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Growth and Survivorship of Meandrina meandrites and Montastrea cavernosa Transplants to an Artificial Reef Environment, and the Effectiveness of Plugging Core Holes in Transplant Donor Colonies

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Dedication

This thesis is dedicated to my family. Thank you for always being there for me. To my parents Tom and Kathy Glynn, thank you for encouraging me to follow my path and for your unending support in so many ways. You have always believed in me and made me believe in myself. To my sisters Sheila and Heather, thank you for being the best friends that I ever could have hoped for and for all of your encouraging words. To Tommy, Will, and Megan, there may be a budding marine biologist in one of you! I am looking forward to enjoying the ocean with each one of you. And finally to the most recent addition to my family, my husband Danny Fahy. Thank you, Danny, for helping me every step of the way. I could never have finished the field work if you weren't there, helping with everything from drilling, to photographing the corals, to driving the boat, to navigating the study sites… and for always giving me a shoulder to lean on during the long writing months. You have been amazing!

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Abstract

The growth and survivorship of two species of scleractinian coral transplants, *Meandrina meandrites* and *Montastrea cavernosa,* were investigated. Identically sized replicate transplants were obtained from the second reef, off Dania Beach, using a hydraulic drill fitted with a 4" core barrel. The transplants were fixed to Reef Ball™ substrates using an adhesive marine epoxy. Drill holes in the donor corals (core holes) were filled with concrete plugs to prevent the detrimental effects of bioeroders. Control corals, of comparable size to both the donor colonies and the transplant corals, were selected for comparison. The transplant corals, donor corals, and controls on the natural reef were monitored for growth and survivorship. The core holes were monitored for tissue regrowth over the surface of the concrete plug, in order to assess the effectiveness of the plugging process. Growth during the transplantation project was defined as an increase in surface area or radius, and was monitored on a quarterly basis using photographic techniques. SigmaScan© Pro4 image analysis software (Jandel Scientific Corporation) was used for the analysis of the photographic data.

The following main hypothesis was tested: species-specific differences will occur in the responses of coral colonies to drilling and transplantation. Additional sub-hypotheses were tested, including: 1) a change in surface area and/ or radius in the experimental corals and the control corals will take place, 2) the survivorship of the experimental corals and their control corals will be similar, 3) a change in surface area and/ or radius of the tissue surrounding the core holes will take place.

Meandrina meandrites transplants exhibited a substantial amount of mortality and displayed significantly less growth (both in surface area and radius change) than *M. cavernosa* transplants, and the *M. meandrites* controls. *Montastrea cavernosa* transplants experienced significantly more growth than their same species controls. All donor corals that experienced drill damage (separate from the drill holes) were able to regenerate the injured tissue in a period of less than three months. No significant difference was found for the change in percent tissue coverage for either donor species when compared with each other and with their same species controls. Tissue did not completely regenerate over the surface of the concrete core hole plugs in either species. However, there was no significant difference between the initial area/ radius of the core holes and the final area/ radius for either *M. meandrites* or *M. cavernosa.* Additionally, there was no significant difference in the total area change of the core holes when the two species were compared. The results of this study indicated that *M. meandrites* did not demonstrate statistically significant survivorship or growth as a transplant coral. The *M. cavernosa* transplants were successful, and displayed a significant increase in surface area. The areas surrounding the core holes did not significantly increase in surface area in either species of donor corals.

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1.0 Introduction to the Coral Transplantation Project

1.1 Overview of hypothesis-based restoration study for the mitigation of the *Memphis* grounding.

1.1.1 Introduction

The *U.S.S. Memphis* ran aground on February 25, 1993, on the second reef offshore of Dania Beach, Florida. The grounding impact caused substantial reef framework damage, resulting in habitat destruction and loss of faunal communities. In April 1997, the State of Florida was awarded a $750,000 settlement to compensate for the *Memphis* grounding damages. A number of restoration plans resulted from this award, including the construction of artificial reef habitats, transplantation of stony corals, and an intended long-term monitoring program (Banks, 1999).

Nova Southeastern University Oceanographic Center (NSUOC) received a contract from Broward County to test hypotheses related to faunal recruitment to artificial habitats and the effects of various attractants in enhancing recruitment. This study has been termed the *Memphis* project. Different levels of fill complexity within the artificial habitats were used to investigate fish recruitment. Three different types of larval attractants (crushed limestone, iron filings, and coral transplants) were used to investigate coral larvae recruitment. Coral transplantation was used to investigate the efficacy of transplanted corals as fish and coral larvae attractants.

The *Memphis* project was designed to use artificial reefs not only to mitigate for lost reef structure, but also to examine restoration strategies. Experiments

were implemented to test: 1) the enhancement of coral recruitment through the use of coral larval attractants, 2) the effect of reef structure on fish assemblages, and 3) the interaction between fish assemblages and coral recruitment and survival.

One hundred and sixty small artificial reef modules (Reef Balls™) were deployed in 13 m of water on a sand flat between the second and third reef tracts adjacent to the *U.S.S. Memphis* grounding site. Reef Balls are 'designed artificial reefs', made of marine friendly concrete, which are intended to imitate natural reef systems (www.reefball.org). The Reef Balls were arranged into 40, 4-module reef units (quads). The separation of individual Reef Balls, within a quad (approximately 1-2 m), was judged sufficient to avoid interaction effects between Reef Balls in terms of coral settlement, but close enough for the 4 balls to function as a single reef unit in terms of fish recruitment (R. Spieler, personal communication). Each quad was situated approximately 30 m from surrounding hard bottom or adjacent quads.

1.1.2 Coral Recruitment

Settlement plates on each Reef Ball were used to test hypotheses on enhancing coral recruitment through the use of larval attractants. The settlement plates attached to each Reef Ball were treated with a potential attractant (iron filings, limestone pieces, coral transplants) and compared with control plates (no attractant) (Table 1).

Table 1: Settlement plate larval attractants

Larval Attractants: Each individual Reef Ball in a quad incorporated one of four different attractants on the settlement plates.	
Iron additive	**Limestone pieces**
Coral transplants	**Control (Concrete)**

Coral recruitment studies have shown increased levels of settlement to substrates treated with iron filings, limestone pieces, and transplanted adult coral colonies (Morse *et al*., 1988; Morse *et al.,* 1991; Oren & Benayahu, 1997) as attractants. The iron and limestone additives were painted onto the settlement plates using the same mixture of concrete as was used for the settlement plate and Reef Ball construction. The coral transplants consisted of 4" diameter cores drilled from large donor colonies, secured to the Reef Ball surface with underwater adhesive epoxy adjacent to the settlement plates. After an initial adjustment period, the settlement plates were monitored for the presence of coral recruits.

1.1.3 Coral Transplantation and Monitoring

Eighty coral cores were transplanted onto the Reef Ball modules (forty cores of each of two different species). One Reef Ball in each quad was designated a 'transplant Reef Ball' and accommodated the two transplant coral cores. Control corals occurring on the natural reef, and of comparable size to the donor corals and transplant corals, were monitored for comparison of growth and mortality. At quarterly intervals the donor corals, coral transplants, and control corals were visually assessed and photographed to provide information on individual colony health, growth, and mortality.

1.1.4 Fish Recruitment

The 40 Reef Ball quads were divided into 4 different levels of structural complexity to test the hypothesis that multiple refuge size and the resultant diverse fish assemblages may affect coral recruitment, survival, and growth.

Table 2 depicts the various fill types for the levels of structural complexity. One set of 10 quads had the void space of all the Reef Balls filled with large refuge structure. One set had the void spaces of all filled with small refuge structure. Another set were mixed and had one Reef Ball empty, one with large refuge, and the last two with small refuge. The final set had the void space of all the Reef Balls empty. Because of the different levels of structural complexity,

Table 2: Structural complexity (fill) for each of the Reef Ball units.

Structural Complexity: Each type of fill will be used for 10 quads.
o Large fill – 4 concrete blocks in each Reef Ball of the quad.
o Small fill – 3/4" plastic mesh in each Reef Ball of the quad.
o Mixed fill – 1 Reef Ball of the quad with blocks, 1 empty and 2 with mesh.
o No fill – all 4 Reef Balls of the quad are empty.

different resultant fish populations should be present. These populations, in turn, may differentially affect the coral transplants. The assemblages of fishes (species, abundance, and size) associated with each quad were recorded every three months by visual census. The results of the coral recruitment and fish recruitment portions of this study are not a part of this thesis. These aspects have been mentioned for the sole purpose of describing the larger scope of the entire study.

1.2 Experimental Site Location

1.2.1 Broward County Reefs

The coastline of Broward County stretches for approximately twenty-two nautical miles from Hallandale, north to Boca Raton (Figure 1).

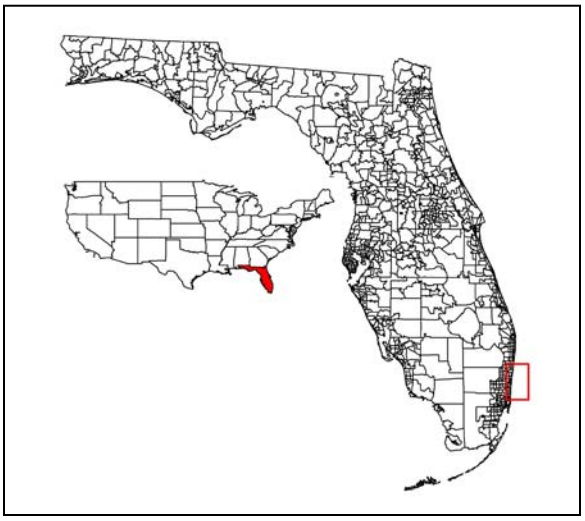


Figure 1: Location of Broward County, Florida. Box indicates area encompassing coastline and offshore of Broward.

Three parallel reef tracts, which comprise the northern portion of the Florida Reef Tract, run approximately parallel to the coast through Dade, Broward, and Palm Beach Counties (Goldberg, 1973). Closest to the shoreline, the inshore reef ranges in depth from about three to nine meters. This zone, known as the back-reef zone, is located approximately 100 meters offshore. It is comprised of mostly patch reefs and undeveloped inshore reefs (Goldberg, 1973). Running from approximately seven to twenty-two meters in depth, the middle reef (2^{nd} reef) is the central habitat. This middle tract is located approximately 800 meters offshore. This zone is comprised of a developed platform and the adjacent slope into deeper waters. The platform rises to a relief of two to three meters in some areas (Goldberg, 1973). The third tract, located

farthest offshore, is comprised of a reef which lies between fourteen to thirty-two meters. This forereef zone consists of the outer reef slope and the deeper reef proper. This tract is the best developed of the three. It is comprised of both a rugged, knoll forming terrain and areas of spurs with shallow grooves. The areas of spurs extend up to a height of eight meters (Goldberg, 1973).

The continental shelf, south of Boca to Miami, is rocky with a thin sediment veneer (Duane and Meisburger, 1969). Between the reef-like ridges (tracts) lying parallel to the shore, are troughs that have accumulated sediment deposits. The sediment in these troughs consists of sand-sized calcareous skeletal fragments (Duane and Meisburger, 1969). Along the coast of southeast Florida, the Anastasia Formation is exposed in low-lying shoals along the shore (Duane and Meisburger, 1969).

The relict reef-framework of the third reef was described by Lighty (1977) as no longer having any active reef-framework accumulation. Radiocarbon dating has shown that the reefs are Holocene in age (130,000 years old); these reefs have since been described as ‘submerged barrier reefs’ (Lighty, 1978).

Despite lower temperatures and increased sedimentation in comparison with Florida Keys reef tracts, Broward reefs are able to support a diverse ecosystem of scleractinian corals and gorgonians (Goldberg, 1973). The major habitat between the tracts is a sandy flat and sloping bottom. Additional substrates in the area include: extensive areas of reef rock, sand and soft sediments, algal mats, and expanses of coral rubble. Much of the reef lies on ledge lines and on low profile hard bottoms (Goldberg, 1973). The reefs of this

area are not as complex as the many reefs of Key Largo and the southern Keys. Despite this difference in complexity, the Broward County reefs are still quite diverse with most major Caribbean species present.

1.2.2 *Memphis* Project Location

The locations for this experimental project were the sandy flat areas

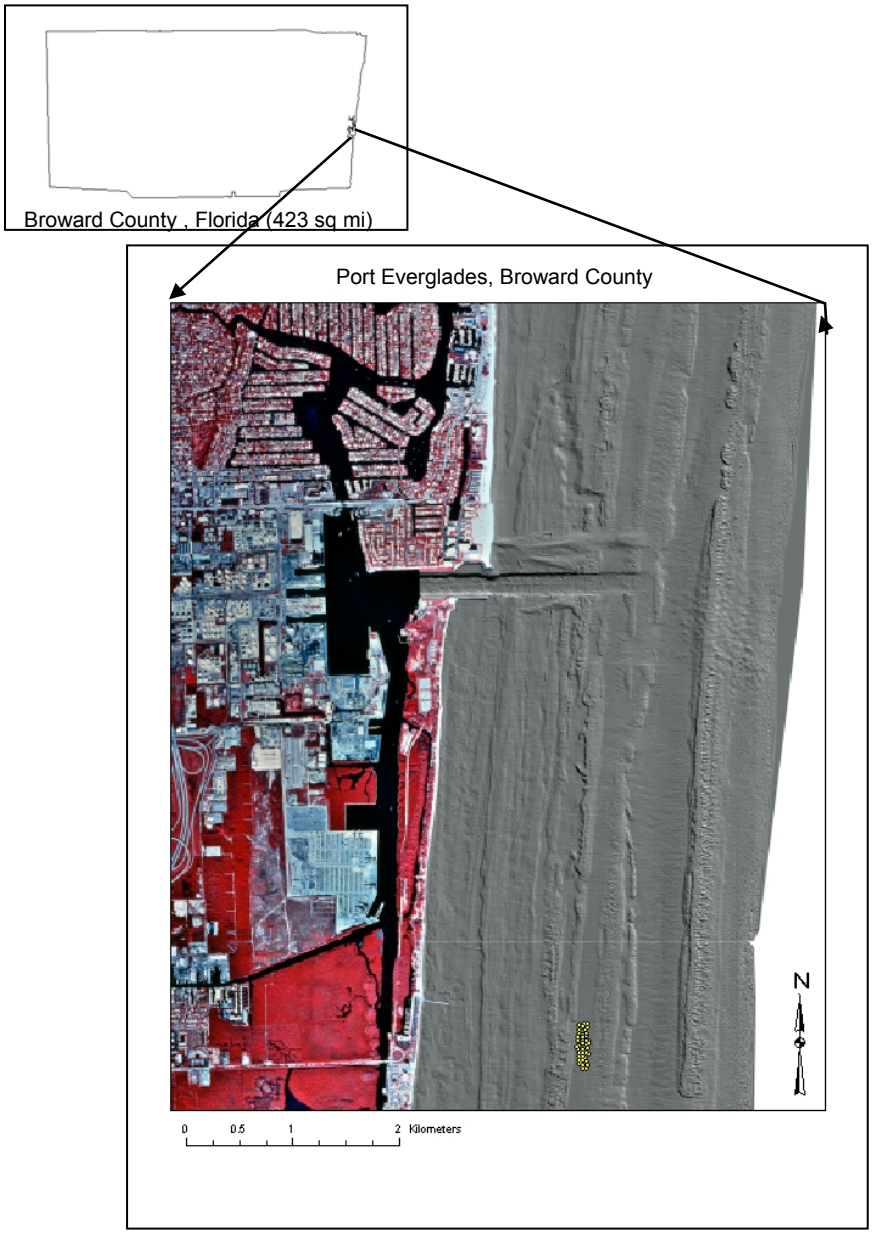


Figure 2: Broward County, Florida to Port Everglades Inlet; Laser Airborne Depth Sounding (LADS) image with sunshaded bathymetry. The Reef Ball coral transplant study site is south of the Inlet and east of Dania Beach Fishing Pier. Reef Ball quads are indicated by dots. The *U.S.S. Memphis* grounding site is directly west of the Reef Balls (Figure 7 shows additional detail of area, including depths and study sites).

between the second and third reefs, and the area surrounding the site of the 1993 *Memphis* grounding, south of the Port Everglades inlet (Figure 2).

1.3 Grounding of the *U.S.S. Memphis*

1.3.1 Grounding Damage and Assessment

On the morning of February 25, 1993 the United States nuclear submarine *U.S.S. Memphis* ran aground in approximately 10 meters water on a coral reef offshore of Dania Beach, Florida (coordinates: 26 03.282N 80 05.870W). The *U.S.S. Memphis* is an SSN–688 Los Angeles class submarine with size specifications as follows, length: 109.73 m; beam: 10 m; displacement: 6,210 metric tons submerged (http://www.fas.org/man/dod-101/sys/ship/ssn-688.htm). After 90 minutes of attempts to dislodge the submarine from the reef, the *Memphis* broke free and was returned to a Naval base for damage evaluation.

Scientists from the Broward County Department of Natural Resource Protection (DNRP) conducted a preliminary damage evaluation of the impacted area the following day. Figure 3 depicts a digitized AUTOCAD map of the

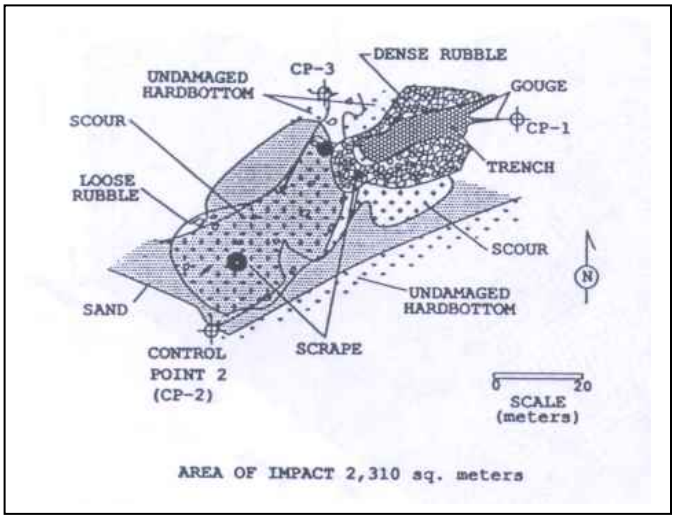


Figure 3: Digitized AUTOCAD map of grounding site (Banks *et al*., 1998).

impacted areas. Inclement weather prevented a detailed assessment immediately following the grounding. Video footage was recorded at that time and an external hydrophone, broken from the hull of the submarine, was recovered at the grounding site (DNRP, 1994). Thorough surveying and underwater mapping of the site was carried out by scientists from the DNRP, the State of Florida (Florida Marine Research Institute), and Nova Southeastern University over the course of subsequent months. Three control pins were established for ease of mapping and future monitoring work. Investigation of the impacted reef in comparison with the adjacent non-impacted reef habitat provided an estimate of the biological influence of the grounding. The grounding caused substantial biological and physical damage to the reef structure and coral community (Banks *et al.*, 1998). Bathymetric surveys identified an eight-foot deep trench in the reef framework, attributable to the grounding incident (Figure 4) (DNRP).

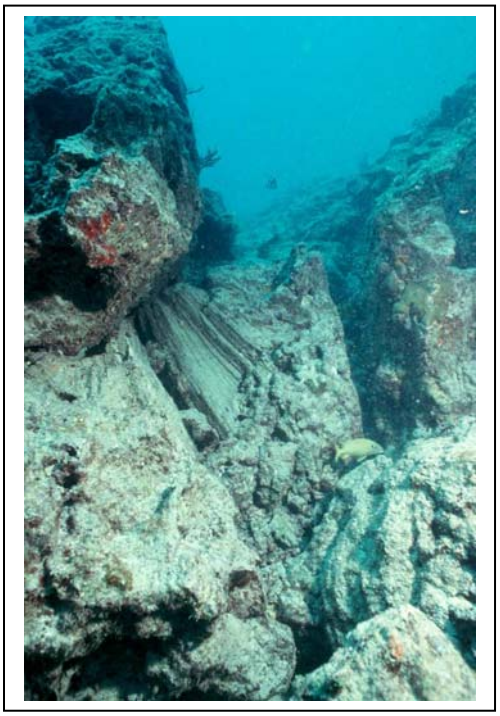


Figure 4: Structural damage and rubble resulting from prop wash (D. Harland).

1.3.2 Impacts to faunal communities & habitat loss

The effects of the grounding to the faunal communities were devastating. An estimated 2,324 stony corals, 10,227 octocorals, and 13,034 sponges were killed as a result of the *Memphis* grounding (DNRP, 1994). Assessment of the grounding site (Figure 5) estimated an impacted area of 2,310m^2; with 1,205m^2 having been totally destroyed.

Figure 5: Keel damage (BC DPEP).

According to DNRP, a damaged area of 1,204 m^2 (100 percent loss) led to the mortality of 2,324 stony coral colonies; this calculates to 23.7 m^2 of live polyp coverage. Using an impacted area of 2,310 m^2 (the size of the area impacted without 100 percent loss) increased the losses to 4,458 stony corals and 45.5 m^2 of live polyp coverage (DEP, 1994). DNRP used the NOAA Habitat Equivalency Model (HEM) to calculate compensatory habitat required as a result of the grounding impact.

Complete restoration is the return to pre-impacted levels of ecological stability and biological diversity. It has been suggested in the literature that the recovery rate for scleractinian coral species acts linearly (DEP, 1994). According

to the DEP (1994), a linear recovery rate over a fifty-year period (with the first two years being used for stabilization and planning) would yield 21,003 m^2 of service years lost due to coral reef community damages, resulting from the *Memphis* grounding.

1.3.3 Claim and Litigation

The State of Florida filed a $2.4 million damage claim against the United States to compensate for the *Memphis* grounding damages. In April 1997, this claim was settled and a $750,000 award was granted to the State of Florida Ecosystem Management Trust Fund. The devised restoration plans resulting from this award included the following: stabilization of the rubble/reef substrate, emplacement of six different types of artificial reef habitats, transplantation of reef-building stony corals, and a long term monitoring program. The compensation awarded to the State of Florida was deemed necessary in order to remunerate for the lost service years, which could not be regained naturally.

Using the information gained from this study, grounding incidents in the future may be dealt with in a more effective manner. No baseline data exists for the grounding site prior to the grounding event, as is usually the case in man-made disturbance events (Pearson, 1981). Aspects of coral ecology including transplantation, survivorship, growth, and recovery have been examined using a hypothesis-based study.

1.4 Impacts to Coral Reefs

1.4.1 The Use and Misuse of Coral Reefs

Worldwide, coral reefs have been recognized for their biological diversity, economic importance, recreational value, and beauty (Jaap, 2000). Reefs have been used for a source of food for millennia (Spalding *et al*., 2001); today the world's coral reefs provide the major source of animal protein to many Pacific Island nations. In addition to the importance of fisheries, reefs also provide an important aspect of tourism and recreation. Reefs shelter, secure, and protect coastlines. The increased aquarium trade and sale of reef products have spurred both enjoyment and exploitation of coral reef ecosystems (Spalding *et al*., 2001). Despite our knowledge and conservation efforts, coral reefs off southeast Florida are exploited by multiple users; resulting in ever-increasing stress and impact on these ecosystems (Jaap, 1984; Causey, 1990).

According to Hughes and Connell (1999), the effect of a specific instance of disturbance is often critically dependent on the impacts of previous disruptions. Natural events, such as violent storms, freshwater inundation, sedimentation, climate change, and Crown of thorn starfish infestations, can be much more devastating than human acts (although many of these 'natural' events may be linked to anthropogenic factors) (Kinsey, 1988; Spalding *et al*., 2001). It is thought that coral communities may require decades to recover from incidents of natural disturbance; the timescale for man-made disturbance is not as clear (Pearson, 1981). However, chronic anthropogenic influences can disrupt the ability of coral communities to recover from natural events (Hughes & Connell,

1999). Man-made disturbances often result in permanent changes to the environment; thereby prolonging, altering, or even preventing the recruitment of coral communities to these damaged areas (Pearson, 1981).

One of the most significant causes of man-induced reef damage is the devastation caused to reefs when ships run aground (Table 3).

Table 3: Selected major types of direct physical damage affecting coral reefs (Spalding, 2001).

Major types of direct physical damage to coral reefs

Activity	Notes
Ship grounding	Direct impact on relatively small areas of shallow reef
Diver damage	Coral breakage or death from frequent handling, only a major problem on very popular dive sites
Direct smothering	Solid waste, and thicker elements of spilled oil can kill corals through contact and direct smothering
Anchor damage	Apart from initial impact, anchors may drag and anchor chains sweep over wide arcs, smashing corals over large areas

In Florida, damage to reef habitats resulting from grounding events is closely followed by damage from dredging for beach renourishment projects as well as channel maintenance (Jaap, 2000). Additional anthropogenic disturbances include the following: pollution from excessive nutrients, sewage, and chemicals; freshwater runoff; and siltation (Rinkevich, 1995). The processes involved in the recovery of the reef system, after a man-induced event, are not completely understood (Gittings, 1988). It is for this reason that further research, on the many aspects of reef recovery, is still needed. As pressures on coral reef ecosystems ever increase, knowledge of the aspects of life history processes of hermatypic corals becomes increasingly important (Bak & Criens, 1981).

1.4.2 Ship Groundings

Shipwrecks and groundings have been common occurrences along the Florida coast since the time of the early Spanish and English explorers (Jaap, 1984). The grounding of pleasure and commercial crafts of less than 100-ft length has increased in the recent past (Jaap, 1984). Since the early 70's, a number of large ships have run aground on Florida reefs. Greater damage to the reef community is likely to result from large tankers or freighters, than from small vessels (Jaap, 1984). The causes of some of these ship groundings have been attributed to failed anchorage, lost vessel steerage, miscalculated navigation, and attempts to avoid sinking (Graham & Schroeder, 1996; Sea Byte Inc. & SSR Inc., 1998; Gittings, 1988; and Jaap, 1984). Much of the resultant damage to the reef framework stems from the original impact, the duration of time that the ship is hard aground, and the removal of the vessel from the reef substrate (Figure 6).

Figure 6: Trench resulting from the *Memphis* grounding (BC DPEP).

With large vessel groundings, the impact of the hull on the top of the reef can have a bulldozing effect, leveling off whatever it comes into contact with (Jaap, 1984). Additional impacts resulting from grounding incidents include: fuel leakage and loss of cargo or other materials on to the reef framework (Jaap, 1984).

Groundings in Florida reef tract, which caused extensive injury to the reef resources, include the *Wellwood, Memphis, Firat, and Hind* groundings (Table 4).

Table 4: Some recent ship groundings in the Florida Keys and Florida reef tract.

Recent ship groundings on the Florida Keys and Northern Florida reef tract				
Vessel	Size	Date	Location	Impact (m^2)
M/V Wellwood	397 ft	1984	Molasses Reef	1,282 m^2
USS Memphis	360 ft	1993	Fort Lauderdale	1,250 m^2
M/V Firat	462 ft	1994	Fort Lauderdale	> 1,000 m^2
C/V Hind	348 ft	1998	Fort Lauderdale	4,516 m^2

In response to the persistent damage caused by ship grounding events, federal and Florida state agencies have developed a number of restoration strategies to repair impacted reefs. The removal of loose debris, the rebuilding of lost three-dimensional structure, and the transplantation of sponges and corals are among some of these techniques (Jaap, 2000). Additionally, the monitoring of all work at damage sites and unimpacted sites has been recommended so that improvements can be made in future restoration work (Jaap, 2000). Several of the groundings in Table 4 are discussed in more detail below.

1.4.2.1 *M/V Wellwood* Grounding

The *M/V Wellwood*, in 1984, grounded on Molasses Reef in a depth of 6-8 meters. The extensive damage was attributed to framework alteration, reef displacement, and sediment production during the initial grounding (Gittings *et al*., 1994). Both during the initial grounding and while the vessel sat hard aground, large coral heads were damaged, toppled over, and/or fractured. The tops of the forereef spurs were flattened and reef rock boulders were plowed to the port side of the ship. Over 1500m^2 of habitat was flattened, with nearly all scleractinian corals in this area completely destroyed. The *Wellwood* stayed aground for 12 days, resulting in severe bleaching of surviving coral colonies. Additional damage resulted from the vessel removal process (Gittings *et al*., 1994).

1.4.2.2 *M/V Firat* Grounding

The 462 ft. Turkish freighter *M/V Firat*, grounded on the nearshore reef off Fort Lauderdale, Florida in November 1994 (Graham & Schroeder, 1996). The grounding was attributed to lost anchorage due to rough seas during tropical storm Gordon. As the freighter was blown aground, its hull impacted the first reef, detaching scleractinian corals and other epibiota along the path. Damage attributable to the grounding included: shearing of the crest of the nearshore reef, patchy scarring of the offshore area of the first reef, and dislodging numerous stony corals. The *Firat* was removed from the grounding site with no further damage to the reef substrate (Graham & Schroeder, 1996).

1.4.2.3 *C/V Hind* Grounding

The 384 foot cargo vessel *C/V Hind* ran aground one half mile north of the Port Everglades Inlet on March 18, 1998. The grounding was caused by the vessel losing steerage while trying to enter the port (Sea Byte Inc. & SSR Inc., 1998). Attempts to stop the vessel failed, and the *Hind* eventually came to rest in approximately ten feet of water (Sea Byte Inc. & SSR Inc., 1998). The path of the vessel caused injury to the nearshore areas in water depths ranging from 10 to 45 feet. Two major types of damage resulted from the grounding. The first type was attributed to the hull of the vessel hitting the reef and scraping along the substrate. The second type occurred as a result of the anchor, which was deployed and then dragged along the bottom (Sea Byte Inc. & SSR Inc., 1998).

All three of the aforementioned grounding events required some form of coral transplantation for the mitigation of the damaged reef ecosystem. The transplantation of corals is typically one of the first priorities of reef restoration after a significant man-induced event.

1.5 Coral Transplantation Projects

1.5.1 Uses of Transplantation

Transplantation of reef biota, including sponges and corals, can benefit local recruitment, accelerate natural recovery processes, and improve aesthetics (Smith & Hughes, 1999). Guzman (1993) cited coral transplantation as the best approach to improve and preserve biodiversity. According to Clark and Edwards (1994), the high costs of transplantation and the need to remove the corals from the natural reef (when dislodged corals are unavailable) are two negative impacts

of transplantation. Reasons for transplanting corals range from accelerating recovery in damaged areas, to saving threatened species, to enhancing the aesthetics of tourist attractions (Edwards & Clark, 1998).

Previous coral transplantation studies have included the reintroduction of corals to a damaged habitat and the movement of threatened corals to a more healthy location (Bak & Criens, 1981; Chou, 1986; Oren & Benayahu, 1997; Lindahl, 1998; and Thornton, Gilliam, & Dodge, 2000). Fragments from branching corals have been transplanted in the hopes of establishing new coral populations in areas of low coral density (Yap, Alino, & Gomez, 1992). In the Philippines, where dynamite blasting of corals has led to reef decimation, coral transplants have been used to augment natural coral colonization (Auberson, 1982). Overexploited commercial dive sites can benefit from transplantation projects, which may alleviate some of the pressures of intense diving tourism (van Treeck & Schuhmacher, 1999). The transplantation of adult corals has been used as a potential means of accelerating the rehabilitation of denuded reefs (Maragos, 1974; Auberson, 1982; Alcala & Gomez, 1979; Birkeland, Randall, & Grimm, 1979).

More recent transplantation studies have involved the transplantation of corals for reasons other than accelerating the recovery of damaged reefs or rescuing threatened corals. Kuffner (2002) transplanted colonies of *Porites compressa* in order to examine the effects of radiation and water motion on the production of mycosporine-like amino acids (MAAs). Raymundo (2001) examined the effects of proximity of dead corals and live conspecifics to

transplants, and the effect on coral growth in the central Philippines. Sabater and Yap (2002) examined the effects of electrochemical deposition of $CaCO_3$ on the growth of *Porites cylindrical*.

The success of transplantation may depend on the selection of transplant species (Auberson, 1982). Coral mortality or transplantation failure may be due to a number of factors, including the stress of transport, the use of unsuitable species, or the movement of corals to an incompatible location (Becker, 2002). Temperature stress may be another possible reason for transplant failure. Transplantation during the cooler months has been more successful than during warmer months with elevated temperatures (Yap & Gomez, 1985).

1.5.1.1 Transplant forms

Examples of forms and sizes of transplant corals vary from fragments/ branches, cores, and nubbins, to whole colonies. There are benefits and drawbacks to each of these transplant forms. The use of whole colonies may be easier than the use of cores simply due to the effort of coring a coral head. Transplantation of whole colonies is also more manageable especially if the colony is small enough so that only divers are required; instead of the use of lift bags or other equipment.

The use of fragments, cores, or nubbins allows for the reseeding of the receiving area while lowering the impact to the reef from which the transplants were obtained. Explantation involves the use of a branch, core, or fragment for the purpose of starting a new colony (Becker, 2002). The term ‘fragment’ has been used to describe portions of coral that have been broken off from an intact

colony. Branching corals often become fragmented through natural processes such as storms or wave action; at that point they have the potential to survive and reproduce, independent of the rest of the colony (Smith & Hughes, 1999). Cores, sampled with a hydraulic or pneumatic drill, can vary in size from very small nubbins (approximately 25mm diameter) to large cores ranging from 2" to 4" diameter (Davies, 1995).

Studies investigating the survivorship of transplants have shown various degrees of success. Whole colony transplantation projects undertaken by Ortiz-Prosper and Bowden-Kirby showed 94% survivorship of coral transplants, ten months after transplantation (Ortiz-Prosper and Bowden-Kerby, 1999). Bouchon, *et al*. found a 64% survival rate for whole colony transplants in the Red Sea (Bouchon *et al*., 1981). Transplant survivorship ranged from 71% to 90% for Kaly's whole colony transplant investigation (Kaly, 1995). A number of transplantation studies have shown poor *Acropora cervicornis* survivorship; including 98% mortality after 5 months, 100% loss after six months, and 92% mortality after 12 months (Knowlton *et al*., 1981; Bak & Criens, 1981; and Cox, 1992). Becker (2002) found no significant difference in survivorship between 2.54cm (diameter) cores and 5.0cm cores of *Montastrea faveolata* (75% survivorship).

1.5.1.2 Transportation methods

Various types of 'exposed' and 'unexposed' techniques are used to transport transplantation corals. In exposed transport, the coral is brought to the surface and stored under a wet tarpaulin or in a cooler during the transport time

(Kaly, 1995; Thornton, 1999; & Becker, 2002). The corals in the cooler are then separated using “bubble wrap”. It appears that the corals survive very well with this technique. In the unexposed technique, the coral is kept in seawater for the duration of the period of transport, thus minimizing the exposure to air. Kaly (1995) reported better survival for corals that were transported wet (i.e. stored in sea water) over those exposed to air. However, the unexposed methods are often more labor intensive and time consuming.

1.5.1.3 Transplantation Methods

Coral transplantation methods include securing fragments or whole coral colonies to the reef substrate using cement, epoxy, hardware (threaded rods or nails), or cable ties (Jaap, 2000). Harold Hudson was one of the first to use cement for coral transplantation (his earlier work involved marking bivalves with cement in growth studies). Hudson cemented corals to the reef with ‘quick-setting, non-toxic, lime-based’ cement (Hudson, 1979). He found that corals cemented to the sea floor, using this method, attracted little attention and were nearly indistinguishable from their natural counterparts (Hudson, 1981). Neeley (1988) described a method using a Portland type II mortar mix. The mixture was combined topside, and then carried in zip-lock bags or tupper-ware to the transplantation site. The surface of the substrate had to be well cleaned in order to ensure proper adhesion of the cement to the substrate. The cement mixture was then built into a mound, into which the transplant coral was inserted (Jaap, 2000). Kaly (1995), reported that attachment of coral fragments using a cement mixture was a superior technique in comparison with the use of nails and cable

ties. Cement allows for greater attachment and reduces the risk of abrasion with other surfaces (Kaly, 1995). Alcala, Gomez, and Alcala (1982) used a different approach consisting of simple and inexpensive nylon threading to tie coral transplants to the reef substrate.

1.5.1.4 Transplantation Studies

Early transplantation experiments began with the work of Vaughan in 1916. Vaughan used cement to attach stony corals to platforms at the Dry Tortugas, Florida and Goulding Cay, Bahamas. Growth rates were then investigated for transplanted corals at two locations (Vaughan, 1916). Maragos (1974) transplanted corals to rehabilitate reefs in Hawaii affected by eutrophication. Birkeland, Randall, and Grimm (1979) did the same for reefs in Guam affected by heated effluent. Coral transplantation has increased in use and popularity following these early studies.

In 1988, Harriott and Fisk reported the results of fourteen years of transplantation studies (1974 to 1988). The transplanted corals had survival rates ranging from 0 to 100% survivorship. Survival rates varied depending upon species, type and shape of transplants, and environmental conditions (Harriott and Fisk, 1988). Plucer-Rosario & Randall (1987) transplanted three threatened species of scleractinian corals in Guam's only commercial harbor. The corals were removed from a polluted harbor and transplanted to a lagoon, unaffected by pollution. In 1997, the National Coral Reef Institute removed and then re-transplanted 271 stony corals growing on a length of Miami-Dade sewage outfall pipe in need of repair. This transplantation project allowed the necessary repairs

to take place on the outfall pipe and provided a unique opportunity to investigate the effects of transplantation in relation to the health and growth of the transplanted specimens (Thornton *et al*., 2000).

Bruckner and Bruckner (2001) examined the growth and survivorship of *Acropora palmata* fragments for 2 years after the *Fortuna Reefer* ship grounding, off Mona Island, Puerto Rico. *Acropora palmata* fragments were secured to reef substrate and dead *A. palmata* using wire. Fragment survivorship was then assessed two years later. Different fragment sizes, orientations, and placements on the reef were examined in this study. The efficacy of the restoration techniques were evaluated by examining the survival rates of whole colonies, as well as the percentage of fragments that were lost due to wire breakage, and the ability of tissue to grow over the wire. It was found that the mean size of fragments that died was significantly smaller than that of the live fragments, with the highest rate of mortality observed among fragments under 50cm in length (Bruckner & Bruckner, 2001).

The transplantation of corals to a controlled artificial habitat provides a unique opportunity for a detailed examination of their optimal niches by means of their survivorship and growth rates (Oren & Benayahu, 1997). The use of juveniles (or cores) in transplantation has been recommended because: (1) adult colonies may come from the survival of those juveniles and (2) most juveniles can be obtained in large numbers and monitored without further damage to the donor reef (Oren & Benayahu, 1997).

1.5.2 Coral Transplantation in Grounding Rehabilitation

After grounding event impacts, a coral reef community can recover in one of two main ways; through natural coral recolonization or through the supplementation of coral fauna. Once the damaged substrate has been stabilized, the natural dispersal and recolonization of larvae may eventually take place. Natural recovery depends upon the 1) growth of undamaged colonies, 2) growth of damaged, but surviving colonies, and 3) recolonization of impacted substrates (Gittings *et al.*, 1988). Alternatively, the recolonization of coral populations can be stimulated through direct manipulation (Maragos, 1974). Coral transplantation is one technique that has been implemented to alleviate the destruction resulting from these groundings.

The transplantation of adult corals has been suggested as an effective technique in the restoration of degraded coral reefs (Clark & Edwards, 1994). In damaged reef areas, where natural recruitment is unlikely to occur, transplantation can be used to strengthen the natural recovery process of the reef community. In heavily damaged areas, transplantation may increase the rate of recovery (Gittings *et al*., 1988). Additionally, transplantation can contribute to rebuilding habitat complexity needed to support reef invertebrate and fish populations (Gittings *et al*., 1994). Ship grounding incidents can provide a 'disaster of opportunity' for studying the effectiveness of transplantation and other remedial efforts. The following are examples of transplantation projects associated with ship grounding events.

1.5.2.1 *Wellwood* use of transplantation

The *Wellwood* grounding incident was used to test the feasibility of stabilizing reef framework and transplanting corals to the damaged area (Hudson & Diaz, 1988). Transplant corals were dislodged from a nearby, undamaged site and transported by boat to the nearby grounding site. Corals were cemented to the cleared substrate using quick-setting underwater cement. Eleven stony coral colonies and thirty soft corals were transplanted at the *Wellwood* site. According to Hudson and Diaz, after a four-year monitoring period, all hard corals appeared to be in excellent health (Hudson & Diaz, 1988). In the case of the *Wellwood* project, coral transplantation efforts were geared towards taking corals from a healthy reef site in order to reseed the coral populations at a damaged reef site.

1.5.2.2 *Firat* use of transplantation

Following the *Firat* grounding, 588 hard coral colonies representing twelve species were transplanted to the impacted area. The corals used for transplantation were individuals that had become dislodged during the grounding incident. In this way, no additional environmental impacts were caused in the removal of hard corals from donor areas (Graham & Schroeder, 1996). Experimental corals and control corals (corals which had not become detached as a result of the grounding) were monitored for a five year period, using observations and qualitative still and video photography. In a report prepared in 2000, for the Florida Marine Research Institute, no difference in the health of the experimental and reference hard corals was accounted (Continental Shelf Associates, Inc., 2000).

1.5.2.3 *Hind* use of transplantation

The *Hind* transplantation project also used corals that had become dislodged during the grounding incident. A total of 387 corals were located at the grounding sites and, using hydraulic cement and epoxy, were fixed to damaged reef substrate. Twelve zones were established, extending from approximately 19 meters water to the inshore depth of 3 meters water. Corals within these zones were mapped and documented, using an Integrated Video Mapping System (IVMS) (Sea Byte Inc. & SSR Inc., 1998). Two years after the grounding, the National Coral Reef Institute (NCRI) located 333 of the original 387 transplants. 157 of these transplants were tagged, photographed, and evaluated for health, bleaching, and signs of disease (Gilliam *et al*., 2000). Of these corals, 74% were still living. Further monitoring and assessment of the *Hind* transplant colonies has enabled the evaluation of reattachment success.

1.5.3 Artificial Substrates and Transplantation

A variety of causative agents have been cited as influential in the recent worldwide decline of coral reefs. Natural impacts including hurricane damage, El Niño events, and thermal stress have contributed to controlling reef development and species diversity (Glynn, 1985; Jaap, 1984). Anthropogenic influences have caused severe damage to coral reefs. Coastal development has led to increased sedimentation and eutrophication. Ship groundings, dredging, and blasting have all been cited as the cause of direct damage to reef framework and reef biota (Grigg & Dollar, 1990). Interest in alleviating this type of damage has led to the development of restoration and rehabilitation programs, aimed at the injured

reefs. The use of artificial substrates, has received special attention over the past few years (Spieler, Gilliam, & Sherman, 2001).

Without intervention, damaged ecosystems may not recover from the stress of anthropogenic factors (Pratt, 1994). Artificial reefs are commonly used to enhance or provide structure lost at damaged reef areas. In the past, much artificial reef research focused on recruitment of fishes to artificial habitats. Artificial habitats such as fish aggregating devices (FADs), have been used by artisanal fishermen for centuries (Polovina, 1991). In areas depleted of fish populations, fisheries and resource management sectors have used artificial reefs as a potential avenue to enhance fish biomass. In areas of turbidity or high sedimentation, artificial reefs have been used to augment the growth of coral colonies (Chou, 1986). In parts of the Maldives, where coral mining for the construction industry has destroyed precious reef systems, artificial habitats have been used to rehabilitate the severely degraded reefs (Clark and Edwards, 1995). The formation of semi-artificial reefs *in situ* via electrolysis has alleviated the pressures of recreational diving on the natural reefs of Aqaba (van Treeck & Schuhmacher, 1999).

One major dilemma resulting from the impact of a coral reef community is the loss of the coral animals; a second problem is the loss of habitat or refuge. An artificial habitat based transplantation project, therefore, may facilitate restoration efforts. The artificial habitat can act as a fixed substrate providing a base for invertebrate settlement. Additionally, the artificial habitat can provide the necessary refuge for fishes and other macro-invertebrates (Spieler, Gilliam, &

Sherman, 2001). Through the establishment of artificial reefs on or adjacent to an impacted area, a substantial jump may be gained on the recovery of these habitats (DEP, 1994). The use of artificial habitats in these situations may result in increased knowledge of the processes related to natural recovery and restoration (Miller *et al*., 1993). The *Memphis* project was designed to test a number of hypotheses relating to fish and coral recruitment to an artificial reef environment. Additionally, this study investigated the success of coral transplantation to artificial habitats.

1.6 Coral Growth

1.6.1 Methods of Monitoring Coral Growth

Darwin's theory of reef development (1842) piqued an early interest in reef structure, the main constructional elements (coral polyps and colonies), and coral reef growth (Buddemeier & Kinzie, 1976). Since that time, an understanding of growth rates, forms, and longevity has been the basis for studies of coral reef ecosystems and their components (Buddemeier & Kinzie, 1976). Growth forms in corals are highly variable, both within and between species, and frequently are connected to environmental factors (Pichon, 1978).

Measurements involving the deposition of coral skeletons are regularly used for coral growth determination (Frank *et al*. 1995). Various measurements of skeletal accretion have been used, including increase in mass, volume, area, various linear dimensions, and uptake of skeletal components such as calcium (Barnes, 1970; Buddemeier & Kinzie, 1976; Dodge & Thomson, 1974). Alizarin Red stain, buoyant weight, x-radiography, and computerized tomography have all

been established as effective methods for measuring skeletal growth (Barnes, 1970; Lamberts, 1978; Dodge *et al*., 1984*a*; Dodge, 1980). Both accretionary (up and out from the center of the colony) (Goreau & Goreau, 1959; Dodge & Thomson, 1974) and encrusting (outwards from the edge of the colony) (Dustan, 1975; Kraemer, 1982) measurements of coral growth have been documented (Gittings, 1988). Skeletal accretion is one means by which an accurate measure of growth may be attained for corals. The following techniques are the more commonly used procedures for skeletal growth determination.

1.6.1.1 Alizarin Red

Barnes described the use of Alizarin Red S to stain coral skeletons in 1970. Initially, the live coral is incubated in a container with the dye in the laboratory, or bagged and stained in the field. Sodium alizarian sulphonate [$C_6H_4COC_6HOH_2(SO_3Na)CO$] becomes incorporated into the skeleton of the coral as a band of color (Barnes 1970, 1972, Dodge 1984*a*). Use of the Alizarin technique provides a visible skeletal time base, from which ensuing growth can then be measured with the sacrifice and sectioning of the coral specimen (Buddemeier & Kinzie, 1976). Alizarin can be used to give a qualitative measure and comparative quantitative measure of calcium deposition (Lamberts, 1978). Staining with Alizarin Red can cause initial, temporary depressions in calcification rates (Dodge *et al*., 1984*a*). The use of Alizarin Red requires the eventual sacrifice of the coral. Thus, this technique is not appropriate for experiments in which the sacrifice of the coral is not desired (i.e., the coral is to remain alive). Additionally, this method has been suggested to be mildly toxic to the coral, and

may affect the coral growth intending to be measured (Lamberts, 1978; Dodge *et al*., 1984*a*).

1.6.1.2 Buoyant weight

The buoyant weight technique involves weighing the coral specimen as it is suspended in a buoyant medium of seawater (Jokiel *et al*., 1978). The coral is measured in water on two occasions, the water density is recorded at each instance and the weight in air is then calculated (Dodge, 1984*a*). Buoyant weight is a simple and flexible method of determining aragonite mass, which does not harm the coral and allows repeated measurements of the same specimen. Despite these advantages, the buoyant weight method necessitates a substantial amount of specimen manipulation, and needs a basis of comparison for weight gain (Buddemeier & Kinzie, 1976). This method does not require the sacrifice of the coral. Further studies have tried to increase the accuracy of this technique by adding a correction for the buoyant weight of the coral tissues (Davies, 1989).

1.6.1.3 X-radiography

Seasonal cycles in skeletal density in scleractinian corals have been studied using X-radiography; a technique similar to studying the distinct yearly bands formed in trees (dendrology). A complete cycle of both high and low-density skeleton is formed on an annual basis (Knutson *et al.,* 1972; Dodge & Thomson, 1974; Hudson *et al*., 1976; Wellington & Glynn, 1983). Using the X-radiography method, age and annual growth rates can be determined by counting the bands and measuring the annual growth increments between them (Hudson, 1981). Whole colonies may be used or colonies are cored (Macintyre,

1975). These are next slabbed and X-radiographed to reveal the banding as described by Knutson *et al*. (1972). The alternating dark and light bands reflect the bulk density of the deposited skeletal material (Knutson *et al*., 1972). This method allows the retrieval of information on long-term growth rates without real time *in situ* experimentation (Knutson *et al*., 1972). The X-radiography technique is also especially effective in examining correlations between coral growth rates and environmental conditions. Again, this method requires the eventual sacrifice of at least part of the coral colony.

1.6.1.4 Alternative methods of measuring growth

Surface area measurements have been used regularly to determine growth for corals (Auberson, 1982). Many of the biological and physical processes affecting an organism can be studied using surface area measurements. Indirect estimates of biomass and other measurements related to photobiology can be obtained using surface area measurements (Myers *et al*., 1999; Lesser *et al*., 2000). Photographic sequences are likely the most common of the surface area measurement techniques used to monitor corals. Other methods for obtaining surface area of corals include the use of aluminum foil, latex rubber, molten wax, or a Methylene Blue dye solution to determine the surface area using a surface area-to mass calibration (Marsh, 1970; Myers *et al.,* 1999; Lesser *et al.,* 2000; Hoegh-Guldberg, 1988). Each of these latter methods can be destructive, since the coral must be taken out of the water for the measurements.

1.6.1.5 Photographic techniques

Photographic monitoring is an *in situ* method that is both facile, highly reproducible, and non-destructive (Vago *et al*., 1994). By repeatedly photographing a coral specimen, at regular intervals from the same distance, coral growth over time can be effectively monitored. Vago *et al*. (1994), described a technique where 35mm slides were digitized to measure both surface area and circumference of the experimental corals. Using this technique, rates of change were plotted against time. In Vago's study, growth was determined over a period of only one month in a specimen of *Favia favus*. According to that study, "While the calculated areas reveal a quantitative picture of the growth of the colony, the sequence of computer drawn images preserves its life history…in this way (this method) can be used as a tool for assessing environmental impacts on the reef (Vago *et al*., 1994)."

Ben-Zion *et al.* (1991) used a similar technique to compare the surface area derived from photographic slides, with the surface area derived from the melted paraffin technique. The calculated surface area from slides deviated from the paraffin method by 6% at most (Ben-Zion *et al*., 1991). Gittings (1988) used a photographic technique to measure coral growth of disturbed coral colonies following the *Wellwood* grounding. Growth stations were established using stainless steel welding rod nails. Colonies were photographed using print film. Colony borders and mouth polyp positions were traced from the prints using mylar drafting material. Border lengths, areas of tissue advance, and areas of tissue retreat were then measured using a digital planimeter (Gittings, 1988).

Surface area measurements (using photographic methods) were selected as the measurable growth parameter in this study because a) they are an obvious measurement of the success of corals in sustaining a reef community, and b) they allow for long-term monitoring of measurements of change (Dodge *et al*., 1984*b*). It must be taken into consideration that the assessment of one growth parameter (surface area in this case), is not equivalent to the evaluation of all parameters (Dodge and Brass, 1984). However the calicoblastic layer, the layer of living tissue over the exoskeleton, is a nearly two-dimensional layer that is responsible for coral growth (Bak, 1977). Accordingly, the use of photography to assess this growth parameter is a justifiable technique.

1.7 Recovery of Injury

1.7.1 Natural Coral Injury Recovery Process

Tissue injury is widespread in reef building corals (Cumming, 2002). Meesters, Wesseling, and Bak (1996) recorded injuries in as much as 68% of (three different species of) Caribbean scleractinian corals. Damage to coral tissue occurs continually from a variety of sources such as fish, invertebrates including molluscs and polychaetes, and human activity (Pearson, 1981; Brown & Howard, 1985). As clonal organisms, corals possess the ability to either overgrow or to defend against overgrowth by neighbors and to regenerate in response to injury (Jackson & Hughes, 1985). These abilities increase with increasing colony size (Jackson & Hughes, 1985); but regeneration depends on the amount of tissue bordering an injury and not the size of the colony (Meesters, Noordeloos, & Bak, 1994). The amount of tissue bordering an injury signifies the

live uninjured tissue surrounding the lesion; which is capable of regeneration (Hall, 2001).

After injury, bare skeleton becomes available for settlement by other organisms (Bak & Steward-Van Es, 1980). In massive corals, boring sponges may contaminate skeleton devoid of tissue; in branching species, denuded skeleton may become more susceptible to breakage (Highsmith *et al*., 1983). Damaged tissue may also be more susceptible to disease (Smith & Hughes, 1999).

Once established, a lesion may become either a permanent feature, or it may recover through regeneration of the tissue and skeleton. All coelenterates are able to regenerate complete functional units, both polyps and medusae (Auberson, 1982). Regeneration rates have been studied in many coral species and have been shown to be species specific (Bak & Steward-Van Es, 1980; Meesters, Noordeloos, and Bak, 1994; Meesters, Bos, and Gast, 1992; Hall, 1997). Morphology can influence the recuperation of injury, branching species have been found to be more susceptible to certain injury types than massives (Hall, 1997).

Tissue regeneration has been described as an 'energy-cost' process, with "the trade-off in energy allocation between regeneration, reproduction, and growth (indicating) that corals are capable of controlling and regulating the energy cost of the regeneration process" (Oren *et al.*, 1997). Subsequent to the injury, colonies may attempt to regenerate missing tissue. Generally, a new tissue layer is formed by surrounding polyps; with new septa emerging in

approximately two weeks (Meesters, Noordeloos, & Bak, 1994). Over time, regeneration rates may decrease; with complete regeneration of the injury site unlikely (Bak & Steward-Van Es, 1980; Meesters, Noordeloos, & Bak, 1994). If regeneration does not take place, bare skeleton may be settled by algae (Cumming, 2002). Polyp mortality may result in partial mortality of the entire colony (Meesters, Noordeloos, & Bak, 1994).

There are at least three ways in which the reduced level of fitness associated with injury can be an important factor in the life history of corals. First, the energy required for regeneration decreases the energy allocated to growth and reproduction. Second, injury can provide a location of pathogen entry. And finally, the injury site reduces tissue area for important processes such as reproduction, photosynthesis, and feeding (Hall, 1997). Recent injury may be more of a predictor of colony fate than old injury (Cumming, 2002). A colony that has been recently injured may direct resources to regeneration; however, a colony that has an old injury may cease to regenerate tissue (Cumming, 2002).

1.7.2 Experiments on injury and tissue regeneration

As concern about the health of coral reefs has continued to grow on a worldwide basis, experiments examining injury and tissue regeneration have become increasingly popular. Some of these studies have addressed topics ranging from the effect of bleaching on resource translocation and tissue regeneration (Fine, Oren, and Loya, 2002); to the recruitment of algae in areas of

bleached tissue (Diaz-Pulido & McCook, 2002); to the response of corals to various injury types (scraping, breakage, or mortality) (Hall, 2001).

Oren *et al.* (1997) artificially inflicted lesions of differing size and shape onto the upper surface of *Favia favus* colonies. The gradual closures of these lesions were monitored monthly using underwater photography. From these images, lesion surface area, perimeters, and perimeter/surface area ratios were calculated**.** The lesions with longer perimeters appeared to build more new tissue than the lesions with shorter perimeters. In this way, the longer perimeter lesions were provided with the greater energy resources for their regeneration. The highest recovery percentages of all lesion types were achieved during the first month interval followed by a significant decrease during the second and third month intervals.

Meesters, Noordeloos, and Bak (1994) examined the regenerative abilities of *Montastrea annularis*. Individuals were inflicted with lesions caused by physical injury. The lesions were followed in order to determine the effects of differing lesion types and the resultant recovery rates. They found regeneration to be fueled by the polyps and the tissue on the border of the lesions (Meesters, Noordeloos, & Bak, 1994). Thus, the amount of tissue bordering the lesion and not the size of the entire colony, dictates the success of regeneration. Patches of bare skeleton (lesion sites), surrounded by living tissue, can develop into permanent states. Meesters, Pauchli, and Bak (1997) found that there is a maximum amount of tissue that can be regenerated (i.e. a maximum lesion size which may fully recover). This maximum size is species specific; for *Montastrea*

annularis, a maximum of 4.7mm^2 of new tissue could be regenerated per mm of lesion perimeter length (Meesters, Pauchli, and Bak, 1997).

Hudson used modeling clay or cement to cover areas cleaned of coral tissue infected by black band disease (Woodley & Clark, 1989). The hope was that the uninfected tissue would then regrow over the cement site (Woodley & Clark, 1989). A similar technique was used in this coral transplantation study. In order to facilitate the recovery of the core hole 'injury' sites, an artificial substrate (a concrete core hole plug) was secured into each core hole. Ideally, coral tissue could then regenerate and expand over the surface of the plugged core hole (see Section 1.7.3). In addition to tissue injury adjacent to the core hole, transplantation itself may have caused injury to the tissue and/ or skeleton of the coral transplants. As such, both of these aspects were examined in this study.

1.7.3 Importance of plugging core holes

Cores of coral skeletons have been used to study past and present climatic changes, which have affected reefs on a worldwide scale. Coral skeletons have also been used as chemical indicators, to study episodes of pollution affecting the local community (Dodge *et al*., 1984*c*). Because the skeleton itself holds this information and because old colonies are often large, corals are drilled to obtain a skeletal sample (see Section 1.6.1.3). Swart, *et al.* (1996) found a 160 year record of salinity and organic matter cycling in one *Solenastrea bournoni* colony in Florida Bay. Information of this kind has helped to determine the effects of canal construction throughout the Everglades, on Florida Bay water quality and fauna (Swart *et al.,* 1996). Other studies like these

have provided invaluable information on climate, salinity, temperature, etc. Time sequences and environmental conditions on the reef have been examined for the "reconstruction of ecological history" of various locations (Hudson, *et al*., 1976). This "physiological-environmental coupling" is the focus of much coral reef research (Dodge & Vaisnys, 1980). By combining the disciplines of ecology and paleobiology, the evolutionary paleoecology of coral reefs has become an advancing field in coral reef research (Aronson & Precht, 1998). This study may provide further information as to the importance of plugging core holes in future coring work.

2.0 Rationale, Statement of Purpose, and Hypotheses

2.1 Rationale

Transplantation of coral species to Reef Balls™ has been shown to be an effective means of establishing coral colonies on an artificial reef environment. The transplantation of massive species, such as *Diploria* spp. and *Montastrea* spp., has shown the potential of a coral to spread over artificial reef structure (Ortiz-Prosper and Bowden-Kerby, 1999). These transplants have enhanced the vertical stratification of the habitat and reduced the time necessary for colony development by jump-starting populations (Ortiz-Prosper & Bowden-Kerby, 1999.)

2.2 Statement of Purpose

This coral transplantation study was designed to assess transplants of two different scleractinian species to artificial reef habitats. Transplant growth and survivorship was measured over a fifteen-month period. Coral transplants were compared to controls on natural reef in order to examine the effects of drilling and transplantation to the experimental corals. Donor corals and controls on the natural reef were compared for survivorship and health in order to examine the effects of drilling on the donor colonies. Regrowth over the core sites was assessed to determine the effects of drilling and the effectiveness of the core plug. Coral transplants in this project were obtained from un-impacted areas, adjacent to the grounding site. Using a hydraulic drill, cores of live tissue were taken from donor colonies to be transplanted onto the artificial habitats. This methodology allowed for replicate transplants and did not denude the donor site

of entire colonies. According to Edwards & Clark (1998), removing coral colonies from one location to transplant to another site is one potential drawback of many transplantation projects. The drilling method used in this project partially eliminated this potential drawback. The donor colonies on the natural reef were impacted, but not removed completely. Additionally, the species chosen were both slow growing massive or encrusting corals. Due to their long-term survival rates, these species are found to be of more benefit in transplantation projects than faster growing, weedy species (Clark & Edwards, 1994).

Transplantation into areas where natural recruitment is substantial may not be the best use of transplantation effort (Edwards & Clark, 1998). The sand flat between the second and third reefs does not provide adequate habitat for coral settlement and recruitment; therefore the transplantation of these corals onto the Reef Balls has created a population that would otherwise not exist there. There are several ways in which the transplants may increase the coral cover on the Reef Ball habitats. The individual transplants may grow and increase in surface area, increasing the coral cover on the Reef Balls. Additionally, these established populations may, stimulate or enhance local recruitment of other coral individuals on the Reef Ball communities (Harriot & Fisk, 1988; Morse *et al*., 1988; Morse *et al.,* 1991). Data collected and conclusions drawn from this study will aid in future transplantation projects.

2.3 Hypothesis

The following are the hypothesis (and sub-hypotheses), to be tested in this study.

- ❖ Main Hypothesis -

Species-specific differences will occur in the responses of coral colonies to drilling and transplantation.

Null-Hypothesis – There will be no species-specific differences in the responses of coral colonies to drilling and transplantation.

Sub-hypotheses -

- Hypothesis 1 – There will be a change in surface area and/or linear radius (tissue regeneration and skeletal growth) in the experimental corals (transplant corals and donor corals) and the control corals.

- Hypothesis 2 –The survivorship of the experimental corals (transplant corals and donor corals) will be similar to their respective control corals.

- Hypothesis 3 – There will be a change in surface area and/ or radius (tissue regeneration and skeletal growth) surrounding the core holes in the donor corals.

Null-Hypothesis 1 – There will be no change in surface area and/or linear radius in the experimental corals (transplant corals and donor corals) and the control corals.

Null-Hypothesis 2 – The survivorship of the experimental corals (transplant corals and donor corals) will not be similar to their respective control corals.

Null-Hypothesis 3 – There will be no change in surface area and/ or linear radius surrounding the core holes in the donor corals.

3.0 Coral Transplantation Project Materials and Methodology

3.1 Construction & Deployment of Reef Ball habitats

One hundred and sixty Reef Ball artificial habitats were constructed at Nova Southeastern University Oceanographic Center in August of 2000. On November 17, 2000, these artificial habitats were deployed between the second and third reef terraces off Dania Beach (see Section and 1.1.1 and Section 1.2.2, Figure 2). The experiment was designed for the Reef Balls to be grouped in quads. One fourth of the Reef Balls (one per quad) were modified for coral transplantation; with two receptacle cups per Reef Ball, for coral transplants. Attempts were made to deploy the transplant Reef Balls so that the receptacle cups were oriented outwards from the quad. Due to the nature of the deployment process, this was not always successful. In general, most of the receptacle cups faced outwards. The orientation (NSEW) of the transplant Reef Ball, in relation to the three other Reef Balls, was haphazard.

3.2 Transplant Species

The transplanted corals were identically sized 4” diameter core replicates. Cores of *Meandrina meandrites* (Linnaeus, 1758) and *Montastrea cavernosa* (Linnaeus, 1766) were used for transplantation. Two cores, one of each species, were affixed to each of the pre-specified transplant Reef Balls. A total of forty cores of each species were sampled.

3.2.1 *Meandrina meandrites*

Meandrina meandrites is a member of the family Meandrinidae. This family includes phaceloid, massive, submassive, columnar, and encrusting forms

(Veron, 2000). Members of Meandrinidae resemble faviids, superficially, but instead have fine non-porous skeletal structures. Both the walls and septa are solid, exsert, and even-spaced (Veron, 2000). Four genera of meandrinids are restricted to the Atlantic, including: *Dendrogyra, Dichocoenia, Eusmilia,* and *Meandrina*. Humann (1993) described *M. meandrites* as forming colonies consisting of both rounded heads and flattened plates. Smooth and widely separated septa create tall ridges. The septa come together forming a thin line along the ridgetops. The colonies are usually tan to yellow-brown in color. *Meandrina meandrites* is common in the South Florida area. It inhabits most reef environments, most specifically on the seaward reefs at a depth of 8-25 meters (Humann, 1993).

According to Chiappone and Peters, *M. meandrites* is not considered greatly threatened; however, both incidence of disease and sensitivity to eutrophication have been reported (http://www.natureserve.org/explorer). There has been a limited amount of information collected on the growth rates and reproductive ecology for this species. As such, *M. meandrites* is a good candidate for growth studies (http://www.natureserve.org/explorer).

3.2.2 *Montastrea cavernosa*

Montastrea cavernosa belongs to the family Faviidae. This family is comprised of twenty-four genera, more than any other family of coral. Characteristics of the faviids include: simple septal structures, columellae forming as an intertwining mass of elongated septal teeth, and walls forming from a combination of thickened cross linkages (Veron, 2000). Corals of the genus

Montastrea have made substantial contributions to reef frameworks throughout the Caribbean (Budd, 1988). *Montastrea cavernosa* colonies are massive, usually forming domes or boulders. The variable corallites are round and exsert. Septa alternate in a long and short pattern, with the septa joined to the columella (Veron, 2000). According to Humann (1993), the surface of the massive colonies of *M. cavernosa* is covered with distinctive blister-like corallites. Colorations vary from green to brown, yellow, red, orange, and gray. *Montastrea cavernosa* is abundant on most Caribbean reefs and is common to abundant in the South Florida area (Szmant, 1991; Humann, 1993). It also inhabits most reef environments, and it is often the dominant coral between the depth range of 13 to 34 meters (Humann, 1993). *Montastrea cavernosa* relies more heavily on heterotrophy than its cogener *M. faveolata* (Lesser *et al*., 2000). This could be an important physiological aspect of the biology of *M. cavernosa,* if feeding by heterotrophic methods allows this species to deal better with periods of stress.

3.2.3 Selection of Transplant Species

These two species were selected on the basis of growth, survivorship or transplantation success, and abundance in the Broward County area. *Meandrina meandrites* and *Montastrea cavernosa* are two of the most abundant corals on the second reef, south of Port Everglades, where the *Memphis* grounding occurred (personal observation). Goldberg (1973) determined that *M. cavernosa* is the dominant scleractinian coral of the northward extension of the Florida reef tract. According to the 2003 Marine Biological Report, *M. cavernosa* accounts for thirteen percent of the coral species in Broward County (Gilliam *et al*., 2002).

Meandrina meandrites ranks as the second most dominant coral along the coast from Miami through Palm Beach County, according to Goldberg (1973). This species comprises over two percent of the scleractinian corals throughout the County (Gilliam *et al*., 2002). Previous transplantation studies have selected transplant species based on importance or ecological dominance of particular species (Yap, Aliño, & Gomez, 1992).

Montastrea cavernosa, in a depth range of 4-28 meters on the reefs of Belize, can attain linear extension rates of 4.36mm/yr (Highsmith *et al*., 1983). Hubbard and Scaturo (1985) reported extension growth rates of 4.5mm/yr. at a depth of 10 meters on the reefs of St. Croix. *Solenastrea bournoni*, another abundant species of Favidae near the Memphis grounding site, has been reported to have a mean annual growth rate of 5.07mm/yr in Florida Bay (Swart *et al*., 1996).

According to Meesters and Bak (1993), *M. meandrites* has a fast tissue regeneration rate (regeneration rate is defined as the mean rate at which tissue lesions recover; it is expressed as area covered per unit of time). Regeneration plays an important role in colony survival (Meesters & Bak, 1993). Miller *et al.,* (1993) assigned *Montastrea cavernosa* to have a high transplant potential (transplant potential refers to the ability of adults to survive transplantation) and *Meandrina meandrites* to have a medium-high transplant potential.

3.2.4 Reproductive Methods and Size of Fecundity

Scleractinian corals are frequently classified by their sexual reproductive method as brooders or spawners. Brooders refer to those corals that brood their

embryos to the planula stage before releasing them (Szmant, 1986). Brooders tend to be of smaller size and have multiple planulating cycles (Szmant, 1986). Most corals do not brood their larvae, but are instead spawners. Spawners tend to have large colony sizes and have short, annual spawning periods (Szmant, 1986). Additionally, corals can be either hermaphroditic or gonochoristic. Hermaphroditic corals house both sexes in the same individual; gonochoristic species are separately sexed. Separately sexed means separate male and female colonies (in solitary species) i.e., separately sexed individuals (Veron, 2000). Most species of scleractinian corals have polyps that are hermaphroditic, but some do have separate sexes (Fadlallah, 1983; Szmant, 1986; Veron, 1986).

Montastrea cavernosa has been classified as a broadcast spawner, with a single gametogenic cycle per year (Szmant, 1986; Acosta & Zea, 1997). Complete data was not available, to date, on the reproductive method of *M. meandrites*. *Dendrogyra cylindrous*, another Atlantic meandrinid species of the same family has been classified as a gonochoristic broadcaster (Szmant, 1986). Transplantation of broadcast spawning species, at a grounding site, has been described as an effective restoration technique. Brooding coral species are often more successful in natural recruitment (Gittings *et al*., 1994). By transplanting coral species that have less successful recruits, coral populations that are not readily reseeded may receive a jumpstart.

Colonies of *M. cavernosa* as small as 20cm^2 are of sexual maturity (Soong, 1993). However, they only exhibit minimal reproductive effort until reaching a circumference of 100cm^2 (approximately 400 polyps) (Szmant, 1991).

Other Caribbean massive species that broadcast gametes (*Diploria strigosa, Montastrea annularis, Siderastrea siderea*) have larger maturation sizes (greater than 100cm^2). All colonies selected for donors in this study had a long axis of greater than 40cm (the average size for the donors was 60cm x 52cm). The transplant corals had an average, initial surface area of 6,100mm^2. Corals in these size ranges were targeted for selection and sampling, in order to maintain the necessary size for reproductive viability.

3.3 Location of Corals

3.3.1 Location of Transplant Corals

The Reef Ball arrays were situated between the second and third reefs (Figure 7). Two transplants (one of each species) were placed on the modified Reef Ball (see Section 3.1) in each of the forty quads (dots). The box to the west of the Reef Balls indicates the location of the donor corals and the control corals (see green box to left and image on next page).

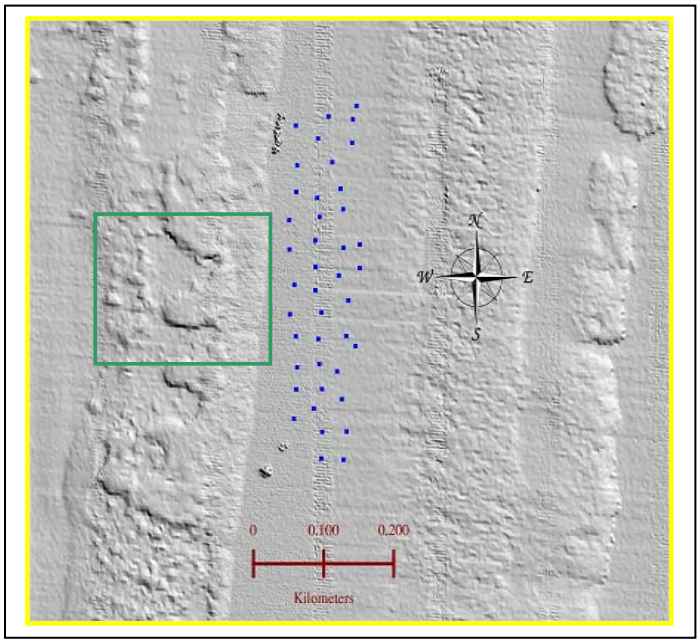


Figure 7: Reef Ball configuration between the second and third reefs. The dots signify the Reef Ball quads. The square outlines the location of the donor and control corals on the natural reef. This box also contains the impact site for the Memphis grounding. The second reef (box) is at a depth of approximately 9 meters with the sandy Reef Ball site (approximately 12 meters), directly offshore.

3.3.2 Location of Donor Corals

Donor corals were selected based on the following criterion: size, health, and proximity to established monitoring sites. Corals were visually assessed prior to selection to ensure that they were of adequate size, with a minimum long axis of 40cm (see Section 3.2.4). Additionally, only corals free of disease, bleaching, or substantial mortality were selected (corals with less than 60% tissue coverage were rejected). All donor corals were located on the second reef, within close proximity of each other and to the west of the Reef Ball site. Previously installed control pins (CP-1, CP-2, CP-3; see Figure 3) at the *Memphis* grounding site were used to establish zones from which donor colonies were located and mapped (Figure 8). Distance and azimuth were measured

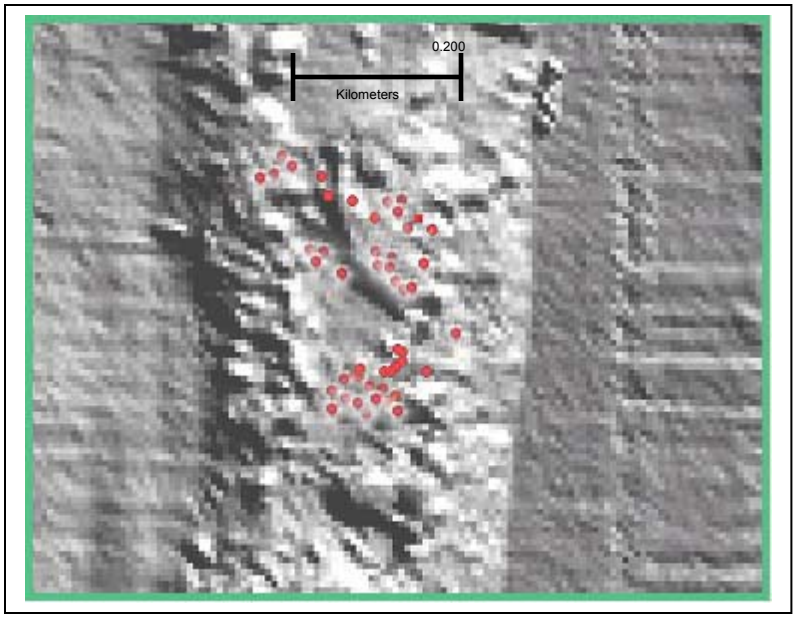


Figure 8: Location of donor and control corals on the natural reef. Total area covered in this image is the same as the outlined green area in preceding image.

from the nearby CPs to the donor corals. Due to the proximity of the donor and control corals around the *Memphis* grounding site (i.e. relative depth, distance

from Port Everglades inlet, and distance from the shoreline), it was assumed that similar factors affected the corals after transplantation.

3.3.3 Location of Control Corals

Two kinds of control corals were selected: controls for cored donor corals (N=20, ten of each species) and controls for transplants (N=20, ten of each species). Individuals of each control type were of similar size to the treatment corals of that type. The purpose of the donor control corals, or large controls (N=20) was to compare the growth and survivorship of corals that have had cores removed (donor corals) to the control corals on the natural reef. This may help to determine the effect of the coring process on mature coral colonies. The purpose of the transplant controls, or small controls (N=20) was to compare the growth and survivorship of cored and transplanted corals to unmolested corals, of comparable size, on the natural reef. These control corals were chosen in the same manner as the donor corals, and mapped using the same methodology (see Figure 8). Table 5 provides a description of all experimental and control corals.

Table 5: Description of experimental corals; including type, species, and number.

	Species	
Experimental Coral Type	*M.m.*	*M.c.*
Transplant Corals (Treatment)	40	40
Donor Corals (Treatment)	20	20
Donor Coral, Core Holes (Treatment)	40	40
Transplant Control Corals	10	10
Donor Control Corals	10	10

3.4 Drilling of Corals and Transplantation

3.4.1 Coral Coring Process and Justification of a 4” Core

A Stanley hydraulic drill and power pack unit, fixed with a 4” core barrel, was used to remove replicate coral cores (Figure 9). Forty cores of each of two species, *Meandrina meandrites* and *Montastrea cavernosa*, were sampled for transplantation. Each donor coral had two cores taken from the colony. This methodology effectively reduced the number of donor corals necessary by 50%.

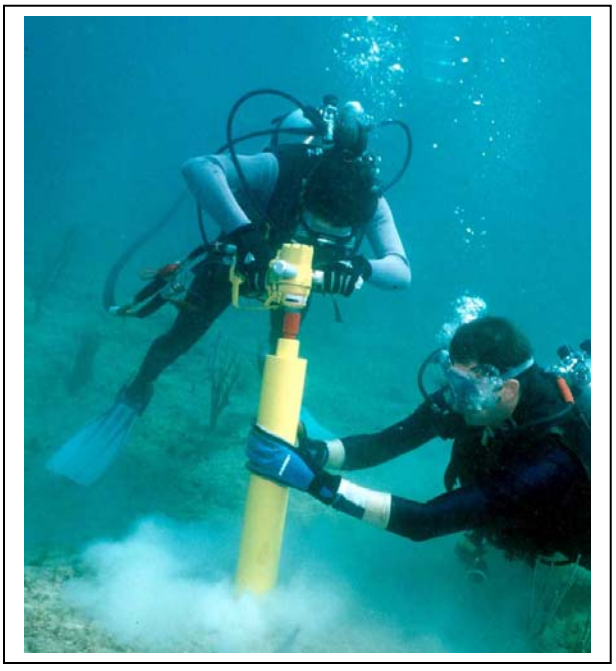


Figure 9: Drilling donor corals for transplantation.

Donor corals in the size range of approximately 50cmx50cm were targeted. Because mortality is inversely related to size (Soong, 1992; Highsmith *et al*., 1980; Hughes & Jackson, 1980; and 1985; Hughes & Connell, 1987), a large size should have better colony survival. Additionally, this size range (50cmx50cm) was readily available at the sampling sites. Both the donor corals

(less the two 4” cores) and the 4” core plug transplants should be of reproductive size/ age (Szmant, 1991).

In drilling cores for transplantation, the potential for injury to both the transplants and to the donor colonies was evident. Efforts to minimize the impact on the coral colonies were taken (including experienced and skilled divers, and stable and accessible coral colonies). Coral transplants were transported and transplanted following well-established methodologies. Cored donor colonies were filled with concrete plugs, as is often the practice in sclerochronology studies (see section 1.7.3).

3.4.2 Coral Collection Permit

A permit was required for the drilling and transplanting of all corals. The permit was issued by the State of Florida, Florida Fish and Wildlife Conservation Commission to Dr. Richard E. Dodge in June of 2000 (Permit #: 00S-535) and renewed in June of 2001 (Permit #: 00S-535A).

3.4.3 Plugging of Donor Corals

Eighty concrete plugs (numbered 1-80) were constructed using Bonsal’s Sure-Mix concrete (commercially available). The plugs were cured in plastic milk crates in the Nova boat basin for approximately five weeks, to allow adequate time for the leaching of toxins (Figure 10). The top edge of each of the concrete

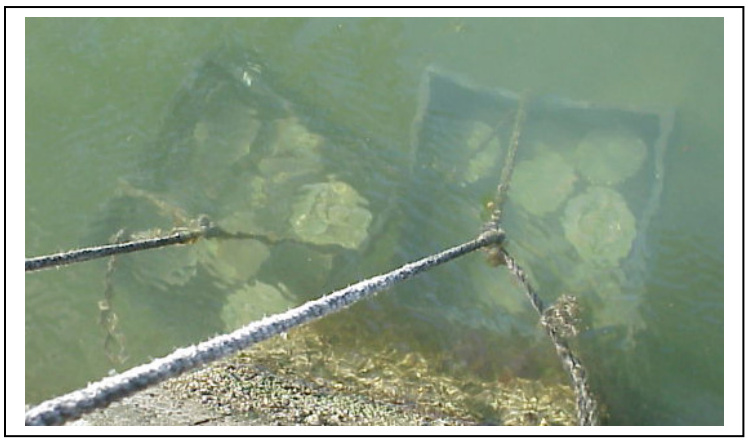


Figure 10: Transplant plugs curing in the Nova boat basin.

plugs was approximately equal to the outside diameter of the core barrel. The concrete plugs were trimmed at the bottom, using a masonry saw, to a height of approximately 10cm. Efforts were made to maintain a flat profile between the surface of the concrete plug and the donor colony. Due to the morphological diversity of the donor colonies, the concrete plug did not always fit flush with the surface of the coral. In cases where the plug was too short, small rocks and shells were used to fill in below the plug to maintain the same relative height as the colony. In cases where the plug was too long, it would generally stick up a few centimeters from the surface of the coral (this was the case for less than 17% of the plugs).

After drilling the core, the appropriate numbered concrete plug was placed into the hole (Figure 11). Underwater marine epoxy (*Aqua-Mend*®, a two-

Figure 11: Concrete core hole plug #30 (June '01, immediately after drilling and filling of Donor 15). Note epoxy around concrete plug.

part stick epoxy) was used to fair in the area (to make smooth and regular) between the plug and the adjacent coral tissue, in order to secure the plug. The

plugs had two functions: 1) it prevented the detrimental effects of bioeroders within the holes, and 2) it provided substrate to facilitate coral tissue regrowth over the core hole.

3.4.4 Transport of Cores (Coral Transplants)

Approximately eight cores were targeted for drilling per day. Once cored, the transplant corals were placed in numbered plastic bags. In this way, the replicates were correctly tagged with the proper transplant number. The cores then were collected in plastic trays, transported to the surface, and stored on the boat in a cooler. The cooler was lined on the bottom with a layer of freezer packs. The freezer packs were separated from the coral cores by layers of bubble wrap packaging material. The cores were wrapped in damp plastic bags and placed on top of the bubble wrap. The transplant corals were kept topside only for the duration of the surface interval (generally less than one hour between dives). Additionally, they were kept out of direct sunlight throughout the entire process. This 'exposed' method of coral transportation has been used successfully with small explants of *Montastrea faveolata* (Mueller, personal communication). The damp plastic bags prevent desiccation of the coral during transport. Once retracted, the coral polyp was no longer in contact with any outside disturbances, thereby reducing the damage to the coral colony (Thornton, 1999). Similar methods have been used successfully in the transport of coral fragments (Kaly, 1995). These methods are low-cost, simple, and time efficient.

3.4.5 Attachment of Cores (Coral Transplants) to Reef Balls

The transplant cores were attached to the Reef Balls using the same epoxy (*Aqua-Mend*®) and methodology as used for core hole plug attachment. Cores were trimmed at the base, topside, using a hammer and chisel. Efforts were made to maintain a flat profile between the surface of the transplant and the Reef Ball. Therefore, the cores were trimmed to a height of approximately 10cm. If the transplant core was in jeopardy of splitting apart, the 10cm height was abandoned in order to maintain the integrity of the transplant. Because of this, some of the transplants did not fit exactly flush with the surface of the Reef Balls.

The transplant cores were then inserted into a pre-fabricated receptacle site in the modified transplant Reef Balls. Underwater epoxy was placed in the space between the core and Reef Ball edge. The epoxy was faired, or joined in a smooth and regular fashion, at these edges in order to secure the plugs and allow tissue growth over the surface of the epoxy. All efforts were made to maintain a stable attachment of the transplant cores to the Reef Ball substrate. Edwards and Clark (1998) cited transplant failure (failure of the transplant to maintain its attachment to the reef substrate) as a main cause of transplant mortality.

3.4.6 Tagging of Donors, Transplants, Controls

A wide variety of techniques have been used for attaching stony corals and their monitoring tags to the reef substrate; including attachment to masonry nails with cable ties, nylon strings, cyanoacrylate glue, and premix cement with retardant - Conplast UW, (Kaly, 1995; Davies, 1995; Alacala Gomez & Alcala,

1982; Yap *et al*., 1998; Clark & Edwards, 1995). Portland type II hydraulic cement has also been cited as an effective adhesive (Neeley, 1988). Portland type I was used for the purposes of this project. Portland type II generally is used for construction purposes (it has a more durable strength), however, this mix is more expensive and not as readily available. For the application of plastic tags to the reef substrate, the Portland type I mix was judged sufficient (Banks, personal communication).

The entire donor colony was tagged using a plastic, pre-numbered luggage tag and zip-tie fixed to the adjacent reef substrate using Portland type I hydraulic cement. The substrate adjacent to the coral colony was scrubbed clean of epibiota, so that the cement could adhere to the area. Each core hole site was tagged using the numbered concrete plugs, which filled the area of drilling (see Figure 11). The transplant cores were tagged using a plastic numbered tag on one of the Reef Balls in the quad. The core hole site and the transplant taken from that hole displayed the same number. The control corals (of both types) on the natural reef were tagged using the same method as the donor colonies. All photographic images taken of the corals contained a reference number in an upper corner of the framer.

3.5 Monitoring of Coral Growth and Recovery

3.5.1 Monitoring of Experimental and Control Corals

Coral transplants, donor corals, and control corals were monitored on a quarterly basis (every three months), barring interference by inclement weather, for a period of fifteen months (eighteen months for the initial March 2001 subset). Monitoring consisted of both a photographic (35mm slide) image of the study coral and *in situ* data recording. *In situ* data recording included the general health and evidence of bleaching or disease, at every sampling period. Photographic images of transplants and core holes (in donor corals) were recorded using a Nikonos V camera with a 28mm lens and close up kit (Figures 12 & 13). The 28mm Nikonos lenses have been shown to be free of optical distortions (±0.1mm) (Done, 1981).

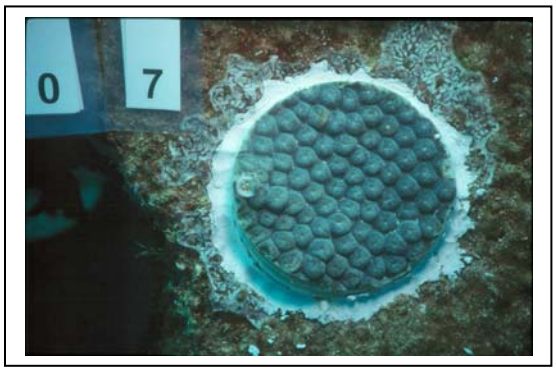


Figure 12: Transplant # 7 epoxied into RB.

Figure 13: Plug # 7 in donor colony # 4.

The close-up framer provided three important functions, a) it established a set distance between the camera and the coral, b) it allowed the photographer to accurately frame the coral, c) it provided a bar to attach the numbers which distinguished each individual coral.

Photographic images of donor and control colonies were recorded using a Nikonos V camera and a 20mm lens with a 0.75m^2 PVC framer marked in 10cm increments (Figures 14 & 15). Replicate photographs over a fifteen-month period were used to determine coral growth.

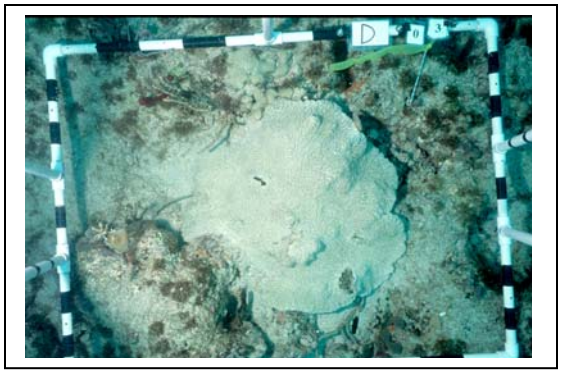


Figure 14: Donor colony # 3, pre-drill.

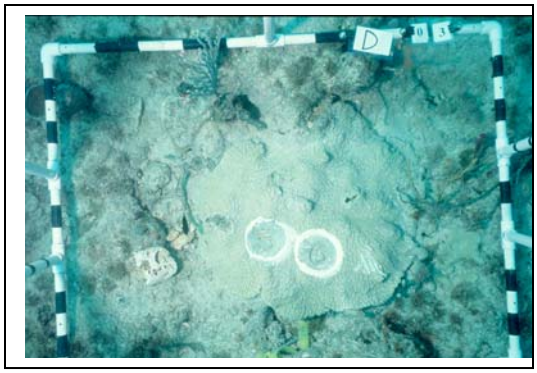


Figure 15: Donor colony # 3, post-drill. (with plugs 5 and 6).

All slides were scanned, using a Hewlett-Packard Photosmart© S20 slide scanner, at a resolution of 900 dpi and saved as jpgs. SigmaScan© Pro4 image analysis software (Jandel Scientific Corporation) was used for the analysis. Individual slides were calibrated using a ruler, included in the image. All transplants, core holes, and small control images were traced and measured in order to determine tissue growth or retreat over time (Figures 16a& 16b).

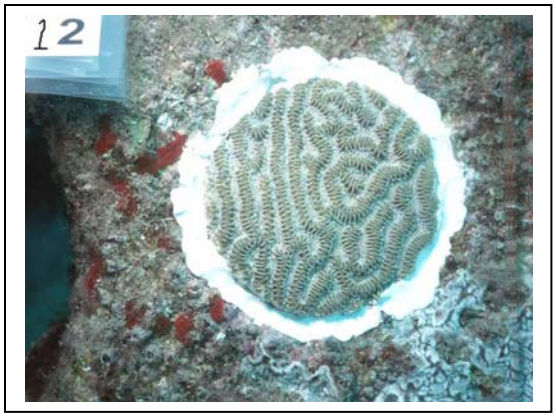


Figure 16a: T12, photographed directly after transplantation in June 2001.

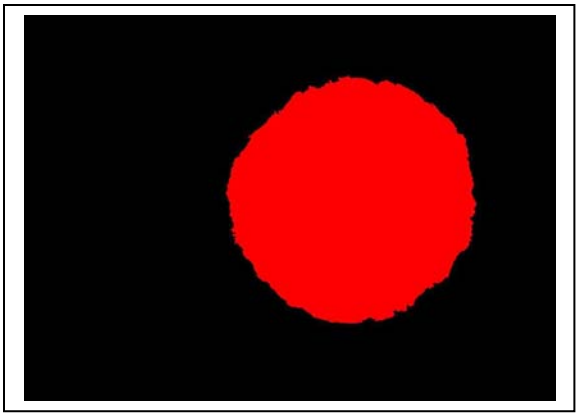


Figure 16b: T12, after coral tissue outline was traced using Sigma Scan software.

All images were traced at 4x magnification of the slide for greater precision (Figures 17a & 17b).

Figure 17a: T12 at 4x magnification (from Figure 16a).

Figure 17b: T12 at 4x magnification (from Figure 16b).

The SigmaScan software defines area as the "sum of the number of pixels defining an object." Once the image was calibrated, the area was calculated using the specified units (SPSS, Inc., 1998). The change in surface area for a specimen was determined from the repeated measurements of surface area for all images. The change in area was standardized to time for comparison purposes. First, the difference in area between the approximately quarterly data sets was calculated. Next, this difference was divided by the actual number of days that passed between photographing the organism to calculate a 'change per day'. Finally, the change per day was multiplied by 90 (90 days per quarter was then used as the standard number of days for comparison). This method of surface area determination was used for the transplants, small controls, and the core holes.

Using the calculated area, the radius length increase (r_i) (change in radius) was determined by means of the following equation: $r_i = r_{Sept2002} - r_{June2001}$ where $r_{June2001} = \sqrt{(A_{June2001}/Pi)}$, and $r_{Sept2002} = \sqrt{(A_{Sept2002}/Pi)}$, A= the two-dimensional surface area of each transplant (Thornton, Gilliam, & Dodge, 2000). Radius length increase has been used to determine radial growth in transplantation studies (Thornton, Gilliam, & Dodge, 2000). It was used as a supplemental growth parameter in this study, in addition to the surface area measurements.

Most of the donor and large control corals were too large to accurately measure the area using Sigma Scan. Because of the larger colony size of the donors and controls, the surface area tracing procedure that was used for the transplants and small controls required adaptation. Instead, the donor and large control corals were assessed quarterly for colony length and width and the change in percent tissue coverage (see following paragraph). The length and width were measured in Sigma Scan by choosing the greatest dimension and naming this the 'length'. The next greatest dimension perpendicular to the length was called the 'width'. The greatest length and next greatest length perpendicular to that have been used in previous studies (Yap, Aliño, & Gomez, 1992). These measurements were determined using the distance measurement in Sigma Scan, which in a calibrated image "uses the Pythagorean theorem to measure the distance between two points on an image." The change in length and width measurements for these large colonies was slight. Slight differences in the camera angle created length differences that were greater than expected growth changes. Growth of the donors using the length and width

measurements, therefore, was not used in the statistical analysis of the large corals.

It was the intent of this project to monitor the survivorship of the larger donors and controls, not the growth. Therefore, the change in percent tissue coverage (as a measure of survivorship) was compared for the duration of the study in order to assess the effect of the coring process on the donors. The percent tissue coverage was estimated from each planar image, using the centimeter marks on the camera framer for reference. Visual estimates of the amount of dead surface on massive corals have been used in previous studies examining reef condition and mortality of reef building corals (Ginsburg *et al*., 2001). Bythell, *et al*. (2001) used a similar photographic technique for the quantitative assessment of partial mortality in corals, and to determine the surface area of larger corals in the field. Using a standard Nikonos camera system, overlapping images were taken of each coral. Photo-Modeler software was used for the processing of the images and in order to build three-dimensional models of the objects (Bythell *et al*., 2001). Although Bythell's method was determined to be both accurate and non-invasive to the study corals, this overlapping method was not used in the monitoring of the corals in this study. The overlapping, repeated photographs technique is both time-consuming and complex (Bythell *et al.,* 2001*).*

3.5.2 Justification of Photographic Technique

The photographic technique described in section 3.5.1 (for the transplants, small controls, and core holes) measured surface area growth over a fifteen-

month period (five quarters), using a non-invasive methodology. This technique is one of the few (planar) growth measurement methods in which the coral colony is not sacrificed. In the past when the coral is to remain *in situ* for growth measurements, the encrusting methods of growth measurements (photographs) have provided accurate data (Gittings, 1988).

Occasionally, images were not used in the data analysis for a particular data set due to incomplete data recording on the slide. Due to the nature of the Nikonos close-up kit, it was necessary to perfectly center the coral transplants (and the concrete plugs and small controls) in the close up kit view. If a portion of the coral was cut off in the image (or if the entire coral was not visible due to lighting difficulties), the coral was re-photographed during a separate sample session. If the image was still incomplete, it was omitted from the data set.

3.5.3 Precision of Tracing

In order to determine the precision of surface area determinations, two coral transplants (one of each species) were repeatedly traced. For the *M. cavernosa* transplant T8, a mean area of 5,569mm^2 was calculated from six samples (with a minimum area of 5,483mm^2 and a maximum area of 5,728mm^2). The standard deviation for this subset was $\pm$108mm^2. Transplant T1 was traced repeatedly for the *M. meandrites* transplants. For this individual, a mean area of 4,982mm^2 was calculated from the six samples (with a minimum area of 4,894mm^2 and a maximum area of 5,044mm^2). The standard deviation for this subset was $\pm$66mm^2. Thus, the Sigma Scan method of tracing the perimeter for surface area determination was precise to approximately 100mm^2 (108mm^2 for

the *M. cavernosa* transplants, and 66mm^2 for the *M. meandrites* transplants). Using a mean area of 5,569mm^2 and standard deviation of 100mm^2, the measurements are precise to within 2% of the Sigma Scan surface area determinations.

3.5.4 Measuring of other abiotic factors

Previous work involving the transplantation of corals has indicated that various environmental parameters may affect the growth and survivorship of coral transplants (Yap *et al.*, 1998). Some of these parameters include water motion, sedimentation, light, temperature, and salinity. Thermographs installed at the Reef Ball site and the *Memphis* grounding site, recorded temperature data for the course of this study. This allowed temperature fluctuations, within the study sites, to be compared with growth data. The two study sites maintain approximately the same distance from the closest port, Port Everglades. Other factors such as water motion, sedimentation, light intensity, and salinity may have varied throughout the *Memphis* grounding study site and the Reef Ball site in a consistent manner. The study of additional parameters such as these was beyond the scope of this project.

3.5.5 Statistical Analyses and Assumptions of ANOVA

Surface area (and radius) determinations of the transplants, small controls, and core holes were tested for normality. Much of the *M. meandrites* transplant growth data was not normally distributed, due to the large amount of partial mortality experienced by the transplants. The data, in these cases, was transformed using both logarithmic transformation and arcsine transformation. In

most cases, these transformations did not change the distribution of the data to normal; because of this, non-parametric tests were performed, in addition to the parametric ANOVA. ANOVA results have been reported along with all non-parametric results (see section 3.5.5.1). ANOVA was used instead of sets of the Student's T-test in order to avoid Type I errors (rejecting the null hypothesis of no difference, when a difference between the means does exist) (Underwood, 1981). The nesting of donors (in the ANOVA analyses) permitted the use of a parametric test, despite the lack of independence.

The non-parametric tests examined the difference in growth without nesting the transplants (avoiding the issue of non-normality). Non-parametric results were included when 1) data was not normally distributed and 2) when data did not exhibit homogeneity of variances. Oftentimes, even when the data was normally distributed, the variances were not homogenous. If the data was normal and displayed equality of variances, only parametric analyses were completed.

The following statistical analyses were employed in this study: Nested ANOVA, Repeated Measures ANOVA, Mann-Whitney U Test, and the Wilcoxon Matched Pairs Test (the Kruskal-Wallis test and the Newman-Keuls post-hoc test were used only on the temperature data). The nested ANOVA was used in order to analyze a design where a "subordinate classification was nested within the higher level of classificiation" (Sokal and Rohlf, 1998); in this case, transplants or core holes were nested within their donors. When the total change in surface area or radius was compared, the nested ANOVA was utilized. The repeated

measures ANOVA is also called the “within-subjects or treatment-by-subject design, in which multiple measurements on the same experimental subject comprise the replicate data” (Zar, 1996). When surface area (or radius) measurements were compared by data set, the repeated measures ANOVA was employed.

The Mann-Whitney U Test is the nonparametric analogue to the two-sample T-test (Zar, 1996). In this test, the actual measurements are not used, but rather the ranks of the measurements. This test is one of the most powerful non-parametric tests. The Man-Whitney U Test was used as the non-parametric comparison to the nested ANOVA (in the cases where two samples were compared). The Wilcoxon Matched Pairs Test is a non-parametric test analogue to the paired sample T-test. It is more powerful than the non-parametric Sign test and was used as the non-parametric comparison to the nested ANOVA (in the cases where one value was compared between each of the two species).

According to Zar (1996), it has been shown that “analyses of variance and T-tests are usually robust enough to perform well even if the data deviate somewhat from the requirements of normality and homoscedasticity.” Additionally, Weinberg and Abramowitz (2002) suggest that violations of the assumptions of normality either do not affect or only minimally affect ANOVA validity. If the sample size is larger than thirty subjects, non-normally distributed data can produce correct results; that is, ANOVA is “robust to violations of the assumptions of normality” (Weinberg and Abramowitz, 2002; Underwood, 1981). Similar arguments have been made for the violations of the assumption of

homogeneity of variance, with minimal or no effects on the validity of ANOVA. With larger sample sizes, whether equal or unequal, there is little distortion in the Type I error (Weinberg & Abramowitz, 2002). In addition, transformations of data to normalize or stabilize heterogeneity are mostly 'not worth doing'. Unless gross violations of normality, etc. are made, transformation may not improve the reliability of the data (Underwood, 1981).

4.0 Results

4.1 Transplants

4.1.1 Transplant Attachment Success

All transplant cores remained attached for the 15-month duration of the study; there was 100% transplant attachment success.

4.1.2 Transplant Total Colony Mortality Determination

At the end of the 15-month sampling period, a total of nine (22.5%) of the original forty *M. meandrites* transplants experienced 'total colony mortality'. Total colony mortality was defined as no live coral tissue on the transplant's entire skeleton. By March 2002, the first *M. meandrites* transplant experienced total colony mortality. Four more individuals died off by June; by September the total was at nine (Figure 18). These nine transplants were drilled from seven

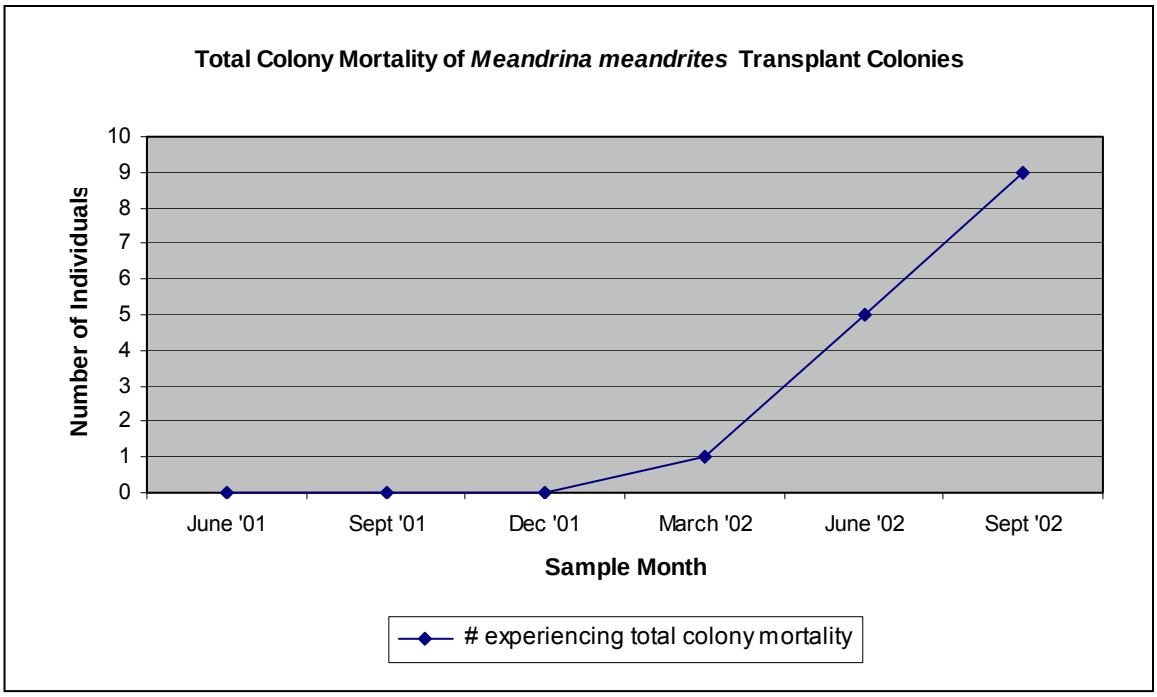


Figure 18: Total colony mortality for *Meandrina meandrites* by month.

individual donor colonies (i.e., two sets of transplants, T47&T48 and T61&T62, came from the same two donor colonies: D20 and D31). In comparison, none of the *M. cavernosa* transplants experienced total colony mortality (Table 6). The total colony mortality for both species of transplants combined was 11% (9/80).

Table 6: Total colony mortality for the transplants by sample month.

Number of Transplants Experiencing Total Colony Mortality		
Month	*M. meandrites*	*M. cavernosa*
June '01	0	0
Sept '01	0	0
Dec '01	0	0
March '02	1	0
June '02	5	0
Sept '02	9	0

4.1.3 Overall Transplant Survivorship

The overall transplant survivorship was determined by comparing the surface area in the initial and the final data sets. The number of transplants experiencing partial die back plus the number of transplants experiencing total colony mortality in the final data set was subtracted from the initial transplantation data set (March and June 2001 – September 2002). Thirty of the forty *M. meandrites* transplants (75%) experienced some form of die back. The thirty *M. meandrites* individuals experiencing die back lost surface area in the range of 200mm^2 to 6,000mm^2. The greatest number of *M. meandrites* transplants experienced partial mortality in the range of 5,000-6,000mm^2 (N=8). In total, *M. cavernosa* transplants had only three of the forty transplants (7.5%) experience partial mortality. The partial mortality for all three of the *M. cavernosa* transplants

was less than 200mm^2. In total, 53.75% of all transplants (both species combined) experienced some form of partial mortality.

4.1.4 Small Controls for Transplants

Small controls of the appropriate size were readily located and monitored for *M. meandrites.* Due to the flat growth morphology of *M. meandrites* on the natural reef, the transplants and the small controls were compared using the change in surface area. Because of the more 'mound-like' nature of the *M. cavernosa* colonies, the comparison of the small controls and transplants was not as suitable. The *M. cavernosa* transplants did not possess the same amount of relief because the transplants were drilled from a generally flat portion of the colony.

Attempts were made to locate small controls that were as close in size as possible, to the transplants (Table 7). The mean size for *M. meandrites* transplants (all sizes refer to initial size after transplantation in June 2001) was 5,875mm^2 (± 421mm^2 s.d.), and for the small controls was 6,497mm^2 (± 1734mm^2 s.d.). The mean size for *M. cavernosa* transplants was 6,328mm^2 (± 438mm^2 s.d.), and for the small controls was 5,725mm^2 (± 1673mm^2 s.d.). All of the small controls (unlike the transplants), for both *M. meandrites* and *M. cavernosa*, survived the duration of the experiment. Originally, ten small controls were selected for monitoring. After the second sample session (September 2001), one of the *M. cavernosa* controls was determined to be too large to photograph using the close up kit. For this reason, nine small controls were assessed for *M. cavernosa* for the duration of the study.

Table 7: Summary statistics for the transplants and small controls, comparing the first data set (June 2001) with the last (September 2002). The only group experiencing a decrease (the mean surface area) was the Mm transplants.

	First Sample Session June 2001			
Coral Type	Transplant	Transplant	Control	Control
Species	*Mm*	*Mc*	*Mm*	*Mc*
Number	40	40	10	9*
Total S. Area (mm^2)	235,004	253,123	64,966	51,524
Mean S. Area (mm^2)	5,875	6,328	6,497	5,725
Standard Deviation	421	438	574	803
% total mortality	0%	0%	0%	0%
% survival	100%	100%	100%	100%

	Sixth Sample Session September 2002			
Coral Type	Transplant	Transplant	Control	Control
Species	*Mm*	*Mc*	*Mm*	*Mc*
Number	40	40	10	9*
Total S. Area (mm^2)	135,463	296,353	76,956	54,930
Mean S. Area (mm^2)	3,387	7,409	7,696	6,103
Standard Deviation	2,669	949	911	1,059
% total mortality	22.5%	0%	0%	0%
% survival	77.5%	100%	100%	100%

4.1.5 Transplant Growth

4.1.5.1 Surface Area Increase/ Change

4.1.5.1.1 Total Transplant S.A. Change

Transplant growth was determined using measurements of surface area and radius. The total change in surface area between the beginning and the end of the study (the last data set – the first) was calculated. The surface area change also was determined on a quarterly basis by subtracting the previous (preceding) data set from the last (latest) data set; this yielded the surface area change between data sets (see Methods section 3.5.1).

Comparison of total area change between two species of transplants

The total area change was calculated by subtracting the initial area in June 2001 from the final area in September 2002. The two species were compared to determine if there was a significant difference in the area change

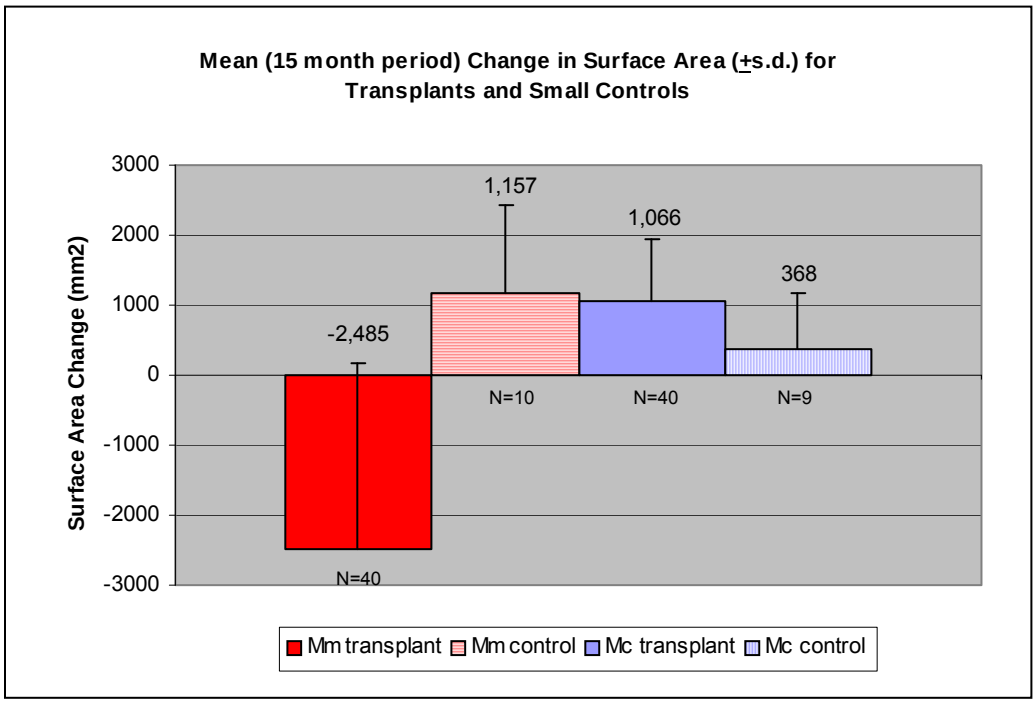


Figure 19: Mean change in surface area of transplants and small controls for the duration of the study. Error bars indicate standard deviation.

between species (Figure 19). Because thirty of the forty *M. meandrites* transplants experienced partial or total mortality (in comparison with three of the forty *M. cavernosa* transplants), a significant difference in surface area change was expected.

A nested ANOVA was performed with the transplant donors nested in species ($F=4.63$ and $p=0.000$). A significant difference existed in the area change when comparing the two species. The donor (number) was nested within the species type because two transplants were taken from each donor coral.

The *M. meandrites* data was not normally distributed, despite all attempts to transform it. Non-parametric results were: Mann-Whitney U-test, U=154, z=-5.98, p=0.000. The ANOVA and the non-parametric Mann-Whitney tests, comparing area change for the duration of the study, showed a highly significant difference between groups, reasonable as the *Meandrina meandrites* transplants exhibited a substantial amount of partial and total mortality.

Comparison of total area change between two species of small controls

No significant difference in total change in area was found between the *M. meandrites* and *M. cavernosa* small controls (ANOVA, F=2.51 and p=0.131). No nesting was necessary for this ANOVA, because the small controls were all independent.

Comparison of total area change in Meandrina meandrites transplants and small controls

A nested ANOVA was performed, with the donors nested in species (F=3.60 and p=0.003). Because the *M. meandrites* transplant area data was not normally distributed, an additional non-parametric test was performed. A Mann Whitney U-test was performed with the following results, U=39, z=-3.87, and p=0.000. Again, this shows a highly significant difference in the change in area of the transplants compared with the small controls for *M. meandrites.*

Comparison of total area change in Montastrea cavernosa transplants and small controls

A nested ANOVA was performed, with the donors nested in species (F=2.95 and p=0.01). Additionally, a non-parametric Mann Whitney U-test analysis was completed, U=101, z=1.89, and p=0.06. The results of the non-parametric test were not significant. This indicates that the difference in total area change (comparing *M. cavernosa* transplants with the controls), although

significant in an ANOVA, was not significant in non-parametric tests. The different outcome in the parametric versus nonparametric tests exemplifies the more robust nature of the parametric test.

Comparison of transplant area between the initial and final data sets within species

The area in the initial data set and the area in the final were compared within species. A nested ANOVA showed a significant difference in the transplant area of the first data set when compared with the transplant area in the final data set for *M. meandrites* (F=4.92 and p=0.00). Using a non-parametric test a significant difference was also determined, in the Wilcoxon Matched Pairs test, Z=4.23 and p=0.00. The difference in area between the first and last data sets showed overall area loss for the *M. meandrites* transplants.

Similarly, differences existed for the comparison of the area in the first and last data sets for *M. cavernosa*. A nested ANOVA showed a significant difference in the transplant area of the first data set when compared with the transplant area in the final data set for *M. cavernosa* (F=2.03 and p=0.02). Using a non-parametric test a significant difference was also determined, in the Wilcoxon Matched Pairs test, Z=5.01 and p=0.00. The difference in area between the first and last data sets showed overall area increase for the *M. cavernosa* transplants.

4.1.5.1.2 Area Change by Sample Period

The change in surface area was determined for each data set and normalized using a three-month time interval (see Section 3.5.1).

Figure 20 depicts the mean surface area of both the transplants and controls for each of the six data sets. The trend-lines indicate the pattern of tissue increase or loss for each of the transplant species (as determined from the

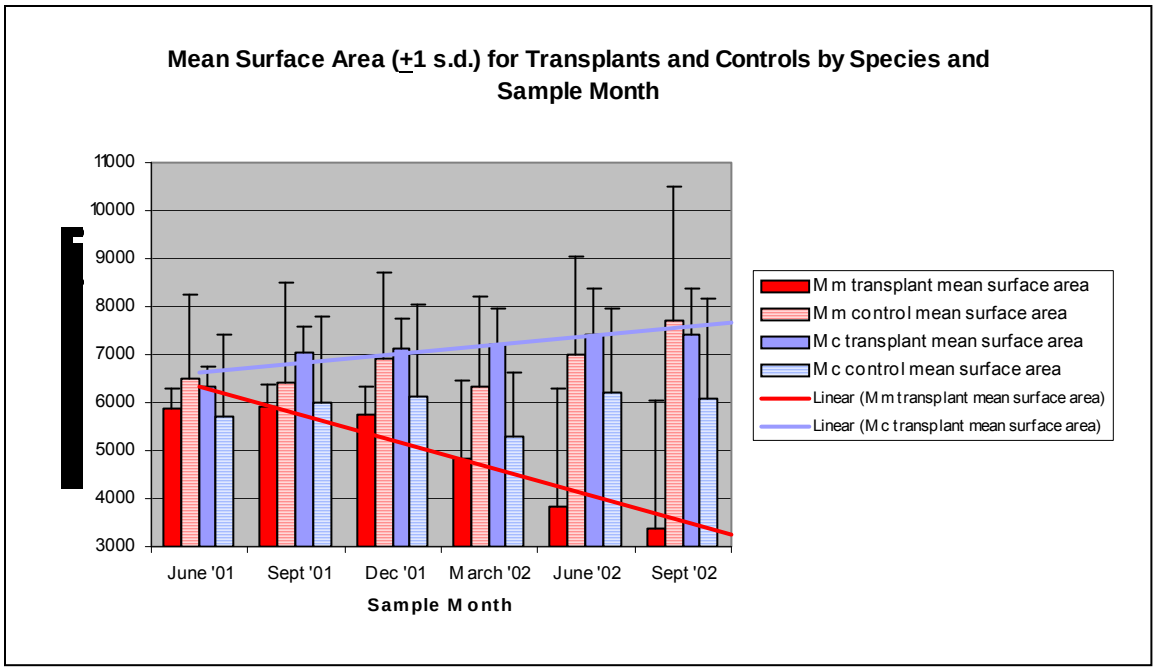


Figure 20: Mean surface area of transplants and small control corals by individual species and sample month. Trend line includes projected area for December 2002. Error bars indicate standard deviation

surface area calculations). Both the gradual increase in surface area for the *M. cavernosa* transplants and the substantial cumulative decrease in surface area for the *M. meandrites* transplants are apparent. The decreases in mean surface area for both of the control species for March 2002 likely may be attributed to the smaller sample size, because some of the images from that data set were not usable (see Section 3.5.2).

Repeated measures comparison of area change between two species of transplants

A repeated measures ANOVA was run on the change in area by data set, comparing *M. meandrites* and *M. cavernosa* transplants. A significant difference was found in the change in area between species, in twenty of the twenty-five

comparisons (F= 4.37 and p=0.002). Table 8 contains select significant results of the Wilcoxon Matched Pairs test.

Table 8: Select significant results of the Wilcoxon matched pairs test (repeated measures) transplant area change by data set and species.

Transplants - *M. meandrites* versus *M. cavernosa*
Repeated Measures (Wilcoxon Matched Pairs Test)

***Mm* Area Chg**	***Mc* Area Chg**	**Z**	**p-level**
Mm area II-I	*Mc* area II-I	4.88	0
Mm area III-II	*Mc* area III-II	2.94	0.003
Mm area IV-III	*Mc* area IV-III	4.06	0
Mm area V-IV	*Mc* area V-IV	4.35	0
Mm area VI-V	*Mc* area VI-V	2.63	0.008

Repeated measures comparison of area change between two species of small controls

The Wilcoxon Matched Pairs test found only one significant difference in the twenty-five comparisons between the two species of small controls.

Repeated measures comparison of area change between <u>Meandrina meandrites</u> transplants and small controls

A significant difference in area between the *M. meandrites* transplants and the controls was found. The Wilcoxon Matched Pairs test found thirteen significant differences in the twenty-five comparisons (Table 9).

Table 9: Select significant results of the Wilcoxon matched pairs test (repeated measures) *M. meandrites* transplant and control area change by data set.

M. meandrites transplant versus control
Repeated Measures (Wilcoxon Matched Pairs Test)

***Mm*T Area Chg**	***Mm*C Area Chg**	**Z**	**p-level**
*Mm*T area IV-III	*Mm*C area IV-III	2.2	0.03
*Mm*T area V-IV	*Mm*C area V-IV	2.37	0.02

Repeated measures comparison of area change between <u>Montastrea cavernosa</u> transplants and small controls

Table 10 shows select significant results of the Wilcoxon matched pairs test, with six of the twenty-five comparisons as significant.

Table 10: Select significant results of the Wilcoxon matched pairs test (repeated measures) *M. cavernosa* transplant and control area change by data set.

M. cavernosa transplant versus control
Repeated Measures (Wilcoxon Matched Pairs Test)

***Mc*T Area Chg**	***Mc*C Area Chg**	**Z**	**p-level**
*Mc*T area II-I	*Mc*C area II-I	2.19	0.03
*Mc*T area IV-III	*Mc*C area IV-III	2.38	0.02

4.1.5.2 Radius Increase/ Change

The radius change was calculated for the entire study (change in radius from the first sample to the last for each transplant) and not for every data set (as the surface area) (see Section 3.5.1 for the radius determination). Statistical analysis of the total radius change was considered an adequate measure, as the surface area change was examined quarterly. The mean radius change for the *M. meandrites* transplants was -9mm (<u>+</u> 11mm s.d.). The mean radius change for the *M. cavernosa* transplants was 2mm (<u>+</u> 2mm s.d.).

Comparison of total radius change between two species of transplants

Again, because thirty of the forty *M. meandrites* transplants experienced mortality (in comparison with three of the forty *M. cavernosa* transplants), a significant difference in (radius change) growth was likely. A significant difference was found for the total change in radius, just as in surface area, between the *M. meandrites* transplants and the *M. cavernosa* transplants (Figure 21).

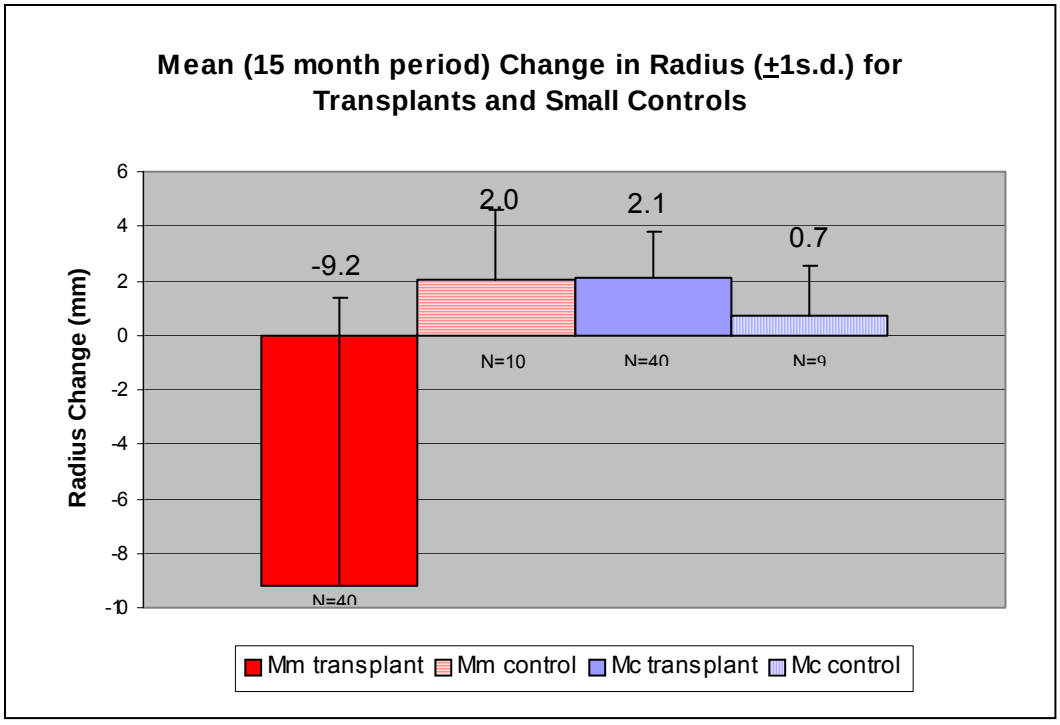


Figure 21: Mean change in radius of transplants and small controls for the duration of the study. Error bars indicate standard deviation.

Because two transplants were taken from each donor colony, a nested ANOVA was performed with the donors nested in species ($F=3.76$, & $p=0.00005$). A significant difference was found in the total radius change for the comparison of both species of transplants; Mann-Whitney U-test, $U=159$, $z=-5.93$, $p=0.000$. This shows a highly significant difference among groups, which seems logical as the *M. meandrites* transplants exhibited a substantial amount of loss.

Comparison of total radius change between two species of small controls

There was no significant difference in total change in radius when comparing the *M. meandrites* and *M. cavernosa* small controls (ANOVA, $p= 0.21$). No nesting was necessary for this ANOVA, as the small controls were all independent.

Comparison of total radius change between <u>Meandrina</u> <u>meandrites</u> transplants and small controls

When comparing the transplants with their controls, a significant difference was found for the total radius change for *Meandrina meandrites.* A nested ANOVA was completed with the donors nested in species (F=2.67 and p=0.015). A Mann-Whitney U –test was conducted with the following results, U=42, Z=-3.795, and p=0.0001. Again, the non-parametric results show a highly significant difference in radius change.

Comparison of total radius change between <u>Montastrea</u> <u>cavernosa</u> transplants and small controls

When the *M. cavernosa* transplants and controls were compared for total change in radius, a significant difference was found using a parametric analysis (nested ANOVA was completed with the donors nested in species F=3.098 and p=0.008); however, no significant difference was found using non-parametric analyses.

These statistically significant results indicate that *M. meandrites* faired better (without treatment) on the natural reef than after exposure to the drilling and transplantation processes. The *M. cavernosa* transplants, however, showed a greater increase in area and radius than their same species controls. The difference for the *M. cavernosa* transplants when compared with the controls was not as substantial as in *M. meandrites*. In fact, it was not significant when analyzed with non-parametric tests. It should be noted that all transplant colonies started at the same total radius; the initial radius for the controls, however, was grouped around the average initial size of the transplants.

The small control corals for the transplants functioned as controls for two different variables. First they acted as a control for the drilling of the coral transplants. Additionally, they acted as a control for the movement of the transplants to the artificial reef habitat. No attempts were made to separate these two variables (the drilling and moving steps). No small control corals were drilled and not transplanted or transplanted without first being drilled; there was no separation for these two 'treatments'. Therefore, it was not feasible to determine which of these two factors may have contributed to the partial and total mortality of the *M. meandrites* transplants. It was, however, possible to point out that the *M. meandrites* donors did not experience a significant amount of additional mortality at the area surrounding the core hole site (see Section 4.3.2.1.1). Significant mortality might have been expected if the drilling alone were the cause of the substantial mortality in the transplants.

4.1.6 Qualitative Observations

4.1.6.1 Side Growth

Although it was not formally assessed in this study, the 'side growth' of tissue along the side of the coral transplants (in a vertical plane) was noted and photographed (Figure 22). This ability to regenerate tissue over either 1) the faired epoxy or 2) the skeletal components that did not previously have tissue (i.e., not within the corallites and along the septo-costae, but rather along the vertical surface of the previously drilled part of the transplant) was noted. In the cases where the coral transplant was flat with the Reef Ball substrate, a number of the corals were able to grow over the epoxy. In the cases where the coral

core was longer, a number of the corals exhibited expanded live tissue along the side of the core.

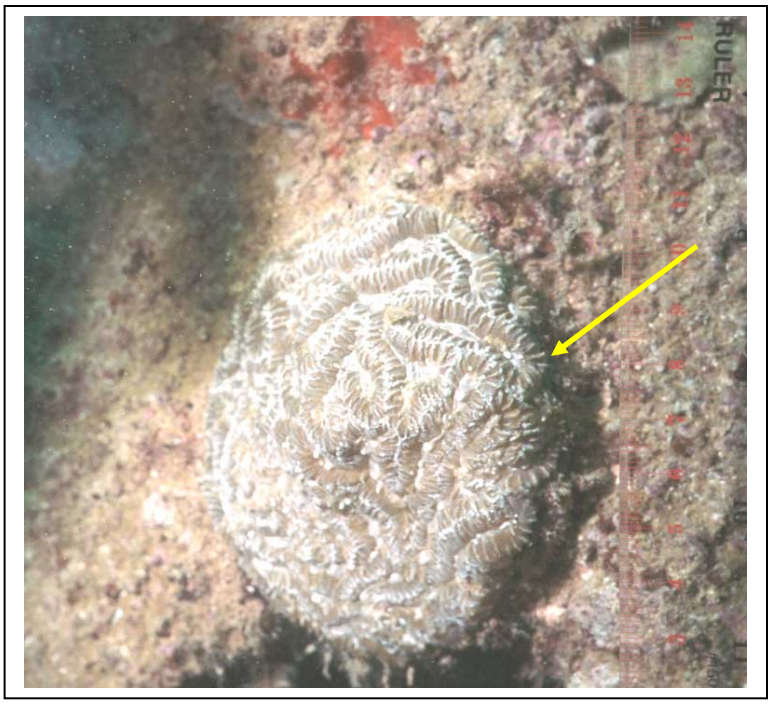


Figure 22: T6 (15 Oct 2001), 7 months after transplantation (transplanted in March '01). Area of 'side growth' along the transplant is indicated by an arrow.

This occurrence displays the regenerative ability of the transplants. It is interesting to note that T6 is a *M. meandrites* colony. Although some of the *M. meandrites* transplants did not survive the duration of the study, (nine of the forty transplants experienced total mortality and thirty of the forty experienced partial mortality) others were able to regenerate tissue in areas of bare skeleton. Ten *M. meandrites* transplants and thirty-six *M. cavernosa* transplants displayed the ability to grow over the epoxy or along the side of the exposed skeleton by the end of the study.

4.1.6.2 Tissue Loss and Bleaching of *M. meandrites*

The tissue loss in many of the *M. meandrites* transplants consisted of a gradual sloughing off of the tissue (Figures 23a-c). The duration for complete colony mortality ranged from nine to fifteen months; with partial mortality initiating in some individuals between the three-month and six-month mark.

Figures 23 a-c: a) T37 in Dec '01, healthy at 6 months after transplantation. b) T37 in March '02, showing signs of tissue deterioration. c) T37 in June '02 showing signs of further mortality. Mortality had progressed further by September '02.

Initial appearance of partial mortality resembled some descriptions of the disease White Plague. White Plague has been described throughout the Caribbean, affecting 33 species of scleractinian coral, including *M. meandrites* (http://www.coral.noaa.gov/coral_disease/white_plague.shtm) (Weil, 2001). Colonies affected by this epidemic exhibit "lesions…radiating outward leaving behind bare white skeleton." (Nugues, 2002). The partial mortality of the *M. meandrites* transplants displayed a somewhat similar pattern; the appearance of tissue necrosis may have been stress-related. No confirmation of the disease presence was made, because the identification of the microorganisms associated with this disease were out of the scope of this project. Inquiries into the possibility of a disease state in the *M. meandrites* colonies were made. It was suggested that the mortality might have been linked to ridge mortality disease (whereby the tissue recedes from the skeletal ridge, leaving only tissue in the

valleys), and/ or damselfish lawn building activity (Esther Peters, personal communication).

Diaz-Pulido and McCook (2002) also describe a sloughing off of tissue (in Pacific scleractinian species on the Great Barrier Reef), similar to that experienced by *M. meandrites*. This tissue sloughing was generally noted in corals that had previously bleached, which then produced a "mucus, sediment, algal layer", which eventually sloughed off (Diaz-Pulido & McCook 2002). In addition to the tissue sloughing, both crustose coralline algae and turf algae were noted on the Reef Balls, adjacent to the transplants. It is possible that the algae may have been in competition with the coral transplants. Yap *et al.* (1998) observed mortality in coral transplants due to algal competition.

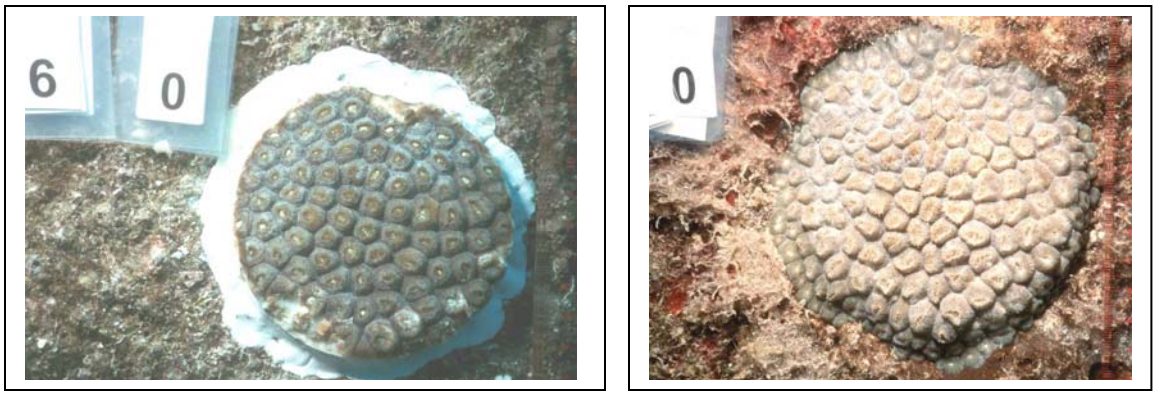
Figure 24: T30, October 2001. Note bleaching of coral and shading settlement plate at lower left.

Another interesting observation was made involving *M. meandrites* transplant # 30. Approximately two months after the corals were transplanted, concrete settlement plates were attached to the top of each Reef Ball. The

settlement plate for the Reef Ball to which T30 was transplanted, hung over the edge and effectively shaded the coral. Three months after transplantation, when the corals were re-photographed for the second sample session, substantial bleaching was noticed on T30 (approximately 30% partial bleaching) (Figure 24). The plate was removed and relocated farther away from the coral, later on that month. By the end of the study, T30 had reached a total surface area of 7,755mm^2. Thus, fifteen months after transplantation, T30 had increased in surface area by 2,048mm^2 and in radius by 64mm. This was the most successful growth of all of the *M. meandrites* transplants. Bleaching was noted in some of the transplants corals; however, there was not a clear pattern of bleaching linked to mortality in either species.

4.1.6.3 Success of *M. cavernosa* transplants

Figures 25a and 25b depict one of the more successful *M. cavernosa*



Figures 25a & 25b: a) T60 in June 2001, with a surface area of 6,462 mm^2. b) T60 in September 2002, with a surface area of 9,151 mm^2. Note that coral tissue surface area increased over the raised portion of the skeleton and down onto the surface of the Reef Ball.

transplants. This individual experienced an increase in surface area of over 2,680mm^2. This surface area increase is an underestimate, as the coral had to grow over the epoxy or the lip of the raised skeleton before it could spread onto

the surface of the Reef Ball. This ability to expand and increase in surface area (as discussed in Section 4.1.6.1) was common in the *M. cavernosa* transplants.

4.1.7 Temperature Data

Two thermographs (Ryan Instruments model RL 100) were placed out at the study sites in order to determine if there was a significant difference between the temperature at the natural reef and the Reef Ball site. One thermograph was placed on Quad 36, centrally located in the Reef Ball array. The second thermograph was place on CP2, the centrally located control pin on the natural reef site. The thermographs were set up to record the temperature at hourly intervals. The average daily temperature was then used to compare the Reef Ball site with the natural reef site, and to compare the annual change in temperature on a monthly basis. Unfortunately the thermograph (#7009563), which was placed at the grounding site on September 25, 2001, flooded shortly after the first three weeks of temperature logging. The Reef Ball thermograph monitored the temperature more or less continuously for the duration of the project (see appendix for graphs of temperature data).

Table 11 lists the deployment and retrieval dates and the average

Table 11: Temperature data for the Reef Balls and natural reef site.

Thermograph Deployment Schedule

Therm #	Location	Deployment	Retrieval	Ave. Temp ©	Std Dev
7009563	CP2 (Natural)	9/25/01	12/12/01	27	1
7009560	Quad 36 (Artificial)	10/2/2001 **	12/16/01	28	3
7009563	Quad 36 (Artificial)	12/14/01	2/26/02	24	1
7009563	Quad 36 (Artificial)	2/26/02	4/19/02	25	1
7009563	Quad 36 (Artificial)	8/9/02	9/28/02	29	1

** Stopped logging on 10/28/01

temperature for that time period, for each thermograph employment. No significant difference was found between the temperatures at the Reef Balls versus the temperatures at the natural reef site for October 2, 2001- October 20, 2001 (the only timeframe when both thermographs were logging temperature data), Mann-Whitney U-test results: Z=-0.66, p-value=0.51. Unfortunately, this comparison was not available for the rest of the study as only one thermograph recorded temperature for the duration of the study.

A significant difference was found for the temperature data (at the Reef Ball site) grouped by months. A Kruskal-Wallis test was run with H (10, N= 271) =251.42 and p =0.000. The Newman-Keuls post-hoc test showed that there was a significant difference in temperature between all months except for the following: (January ’02 & February ’02; June ’02 & July ’02; June ’02 & October ’01; July ’02 & October ’01; August ’02 & September ’02). The greatest mortality in *M. meandrites* transplants occurred between March and June 2002, followed by December 2001 thru March 2002. These months recorded the coldest water temperature for the duration of the study. The *M. cavernosa* transplants, however, continued to increase in mean surface area during the same timeframe.

4.2 Donors

4.2.1 Donor Survivorship

All forty of the donor colonies survived the duration of the project. Partial mortality (a change in tissue coverage) was observed on some specimens. Both the drill damage (separate from hole) and the change in percent tissue coverage,

which was not associated with the core holes, were monitored (see Section 4.2.3).

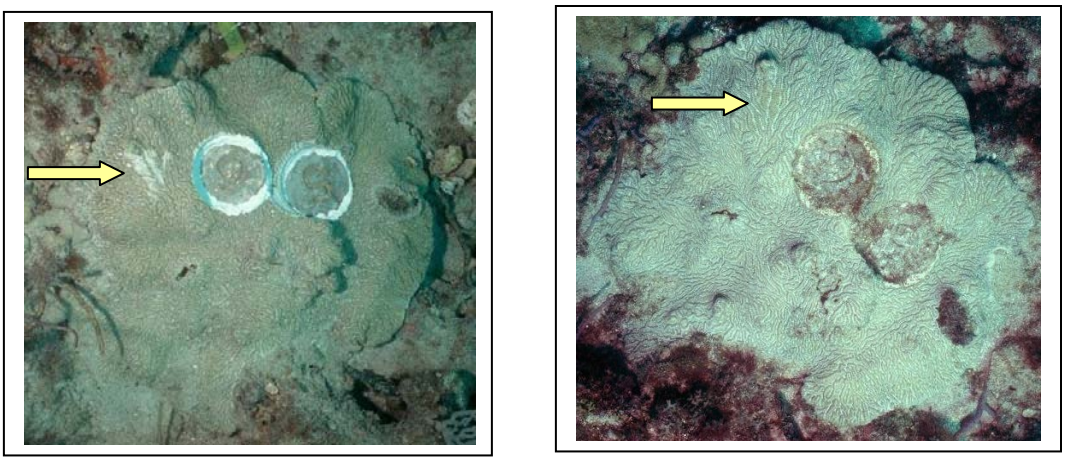
4.2.2 Large Controls for Donors

The change in percent tissue coverage for the large controls was determined from a planar image for each colony. This percent tissue coverage was then compared for each data set. Large sized donors and controls consisted of corals greater than 40cm X 40cm. By using larger colonies for donors, it was thought that the coring process might have less of an effect on the colony. Larger colonies oftentimes invest more energy into reproduction than growth (Jackson & Hughes, 1985). Only a small amount of growth was expected for these colonies, throughout the duration of this study. Because of this fact, but in order to monitor the health and survivorship of these organisms, the tissue loss/ gain was examined.

4.2.3 Drill Damage

Only three out of forty donor corals experienced any 'drill damage' during the drilling process. For the purposes of this study, 'drill damage' was defined as a scrape or gouge of the coral tissue area that was caused by the drilling process and separate from the core hole site itself. The three corals experiencing 'drill damage' were all *M. meandrites* colonies.

The cause of the damage was likely the skipping of the drill before it bit into the coral skeleton (Figures 26a and 26b). *Meandrina meandrites* has a more delicate skeleton (the corallites are thinner than in *M. cavernosa*) and as a result,



Figures 26a & 26b): a) *on left:* Donor 3 on 14 March 2001 (note scar at top left).
b) *on right:* Donor 3 on 11 January 2002 (note lack of scar).

a small number of the drilled colonies experienced some damage. Of these three colonies, all experienced an additional partial mortality of 5% or less (a range of 2% to 5%) over the course of the next few months. This drill damage recovered in all three colonies (i.e., the live tissue grew back over the abraded skeleton) within a year's time. Therefore, none of the drill damage data was included in the statistical analyses. The remaining change in tissue coverage was divided into two further categories.

4.2.4 Change in Percent Tissue Coverage

The change in percent tissue coverage for the donors and large controls was assessed for the duration of the study (Figure 27). This included the change in tissue coverage that was apparent on the colony from the start of the study, and not the change that associated with (adjacent to) the core holes (see Section 4.3).

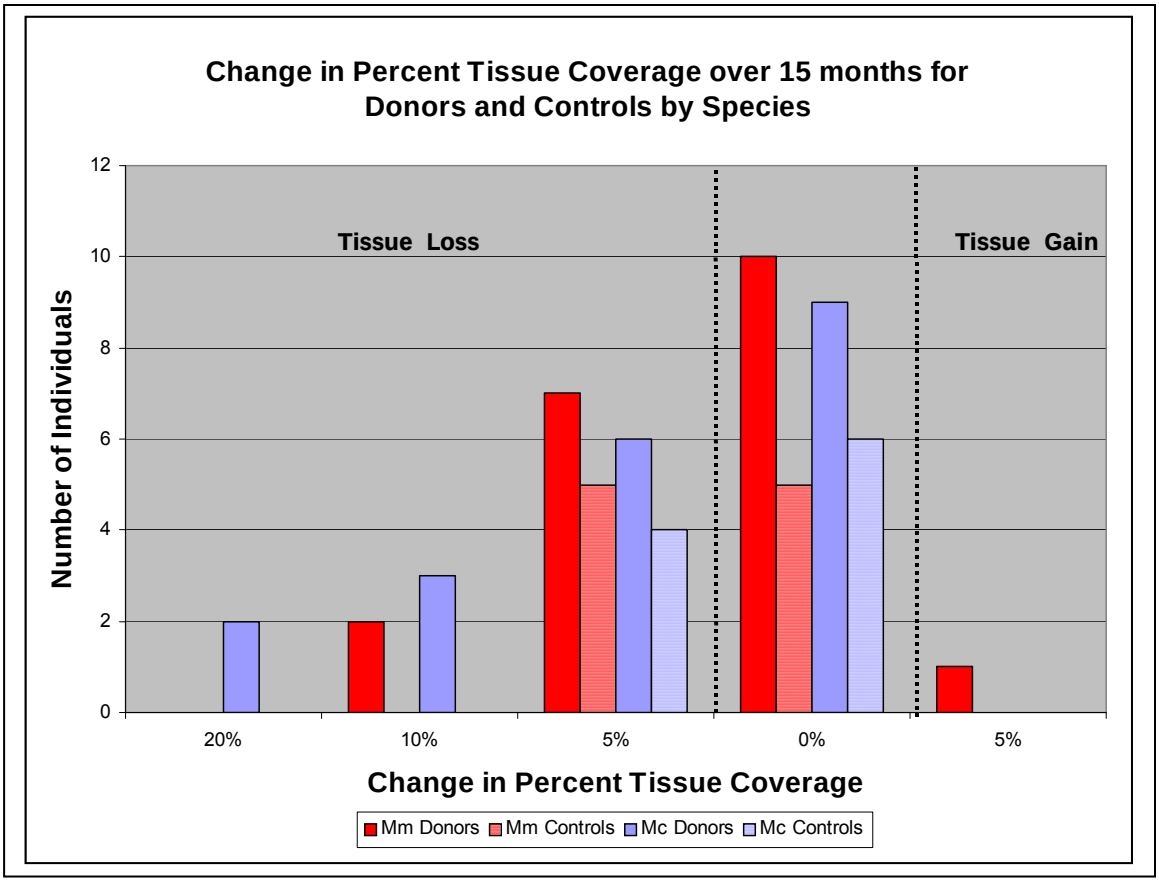


Figure 27: Change in percent tissue coverage in donor and control corals, for the duration of the study.

This change in percent tissue coverage was assessed as follows: the existing skeletal surface area without live tissue was estimated to the nearest 5% using the photographic planar image from each sample session; the change between sample sessions was then estimated. This was calculated for the difference between each data set, and the difference for the entire study (from start to finish). Figure 27 depicts this tissue change. Over half of all donors and controls demonstrated either no change or minimal change (5%) in surface area of live tissue.

Comparison of change in % tissue coverage between two species of donors

Ten of the twenty *M. meandrites* donors experienced change in tissue coverage. One individual had a 5% increase in tissue coverage (natural regeneration of tissue in an area previously devoid of tissue). Nine others ranged from a decrease in tissue coverage of 5% (N=7), to a decrease of 10% (N=2). The *M. cavernosa* donors experienced similar patterns. Eleven of the twenty colonies experienced a change in tissue coverage. This change ranged from a decrease in tissue coverage of 5% (N=6) to 10% (N=3) and up to 20% (N=2). A Mann-Whitney U-test was performed in order to compare the change in percent tissue coverage between species. No significant difference was found between the two species, (U=165.50, z=-0.933, and p=0.35).

Comparison of change in % tissue coverage between donors and large controls

The change in percent tissue coverage for the donors was then compared with that in the large controls. *Meandrina meandrites* controls experienced little change in percent tissue coverage for the duration of the project. Half of the *M. meandrites* controls experienced no change at all. *Montastrea cavernosa* controls experienced similar change in loss of tissue to that of *M. meandrites* controls. Over half of the *M. cavernosa* controls experienced no change at all (six of the ten).

When each individual species was compared with its control, no significant difference was found for the change in percent tissue coverage. For *M. meandrites,* the Mann-Whitney U-test resulted in U=94, z=0.26, and p=0.79. For *M. cavernosa*, the Mann-Whitney U-test resulted in U=75, z=1.099, and p=0.27.

These analyses have shown that there is no significant difference in the naturally occurring change in tissue coverage in the study corals, regardless of if they were impacted by the drill or not manipulated (as in the case of the controls).

4.3 Core Holes

4.3.1 Concrete Plug Attachment Success

Two of the eighty concrete plugs failed to maintain attachment to the donor corals. These two plugs (plugs 13 and 15) became unattached because they were located on the outer edge of the colony and were too heavy for the epoxy to hold. These core holes were photographed for the duration of the project, but the data was not included in the final analyses.

4.3.2 Recovery of Core Holes

4.3.2.1 Core Hole Surface Area Increase/Change

4.3.2.1.1 Total Core Hole Surface Area Change

Following a similar methodology to the transplant and small control monitoring, the monitoring of the core holes compared the surface area (surface area of concrete plug and the surrounding area devoid of coral tissue) for individual data sets, and the final change in surface area and radius for the entire study. The mean initial surface area of the core holes in *M. meandrites* colonies for June 2001 was 8,841mm^2 ($\pm$ 574mm^2 s.d.); by September 2002 this had increased to 8,986mm^2 ($\pm$ 911mm^2 s.d.). For the *M. cavernosa* colonies, the mean initial surface area of the core holes for June 2001 was 9,197mm^2 ($\pm$ 803mm^2 s.d.); by September 2002 this had increased to 9,611mm^2 ($\pm$ 1,059mm^2 s.d.).

Comparison of total area change of donor core holes between two species

Figure 28 depicts the total change for the surface area surrounding the core holes for both *M. meandrites* and *M. cavernosa*. Over the course of the study, the coral tissue never completely regenerated over the surface of the concrete plug for any of the core holes. However, noticeable tissue advances

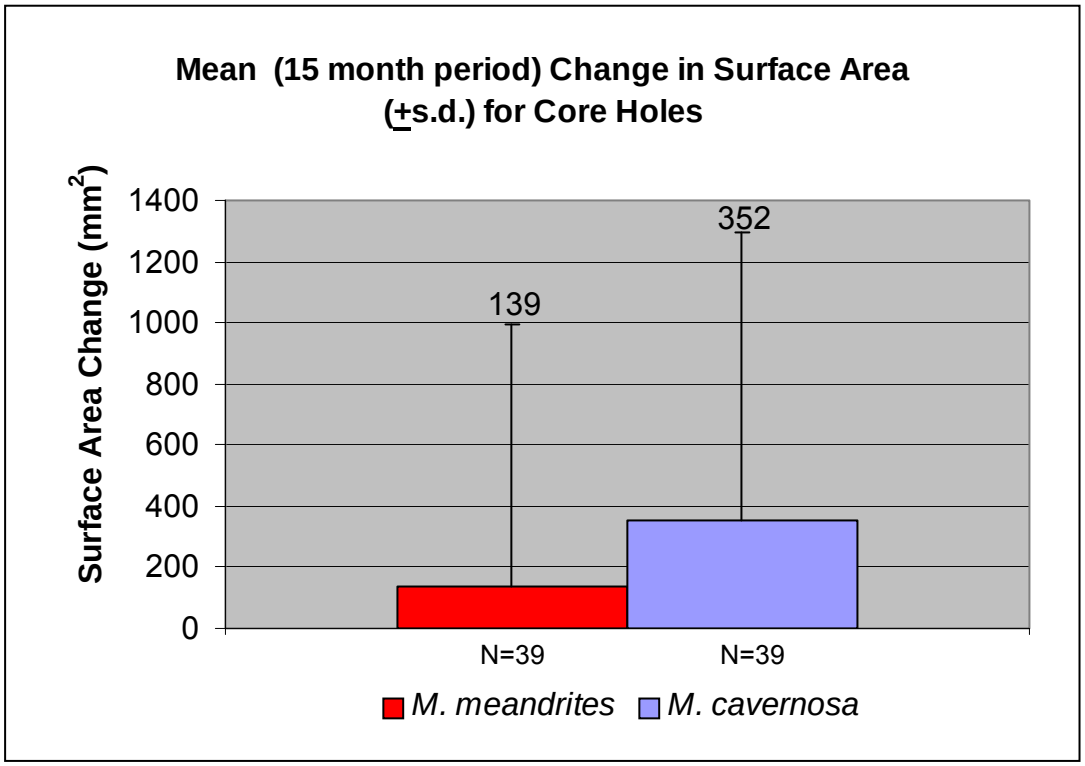
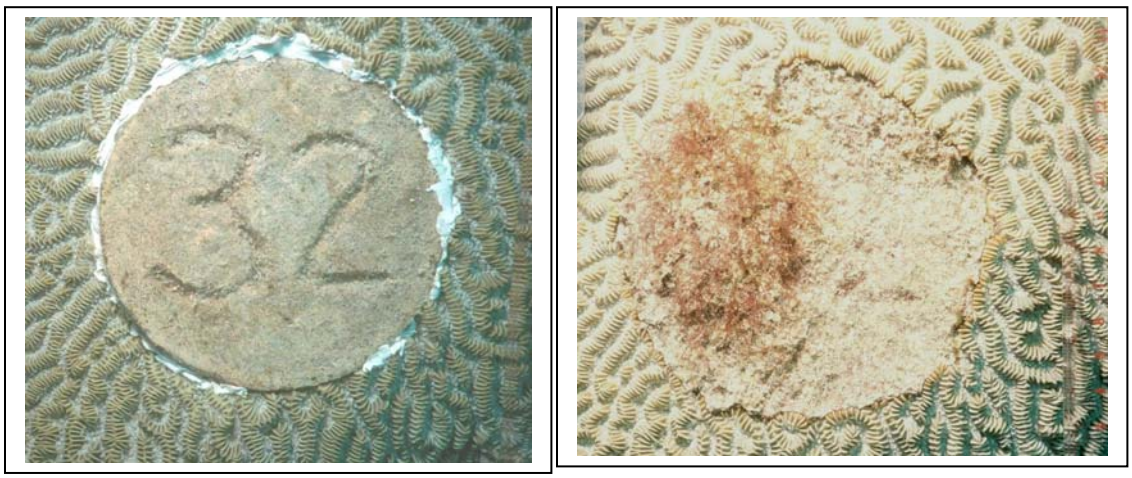


Figure 28: Mean change in surface area for the core holes. Error bars indicate standard deviation.

over the concrete plug did take place in a number of the donor colonies (Figures 29a & 29b). No comparison of recovery of plugged versus unplugged core holes was possible, because the Permit required the plugging of all drilled corals.



Figures 29a & 29b: (a) Plug 32 in June 2001 with a surface area of 8,471mm^2, and (b) in September 2002 with a surface area of 8,361mm^2. Note regrowth of tissue over the surface of the concrete plug.

The total change in core hole area (from June 2001 to September 2002) was compared between the two species. Using a nested ANOVA, no significant difference was found in the change in area between species for the duration of the study (F=1.35 and p=0.19). When the total change in core hole area was analyzed using the non-parametric Mann-Whitney U-test, once again no significant difference was found between species, U=654, z=-0.71, p=0.48.

Comparison of core hole area in first and last data sets within species of donors

The first and last data sets were compared to determine the significance of area change (area of the core hole) by species instead of between species. For *M. meandrites*, a nested ANOVA found that there was no significant difference found between the initial area of the core holes in June of 2001 and the final area of the core holes in September of 2002 (F=0.98 and p=0.44). Using a non-parametric test, the Wilcoxon Matched Pairs test (Z=1.57, p=0.12), no significant difference between the initial area of the core holes and the final area of the core holes was found for *M. meandrites* (Figures 29a & 29b).

For *M. cavernosa*, a nested ANOVA found that there was also no significant difference between the initial area of the core holes in June of 2001 and the final area of the core holes in September of 2002 (F=1.7 and p=0.08). Using a non-parametric test, the Wilcoxon Matched Pairs test (Z=1.02, p=0.31), no significant difference between the initial area of the core holes and the final area of the core holes was found for *M. cavernosa.*

4.3.2.1.2 Core Hole Surface Area Change by Sample

Repeated measures comparison of area change of donor core holes between two species

A repeated measures ANOVA was run on the change in area by data set, comparing *M. meandrites* and *M. cavernosa* core holes. A significant difference was found in the change in area between species (F= 3.38 and p=0.01). A significant difference was found, using the Wilcoxon match pairs test, in nine of the twenty-five comparisons (Table 12).

Table 12: Selected significant results of the Wilcoxon matched pairs test (repeated measures) core hole area change by data set and species

Core Holes (Plugs)

M. meandrites versus *M. cavernosa*

Repeated Measures (Wilcoxon Matched Pairs Test)

***Mm* Area Chg**	***Mc* Area Chg**	**Z**	**p-level**
*Mm*P area III-II	*Mc*P area III-II	3.04	0.002
*Mm*P area VI-V	*Mc*P area VI-V	2.15	0.03

The mean surface area of the core holes for both species did gradually increase over the course of the study. Because there was no significant difference in the initial surface area versus the final surface area of core holes for either species, the change in surface area was further investigated for each sampling period. This change was examined using the repeated measurements from each data set. This enabled a closer examination of the change on a smaller scale (three month intervals). Much of the work done on injury recovery in scleractinian corals has used smaller time scales (on the magnitude of days after the injury event) (Meesters & Bak, 1993; Meesters, Noordeloos, & Bak, 1994; Meesters, Pauchli, & Bak, 1997; Hall, 1997; Hall, 2001).

Figure 30 depicts the mean surface area of the core holes for each data set. The development of a gradual increase in surface area can be seen

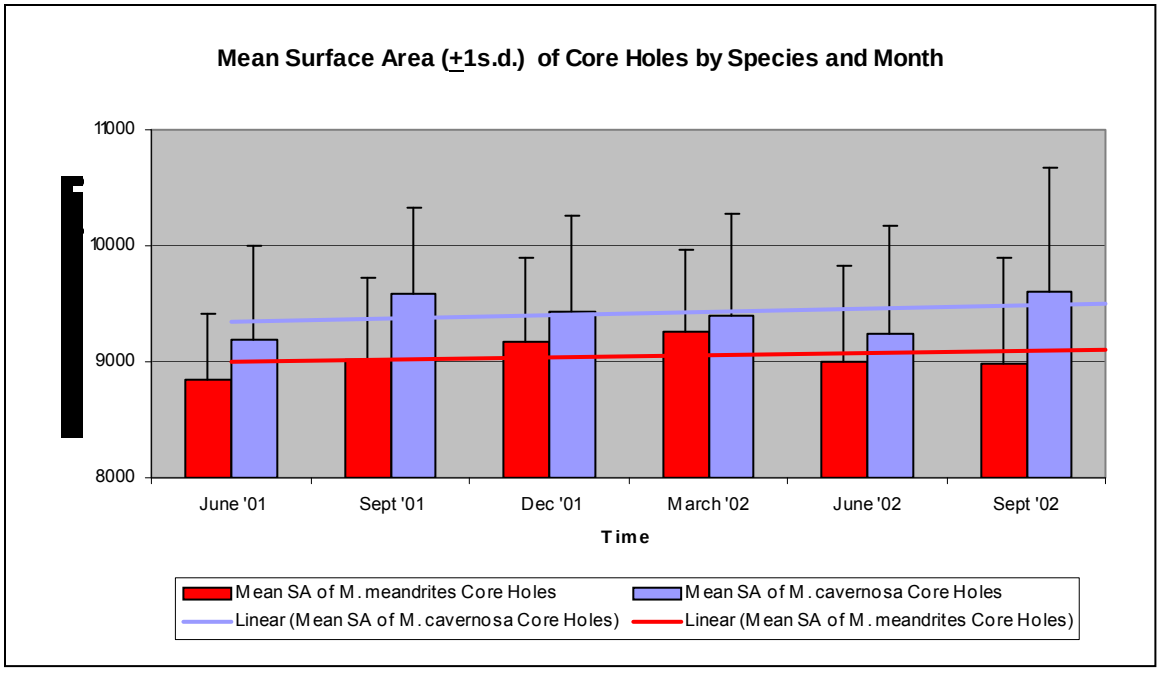


Figure 30: Mean surface area of core holes by species and data set. Trend line indicates projected area of core holes for December 2002. Error bars indicate standard deviation.

following the trend lines. Some core holes did experience regeneration over the plugs, however, the mean surface area did display an increase that was not significant when comparing the initial and final results.

4.3.2.2 Core Hole Site Radius Increase/ Decrease

Comparison of total radius change of donor core holes between two species

The total change in core hole radius was compared between two species. Figure 31 depicts the mean annual change in radius for the core holes. Although this change was not significant in either species, *M. cavernosa* core holes did experience a slightly greater change in radius during the duration of this study; the mean average increase in *M. cavernosa* core hole site radius was 0.5mm (±1.5mm s.d.), for *M. meandrites* this increase was only 0.2mm (± 1.4mm s.d.).

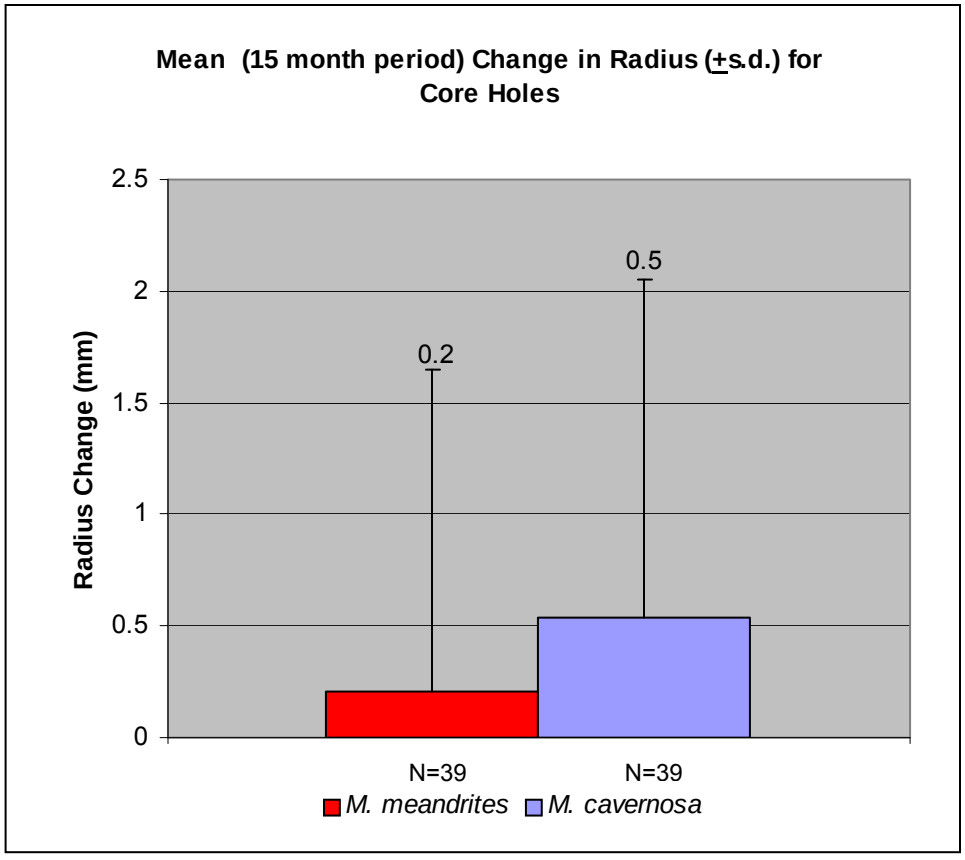


Figure 31: Mean change in radius for core holes. Error bars indicate standard deviation

Using a nested ANOVA, no significant difference was found in the change in radius between species for the duration of the study (F=1.35 and p=0.18). When the total change in core hole radius was analyzed using the non-parametric Mann-Whitney U-test, once again no significant difference was found between species, U=659.00, z=-0.65, p=0.51.

Comparison of total existing radius change of donor core holes and no change in radius of donor core holes

The total change in core hole radius was compared with a theoretical no change in radius for each species, in order to determine if the existing radius change was significantly different from zero. No significant difference was found between the existing radius change and zero for the *M. meandrites* core holes, using the Mann-Whitney U-test (U=680.00, z=0.80, p=0.42). Additionally, no significant difference was found between the existing radius change and zero for the *M. cavernosa* core holes, using the Mann-Whitney U-test (U=600.00, z=1.60, p=0.12). These results indicate that even though most of the corals did not grow over the concrete plugs, the change in radius for core holes for the duration of the project was not statistically significant from zero change (with zero being no change at all).

Comparison of core hole radius in first and last data sets within species of donors

The first and last data sets were compared to determine the significance of radius change (radius of the core hole site) by species instead of between species. For *M. meandrites*, a nested ANOVA found that there was no significant difference found between the initial radius of the core holes in June of 2001 and the final radius of the core holes in September of 2002 (F=0.90 and p=0.62).

Using a non-parametric test, the Wilcoxon Matched Pairs test (Z=0.91, p=0.36), no significant difference between the initial radius of the core holes and the final radius of the core holes was found for *M. meandrites.* For *M. cavernosa*, a nested ANOVA found that there was no significant difference between the initial radius of the core holes in June of 2001 and the final radius of the core holes in September of 2002 (F=1.30 and p=0.21). Using a non-parametric test, the Wilcoxon Matched Pairs test (Z=1.72, p=0.09), no significant difference between the initial radius of the core holes and the final radius of the core holes was found for *M. cavernosa.*

5.0 Discussion

5.1 Transplantation & Transplant Corals

5.1.1 Success of Transplantation: Attachment

The attachment part of the transplantation process itself was a success. All of the transplanted colonies remained attached for the duration of the study. The use of AquaMend® repair epoxy did not appear to be harmful to the transplants. The AquaMend® epoxy was pliable enough that it could be manipulated into small spaces, which was the case for the transplants in the Reef Balls and for the concrete plugs in the core holes. Pre-fabricated notches were created in modified Reef Balls and successfully used as receptacle sites for transplant corals.

5.1.2 Success of Transplantation: Survivorship

Success as defined by survivorship and growth of the transplants was variable. Ideally, in a successful transplantation project, the transplanted corals will survive and grow in a manner similar to that of naturally occurring corals (Yap, Aliño, & Gomez, 1992). According to Yap, Aliño, & Gomez (1992), by comparing the transplants (and donor corals) with control corals, the extent of distress from the transplantation process itself may be inferred.

Total colony mortality has been shown to be inversely related to colony size (Soong, 1992; Highsmith *et al*., 1980; Hughes & Jackson, 1980; and 1985; Hughes & Connell, 1987). Early in life, corals have very high mortality rates. With larger colony size, corals develop an increased survival rate (Birkeland, 1976). Additionally, fecundity has been shown to increase with colony size

(Soong, 1993). Growth, competition, and regenerative abilities also have been shown to increase with increasing colony size (Soong, 1993; Buss, 1980; Hughes, 1984; Jackson & Coates, 1986; Lang & Chornesky, 1990). The use of a larger sized core (4 inch) may have increased the ability of the coral transplants to compete for space (Lindahl, 1998). The use of plugs of coral tissue (instead of the entire coral colony) allowed for the perpetuation of the donor corals at the donor site.

The cause of the decline of *M. meandrites* transplants was not determined. The appearance of mortality on the *M. meandrites* transplants (see Section 4.1.6.2) did not follow the pattern of any illustrated diseases. Since only the transplants experienced significant mortality, and not the donor corals or core holes, it may be inferred that the drilling was not the sole contributing factor involved in the decline of the *M. meandrites* transplants. The decrease in colony size, that took place in the transplants when removed from the donor colonies, may have affected the transplant survivorship.

Possibly, the mortality of the *M. meandrites* transplants was associated with the change in light regime experienced by the transplants. The transplants were originally located at a depth of 8-10 meters. After transplantation, they were located at a depth of approximately 12 meters. On the natural reef, the *M. meandrites* corals were naturally situated in a horizontal manner. Once transplanted, the corals were moved to an angle of approximately 45 degrees. It is possible that this new depth and angle, and thus light penetration, caused additional stress on these transplants. A decrease in coral growth has been

reported for a depth increase as little as 6 meters (Rezak & Bright, 1981; Dodge & Lang, 1983). *Meandrina meandrites* is commonly found at 12 meters depth, and at greater depths along the third reef in Broward County. Thus, it is not uncommon for the species to flourish in a reduced light regime. Perhaps these individuals could not adapt to the new depth and light transition. Bleaching was observed in some stressed transplants that had been shaded by the settlement plates.

The *M. meandrites* small controls for the transplants (which did not experience the same amount of significant mortality as the transplants) were located at a slightly shallower depth. Reef adjacent to the Reef Ball site was chosen for the controls. The difference in depth for these two sites was only 3-4 meters. It is possible, but unlikely (due to the slight depth difference) that the comparison of the small controls and the transplants may have been confounded by the depth difference (Diaz-Pulido & McCook 2002). It is more likely that the transplants may have been affected by the change in angle and light regime.

On the other hand, the *M. cavernosa* transplants did not experience a similar amount of mortality. These individuals came from the same reef locale as the *M. meandrites* transplants. The general growth form of *M. cavernosa* colonies is more vertical than *M. meandrites,* which tends to grow in a more horizontal and encrusting fashion. Frequently, *M. cavernosa* transplants were drilled from the side of the colony where the colony is not as thick. Thus, these corals were already acclimated to the 45-degree angle of exposure to penetrating

light. It is possible that this was an additional favorable factor, which led to the success of the *M. cavernosa* colonies.

The negative effects of sedimentation might have been a possible factor involved with the partial mortality. As sediment loading increases, coral growth has been found to decrease (Dodge & Vaisnys, 1977). The coral transplants were relocated at a height of over one meter off the seafloor, and tilted at an angle. This makes sediment overload an unlikely cause of the mortality. Transplants subjected to a solely horizontal incline or closer to the substrate, would have been more likely to suffer from sediment overload.

Some species of hermatypic corals have different light-level requirements (Dodge & Vaisnys, 1980; Huston, 1985); perhaps *M. meandrites* was acclimated at its donor depth (of approximately 9 meters), but it could not survive relocation to the transplant depth (of approximately 12 meters). A very small change in depth such as this is unlikely to be the sole cause of the mortality. Mortality of reef-building corals in the Florida Keys has been attributed to reduced light levels (due to sediment resuspension or nutrient enrichment) (Yentsch *et al*., 2002). More specific studies may be needed to determine the particular cause of the mortality experienced in the transplants of this species. Similar mortality patterns to those observed in the *M. meandrites* transplants have been observed both in transplantation projects in the field, and in aquarium maintained individuals (personal observation).

Oren and Benayahu (1997) found that low survivorship of transplanted corals may have been correlated to insufficient light requirements (the corals

were transplanted to a location that did not meet their light requirements and therefore, suffered mortality). Smith and Hughes (1999) found that transplants moved to a greater depth (an increase of 8-9 meters), were subject to lower levels of light intensity. The reduced light likely reduced the growth rate of those transplants that did survive (Smith and Hughes, 1999). Yap and Gomez also found that transplants were affected by the altered light regimes of the transplant site. Transplanted branches of *Acropora pulchra,* once subjected to altered light, changed their growth pattern to orient towards the light (Yap & Gomez 1984, 1985). Alcala, Gomez, and Alcala (1982) found annual survival in transplanted corals to range from 18-100% (with a 100- 800 cm^2 area increase). The factors that contributed to the low survival rates were "unknown". Factors such as the stress of the transplantation process itself, may have contributed. The faster growth rates may have been due to the greater sunlight exposure at the transplant site (Alcala, Gomez, & Alcala, 1982).

5.1.3 Transplant Null-Hypothesis Rejection

Main Null-Hypothesis *[There will be no species-specific differences in the responses of coral colonies to drilling and transplantation]* was rejected. Differences were found in the growth and survivorship between species of transplanted corals. These differences have been more specifically addressed in the following sub-hypotheses:

Null-Hypothesis 1 *[There will be no change in surface area and/or linear radius in the experimental corals and the control corals]* was rejected. A significant difference was found in the total area and radius change when

comparing both transplant species. A significant difference was also found within species when comparing the first and last data sets (this difference was negative in the case of the *M. meandrites* mortality and positive in the case of the *M. cavernosa* growth). These differences within species were also apparent in the repeated measures analyses. No significant difference was found for the total area/ radius change (or the repeated measures area change) when the two species of controls were compared. Significant differences between the transplants and their controls also were encountered (see null-hypothesis 2).

Null-Hypothesis 2 *[The survivorship of the experimental corals (transplant corals and donor corals) will not be similar to their respective control corals]* failed to be rejected for *M. meandrites*. A significant number of the transplants did not survive the duration of the project, while all of the controls survived. A significant difference was found when the *M. meandrites* transplants and controls were compared using total area and radius change, and using the repeated measures method. For all of these comparisons, the *M. meandrites* controls displayed a greater amount of growth than the transplants.

Null-hypothesis 2 was rejected for *M. cavernosa*, as the majority of the transplants and all of the controls survived the duration of the project. A statistical difference between the transplants and the controls was found using total area and radius change, and using the repeated measures method. These differences were not as substantial as in *M. meandrites*. Even though there was a statistical difference in the amount of growth (the *M. cavernosa* transplants exhibited a greater amount of growth than the controls), there was no difference

in the fact that both groups exhibited growth. Therefore, a positive association was found between the *M. cavernosa* transplants and controls.

5.1.4 Photographic Methodology

Single photographs can provide a very limited perception of depth; even very small shifts in the camera may cause 'apparent' shifts in colony image and shape (Done, 1981). With a fixed coral the photographer must ensure that the coral was photographed at the same angle during each sample event in order to continually monitor the health and growth of that organism. Occasionally, images were rejected from the coral transplantation data sets because they were not comparable to previous photos, due to lighting differences (the flash failed) or due to lack of a complete image (a portion of the coral was cut off in the framer). The camera angle remained virtually the same in all images, due to the fixed distance between the framer and the subject. Corals on the natural reef were photographed in a northward direction to maintain the same angle on the subject (the diver positioned herself on the southern side of the control when photographing).

The growth of the *M. cavernosa* transplants was assessed as the transplant tissue grew in a horizontal fashion. Upward growth was not assessed using the planar imagery. The significant difference in growth for the *M. cavernosa* transplants versus the small controls (with greater growth in the transplants) may be attributed to this discrepancy. It is unlikely that the small controls did not grow during the 15-month monitoring period. A reasonable explanation might be that they exhibited a small amount of upward growth.

5.2 Donor Corals

5.2.1 Donor Survivorship

All of the donor and large control corals survived the duration of the project. Only three donor colonies out of forty experienced tissue mortality due to the act of an error during drilling. All three of the colonies regenerated tissue over that area damaged by drilling (see Section 4.2.3).

5.2.2 Change in Percent Tissue Coverage

The mortality present on the donor and large control corals, prior to the drilling, was pre-existing mortality from natural causes. The change in percent tissue coverage for the donor corals was not significantly different from the change for the controls of the same species. This change was minor (it ranged from a increase in tissue coverage of 5%, to a decrease in tissue coverage of 20%). It is likely that the change in tissue coverage was natural. The coring process did not appear to exacerbate the change in tissue coverage, as the donors and controls exhibited similar amounts of change.

5.2.3 Donor Null-Hypothesis Rejection

Null-Hypothesis 2 *[The survivorship of the experimental corals (transplant corals and donor corals) will not be similar to their respective control corals]* was rejected. Both the donor colonies and the large control colonies survived the duration of the project. No significant difference was found for the change in percent tissue coverage when the donors were compared with their same species controls. Additionally, no significant difference was found when the

original and final tissue coverages were compared (there were not control comparisons for this portion of the study).

5.3 Core Holes

5.3.1 Success of Plugging Core Holes: Attachment

The attachment part of the process of plugging the core holes was successful. Seventy-eight of the eighty concrete plugs remained attached for the duration of the study. The use of AquaMend® repair epoxy did not appear to be harmful to the donor colonies.

5.3.2 Success of Plugging Core Holes: Effect on Donors

Recent injury is more of a predictor of colony fate than old injury (Cumming, 2002). Because the core holes did not show significant die back after the initial fifteen-month study period, it is possible that tissue injury will not progress further. Both *M. cavernosa* and *M. meandrites* were shown to be suitable species for drilling projects. The two species also were able to retain concrete plugs within the core holes.

Whether plugging the core holes was beneficial or detrimental was not determined due to the lack of comparable controls. The change in core hole area was not significant when comparing species, indicating that neither *M. meandrites* nor *M. cavernosa* differed in their response. Additionally, there was no significant difference in the initial area/ radius and the final area/ radius of the core holes for either species, indicating that the use of concrete plugs did not cause significant mortality in the adjacent area surrounding the core holes.

The lack of significant mortality surrounding the core holes suggests that this practice may be worthwhile in studies where a sample of coral is necessary. Further examination of the regenerative abilities in coral species with varying growth rates may provide more information on the success of plugging core holes. Additionally, a longer monitoring period for the core holes may provide information on the long-term recovery of these areas. Due to the slow growth rates of scleractinians at this high latitude environment, it is still possible that the core holes may eventually completely recover.

5.3.3 Core Hole Null-Hypothesis Rejection

Null-Hypothesis 3 *[There will be no change in surface area and/ or radius (tissue regeneration) of the tissue surrounding the core holes]* failed to be rejected. Although the mean area/ radius of the core holes did exhibit a total area change for each species, for the original versus the final area/ radius, and for the repeated measures test; none of these were statistically significant.

6.0 Conclusions

Differential species-specific survivorship and growth rates can provide important information for future transplantation studies. *Montastrea cavernosa* was shown to be a hardy coral, able to withstand both coring and transplantation. Once transplanted onto the Reef Ball substrates, the *M. cavernosa* corals displayed the ability to successfully increase in surface area and annual radius. *Meandrina meandrites* was shown to be a relatively sensitive coral. Again, this species was able to handle the effects of both coring and transplantation. Although 100% of the donor colonies survived coring, the transplants did not fair as well. The experimental process negatively affected the *M. meandrites* transplants, with 30 of the 40 transplants experiencing some degree of partial mortality. The *M. meandrites* transplants may have succumbed to stress. Whether this stress was "internal (physiological) or external (environmental)" (Yap, Aliño, & Gomez, 1992) was never determined.

Numerous stressors (coral disease; sedimentation, nutrient, and temperature stresses; and competition with other organisms) are currently affecting coral reefs worldwide and causing an increase in natural mortality. As both the controls and the experimental corals experienced mortality, it is unlikely that all mortality could have been attributed to an effect of the transplantation process. Varying levels of mortality in colonies of *M. cavernosa* have been attributed to genetic variation, disturbance, and differences in both positions on substrate and amount of shading (Amaral, 1994). Natural mortality in

scleractinian species has been observed in other monitoring projects throughout Broward County (personal observation).

Growth (defined as an increase in surface area and linear radius) in two species of scleractinian coral was monitored using photographic methodology. Sigma Scan Pro4 image analysis software effectively provided surface area and radius measurements from planar photographs. Using this software, differential growth and survivorship of two scleractinian species were assessed.

Meandrina meandrites and *M. cavernosa* were investigated for their effectiveness as transplantation species. The growth and survivorship rates of *M. cavernosa* indicated that this species was more successful for transplantation than the less hardy *M. meandrites.* *Montastrea cavernosa* was shown as a suitable species for a transplantation project in a high-latitude reef environment. Control corals provided a reference for comparison to the treated transplants and donor corals.

Both *M. meandrites* and *M. cavernosa* were shown to efficiently handle the drilling of transplants (although the transplants themselves did not show statistically successful survivorship or growth in *M. meandrites*). Additionally, both species of the donors were shown to handle the drilling process successfully, as seen in the donor survivorship and the effectiveness of plugging the core holes. This information may prove useful in studies where corals are drilled for age/ growth and climate information, as the use of concrete core hole plugs appeared effective in preventing the detrimental effects of bioeroders.

The results from this study may provide useful insight in future coral transplantation and restoration projects. Specific information on transplant, donor, and control growth and survivorship for *Montastrea cavernosa* and *Meandrina meandrites* may prove helpful in the case of ship grounding events and experimental studies. The core hole tissue regeneration results may encourage the use of concrete core hole plugs in future coral drilling work.

7.0 Literature cited

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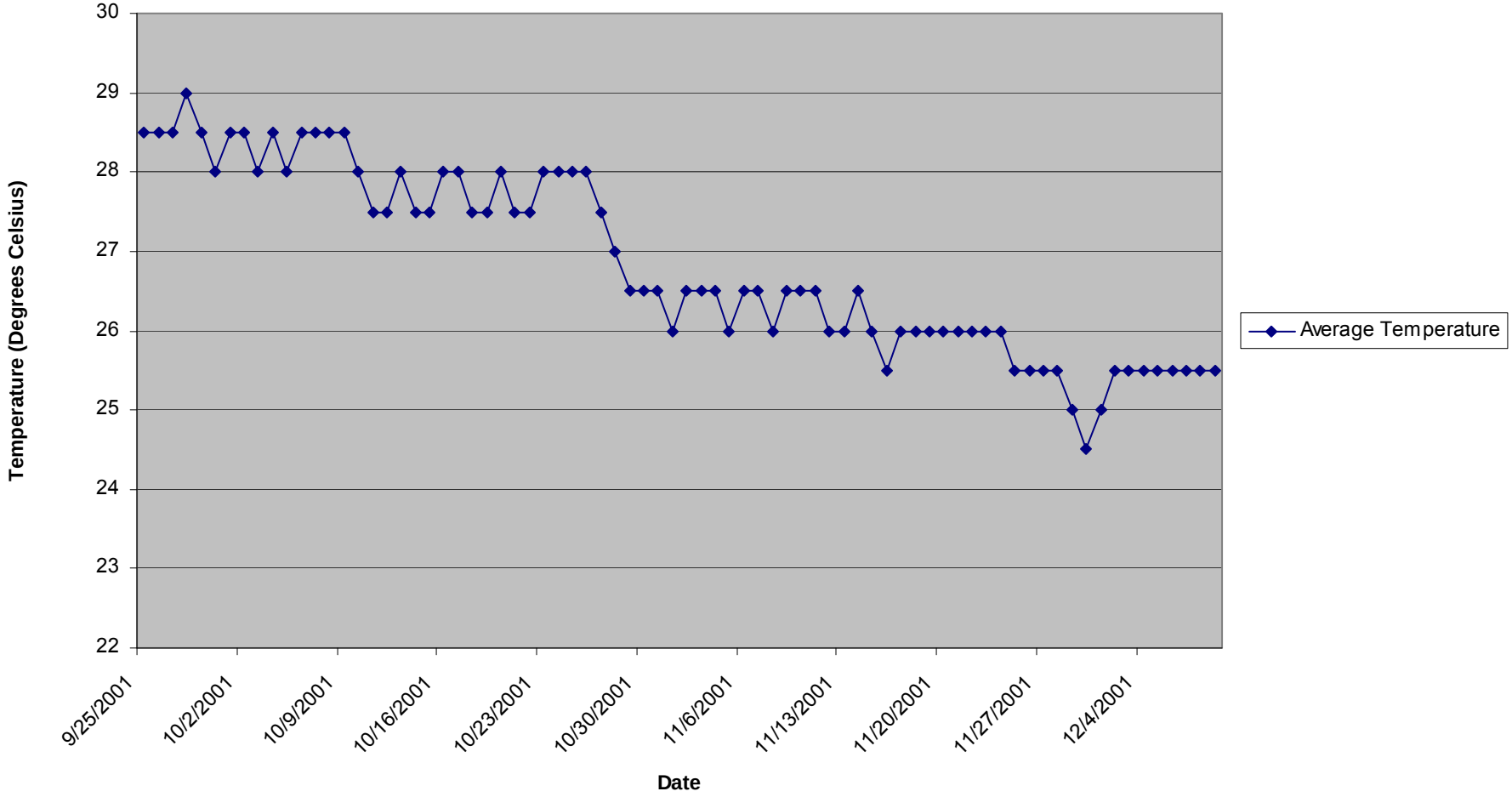
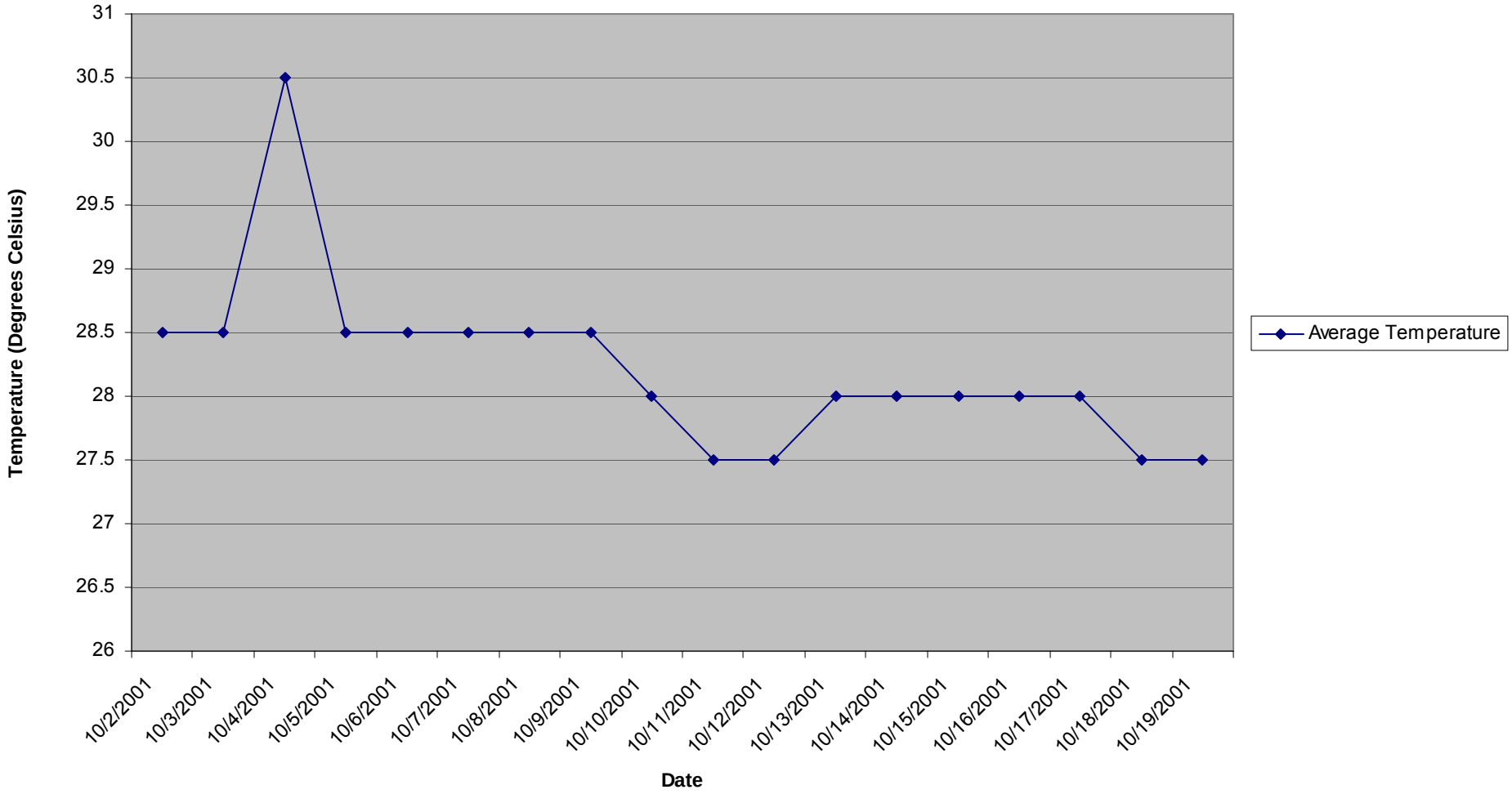
Coral list discussion group and archived list-server.
coral-list@coral.aoml.noaa.gov

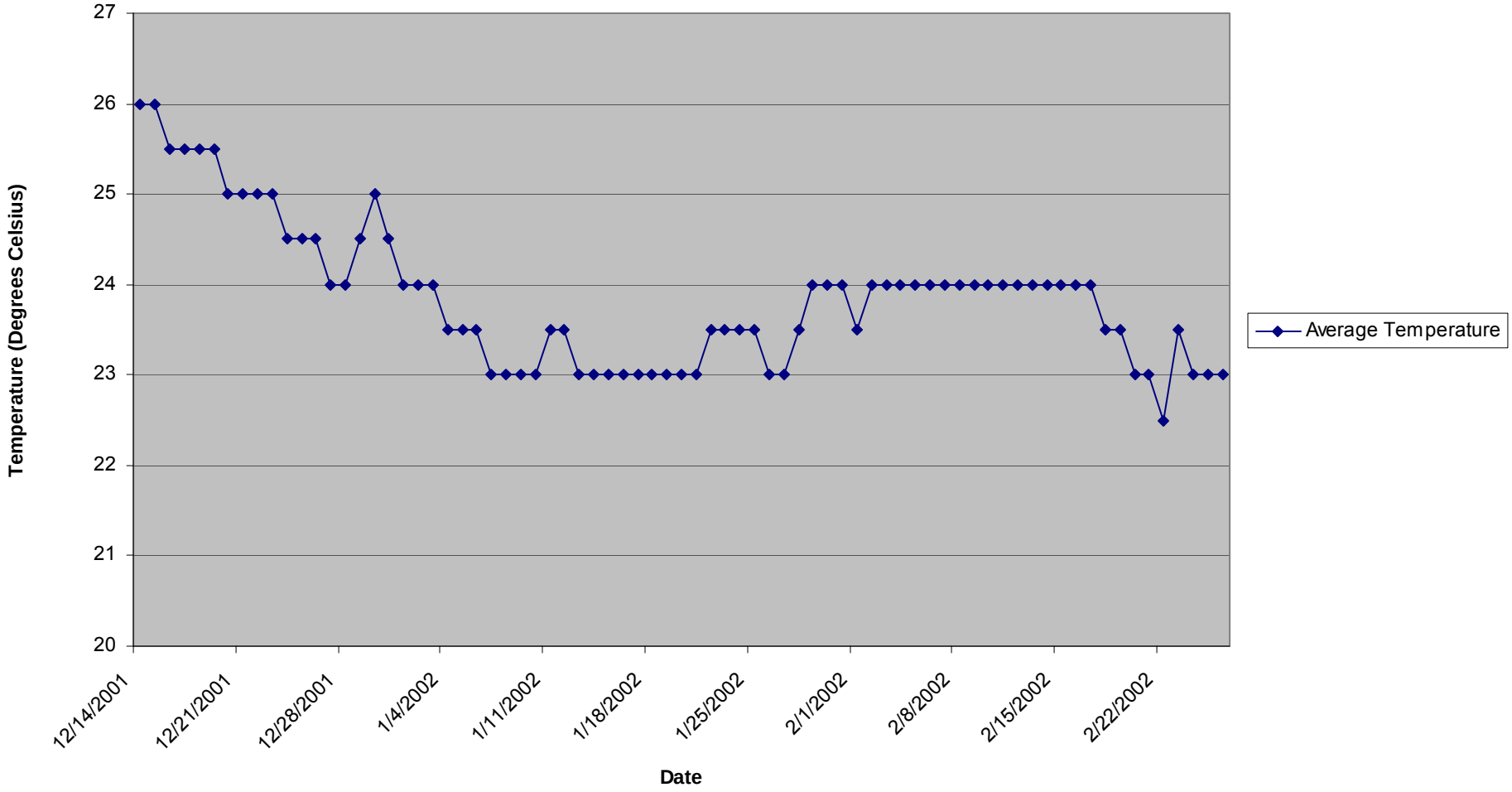
Appendix: (Figure 1) Average Daily Temperature at Natural Reef Site (Sept-Dec 2001)



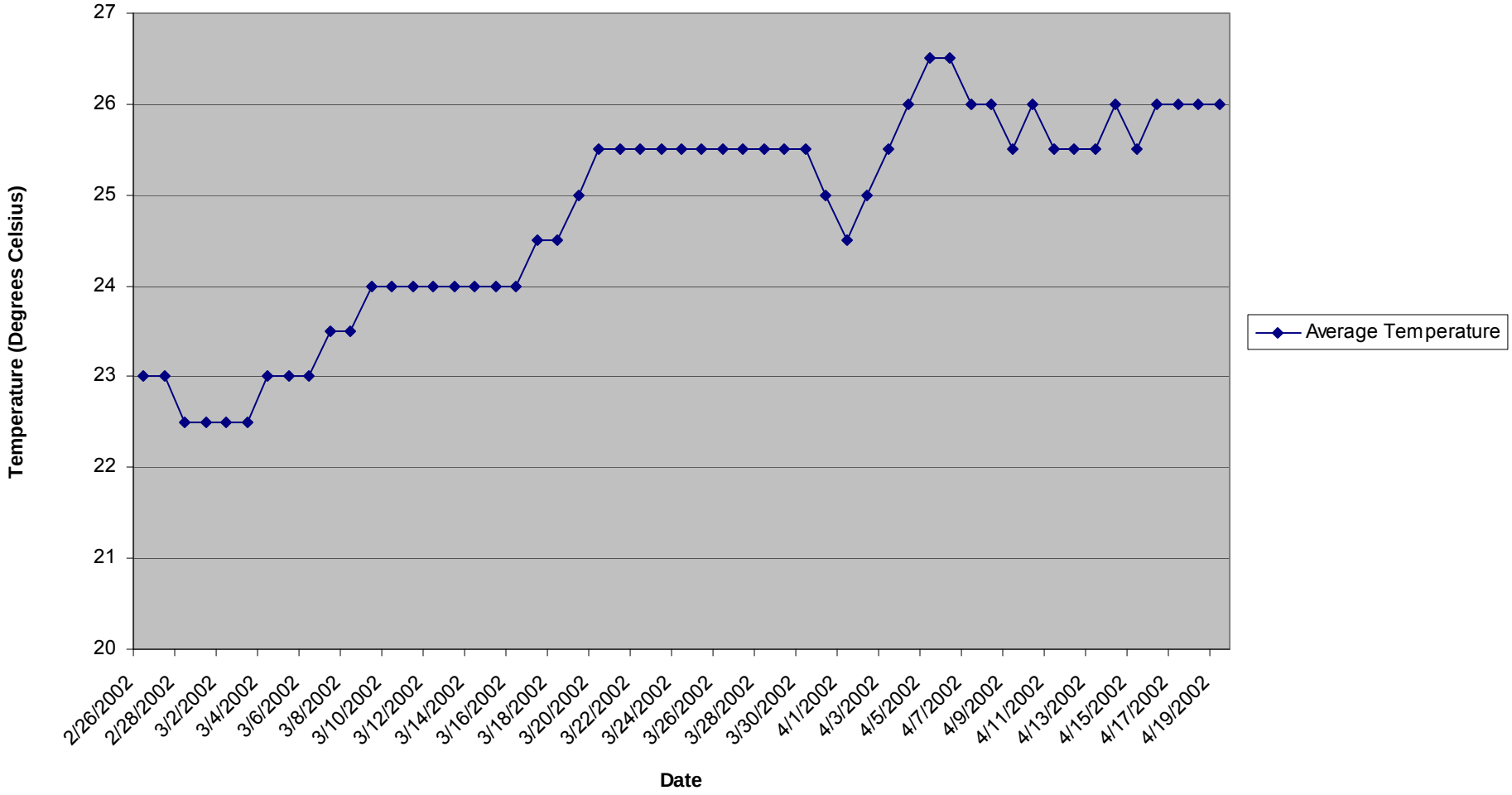
Appendix: (Figure 2) Average Daily Temperature at the Reef Balls (October 2001)



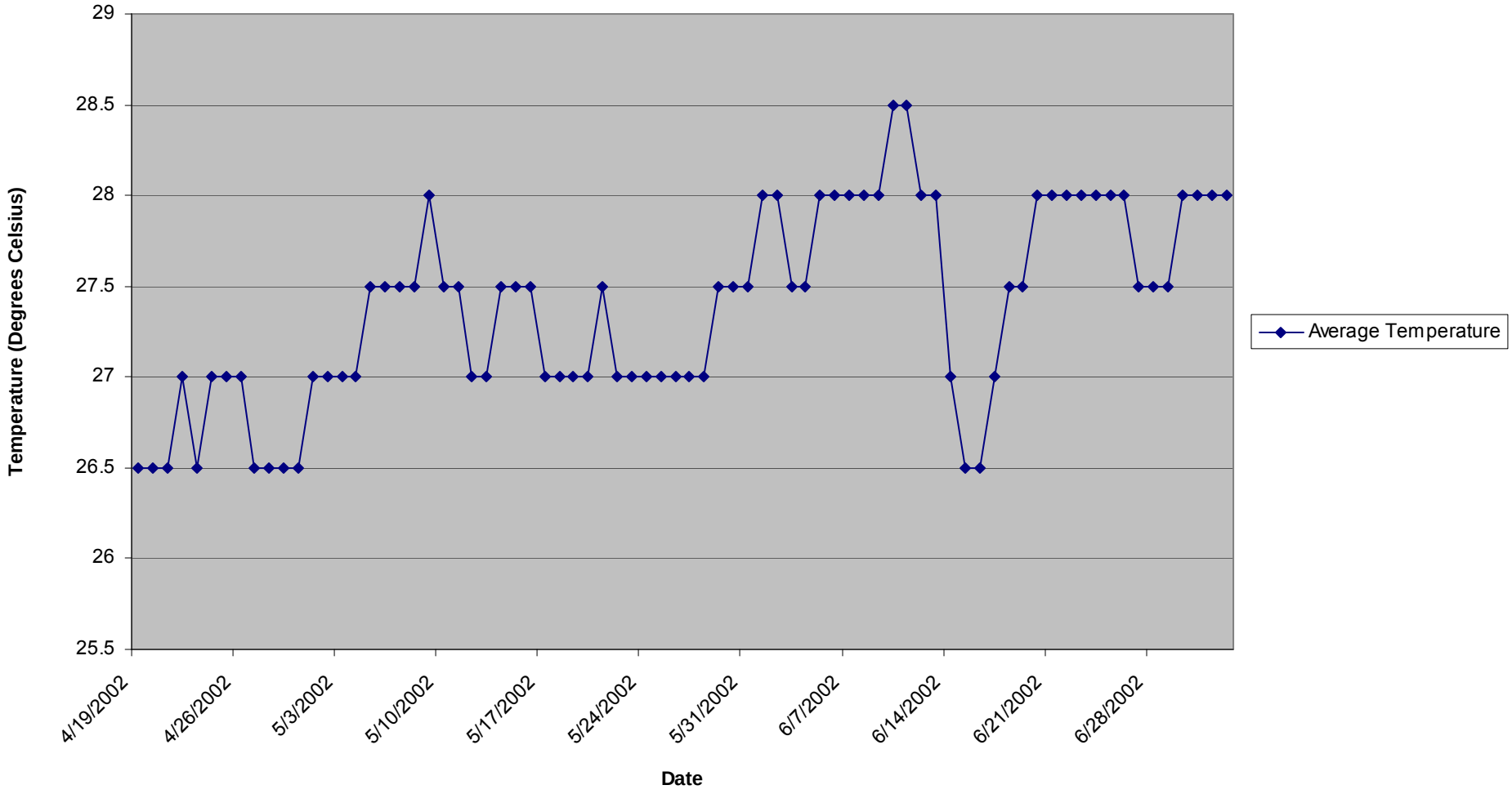
Appendix: (Figure 3) Average Daily Temperature at the Reef Balls (Dec 2001 - Feb 2002)



Appendix: (Figure 4) Average Daily Temperature at the Reef Balls (Feb - April 2002)



Appendix: (Figure 5) Average Daily Temperature at the Reef Balls (May-June 2002)



Appendix: (Figure 6) Average Daily Temperature at the Reef Balls (July-Sept 2002)