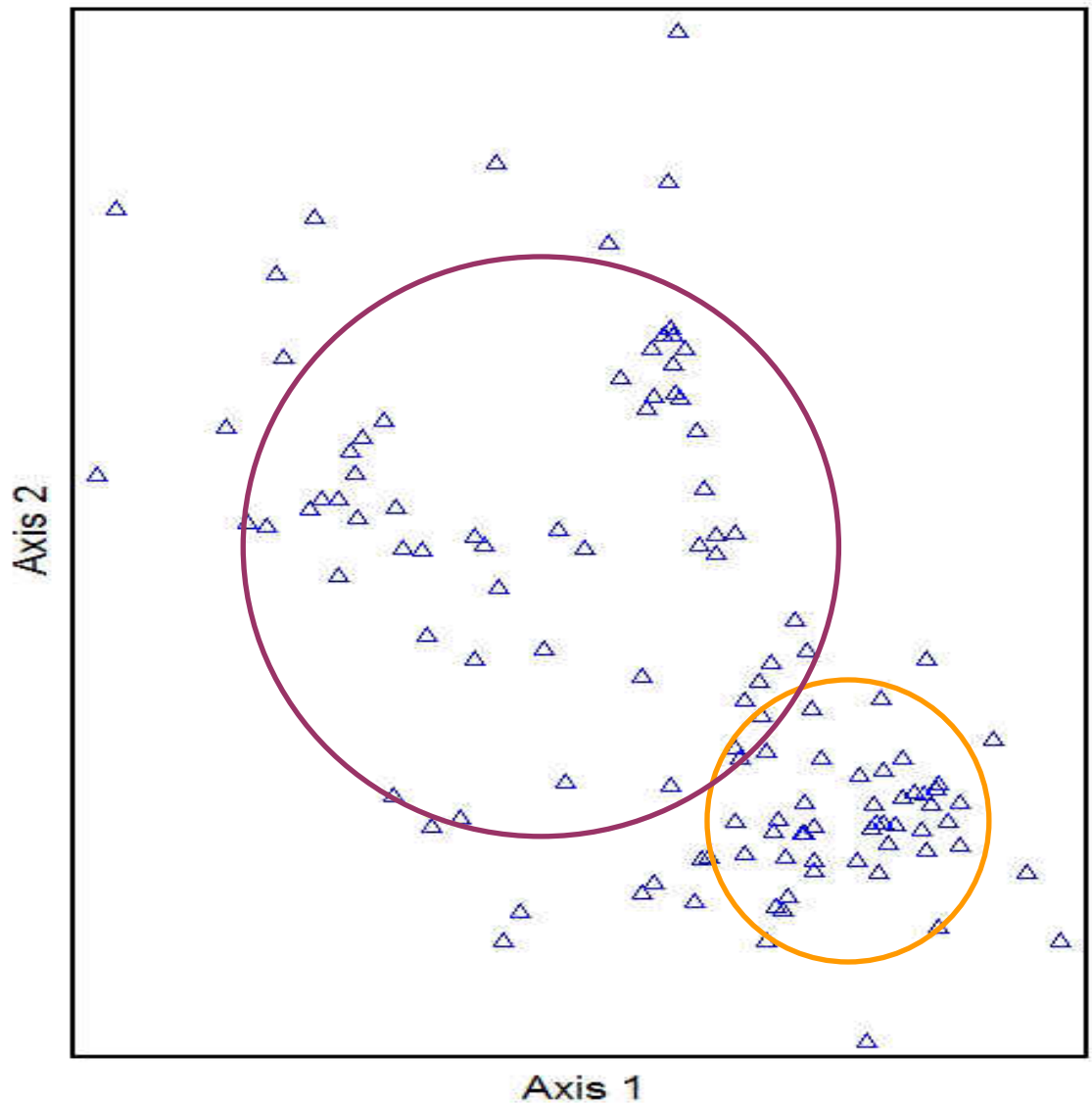


Figure 30: NMS ordination graph for change in biodiversity from July to August
The orange circle contains the majority of lift nets which had restoration mats placed in them. The purple circle contains the majority of lift nets which had Vexar only placed in them. Here, biodiversity in lift nets were significantly different.

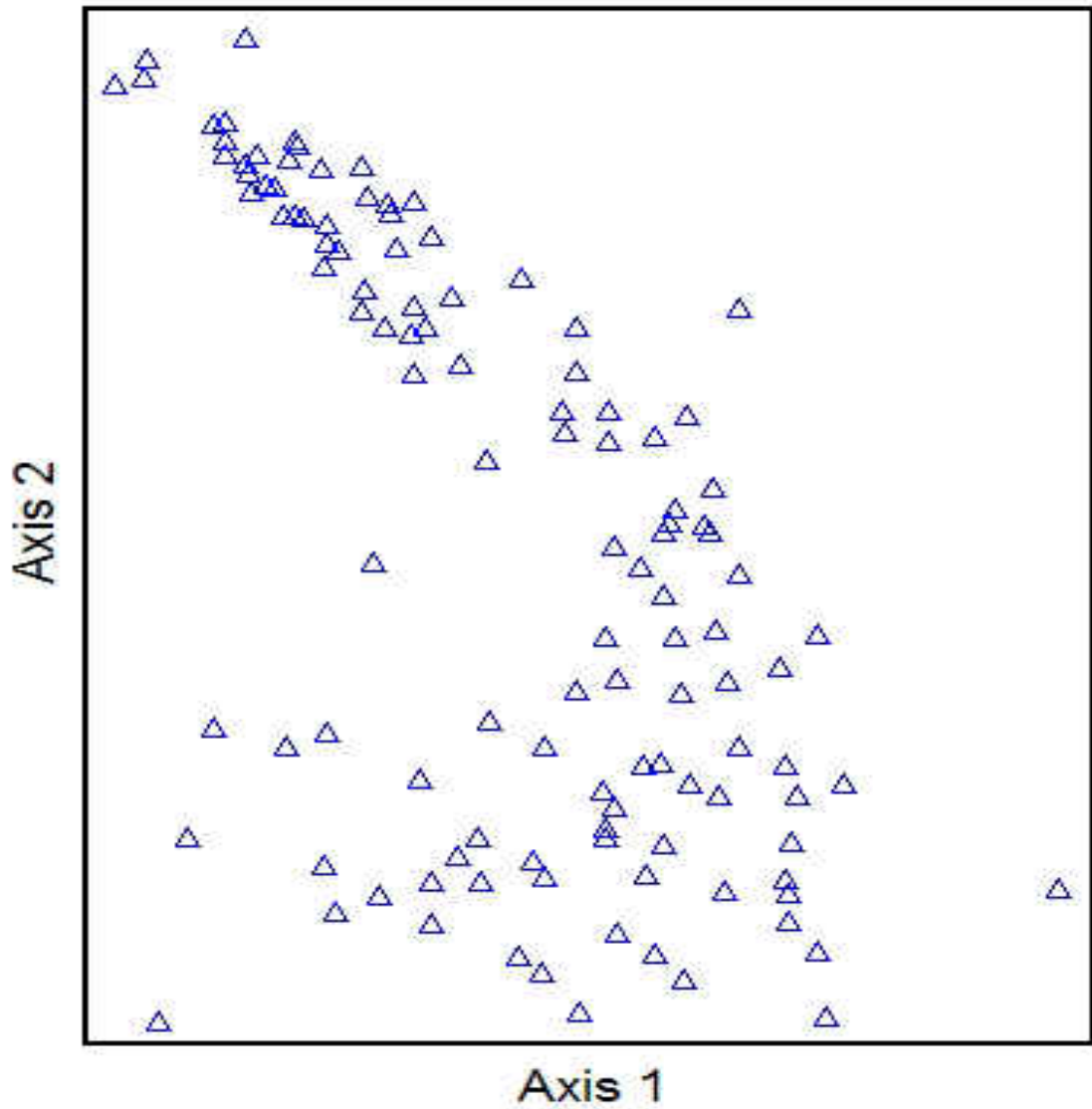


Figure 31: NMS ordination graph for change in biodiversity from August to September

Biodiversity in lift nets were not significantly different.

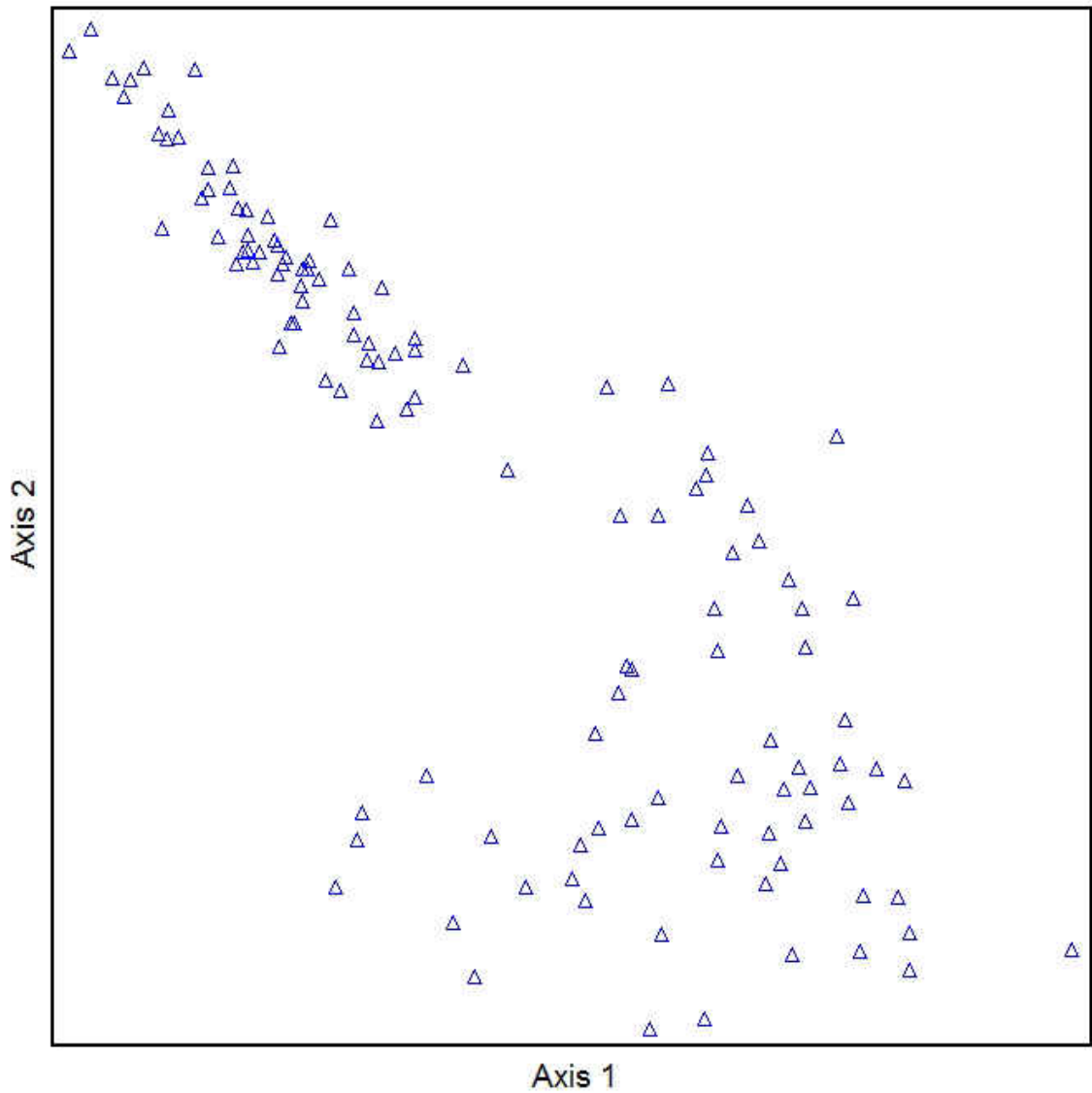


Figure 32: NMS ordination graph for change in biodiversity from September to October

Biodiversity in lift nets were not significantly different.

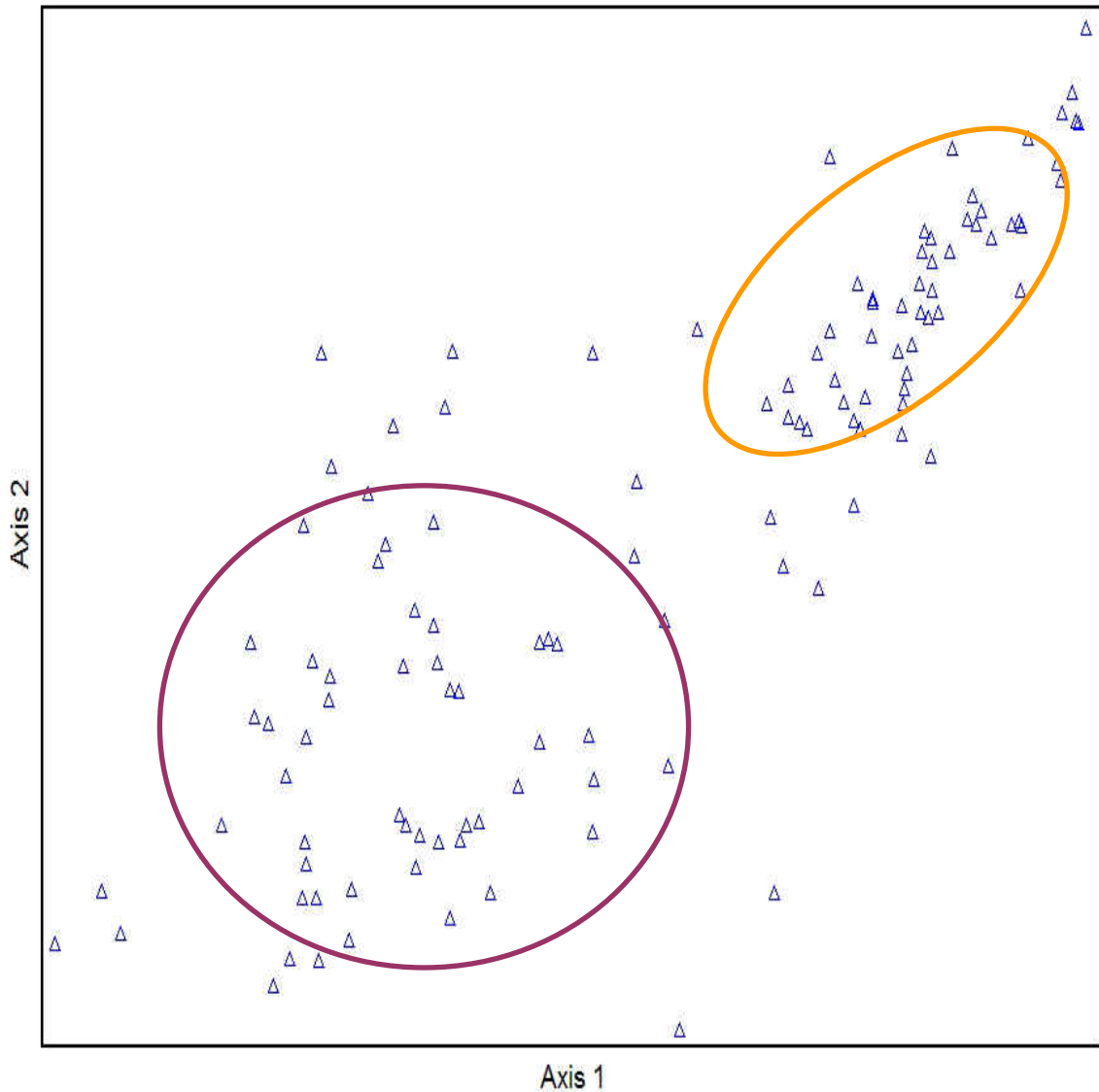


Figure 33: NMS ordination graph for change in biodiversity from October to November

The orange oval contains the majority of lift nets which had restoration mats placed in them. The purple circle contains the majority of lift nets which had Vexar only placed in them. Here, biodiversity in lift nets were significantly different.

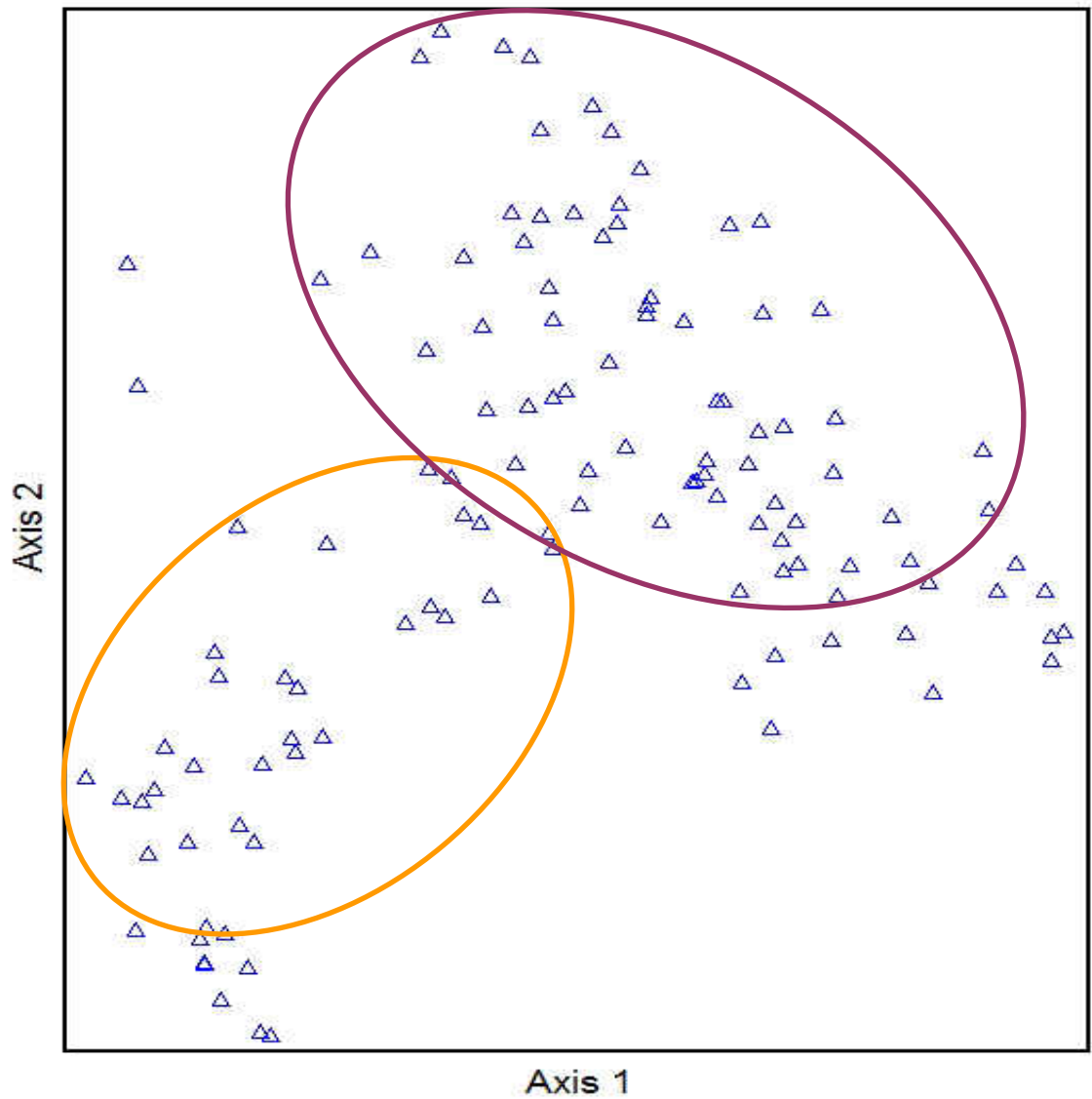


Figure 34: NMS ordination graph for change in biodiversity from November to December

The orange oval contains the majority of lift nets which had restoration mats placed in them. The purple oval contains the majority of lift nets which had Vexar only placed in them. Here, biodiversity in lift nets were significantly different.

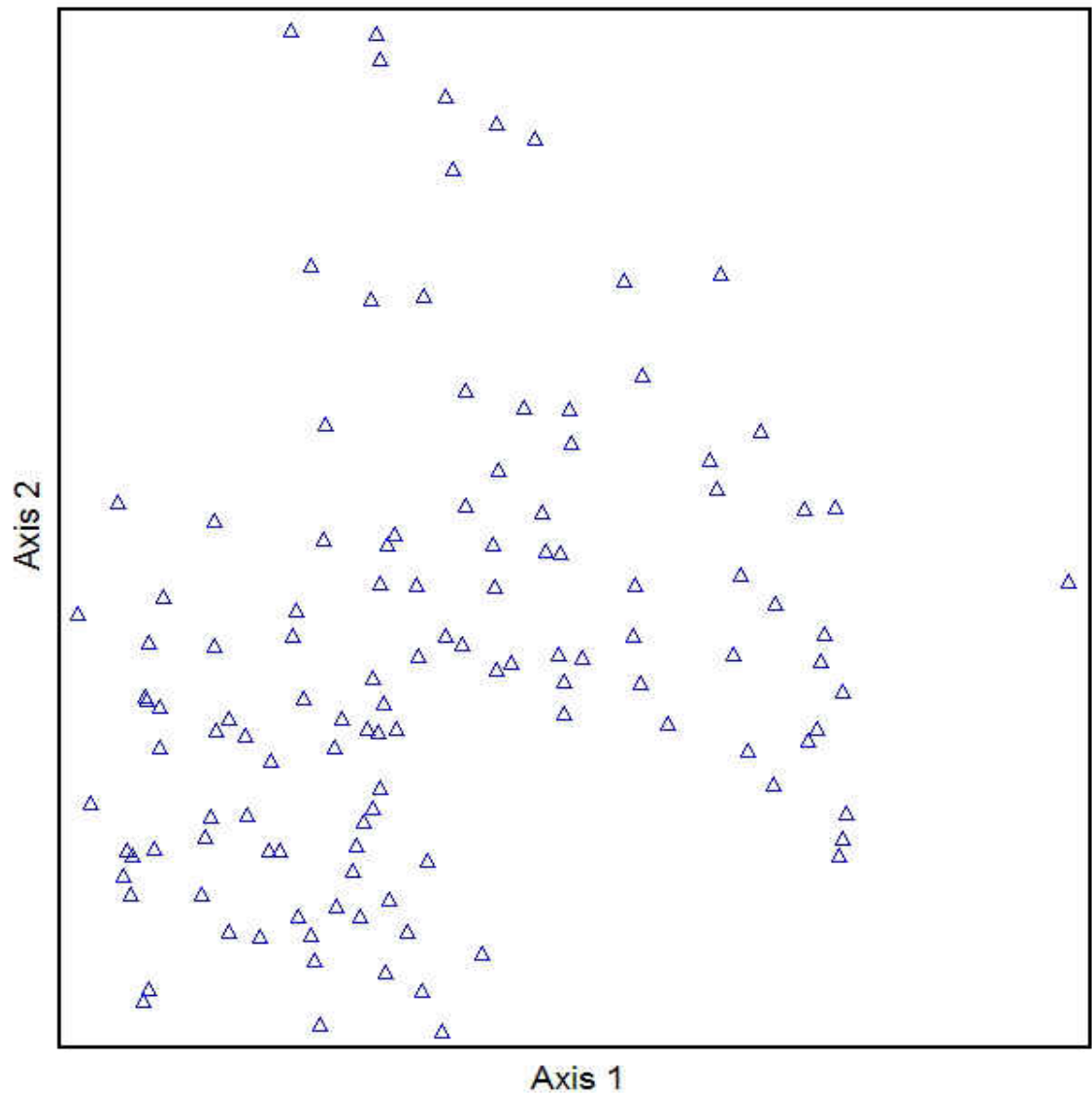


Figure 35: NMS ordination graph for change in biodiversity from December to January

Biodiversity in lift nets were not significantly different.

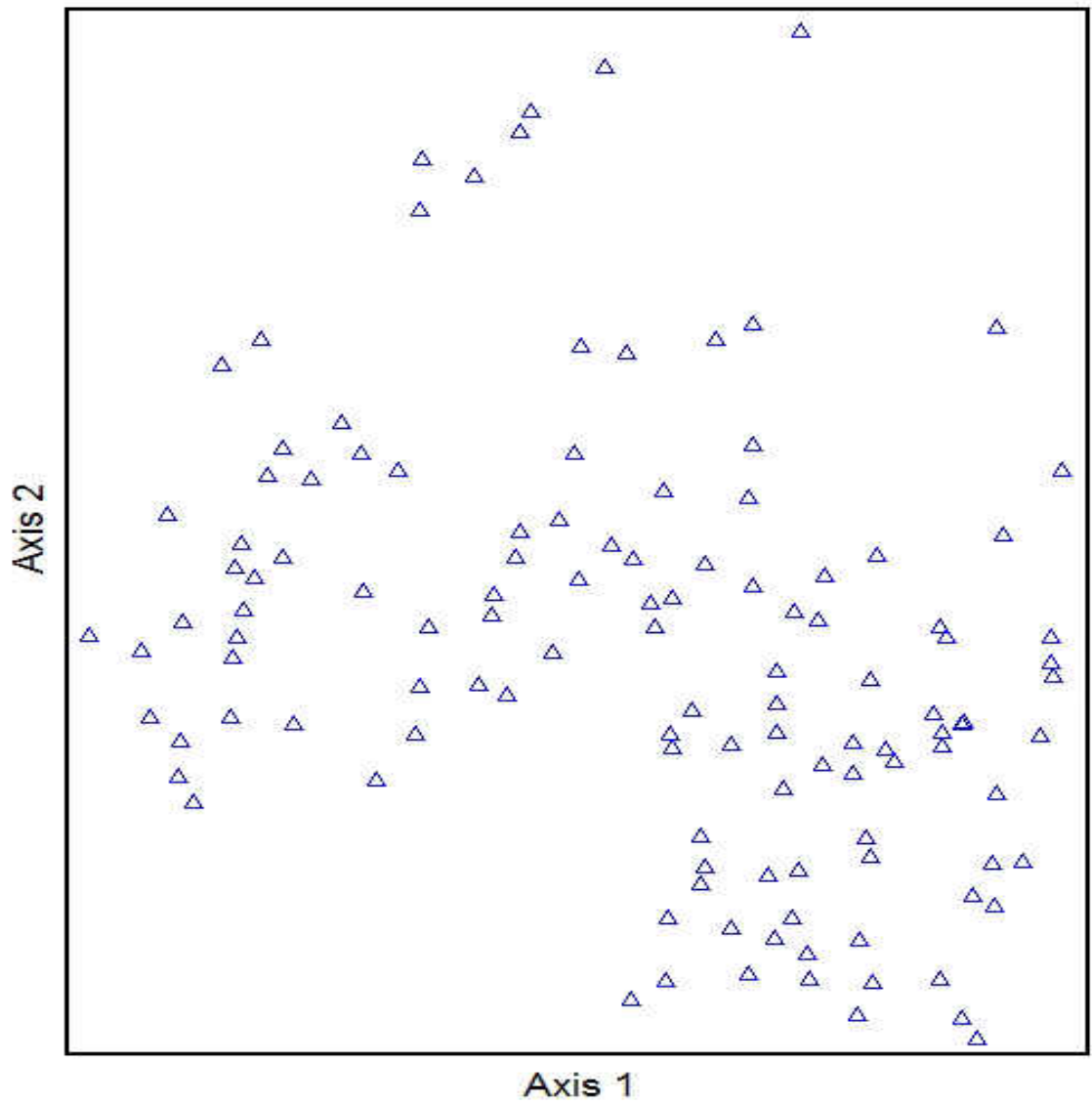
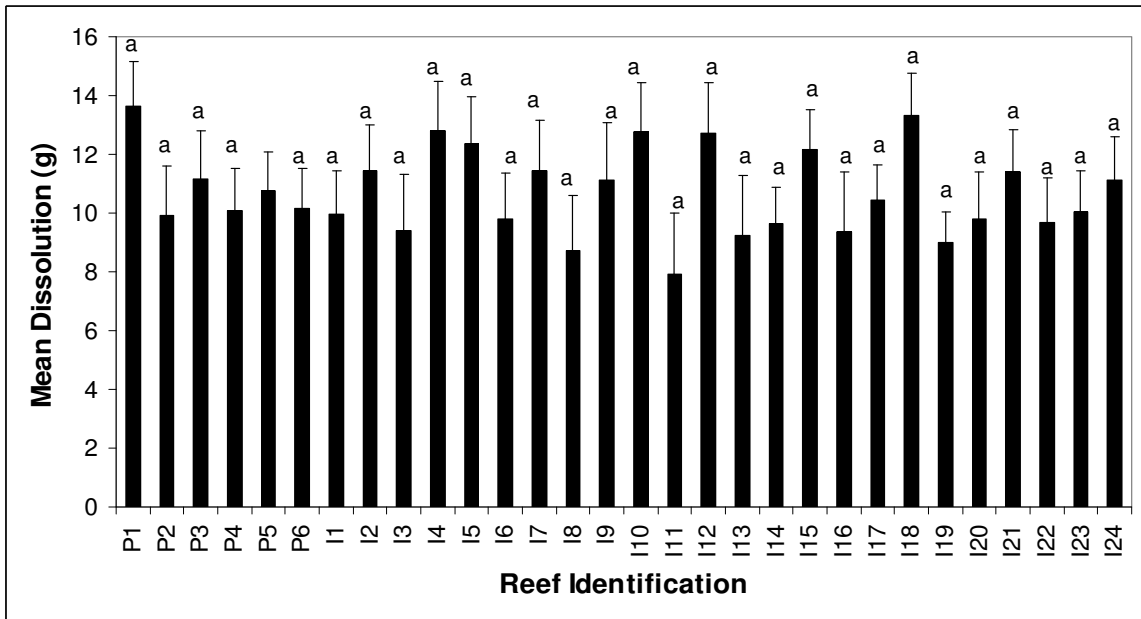


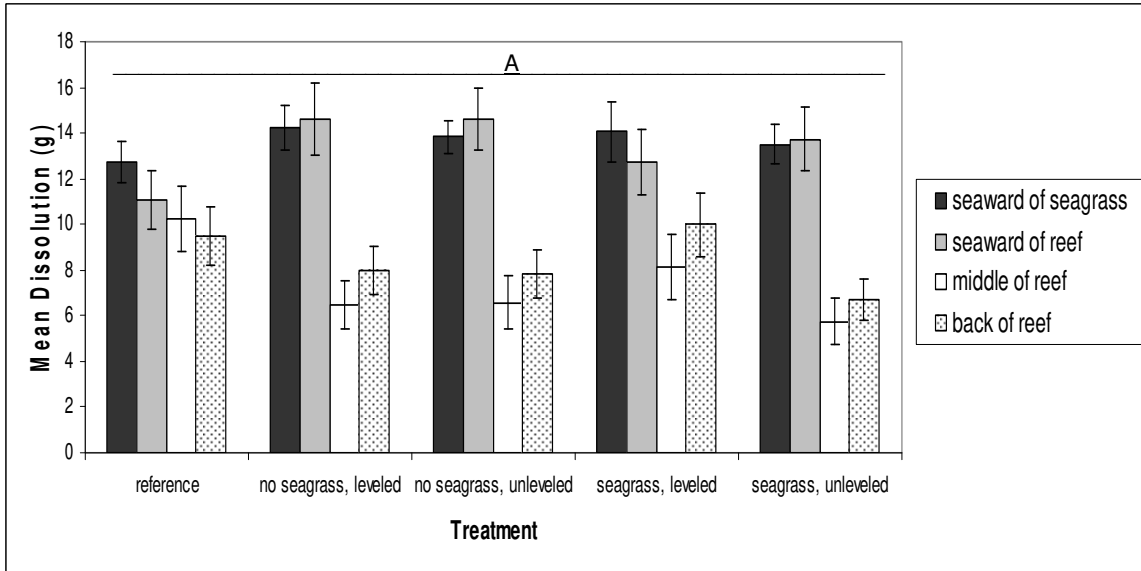
Figure 36: NMS ordination graph for change in biodiversity from January to February

Biodiversity in lift nets were not significantly different.

a)



b)



c)

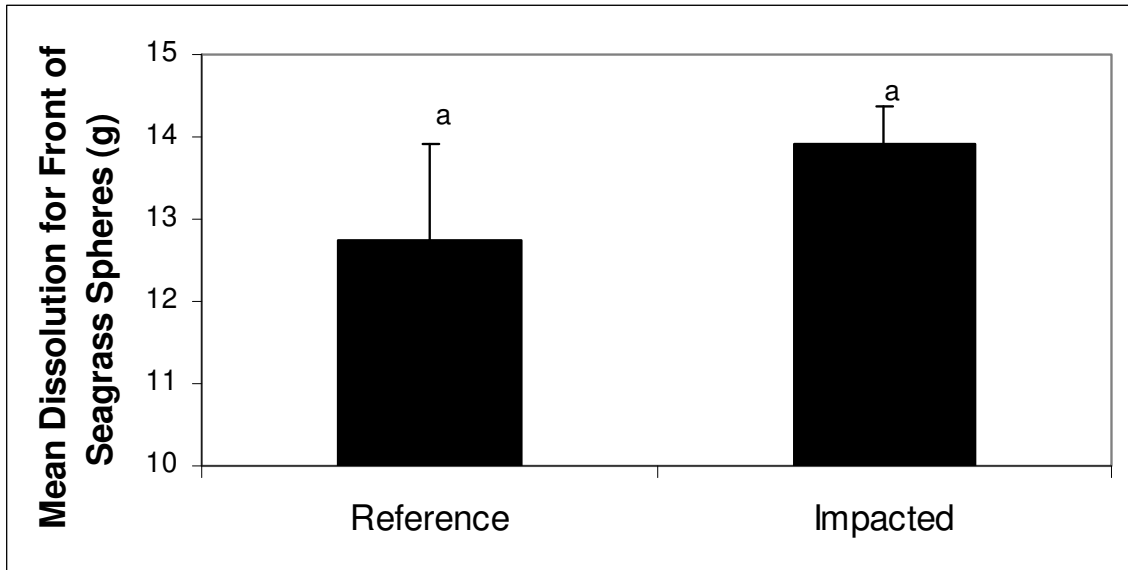


Figure 37: Mean dissolution (\pm SE) of plaster-of-Paris spheres

Data presented for a) the 30 reefs, b) treatments and location, and c) reference and impacted reefs. Lower and upper case letters represent means that are significantly different between sphere dissolution rates at the $p \leq 0.05$ level.

APPENDIX B – TABLES

Table 1: Dependent *t*-test results for comparison of before leveling and after leveling for those treatments that were leveled.

	Paired Differences			t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean			
Pair 1 before leveling height - after leveling height	11.10000	6.94040	2.0035	5.540	11	< 0.001

Table 2: Repeated measures ANOVA tests of within-subject effects for reef height. Results for the average of the five maximum heights of all reefs for month 0 or after leveling if the reef was leveled, month 4, month 8, and month 12.

Source	df	Mean Square	F	Sig.
TIME	3	69.208	4.597	0.005
TIME * TREATMENT	12	33.031	2.194	0.020
Error(TIME)	75	15.056		

Table 3: Repeated measures ANOVA tests of within-subject contrasts for reef height.

Source	TIME	df	Mean Square	F	Sig.
TIME	Quadratic	1	131.545	9.715	0.005
TIME * TREATMENT	Linear	4	81.776	3.987	0.012
Error(TIME)	Linear	25	20.508		
	Quadratic	25	13.540		

Table 4: Repeated measures ANOVA simple contrasts of treatment for reef height.

TREATMENT Simple Contrast		Averaged Variable
no seagrass, leveled vs. reference	Std. Error	5.866
	Sig.	0.732
no seagrass, unleveled vs. reference	Std. Error	5.866
	Sig.	0.041
seagrass, leveled vs. reference	Std. Error	5.866
	Sig.	0.365
seagrass, unleveled vs. reference	Std. Error	5.866
	Sig.	0.459

Table 5: Repeated measures ANOVA results of within-subject effects for spat recruitment.

Values corrected with Greenhouse-Geiser epsilon.

Source	df	Mean Square	F	Sig.
time	1.330	8534.655	5.924	0.010
time * treatment	5.319	842.067	0.585	0.722
time * reef location	2.659	3243.812	2.252	0.094
time * treatment * reef location	10.637	773.584	0.537	0.869
Error(time)	99.726	1440.622		

Table 6: Pairwise comparisons with Bonferroni corrections for 4 month, 8 month and 12 month spat recruitment

TIME	TIME	Mean Difference	Std. Error	Sig.
4 months	8 months	-1.400	4.476	1.000
	12 months	-14.399	5.912	0.052
8 months	4 months	1.400	4.476	1.000
	12 months	-12.999	2.978	0.000
12 months	4 months	14.399	5.912	0.052
	8 months	12.999	2.978	0.000

Table 7: Repeated measures ANOVA between-subject effects tests for spat recruitment.

Source	df	Mean Square	F	Sig.
Intercept	1	338776.716	89.852	0.000
treatment	4	6794.893	1.802	0.137
reef location	2	54145.194	14.361	<0.001
treatment * reef location	8	3758.904	0.997	0.446
Error	75	3770.391		

Table 8: Spat recruitment Games-Howell *post hoc* tests for repeated measures ANOVA.

fore, mid, or back reef	fore, mid, or back reef	Mean Difference	Std. Error	Sig.
fore reef	mid reef	48.4419	7.46420	<0.001
	back reef	30.9200	11.01635	0.019
mid reef	fore reef	-48.4419	7.46420	<0.001
	back reef	-17.5219	9.13703	0.148
back reef	fore reef	-30.9200	11.01635	0.019
	mid reef	17.5219	9.13703	0.148

Table 9: Repeated measures ANOVA results for shell coverage of restoration mats. P-values are given for the Greenhouse-Geiser test because data failed to meet assumption of sphericity.

Source	df	Mean Square	F	Sig.
time	1.699	35.504	0.091	0.884
time * treatment	6.795	115.275	0.297	0.951
time * reef location	3.397	1663.834	4.286	0.005
time * treatment * reef location	13.589	260.541	0.671	0.794
Error(time)	127.399	388.197		

Table 10: Shell coverage repeated measures ANOVA between-subject effects tests.

Source	df	Mean Square	F	Sig.
Intercept	1	83547.223	127.430	<0.001
treatment	4	4893.043	7.463	<0.001
reef location	2	6456.229	9.847	<0.001
treatment * reef location	8	1320.360	2.014	0.056
Error	75	655.635		

Table 11: Shell coverage repeated measures ANOVA treatment contrasts.

TREATMENT Simple Contrast		Averaged Variable
no seagrass, leveled vs. reference	Std. Error	4.928
	Sig.	0.000
no seagrass, unleveled vs. reference	Std. Error	4.928
	Sig.	0.000
seagrass, leveled vs. reference	Std. Error	4.928
	Sig.	0.000
seagrass, unleveled vs. reference	Std. Error	4.928
	Sig.	0.047

Table 12: Shell coverage repeated measures ANOVA, Games-Howell *post hoc* comparisons of reef location.

fore, mid, or back reef	fore, mid, or back reef	Mean Difference	Std. Error	Sig.
fore reef	mid reef	-16.2370	4.71636	0.004
	back reef	-3.9379	3.53144	0.509
mid reef	fore reef	16.2370	4.71636	0.004
	back reef	12.2991	5.10461	0.051
back reef	fore reef	3.9379	3.53144	0.509
	mid reef	-12.2991	5.10461	0.051

Table 13: Animal species collected in lift nets on oyster reefs within Mosquito Lagoon, Florida
Overall total and monthly totals of number of individuals of each species in lift nets.

Category	Species	Common Name	Total	Date of Collection									
				5/06	6/06	7/06	8/06	9/06	10/06	11/06	12/06	1/07	2/07
Mollusks	<i>Argopecten gibbus</i>	Atlantic calico scallop common	1	0	0	0	0	0	0	1	0	0	0
	<i>Bulla striata umbilicata</i>	Atlantic bubble	6	2	1	1	0	0	0	0	2	0	0
	<i>Urosalpinx cinerea</i>	Atlantic oyster drill	39	5	4	1	0	2	6	12	4	1	4
	<i>Crepidula fornicata</i>	Atlantic slipper shell	414	71	58	81	46	40	66	28	15	4	5
	<i>Anadara ovalis</i>	blood ark	4	1	3	0	0	0	0	0	0	0	0
	<i>Fasciolaria hunteria</i>	banded tulip	2	0	0	1	0	0	1	0	0	0	0
	<i>Cronia contracta</i>	marine snail	2	0	0	1	0	0	1	0	0	0	0
	<i>Chione elevata</i>		1	0	0	1	0	0	0	0	0	0	0
	<i>Crepidula convexa</i>	convex slipper	47	17	4	9	1	9	3	2	0	2	0
	<i>Cerithium atratum</i>	Florida cerith	18	1	2	3	0	0	7	2	2	0	1
	<i>Pleuroploca gigantean</i>	Florida horse conch	13	0	0	0	0	13	0	0	0	0	0
	<i>Balanus eburneus</i>	ivory barnacle	96267	1255	338	3302	10397	29742	28146	22745	240	24	78
	<i>Tangelus divisus</i>	jackknife clam	1	1	0	0	0	0	0	0	0	0	0
	<i>Anomia simplex</i>	jingle shell	48	1	0	1	0	38	3	5	0	0	0

Category	Species	Common Name	Total	Date of Collection									
				5/06	6/06	7/06	8/06	9/06	10/06	11/06	12/06	1/07	2/07
	<i>Doriopsilla pharpa</i>	lemon drop sea slug	14	0	0	0	0	0	0	10	3	0	1
	<i>Nassarius vibex</i>	mottled dog whelk	171	7	17	4	63	22	37	5	9	5	2
	<i>Cerithiopsis greeni</i>	awl miniature cerith	34	0	12	3	19	0	0	0	0	0	0
	<i>Mercenaria mercenaria</i>	hard clam	8	4	1	0	0	3	0	0	0	0	0
	<i>Mytella charruana</i>	charru mussel	7	0	0	4	0	2	1	0	0	0	0
	<i>Balanus amphitrite</i>	purple striped barnacle	17086	14	1	806	4127	5645	3729	2748	11	2	3
	<i>Geukensia demissa</i>	ribbed mussel	18	1	0	3	8	4	0	1	1	0	0
	<i>Brachidonetes exuctus</i>	scorched mussel	12	1	1	8	0	0	0	0	2	0	0
	<i>Sphenia fragilis</i>	fragile sphenia	70	1	0	3	4	46	16	0	0	0	0
	<i>Stramonita haemastoma</i>	florida rock snail	1	0	1	0	0	0	0	0	0	0	0
	<i>Aplysia brasiliiana</i>	sooty sea hare	1	1	0	0	0	0	0	0	0	0	0
	<i>Crassostrea virginica</i>	Eastern oyster	10061	6693	1520	208	9	1043	458	114	5	7	4
	<i>Martesia cuneiformis</i>	striated wood paddock	1	0	1	0	0	0	0	0	0	0	0
	<i>Eupleura caudate</i>	thick lipped oyster drill	4	0	0	0	0	0	1	0	2	0	1
	<i>Trachycardium moricatum</i>	yellow cockle	1	1	0	0	0	0	0	0	0	0	0
	<i>Tellina squamifera</i>	crenulate tellin	5	1	0	4	0	0	0	0	0	0	0

Category	Species	Common Name	Total	Date of Collection									
				5/06	6/06	7/06	8/06	9/06	10/06	11/06	12/06	1/07	2/07
Crustaceans	<i>Anadara transversa</i>	transverse ark	84	30	41	5	0	5	0	0	2	0	1
	<i>Fasciolaria tulipa</i>	true tulip	5	1	4	0	0	0	0	0	0	0	0
	<i>Crepidula atrasolea</i>	Eastern white slipper shell	5093	381	577	817	255	526	1316	718	335	124	44
	<i>Busycon spp.</i>	case	6	1	0	0	5	0	0	0	0	0	0
	<i>Panopeus herbstii</i>	Atlantic mud crab	563	51	45	56	109	78	77	43	50	36	18
	<i>Alpheus heterochaelis</i>	big claw snapping shrimp	1439	178	173	157	96	220	159	120	111	107	118
	<i>Callinectes sapidus</i>	blue crab	140	9	7	5	19	9	21	33	26	9	2
	<i>Eurypanopeus depressus</i>	flat mud crab	67	12	12	5	13	2	2	5	7	3	6
	<i>Palaemonetes vulgaris</i>	grass shrimp	670	162	83	43	0	2	5	6	180	154	35
	<i>Rhithropanopeus harristii</i>	Harris' mud crab	1059	182	132	102	41	62	132	89	131	120	68
	<i>Clibanarius vitatus</i>	striped hermit crab	234	128	28	15	4	12	14	12	9	9	3
	<i>Hexapanopeus angustifrons</i>	narrow mud crab	613	46	46	58	131	94	77	54	59	32	16
	<i>Penaeus duorarum</i>	pink shrimp	93	35	1	5	11	6	2	7	18	7	1
	<i>Petrolisthes armatus</i>	green porcelain crab	2816	351	312	266	470	166	264	248	292	269	178
	<i>Libinia dubia</i>	doubtful spider crab	71	17	13	13	2	7	5	10	2	2	0

Category	Species	Common Name	Total	Date of Collection									
				5/06	6/06	7/06	8/06	9/06	10/06	11/06	12/06	1/07	2/07
Other Invertebrates	<i>Menippe mercenaria</i>	stone crab common	19	1	1	2	7	3	2	1	0	1	1
	<i>Bugula neritina</i>	bryozoan	4855	3402	905	22	1	5	23	134	107	207	49
	<i>Halichondria melandocia</i>	black volcano sponge	8	3	3	1	0	0	0	1	0	0	0
	<i>Botryllus planus</i>	royal tunicate	128	0	7	12	3	32	40	33	0	1	0
	<i>Cliona spp.</i>	boring sponge	1	0	1	0	0	0	0	0	0	0	0
	<i>Botryllus scholserri</i>	golden star tunicate	673	110	85	73	19	184	178	19	1	2	2
	<i>Didemnum spp.</i>		40	1	35	1	0	0	3	0	0	0	0
	<i>Sabella spp.</i>	feather duster worm	12	0	0	0	0	4	8	0	0	0	0
	<i>Hippoprina verrilli</i>		306	7	106	22	19	6	129	17	0	0	0
	<i>Hydroides spp.</i>	tube worms	43121	4017	12960	17509	852	1726	3670	2005	164	76	142
	<i>Conopeum spp.</i>	lacy crust bryozoan	981	164	180	134	101	77	68	35	85	44	93
	<i>Maiphysa sanguinea</i>		6	1	0	0	2	0	0	1	2	0	0
	<i>Ophionereis reticulate</i>	reticulated brittle star	2	0	0	0	1	0	0	0	0	1	0
	<i>Mogula manhattensis</i>	sea grape	127	0	3	3	0	2	69	50	0	0	0
	<i>Sigalionidae</i>	polychaete worms	1	0	0	1	0	0	0	0	0	0	0
	<i>Hymeniacion heliophila</i>	sun sponge	10	0	6	1	0	3	0	0	0	0	0
	<i>Styela plicata</i>	rough sea squirt	159	11	19	1	0	10	9	7	46	43	13
	<i>Zoobotryon verticillatum</i>	common moss bryozoan	28	4	3	2	0	0	0	0	18	1	0

Category	Species	Common Name	Total	Date of Collection									
				5/06	6/06	7/06	8/06	9/06	10/06	11/06	12/06	1/07	2/07
Fishes	<i>Gobiosoma robustum</i>	code goby	173	5	44	21	2	6	36	46	2	5	6
	<i>Gobionellus boleosoma</i>	darther goby	63	0	0	0	2	5	18	35	0	3	0
	<i>Chasmodes saburrae</i>	Florida blenny	53	1	10	7	3	6	7	5	7	6	1
	<i>Bathygobius soporator</i>	frillfin goby	38	3	6	4	0	0	0	8	8	3	6
		fish eggs	95	95	0	0	0	0	0	0	0	0	0
		fish larvae	3	3	0	0	0	0	0	0	0	0	0
	<i>Mycteroperca microlepis</i>	gag grouper	1	0	0	1	0	0	0	0	0	0	0
	<i>Floridichthys carpio</i>	goldspotted killifish	1	0	0	1	0	0	0	0	0	0	0
	<i>Fundulus grandis</i>	gulf killifish	34	0	2	6	0	0	0	0	16	3	7
	<i>Lutjanus griseus</i>	gray snapper	110	1	0	12	19	14	16	20	6	16	6
	<i>Lutjanus spp.</i>	snapper	2	0	1	0	0	0	0	1	0	0	0
	<i>Gobiosoma bosc</i>	naked goby	1504	68	166	189	42	103	254	205	248	131	98
	<i>Orthopristis chrysoptera</i>	pigfish	22	5	5	6	2	4	0	0	0	0	0
	<i>Lagodon rhomboids</i>	pinfish	64	49	6	5	2	1	1	0	0	0	0
	<i>Sygnathus scovelli</i>	Gulf pipefish	18	5	2	3	0	0	1	2	2	3	0
	<i>Lutjanus campechanus</i>	red snapper	10	6	2	2	0	0	0	0	0	0	0
		rainwater killifish	30	8	5	9	0	0	0	0	6	2	0
	<i>Lucania parva</i>												
	<i>Archosargus probatecephalus</i>	sheepshead	69	7	11	11	6	8	11	3	5	7	0

Category	Species	Common Name	Total	Date of Collection									
				5/06	6/06	7/06	8/06	9/06	10/06	11/06	12/06	1/07	2/07
	<i>Cyprinidon variegates</i>	sheepshead minnow	17	2	0	2	10	0	1	1	0	0	1
	<i>Poecilia latipinna</i>	sailfin molly	43	0	0	4	6	0	0	2	14	10	7
	<i>Bairdiella chrysoura</i>	silver perch	11	0	0	0	0	0	2	8	1	0	0
	<i>Myrophis punctatus</i>	speckled worm eel	18	2	1	3	5	4	2	1	0	0	0
	<i>Eucinostomus argenteus</i>	spotfin mojarra	3	0	0	1	0	0	0	2	0	0	0
	<i>Opsanus tau</i>	oyster toadfish	87	29	9	22	7	8	3	7	2	0	0
	<i>Symphurus plagiusa</i>	blackcheek tonguefish	1	0	0	0	0	0	0	0	1	0	0
	<i>Mugil curema</i>	white mullet	1	0	0	0	1	0	0	0	0	0	0

Table 14: Wet weights (g) of macroalgal species collected in lift nets on oyster reefs in Mosquito Lagoon, Florida

Species	Date of Collection									
	5/06	6/06	7/06	8/06	9/06	10/06	11/06	12/06	1/07	2/07
<i>Chondria</i>	57.3	0	0	0	0	15.8377	0	7.7653	0	0
<i>Dasya crouaniana</i>	412.4	309.6025	468.4937	10.405	58.0549	59.7585	64.3589	61.6084	136.9915	1.099
<i>Derbesia turbinata</i>	39.3	21.0593	0	0	0	00.1121	6.2177	70.5976	91.6925	69.6823
<i>Diatom</i>	54.8	0.4589	0	0	0	2.4307	48.3538	262.7762	386.7851	1345.0611
<i>Enteromorpha flexuosa</i>	0.2	0.4086	1.9245	0.0504	0	0.0088	11.9461	1674.2788	1739.0851	195.6931
<i>Gracilaria lemaeniformes</i>	2.5	0.8103	0	0	5.1518	0	0.0408	2.5851	3.5174	3.5677
<i>Gracilaria tikvahiae</i>	40.4	12.3829	24.2521	0.0616	29.5459	11.8268	84.7152	54.6587	157.7221	19.879
<i>Acanthophora spicifera</i>	0.1	23.0143	11.7266	6.7462	2.8295	26.4741	40.4044	0.1325	14.3577	0.8222
<i>Codium decortcatum</i>	0.1	0	0	0	0	0	0	0	0	0
<i>Gracilaria cervicornis</i>	0	0.6327	0	0	0	0	0	0	0	0
<i>Ulva faciata</i>	0	0	0	0	0	0	0	0	2.2276	0

Table 15: Dry weights (g) of macroalgal species collected in lift nets on oyster reefs in Mosquito Lagoon, Florida

Species	Date of Collection									
	5/06	6/06	7/06	8/06	9/06	10/06	11/06	12/06	1/07	2/07
<i>Chondria</i>	3.5482	0	0	0	0	0.6166	0	2.5367	0	0
<i>Dasya crouaniana</i>	55.639	40.2003	97.3178	9.2032	6.8892	9.7651	11.6799	10.3209	19.269	0.1499
<i>Derbesia turbinata</i>	1.4613	2.0441	0	0	0	0	0.7632	7.0962	9.2684	10.6818
<i>Diatom</i>	4.3979	0.0691	0	0	0	0.3261	3.0106	66.5465	66.4471	273.6803
<i>Enteromorpha flexuosa</i>	0.0154	0.0321	0.1003	0.0134	0	0.001	1.2546	268.4908	198.5264	32.6986
<i>Gracilaria lemaeniformes</i>	0.3306	0.1166	0	0	0.7403	0	0.016	0.1159	0.3491	0.4195
<i>Gracilaria tikvahiae</i>	4.7651	1.8817	3.1714	0.0151	3.53	1.5776	11.6232	6.2014	16.6161	1.7851
<i>Acanthophora spicifera</i>	0.0035	1.9623	0.708	0.7162	0.2457	2.2529	4.7053	0.0175	1.1885	0.0843
<i>Codium decortatum</i>	0.0068	0	0	0	0	0	0	0	0	0
<i>Gracilaria cervicornis</i>	0	0.0717	0	0	0	0	0	0	0	0
<i>Derbesia ...</i>	0	0	0	0	0	0.0019	0	0	0	0
<i>Ulva fasciata</i>	0	0	0	0	0	0	0	0	0.2315	0

Table 16: Kruskal-Wallis comparison between treatments of species richness in lift nets

Presented by month. a) May, b) June, c) July, d) August, e) September, f) October, g) November, h) December, i) January, and j) February.

a) May

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	5.624	4	0.229
Vexar Only	2.052	4	0.726

b) June

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	2.171	4	0.704
Vexar Only	2.003	4	0.735

c) July

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	4.292	4	0.368
Vexar Only	2.314	4	0.678

d) August

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	7.022	4	0.135
Vexar Only	0.276	4	0.991

e) September

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	6.934	4	0.139
Vexar Only	7.485	4	0.112

f) October

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	6.300	4	0.178
Vexar Only	5.202	4	0.267

g) November

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	6.107	4	0.191
Vexar Only	3.137	4	0.535

h) December

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	7.904	4	0.095
Vexar Only	5.654	4	0.226

i) January

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	9.336	4	0.053
Vexar Only	8.530	4	0.074

j) February

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	10.405	4	0.034
Vexar Only	9.528	4	0.049

Table 17: Bonferroni corrections for species richness in lift nets for February complete restoration mat

Treatments are labeled below with NSL (no seagrass and leveled), NSUL (no seagrass and unlevelled), SL (seagrass and leveled), and SUL (seagrass and unlevelled).

TREAT	TREAT	Mean Difference	Std. Error	Sig.
reference	NSL	-.1667	1.43450	1.000
	NSUL	-3.6667	1.43450	0.170
	SL	1.3333	1.43450	1.000
	SUL	.1667	1.43450	1.000
NSL	pristine	.1667	1.43450	1.000
	NSUL	-3.5000	1.43450	0.221
	SL	1.5000	1.43450	1.000
	SUL	.3333	1.43450	1.000
NSUL	pristine	3.6667	1.43450	0.170
	NSL	3.5000	1.43450	0.221
	SL	5.0000	1.43450	0.018
	SUL	3.8333	1.43450	0.131
SL	pristine	-1.3333	1.43450	1.000
	NSL	-1.5000	1.43450	1.000
	NSUL	-5.0000	1.43450	0.018
	SUL	-1.1667	1.43450	1.000
SUL	pristine	-.1667	1.43450	1.000
	NSL	-.3333	1.43450	1.000
	NSUL	-3.8333	1.43450	0.131
	SL	1.1667	1.43450	1.000

Table 18: Kruskal-Wallis comparison between treatments of number of individuals in lift nets

Presented by month. a) May, b) June, c) July, d) August, e) September, f) October, g) November, h) December, i) January, and j) February.

a) May

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	4.602	4	0.331
Vexar Only	2.386	4	0.665

b) June

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	4.439	4	0.350
Vexar Only	3.008	4	0.556

c) July

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	3.144	4	0.534
Vexar Only	1.761	4	0.780

d) August

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	0.541	4	0.969
Vexar Only	2.584	4	0.630

e) September

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	4.615	4	0.329
Vexar Only	4.663	4	0.324

f) October

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	5.888	4	0.208
Vexar Only	9.388	4	0.052

g) November

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	4.572	4	0.334
Vexar Only	6.217	4	0.184

h) December

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	7.916	4	0.095
Vexar Only	4.673	4	0.323

i) January

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	5.398	4	0.249
Vexar Only	5.945	4	0.203

j) February

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	4.884	4	0.299
Vexar Only	7.411	4	0.116

Table 19: Kruskal-Wallis comparison between treatments of maroalgal wet weights in lift nets

Presented by month. a) December and b) January.

a) December

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	0.728	4	0.948
Vexar Only	3.497	4	0.478

b) January

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	5.935	4	0.204
Vexar Only	1.567	4	0.815

Table 20: Kruskal-Wallis comparison between treatments of macroalgal dry weights in lift nets

Presented by month. a) December and b) January.

a) December

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	0.719	4	0.949
Vexar Only	4.493	4	0.343

b) January

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	3.549	4	0.470
Vexar Only	1.735	4	0.784

Table 21: Kruskal-Wallis comparison between treatments of macroalgal species richness in lift nets for December

Presented by month. a) December and b) January.

a) December

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	1.232	4	0.873
Vexar Only	2.796	4	0.593

b) January

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	1.834	4	0.766
Vexar Only	1.495	4	0.827

Table 22: Kruskal-Wallis comparison between treatments of species evenness in lift nets

Presented by month. a) May, b) June, c) July, d) August, e) September, f) October, g) November, h) December, i) January, and j) February.

a) May

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	2.525	4	0.640
Vexar Only	2.711	4	0.607

b) June

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	2.009	4	0.734
Vexar Only	2.442	4	0.655

c) July

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	3.080	4	0.545
Vexar Only	3.892	4	0.421

d) August

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	0.082	4	0.999
Vexar Only	10.683	4	0.030

e) September

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	1.484	4	0.829
Vexar Only	2.551	4	0.636

f) October

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	7.518	4	0.111
Vexar Only	4.065	4	0.397

g) November

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	6.108	4	0.191
Vexar Only	8.764	4	0.067

h) December

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	2.774	4	0.596
Vexar Only	1.226	4	0.874

i) January

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	2.310	4	0.679
Vexar Only	1.444	4	0.837

j) February

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	2.034	4	0.729
Vexar Only	1.770	4	0.778

Table 23: Kruskal-Wallis comparison between treatments of Shannon-Wiener Index in lift nets

Presented by month. a) May, b) June, c) July, d) August, e) September, f) October, g) November, h) December, i) January, and j) February.

a) May

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	1.200	4	0.878
Vexar Only	0.223	4	0.695

b) June

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	1.948	4	0.745
Vexar Only	1.434	4	0.838

c) July

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	2.551	4	0.636
Vexar Only	2.890	4	0.576

d) August

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	0.710	4	0.950
Vexar Only	5.004	4	0.287

e) September

Lift Net	Chi-Square	df	Asymp. Sig.
Restoration Mat	1.832	4	0.767
Vexar Only	0.654	4	0.957

f) October

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	6.103	4	0.192
Vexar Only	1.303	4	0.861

g) November

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	6.344	4	0.175
Vexar Only	3.752	4	0.441

h) December

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	6.938	4	0.139
Vexar Only	5.628	4	0.229

i) January

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	8.120	4	0.087
Vexar Only	7.415	4	0.116

j) February

	Chi-Square	df	Asymp. Sig.
Lift Net			
Restoration Mat	8.473	4	0.076
Vexar Only	8.311	4	0.81

Table 24: Bonferroni corrections for species richness in lift nets for February incomplete restoration mat

Treatments are labeled below with NSL (no seagrass and leveled, NSUL (no seagrass and unlevelled), SL (seagrass and leveled), and SUL (seagrass and unlevelled).

TREAT	TREAT	Mean Difference	Std. Error	Sig.
pristine	NSL	.1667	0.99666	1.000
	NSUL	-1.8333	0.99666	0.778
	SL	1.6667	0.99666	1.000
	SUL	.0000	0.99666	1.000
NSL	pristine	-.1667	0.99666	1.000
	NSUL	-2.0000	0.99666	0.557
	SL	1.5000	0.99666	1.000
	SUL	-.1667	0.99666	1.000
NSUL	pristine	1.8333	0.99666	0.778
	NSL	2.0000	0.99666	0.557
	SL	3.5000(*)	0.99666	0.017
	SUL	1.8333	0.99666	0.778
SL	pristine	-1.6667	0.99666	1.000
	NSL	-1.5000	0.99666	1.000
	NSUL	-3.5000(*)	0.99666	0.017
	SUL	-1.6667	0.99666	1.000
SUL	pristine	.0000	0.99666	1.000
	NSL	.1667	0.99666	1.000
	NSUL	-1.8333	0.99666	0.778
	SL	1.6667	0.99666	1.000

Table 25: Bonferroni corrections for species evenness in lift nets for August incomplete restoration mat

Treatments are labeled below with NSL (no seagrass and leveled, NSUL (no seagrass and unlevelled), SL (seagrass and leveled), and SUL (seagrass and unlevelled).

TREAT	TREAT	Mean Difference	Std. Error	Sig.
pristine	NSL	-.0249	0.09386	1.000
	NSUL	.2812	0.09386	0.061
	SL	-.0318	0.09386	1.000
	SUL	-.0326	0.09386	1.000
NSL	pristine	.0249	0.09386	1.000
	NSUL	.3061	0.09386	0.032
	SL	-.0069	0.09386	1.000
	SUL	-.0076	0.09386	1.000
NSUL	pristine	-.2812	0.09386	0.061
	NSL	-.3061	0.09386	0.032
	SL	-.3130	0.09386	0.027
	SUL	-.3138	0.09386	0.026
SL	pristine	.0318	0.09386	1.000
	NSL	.0069	0.09386	1.000
	NSUL	.3130	0.09386	0.027
	SUL	-.0007	0.09386	1.000
SUL	pristine	.0326	0.09386	1.000
	NSL	.0076	0.09386	1.000
	NSUL	.3138	0.09386	0.026
	SL	.0007	0.09386	1.000

Table 26: ANOVA comparison of lift nets with restoration mats and Vexar only from NMS vectors

Month	Source	df	Mean Squares	F	P-value
May to June	Between Groups	1	0.912	5.956	0.018
	Within Groups	58	.0153		
	Total	59			
June to July	Between Groups	1	1.157	5.280	0.025
	Within Groups	58	0.219		
	Total	59			
July to August	Between Groups	1	2.406	30.999	<0.001
	Within Groups	58	0.078		
	Total	59			
August to September	Between Groups	1	0.215	0.898	0.347
	Within Groups	58	0.239		
	Total	59			
September to October	Between Groups	1	0.319	3.831	0.055
	Within Groups	58	0.083		
	Total	59			
October to November	Between Groups	1	0.358	4.112	0.047
	Within Groups	58	0.087		
	Total	59			
November to December	Between Groups	1	5.538	31.168	<0.001
	Within Groups	58	0.178		
	Total	59			
December to January	Between Groups	1	0.516	1.305	0.258
	Within Groups	58	0.395		
	Total	59			
January to February	Between Groups	1	0.094	0.254	0.616
	Within Groups	58	0.370		
	Total	59			

Table 27: Plaster-of-Paris dissolution rates as a proxy for relative water motion.

Results of a univariate ANOVA with reef as a fixed factor.

Source	df	Mean Square	F	p - value
Corrected Model	30	3.893	2.356	<0.001
Intercept	1	7.224	4.371	0.037
initial weight	1	90.168	54.563	<0.001
reef	29	0.956	0.579	0.963
Error	569	1.653		
Total	600			
Corrected Total	599			

Table 28: Plaster-of-Paris dissolution rates as a proxy for relative water motion.

Results of a two-way ANOVA with treatment and location as fixed factors.

Source	df	Mean Square	F	Sig.
Corrected Model	20	9.897	6.670	<0.001
Intercept	1	11.154	7.517	0.006
initial	1	104.696	70.558	<0.001
location	3	29.028	19.563	<0.001
treatment	4	0.472	0.318	0.866
location * treatment	12	1.690	1.139	0.325
Error	579	1.484		
Total	600			
Corrected Total	599			

Table 29: Plaster-of-Paris dissolution rates as a proxy for relative water motion.

Results of a one-way ANOVA with reference versus impacted reefs as a fixed factor for front of seagrass spheres.

	df	Mean Square	F	Sig.
Between Groups	1	6.555	1.094	0.305
Within Groups	28	5.994		
Total	29			

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