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Staghorn Coral, Acropora cervicornis, Restoration in South Florida: Growth and Survivorship of Outplanted Nursery Corals

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STAGHORN CORAL, *ACROPORA CERVICORNIS*, RESTORATION IN SOUTH FLORIDA: GROWTH AND SURVIVORSHIP OF OUTPLANTED NURSERY CORALS.

By

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ABSTRACT OF THE THESIS

Staghorn Coral, *Acropora cervicornis*, Restoration in South Florida: Growth and Survivorship of Ouplanted Nursery Corals

This thesis provides a detailed analysis of the growth and survivorship of outplanted *Acropora cervicornis* corals from underwater nurseries within three regions of the Florida Reef Tract. Substantial loss of stony coral cover on Florida's coral reefs, including the branching staghorn coral, *Acropora cervicornis*, has occurred for decades due to disturbances such as disease, temperature induced bleaching, hurricanes, sedimentation, and pollution. This rapid population decline contributed to *A. cervicornis* being listed as a threatened species under the U.S. Endangered Species Act in May 2006. To aid in the recovery of the species, coral fragments were grown in underwater nurseries and outplanted to selected sites located within unique cross-shelf zones in the Upper Florida Keys, Lower Keys, and Biscayne regions. This study evaluated the regional and zonal variation in growth and survivorship of known genotypes of outplanted *A. cervicornis* corals to better inform future large-scale restoration projects. The zone in which corals of *A. cervicornis* were outplanted to had a more significant effect on growth than the coral genotype. The forereef zone within the Upper and Lower Keys regions and the mid-channel zone in the Biscayne region had significantly higher mean growth rates. When comparing growth rates of genotypes that performed best, high growth, in the Lower Keys nursery, these same genotypes did not perform the best at any of the outplant sites. Survivorship was not significantly different in any of the regions. Based on these results, future coral outplantings focused in the forereef and mid-channel zones would maximize

growth. Choosing coral genotypes based on their high growth rates in the nursery does not ensure the same high growth rates when outplanted.

Key Words: *Acropora cervicornis*, staghorn, coral reef, restoration, coral nursery

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1.0 INTRODUCTION

1.1 IMPORTANCE OF CORAL REEFS

Coral reefs provide coastal protection, are centers of high biodiversity, and support a wide range of recreational and commercially important species of fish and invertebrates. Although they make up only a small percent, 0.02%, of the marine environment (Veron et al. 2009), it is estimated that they are home to one third of all known marine species (Reaka-Kudla 1997, 2001). More than 100 coastal countries are protected by coral reefs (Moberg and Folke 1999) shielding from coastal erosion. The 3-dimensional structures provide habitat and act as protective breakwaters to adjacent shorelines (Perry et al. 2013). Coral reefs protect mangrove and shallow marine habitats that support many life stages of important marine species (Johnson and Marshall 2007). These ecosystems also support fishery resources that provide a significant source of food for humans. In addition to these physical resources that coral reefs provide, their intrinsic beauty draws and supports billions of dollars in tourism each year. In Florida, tourism and recreation generated the highest amount of sales, over $62 billion, in 2005 (Waddell and Clarke 2008). In South Florida, during a 12-month period from June 2000-May 2001, coral reef related expenditures generated $1.3 billion in sales in Miami-Dade County and $504 million in Monroe County (Hazen and Sawyer 2003). These expenditures provided thousands of jobs supporting tourism and fisheries each year, over $1.2 billion in the Florida Keys alone (USCRTF 2000). As the coral reef ecosystem declines due to numerous stresses, ecosystem services that humans depend on will also quickly diminish.

1.2 CORAL REEF DECLINE

Significant declines in living coral coverage worldwide have occurred in recent decades due to disturbances such as coastal development, sedimentation, invasive species, bleaching, disease, and pollution (Gardner et al. 2003; Hughes et al. 2003; Grimsditch and Salm 2006). It is estimated that 35% of the world's coral reefs are threatened, and 19% have already been lost (Wilkinson 2008). Additional local and global stressors include overfishing and eutrophication (Hughes et al. 2003). Global climate change is one of the major threats to coral reefs around the world. Predictions for increased ocean acidification, sea level rise, temperature induced bleaching and disease will only add to the already existing stressors (Hoegh-Guldberg 1999; Buddemeier et al. 2004; Veron et al. 2009). Coral reefs are already extremely sensitive to climate change induced changes in the marine environment (Baker et al. 2008). Large scale disturbances such as coral bleaching and mortality have already occurred over the last few decades because of increasing sea temperatures (Lough 2000; Wilkinson 2000; Baker et al. 2008). As the oceans continue to warm due to climate change, these events will become more common. Historic sea surface temperature measurements in the Florida Keys suggest a 0.8°C increase over the last century (Kuffner et al. 2015). Other extreme weather events, such as the cold-water event that in occurred in Florida in 2010, have also caused major coral mortality within a short period of time (Lirman et al. 2011). Due to all of these stressors combined, it is currently estimated that one-third of all reef building corals are at risk of extinction (Carpenter et al. 2008).

1.3 DECLINE AND IMPORTANCE OF *ACROPORA CERVICORNIS*

One particular reef building coral at risk of extinction is *Acropora cervicornis*, or staghorn coral (Lamarck 1816). The *Acropora* genus is the most diverse genus of coral, with more than 120 extant species (Wallace 1999; Vernon 2000; Wallace and Rosen 2006). *Acropora* is found in the central Indo-Pacific (Vernon 1995; Wallace 1999), and throughout much of the Caribbean (Greenstein et al. 1998; Vargas-Angel et al. 2003), however it is still threatened. These large branching corals are mainly found in depths of 0-30 meters (Goreau 1959; Adey and Burke 1977; Neigell and Avise 1983) in the fore and back reef areas. *Acropora cervicornis* is only one of three *Acropora* species found throughout the Caribbean. Prior to the 1980's, these fast growing corals, up to 11cm/year in Florida, formed dense three dimensional thickets, contributing significantly to reef growth, island formation, coastal protection, and fisheries habitat diversity (Shinn 1966; Bruckner 2002). The unique open framework of these densely populated staghorn thickets provide essential habitat for fish, turtles, lobsters, crabs, echinoids, and gastropods (Bruckner 2002). Juvenile reef fish, schooling bait fish, large herbivores and predatory reef fish, and invertebrates are associated with staghorn and elkhorn thickets on reefs (Lirman 1999). Recent work by Coker et al. (2012) suggests that levels of live coral cover have a stronger influence on abundance and diversity of fish than the complexity of the reef.

Various disturbances such as hurricanes, bleaching, and disease have occurred over the last 25 years, changing the landscape of Caribbean reefs, especially increased mortality of *Acropora* spp. (Aronson and Precht 2001). Major present threats affecting *A. cervicornis*

include: storms, thermal stress, nutrients, white band disease, sedimentation, and predation (Aronson and Precht 2001; Vargas-Angel et al. 2003). Specifically, white band disease has caused significant decline in the *Acropora* corals, and highly demonstrates the ecosystem effects of coral disease on coral reefs (Aronson and Precht 2001; Vollmer and Kline 2008; Gignoux-Wolfsohn et al. 2012). Due to a rapid and significant population decline, both *A. cervicornis* and *A. palmata* were listed as threatened species under the U.S. Endangered Species Act in May 2006 (Hogwarth 2006) and critically endangered in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species in 2008 (Aronson et al. 2008, Carpenter et al. 2008). These designations provide additional protection, and development of a recovery plan to aid in recovery of the species (National Marine Fisheries Service 2015). Due to the multitude of threats to *Acropora* spp., the local management of reefs and nearby lands is crucial to maintain net production in coral nurseries and aid in recovery of the species (Hernandez et al. 2014)

1.4 REPRODUCTION OF *ACROPORA CERVICORNIS*

Acropora cervicornis reproduces both sexually by broadcast spawning and asexually via fragmentation. Broadcast spawning of this species in south Florida is known to occur annually, within a few days of the July or August full moon (Vargas-Angel et al. 2006). However, as little as 5% of all coral recruits that settle onto the reef survive to a mature state (Soong and Chen 2003). Although sexual reproduction does occur, this species relies heavily upon asexual reproduction via fragmentation, and has shown very little success of sexual recruitment due to low population numbers (Knowlton et al. 1990;

Vargas-Angel et al. 2003). Reproduction through only asexual fragmentation may limit the number of genetically distinct colonies that occur throughout the species range. While exact population numbers for this species are not available, recent studies suggest that there is high genetic diversity within south Florida for *A. cervicornis* (Hemond and Vollmer 2010). While this may be true, unless coral colonies are within close enough proximity to each other to sexually reproduce, this genetic diversity may not increase or even be maintained over time. By increasing population numbers through transplanted coral fragments, sexual recruitment should increase, aiding in the recovery of the species. Documented fast growth rates (5cm/year for every cm of coral), coupled with high productivity from fragmenting corals in nurseries, make *Acropora cervicornis* a great candidate for species restoration (Lirman et al. 2014).

1.5 RESTORATION OF *ACROPORA CERVICORNIS*

Coral reef restoration has historically been done in response to damage caused by human activities such as ship groundings, blast fishing and dredging (Hudson et al. 1989; Bruckner and Bruckner, 2001; Fox et al. 2005). Restoration focused on restoring the structural integrity and topographical complexity to reefs (Precht 2006) versus coral populations. Due to the continuing decline of coral reefs and a lack of recovery of *Acropora* spp., there has been a heightened interest in coral species restoration. Techniques, such as coral transplantation, previously used to restore reef areas after being destroyed by human activities, are now being used to restore reef areas declining due to a multitude of stressors and disturbances. Recommendations for *A. cervicornis* coral restoration techniques were identified at the 2002 *Acropora* Workshop held in Miami,

Florida. It was noted that "coral mariculture, aquaculture, and other propagation techniques, along with transplantation, and reattachment of dislodged *Acropora* fragments may provide a feasible strategy to rebuild degraded *Acropora* populations" (Bruckner 2002). Coral restoration techniques have the potential to reverse the population decline and accelerate the re-growth of a reef after a disturbance. These efforts may be especially useful in areas where natural recovery is unlikely due to the lack of sexually reproductive colonies.

Various coral restoration techniques have been tested throughout the Caribbean and other areas around the world. The concept of "coral gardening" or "coral nurseries" where corals are grown in underwater "nurseries" and transplanted back onto degraded reefs has proven to be successful (Rinkevich 2000; Shafir 2006; Amar and Rinkevich 2007). Many methods of coral nursery construction exist including the use of: floating lines, wire frames, and cement platforms (Rinkevich 2000; Bowden Kerby 2001; Shafir 2006). Each of these methods may be better suited to different reef areas based on environmental conditions such as: depth, wave action, and proximity to shore. Restoration practitioners in Florida developed an *Acropora* restoration guide, to outline these different methods and best practices (Johnson et al. 2011). These underwater nurseries can provide a significant amount of coral with limited time and resources (Bowden Kerby 2001; Herlan and Lirman 2008). Coral nurseries can also serve as genetic repositories for corals that may otherwise be lost in the wild due to storms or extreme temperature events (Schopmeyer et al. 2011). A recent study by Young et al. (2012) outlined advantages and concerns associated with coral reef restoration and low-cost methods (e.g. coral

gardening) and fragment stabilization were ranked as most effective. Although growing corals in underwater nurseries has been proven to be successful, determining which areas of the reef to transplant nursery reared corals to has been less documented. Identifying the habitat features and localized interactions at a transplantation site is thought to determine areas which would support the best survivorship (Raymundo 2001; Dizon and Yap 2006).

1.6 PROJECT OVERVIEW

In December 2004, The Nature Conservancy, an environmental non-profit organization, initiated a staghorn coral restoration project funded through the TNC-NOAA Community-based Restoration Program (CRP) which focused on restoring degraded reefs in the Upper Florida Keys through the development of an underwater coral nursery. At the center of this project was a partnership with Sea Life Inc. who privately owned a permitted live rock farm site within Florida Keys National Marine Sanctuary waters where genetically unique coral fragments of naturally occurring staghorn coral had been propagated since 2000. Clones of those parent colonies along with fragments collected from wild colonies in the Upper Keys region were grown out in this underwater coral nursery. After one year, the corals were then transplanted to selected sites located within unique cross shelf zones within the Upper Keys region and monitored for growth and survivorship by Sea Life Inc. and Nature Conservancy staff. These zones were determined using The Nature Conservancy's Florida Reef Resilience Program's spatial framework (Wagner et al. 2010). This framework was developed utilizing existing spatial data sets (including the underlying geology, existing bio-regional characterization,

and mapped reef and hard-bottom areas) combined with expert knowledge, depicting 14 different regions containing reef and hard-bottom habitats. Eleven of these regions were classified further to delineate zones based on cross-shelf position creating a total of 52 classifications.

In August 2006, additional funding was received through the TNC-NOAA Community-based Restoration Program to expand the Upper Keys restoration efforts to be replicated in three more regions of the Florida Reef Tract (Lower Keys, Biscayne, and Broward). At each site, an existing nursery site was established along with partner institutions for coordinating field work linked to the relevant management agency (Broward- Nova Southeastern University/Broward Department of Environmental Protection; Biscayne- University of Miami /Biscayne National Park and Lower Keys- Mote Marine Laboratory/Florida Keys National Marine Sanctuary).

The overall study design for each region was to collect fragments from naturally occurring colonies of *A. cervicornis* that were genetically distinct, grow them in an underwater nursery for one year to produce more fragments, and compare their genetic fitness once outplanted within different zones. These corals were monitored in both the nursery and outplant sites for condition and survivorship, and measured for linear extension (growth).

My thesis used the Upper Keys, Lower Keys and Biscayne regions outplant datasets due to these regions having the same cross-shelf zones (inshore, mid-channel, offshore,

forereef). The Lower Keys nursery dataset was also used to compare differences in growth (linear extension) between genotypes in the nursery. Each of these regions datasets were collected by the managing organizations: 1) Upper Keys (Sea Life Inc. and The Nature Conservancy), 2) Lower Keys (Mote Marine Laboratory) and 3) Biscayne (University of Miami RSMAS). This study, which stems from these unique projects, took advantage of these datasets and focused on the growth and survivorship data of the outplanted nursery corals.

2.0 RATIONALE, GOALS AND HYPOTHESES

2.1 RATIONALE

Due to its threatened status and importance to the health of coral reef ecosystems, recovery of *A. cervicornis* populations is necessary. Natural recovery appears to be limited, therefore, active restoration activities will aid in the recovery of declining populations throughout its range. Understanding what factors influence coral outplant success, will better advance coral restoration efforts.

2.2 GOALS AND HYPOTHESES

For my thesis I used data collected from the Lower Keys, Upper Keys and Biscayne outplanting sites and Lower Keys nursery. The goals were to determine if corals outplanted from a nursery site in each region would experience differences in growth and survivorship based on outplant location (zone) and genotype. Also, I examined whether or not there would be differences in growth in the Lower Keys nursery based on

genotypic differences and if so, compare them to the Lower Keys outplant data. The following hypotheses were tested.

2.2.1 HYPOTHESES

2.2.1.1 OUTPLANTED CORALS WITHIN EACH REGION

Ho 1: There will be no significant difference in growth rate (linear extension) among zones within each region.

Ho 2: There will be no significant difference in growth rate (linear extension) among genotypes within each region.

Ho 4: There will be no significant difference in growth rate (linear extension) among genotypes within the Lower Keys nursery.

Ho 5: There will be no significant difference in survivorship among zones within each region.

2.2.1.2 OUTPLANTED CORALS WITHIN ALL REGIONS

Ho 6: There will be no significant difference in growth rate (linear extension) among zones within all regions.

Ho 7: There will be no significant difference in growth rate (linear extension) between regions.

Ho 8: There will be no significant difference in survivorship among zones within all regions.

3.0 MATERIALS AND METHODS

3.1 NURSERY SET UP

The first staghorn coral, *Acropora cervicornis*, nursery along the Florida Reef Tract was established at Sea Life Inc.'s permitted live rock farm within the Upper Keys region of the Florida Keys National Marine Sanctuary off Tavernier Key, Florida (N24° 58.931', W80° 26.191') in December 2004 ("Sea Life Inc.", 2015). The depth at this nursery is approximately 9m, and is located on a sand bottom habitat. This nursery consists of 22 cement blocks buried into the sand bottom 7-10cm. The blocks were arranged into three rows, with each block spaced 1m apart and 3m between the rows (Figure 1). The blocks were stabilized with 1m rebar stakes secured to both sides of the block with heavy-duty zip ties.

Figure 1: Upper Keys coral nursery block arrangement.

Additional coral nurseries were established in June 2007 in the Biscayne and Lower Keys regions of the Florida Reef Tract (Figure 2).

Figure 2: Map of the Lower Keys, Upper Keys and Biscayne coral nurseries.

All three nursery sites were chosen after considering location depth, proximity to shore, protection from storms and proximity to coral reefs which have or had a historical presence of *A. cervicornis*. Although smaller, similar set-up methods used for the Upper Keys nursery were applied at each of these two additional coral nurseries. The Lower Keys region nursery was established by Mote Marine Laboratory within the waters of the Florida Keys National Marine Sanctuary off Big Pine Key, Florida (N24° 34.171', W81° 19.816). The depth of this nursery is approximately 8m, and is located on a sand bottom

habitat. Ten blocks were buried into the sand bottom 7-10cm and were arranged into two rows with each block spaced approximately 1m apart and 3m between rows (Figure 3). One meter rebar stakes were hammered on both sides of the block and secured using heavy-duty zip ties.

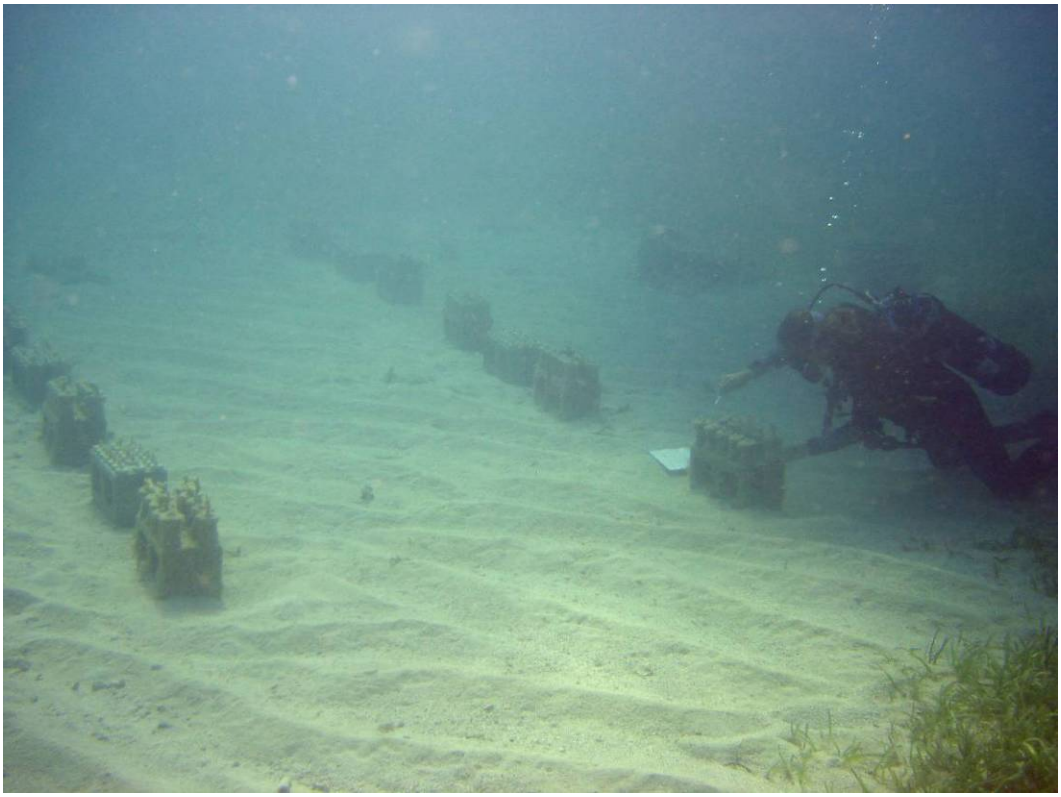

Figure 3: Lower Keys coral nursery block arrangement.

The Biscayne region nursery was established by the University of Miami Rosenstiel School of Marine and Atmospheric Science (RSMAS) within Biscayne National Park off Miami, Florida (N25° 21.753', W80° 09.985'). The depth of this nursery is approximately 6m, and located on a sand bottom habitat. Ten blocks were buried into the sand bottom 7-10cm and were arranged into two rows with each block spaced

approximately 2m apart and 2m between the rows (Figure 4). One meter rebar stakes were hammered on both sides of the block, and secured using heavy-duty zip ties.

Figure 4: Biscayne coral nursery block arrangement.

3.2 PREPARATION OF NURSERY MATERIALS

In preparation for the collection of *A. cervicornis* branch fragments to populate the nurseries, cement pedestals and disks were created for each fragment. Equal parts of white Portland cement and sand were mixed together with water and poured into 9 ounce plastic cups. Cups were filled full to create the pedestals and ¼ full to create the disks. The pedestals and pucks are visible on the top of the blocks in Figures 1, 3 and 4. After the disks had dried, each disk was pre-labeled using a Sharpie permanent marker and then

covered with a 2-part epoxy to preserve the label. The labeling system consists of two numbers separated by a hyphen (Figure 5). The first number refers to the fragment parent colony number, and the second number refers to the fragment number. The labeling system permitted tracking and monitoring of each fragment.

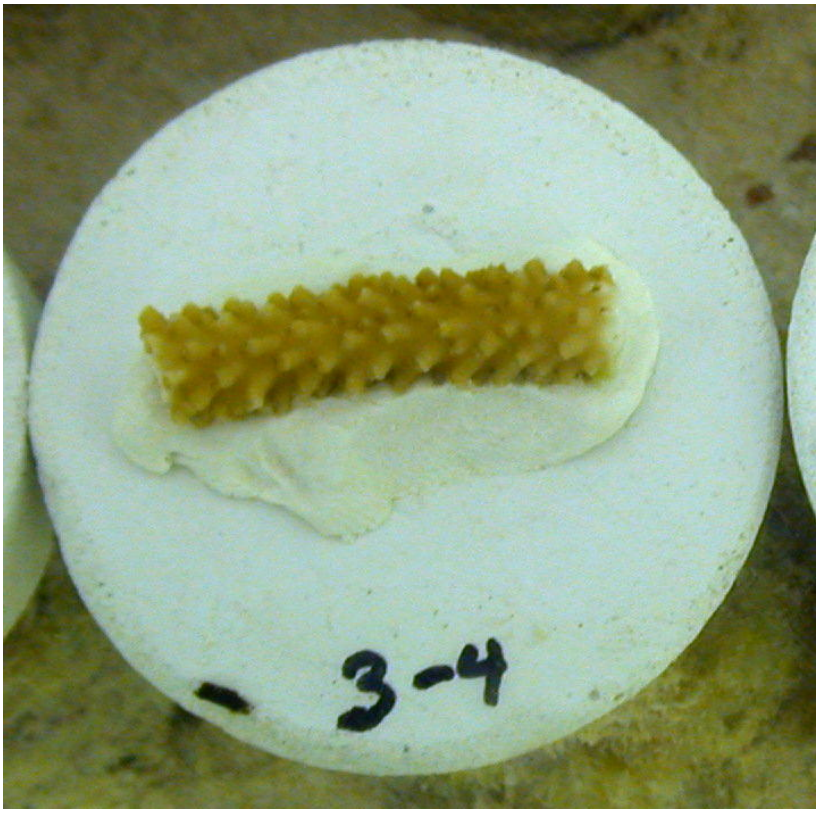

Figure 5: Two number fragment puck labeling system. This example is fragment 4 from parent colony 3.

3.3 CORAL COLLECTIONS

To populate the Upper Keys nursery, 22 wild *A. cervicornis* colonies were sampled in November 2005 within the Upper Keys region (Table 1). Three 10cm coral branch fragments were collected from each of the 22 colonies using pruning shears and placed in labeled plastic containers with lids. The containers were then brought onto the boat and

placed in coolers filled with seawater. The fragments were then transported to the nursery.

Table 1: Upper Keys region parent colony coral collection sites

Region	Parent Colony #	Latitude (dd ddddd)	Longitude (dd ddddd)
Upper Keys	1	25.01848	-80.43943
Upper Keys	2	25.01830	-80.43995
Upper Keys	3	25.00782	-80.44777
Upper Keys	4	25.00782	-80.44777
Upper Keys	5	24.98847	-80.44987
Upper Keys	6	24.98740	-80.45192
Upper Keys	7	25.00955	-80.41497
Upper Keys	8	24.98025	-80.44273
Upper Keys	9	24.94297	-80.49650
Upper Keys	10	24.94297	-80.49650
Upper Keys	11	24.98713	-80.45288
Upper Keys	12	24.95160	-80.48870
Upper Keys	13	24.95210	-80.48876
Upper Keys	14	24.95070	-80.49007
Upper Keys	15	24.95070	-80.49007
Upper Keys	16	24.99163	-80.46368
Upper Keys	17	24.98908	-80.46655
Upper Keys	18	24.98942	-80.46673
Upper Keys	19	24.98787	-80.46785
Upper Keys	20	24.98555	-80.46937
Upper Keys	21	24.89243	-80.54523
Upper Keys	22	24.89312	-80.54822

The three 10cm fragments were fragmented again into 2-3cm fragments and mounted horizontally onto each labeled disk using All-Fix® underwater epoxy (Figure 6). In total, 10 (2-3cm) fragments were collected from each parent colony. Ten pedestals were attached to each of the 22 blocks using All-Fix® underwater epoxy. The disk containing each fragment was then epoxied to its corresponding pedestal. Each cement block then contained 10 nursery colonies (once a branch fragment is mounted in the nursery it is referred to as a nursery colony) each from the same parent colony which facilitated monitoring (Figure 6).

Figure 6: One nursery block containing 10 recently mounted nursery colonies (numbers 1-10) from the same parent colony (number 3).

To create the Lower Keys region nursery, ten wild parent *A. cervicornis* colonies were sampled in June 2007 at ten sites within the Lower Keys region. To create the Biscayne

region nursery, sixteen wild parent *A. cervicornis* colonies were sampled from 11 sites within the Biscayne region (Table 2). The methods used for the Upper Keys collections were replicated in the Lower Keys and Biscayne regions.

Table 2: Lower Keys and Biscayne region parent colony coral collection sites

Region	**Parent Colony #**	**Latitude (dd ddddd)**	**Longitude (dd ddddd)**
Lower Keys	1	24.56937	-81.33028
Lower Keys	2	24.56832	-81.33357
Lower Keys	3	24.56307	-81.401
Lower Keys	4	24.56935	-81.38168
Lower Keys	5	24.52257	-81.51978
Lower Keys	6	24.52298	-81.52043
Lower Keys	7	24.56038	-81.50137
Lower Keys	8	24.58353	-81.43298
Lower Keys	9	24.61440	-81.37895
Lower Keys	10	24.61515	-81.37917
Biscayne	1	25.33911	-80.19263
Biscayne	2	25.3129	-80.19881
Biscayne	3	25.30911	-80.200183
Biscayne	4	25.30686	-80.20245
Biscayne	5	25.31895	-80.1854
Biscayne	6	25.50876	-80.12053
Biscayne	7	25.36315	-80.1658
Biscayne	8	25.59000	-80.09666
Biscayne	9	25.38906	-80.16571
Biscayne	10	25.49521	-80.14368
Biscayne	11	25.38886	-80.16275

Orientation of the fragments on the disks was changed at the Lower Keys and Biscayne nurseries to potentially test for differences in growth and branching. Seventy-five percent of the fragments (n = 75 were mounted on the pucks vertically and 25% (n = 25) percent were mounted horizontally to test these differences which are not included in this study.

3.4 PARENT COLONY GENOTYPE ANALYSES

Less than 1cm of tissue was collected from each of the parent colonies from all regions using pruning shears and placed in 2ml criovials filled with ethanol. The samples were sent to Penn State University for genotype analysis by Dr. Iliana Baums. Analysis was completed using two PCR (Polymerase Chain Reaction) multiplexes, using 4-5 microsatellite loci (Baums et al. 2005). This data was later used to organize and identify the corals in the nursery and to assist with the selection of corals for outplanting.

3.5 NURSERY MONITORING AND MAINTENANCE

Fragment size (length in cm) and number of branches was recorded immediately after mounting in each of the nurseries. Following these initial measurements, monitoring and maintenance of each nursery colony was conducted monthly for a one-year period. During each visit, nursery colony breakage and presence/absence of disease and/or bleaching were recorded. Growth measurements were taken for each nursery colony measuring linear extension (the sum of all branch lengths), and the number of branches was also recorded. All branches 5mm and greater in length were measured and summed for a linear extension measurement per nursery colony (Figure 7).

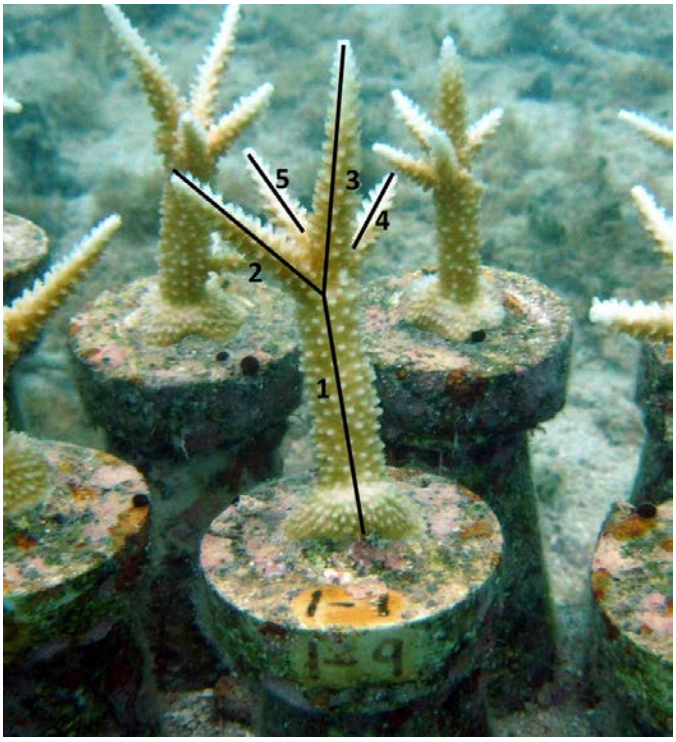

Figure 7: Nursery colony (fragment 9 from parent colony 1) linear extension measurements. Linear extension is the sum of the branch lengths (lines 1-5).

Linear extension measurements were made using calipers or flexible plastic rulers. Maintenance included cleaning all pedestals and disks with wire and nylon brushes to remove algae, sponges, and other types of overgrowth, and removing dead nursery colonies from the blocks. Identifiable broken fragments from nursery colonies were reattached to their corresponding disk and noted as such. Each block was also photographed during each visit for reference. All data was collected on underwater paper and entered into a standardized excel spreadsheet.

3.6 OUTPLANTING

Within each region by taking corals were removed from the nursery and outplanted to reef sites within each of the four zones (inshore, offshore, mid-channel and forereef). Parameters considered for choosing outplant sites in each zone included: depth, historical and/or current presence of *A. cervicornis*, and an adjacent area of sand. Historical and/or current presence of *A. cervicornis,* were considered to increase their chance of survival based on where wild colonies grow currently or in the past. Reef sites adjacent to sand were chosen in the Upper Keys, so that corals could be outplanted to EcoReef® modules that were installed in the sand. Fragments were cut from nursery corals using pruning shears approximately two weeks prior to outplanting. The fragments were then mounted onto new labeled cement disks, and temporarily epoxied to cement blocks. This allowed the recently fragmented corals time to recover from stress prior to being transported to the outplant sites. In the Upper Keys region, four outplant sites were chosen, one within each of the four Upper Keys zones: inshore, mid-channel, offshore, forereef (Table 3; Figure 8).

Table 3: Upper Keys ouptlant sites.

Region	Zone	Site #	Latitude dd ddddd	Longitude dd ddddd	# of Corals Outplanted
Upper Keys	Inshore	1	25.01253	-80.48107	19
Upper Keys	Mid-Channel	2	24.98615	-80.44947	21
Upper Keys	Offshore	3	24.97582	-80.44197	16
Upper Keys	Forereef	4	24.97067	-80.43743	18
Upper Keys	ALL	ALL	ALL	ALL	74

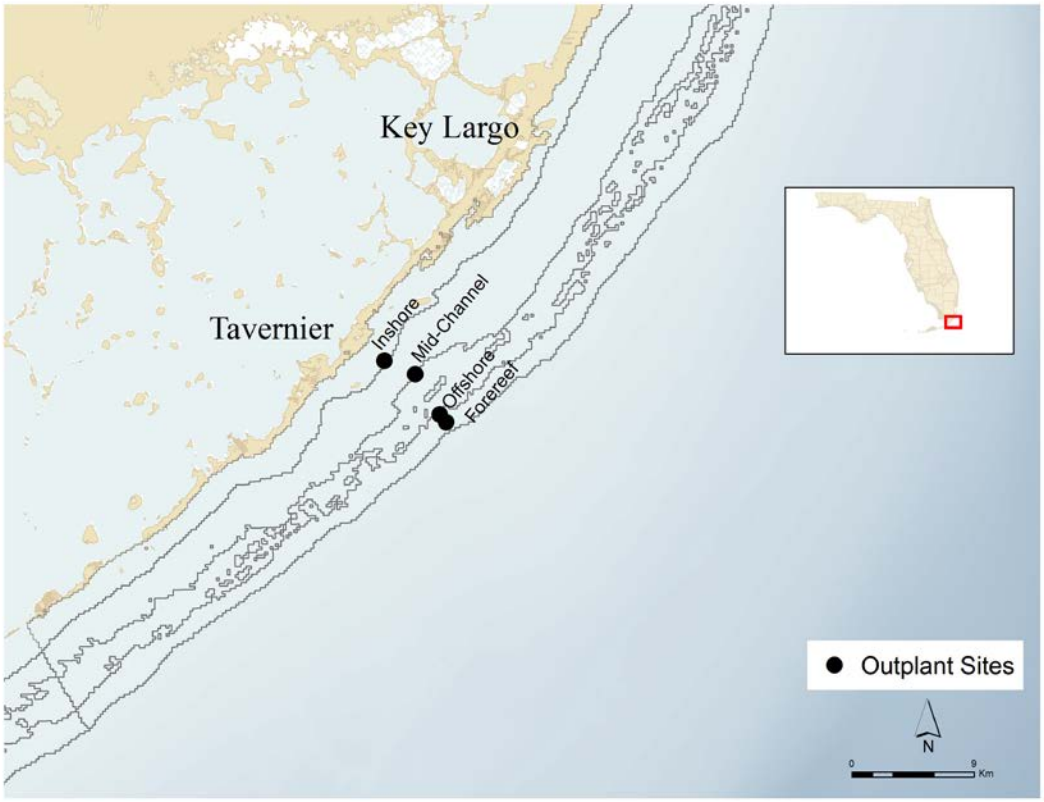

Figure 8: Map of the Upper Keys region outplant sites

In April 2007, five EcoReef® modules were anchored into sand with a 1m wire duck-billed anchor adjacent to a patch reef at each of the four outplant sites in the Upper Keys. EcoReef® modules were used to provide an artificial platform for the outplanted coral to grow on that was similar in structure to *A. cervicornis* versus attaching them directly to natural bare substrate. These modules were also used to avoid predation or interaction with reef algae during this study. In May 2007, 74 coral fragments 8-28cm in length, were then transported to four outplant sites. The disks on which the coral fragments were attached in the nursery were pried from their pedestal using a blunt tip knife, brought to the water's surface in a large plastic bin, and placed in coolers of seawater on the boat. Coral fragment disks were then epoxied to each of the EcoReef modules (Figure 9). Each outplant site contained at least two replicates of seven distinct genotypes. This provided a total of 74 corals of 7 genotypes that were monitored as part of this study (Table 3).

Figure 9: Six coral fragments epoxied to the EcoReef modules in the Upper Keys.

In the Lower Keys region, four outplant sites were also chosen, one within each of the four reef zones: inshore, mid-channel, offshore, forereef (Table 4; Figure 10).

Table 4: Lower Keys outplant sites

Region	**Zone**	**Site #**	**Latitude dd ddddd**	**Longitude dd ddddd**	**# of Corals Outplanted**
Lower Keys	Inshore	1	24.62163	-81.36238	30
Lower Keys	Mid-Channel	2	24.59598	-81.37146	28
Lower Keys	Offshore	3	24.56306	-81.40100	29
Lower Keys	Forereef	4	24.55206	-81.38368	26
Lower Keys	ALL	ALL	ALL	ALL	113

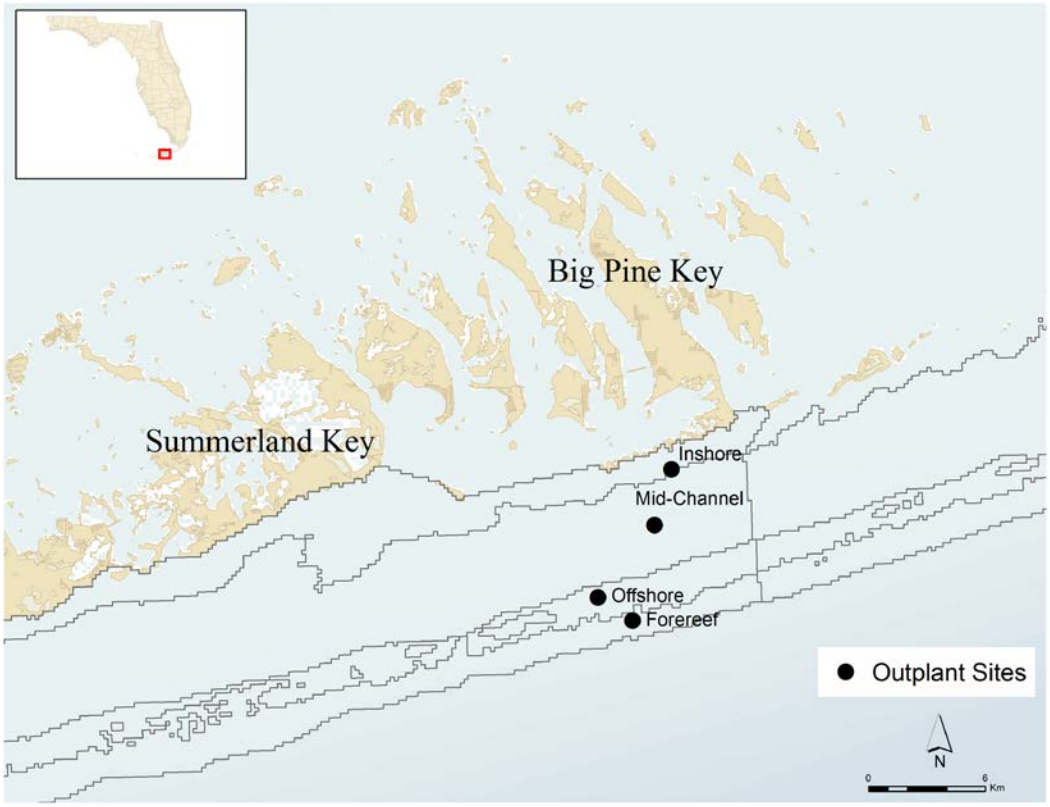

Figure 10: Map of the Lower Keys region outplant sites.

In May 2008, approximately 30 coral fragments 3-5cm in length, were transported to each ouptlanting site and epoxied directly to the reef. At each of the four outplant sites, outplant corals were arranged in three clusters of 10 corals (Figure 11) with each cluster containing a replicate of the 10 distinct genotypes. To determine each outplant coral location and placement within each cluster, each was assigned a number and randomly placed using a random number generator. Clusters were spaced approximately 1m apart and corals were spaced approximately 10cm apart (Figure 11).

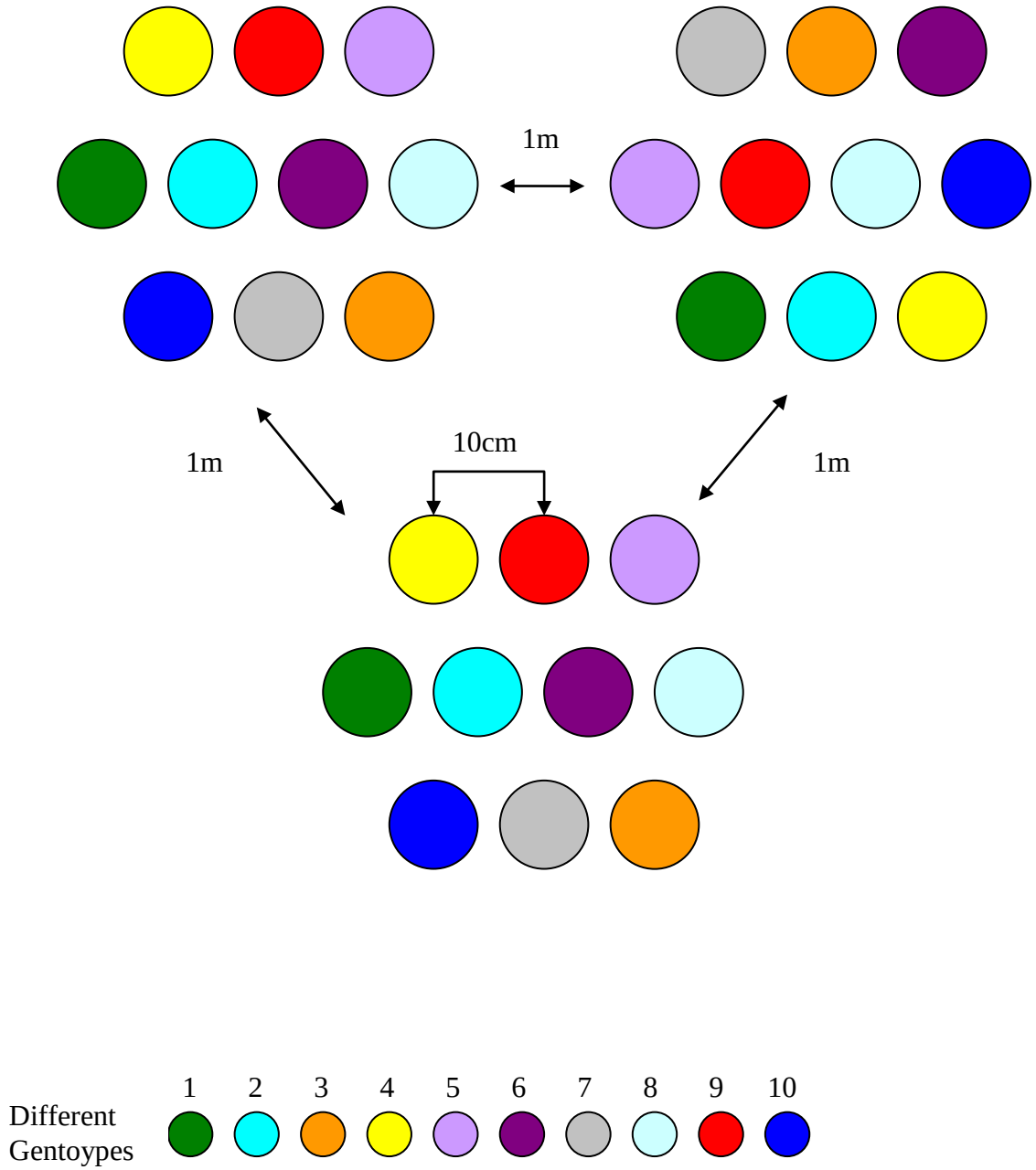

Figure 11: Three cluster arrangement at each outplant site in the Lower Keys. Each coral is approximately 10cm apart, and clusters are approximately 1m apart.

In the Biscayne region, four outplant sites were also chosen, one within each of the four reef zones: inshore, mid-channel, offshore, forereef (Table 5; Figure 12).

Table 5: Biscayne region outplant sites

Region	**Zone**	**Site #**	**Latitude dd ddddd**	**Longitude dd ddddd**	**# of Corals Outplanted**
Biscayne	Inshore	1	25.36131	-80.18851	24
Biscayne	Mid-Channel	2	25.36431	-80.17638	24
Biscayne	Offshore	3	25.36256	-80.16641	24
Biscayne	Forereef	4	25.37316	-80.13655	24
Biscayne	ALL	ALL	ALL	ALL	96

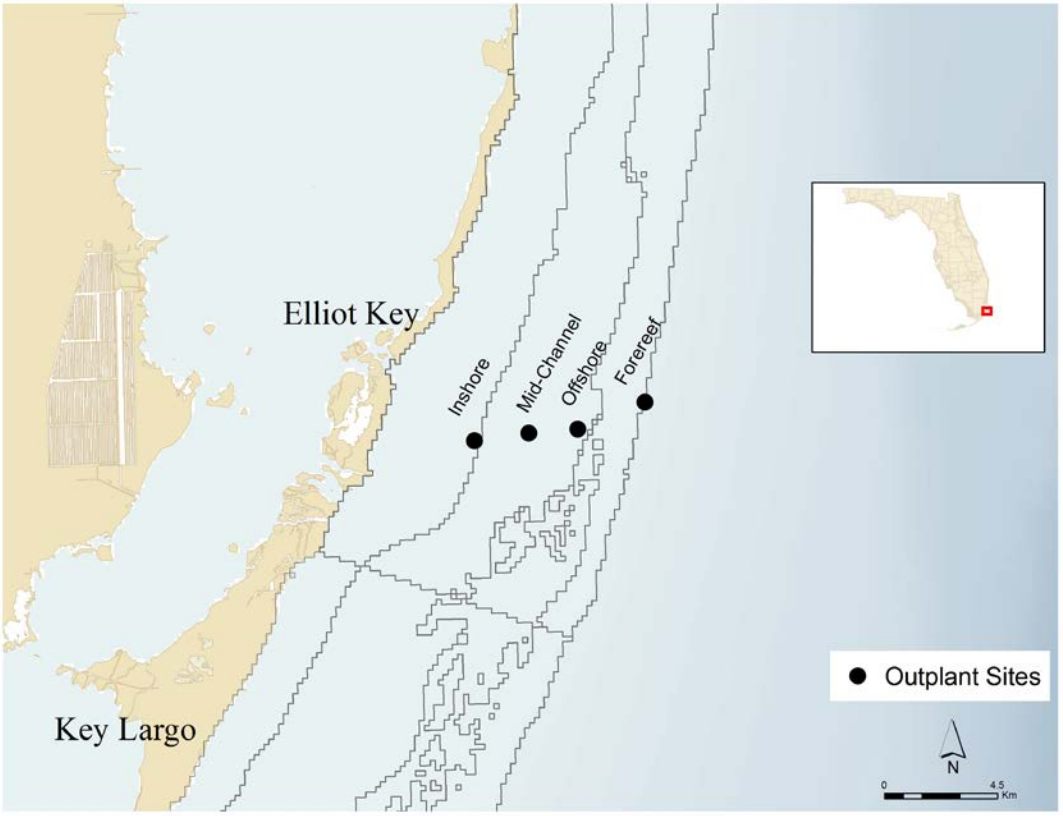

Figure 12: Map of the Biscayne region outplant sites.

In July 2008, 24 coral fragments 7-10cm in length, were transported to each ouptlant site and epoxied directly to the reef. The corals were also arranged in three clusters of 8 corals (Figure 13) with each cluster containing a replicate of the 8 distinct genotypes. To determine each coral fragment's outplanting location and placement within each cluster, each fragment was assigned a number and randomly placed using a random number generator. The spacing between the clusters and corals were different than the Lower Keys and Upper Keys region. Clusters were spaced approximately 2m apart and corals were spaced approximately 50cm apart (Figure 13).

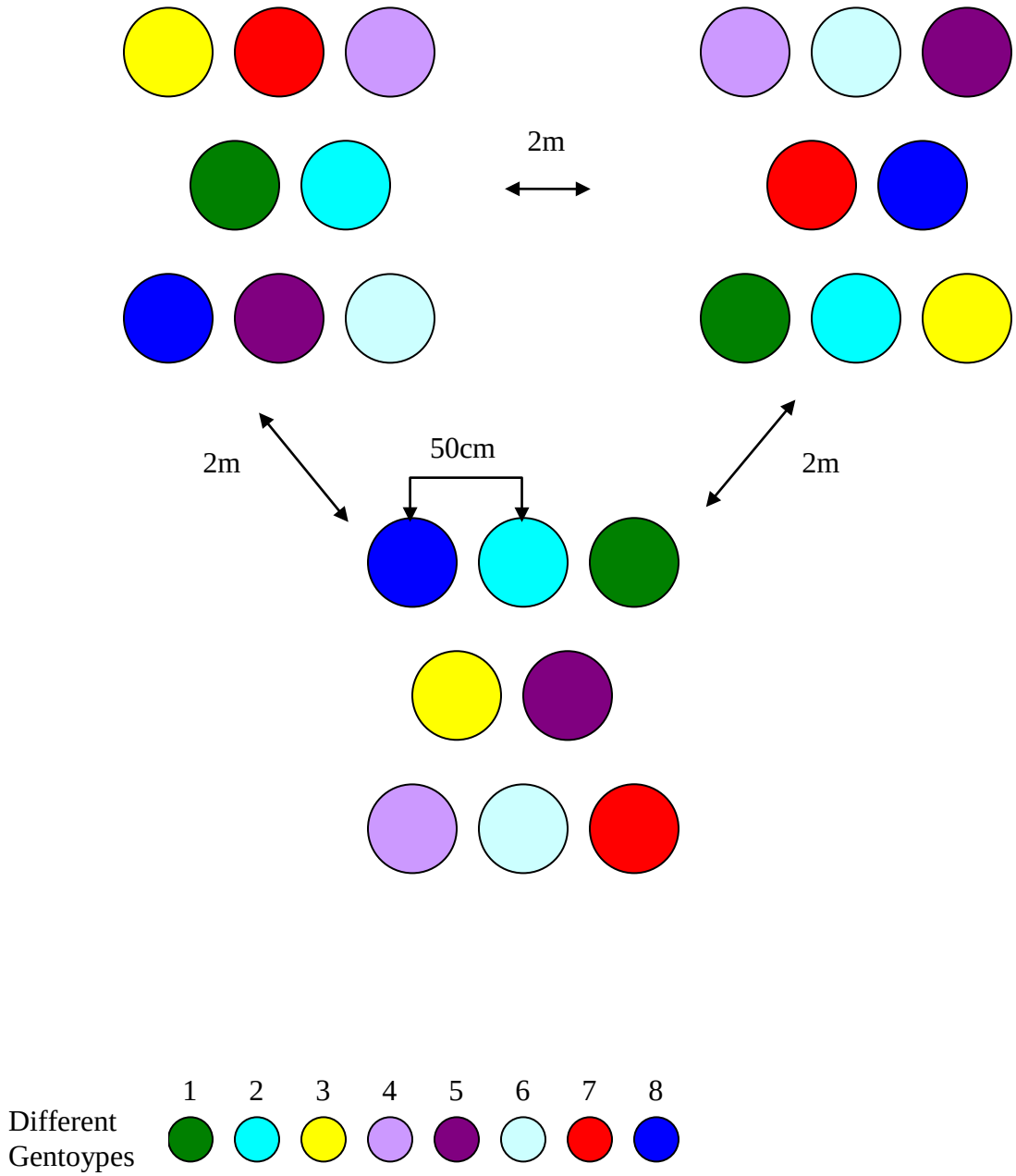

Figure 13: Three cluster arrangement at each outplanting site in Biscayne. Each coral is approximately 50cm apart, and clusters are approximately 2m apart.

3.7 OUTPLANT SITE MONITORING AND MAINTENANCE

In all regions, outplant monitoring consisted of each coral being monitored for breakage and presence of disease and/or bleaching. Linear extension was measured using calipers or flexible plastic rulers and number of branches was recorded. All coral disks were cleaned with wire and nylon brushes to remove algae and sponge overgrowth. Any broken outplanted corals that could be identified were re-attached to their corresponding disk. Each coral fragment was also photographed during each visit for reference. All data was collected on underwater paper and entered into a standardized excel spreadsheet.

In the Upper Keys region, the outplant sites were monitored and maintained in May 2007 and November 2007. Total linear extension of each coral was recorded immediately after the corals were mounted on the EcoReef® modules. In the Lower Keys region, the outplant sites were monitored and maintained every three months from May 2008 to November 2008. Initial measurements of each coral were recorded immediately after the corals were epoxied onto the reef. In the Biscayne region, the outplant sites were monitored and maintained in July 2008 and November 2008. Initial measurements of each coral were recorded immediately after the corals were epoxied onto the reef, similar to the Lower Keys region.

3.8 STATISTICAL ANALYSES

All data was analyzed using JMP 9.0 statistical software. Growth (linear extension) rate was calculated in cm per month prior to statistical analysis, for each coral, within each of the regions (Upper Keys, Lower Keys and Biscayne) using Excel. Linear extension rate was determined for each coral by subtracting the initial linear extension measurement from the final linear extension measurement for each coral, and dividing that number by the number of months between measurements. To determine growth rate significance by zone within each region, a one-way ANOVA was used for each dataset including and excluding corals that experienced breakage and/or complete mortality. Corals experiencing breakage were included in the first set of analyses for each region to determine the effect breakage had on zone linear extension rates. To determine significant differences in growth rates among genotypes within each region (Upper Keys, Lower Keys, and Biscayne) a one-way ANOVA was used for the dataset which only included outplant colonies which did not experience breakage or mortality. A one-way analysis and means comparison (using Tukey-Kramer HSD) was used to compare mean growth rates among zone within each region including and excluding outplant colonies which experienced breakage and/or mortality. A one-way analysis and means comparison (using Tukey-Kramer HSD) was used to compare mean growth rates in each region by genotype, only excluding corals with breakage and complete mortality. A final one-way analysis and means comparison (using Tukey-Kramer HSD) was used to compare genotype mean growth rates in the Lower Keys nursery only including nursery colonies which did not experience breakage and/or mortality.

Percent survivorship was determined in each of the regions Excel spreadsheets and entered into simple tables, showing the original and final (n). Since there was only one monitoring event (6 months) in the Upper Keys and Biscayne (4 months), and two monitoring events in the Lower Keys (3 and 6 months), a survival curve analysis could not be completed.

4.0 OUTPLANT RESULTS

4.1 UPPER KEYS REGION

4.1.1 GROWTH

Fragments from nursery corals were outplanted to four reef sites in the Upper Keys region with one site in each of four zones (inshore, mid-channel, offshore and forereef) in May 2007. Once relocated to an outplant site, fragments are referred to as outplant corals. All outplanted corals (n=74) were measured in May 2007 for linear extension immediately after outplanting, and in November 2007. The nursery coral fragments (outplant corals) were 8- 28 cm in length. The range of sizes collected from nursery colonies was large at this nursery site. The size of the nursery coral fragment, and therefore the initial size of the outplant colony, was determined by the size of the nursery colony. Larger fragments were removed from larger nursery colonies. Several genotypes in the nursery had larger colonies. Due to these size differences, a significant difference in mean fragment (outplant colony) size was determined among genotypes ($p<0.0005$; $F=4.6693$; $df=6$).

Table 6: Upper Keys region genotype mean fragment linear extension of corals outplanted from the nursery.

Genotype	Mean Fragment Size (cm)	# Corals (n)
1	15.41 ± 6.82	17
2	11.47 ± 5.89	9
3	14.11 ± 4.49	9
4	19.78 ± 4.10	8
5	8.53 ± 4.25	9
6	26.29 ± 13.82	11
7	17.22 ± 12.41	10

A total of 74 coral fragments were outplanted to four sites, representing seven different genotypes. Each of these seven genotypes was replicated with at least two corals at each site (Table 7). These seven genotypes (G1-G7) are not the same as genotypes (G1-G7) in the Lower or Biscayne regions, but distinct genotypes in the Upper Keys. Throughout the monitoring period (May 2007-November 2007) nine corals were broken due to storms or other unknown causes (Table 7). Eight corals experienced complete mortality. These factors negatively affect linear extension (measured growth rate), and therefore, two data sets were analyzed: 1) including all corals (n=74) and, 2) corals without complete mortality and/or breakage (n=56). The first data set represents a more natural-common population that experiences fragmentation and mortality while the second data set permitted an evaluation of growth during an essentially ideal situation.

Table 7: Upper Keys region number of each genotype outplanted to each zone and the number of broken corals and corals with complete (dead) mortality identified though the monitoring period (May 2007- November 2007) (G=genotype).

Zone	G1	G2	G3	G4	G5	G6	G7	Total	# Broken	# Dead
Inshore	3	3	3	2	2	3	3	19	2	1
Mid-channel	6	2	3	2	2	3	3	21	4	1
Offshore	4	2	2	2	2	2	2	16	2	5
Forereef	4	2	2	2	3	3	2	18	2	1
Total	**17**	**9**	**10**	**8**	**9**	**11**	**10**	**74**	**10**	**8**

The overall mean growth rate was 1.89 ± 1.76 cm/month for all corals (n= 74). There were significant differences in growth rates among zones ($p<0.0027$; $F= 5.1955$; $df=3$). The forereef (2.83 ± 2.08 cm/month) had significantly higher mean growth rates than the

offshore (1.15 ± 1.24 cm/month) and inshore (1.08 ± 0.84 cm/month) zones, but not the mid-channel (2.34 ± 1.92 cm/month) zone (Figure 14; Table 8).

After excluding corals with complete mortality and/or breakage (n=56), significant differences among zones was still determined ($p<0.0042$; $F= 4.9346$; $df=3$) (Figure 14; Table 9). This data showed that the forereef (3.10 ± 2.14 cm/month) and mid-channel (2.95 ± 1.67 cm/month) zones had a significantly higher mean growth rate than the inshore (1.26 ± 0.77 cm/month), but not the offshore (1.80 ± 1.18 cm/month) zone (Figure 14; Table 9).

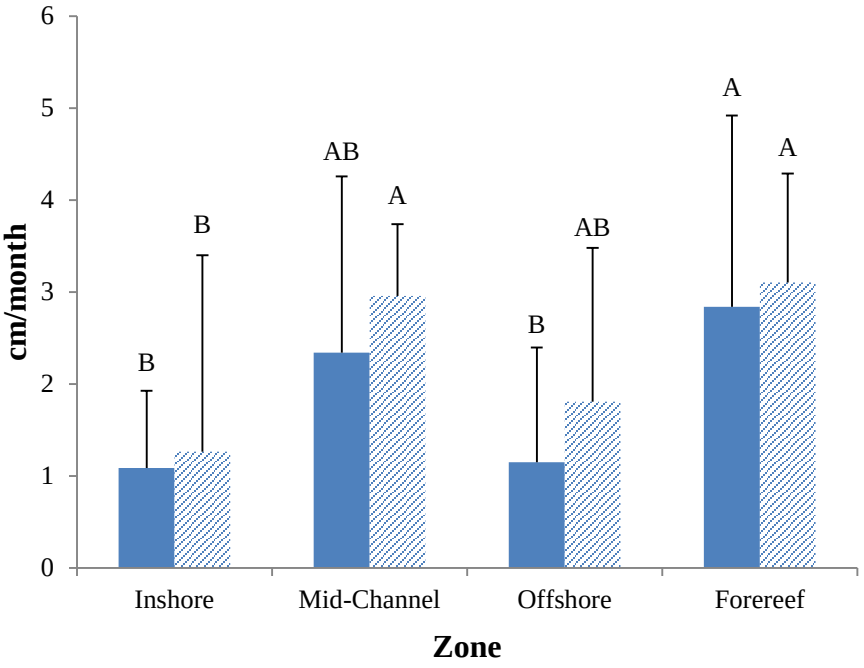

Figure 14: Upper Keys region mean growth rate (cm/month ± SD) by zone, May 2008 - November 2008. Means with different letters are significantly different from others in the same group (Tukey's HSD $p<0.05$). Solid bars include all corals. Hashed bars only include corals without breakage or complete mortality.

Table 8: Upper Keys region statistic post-hoc test (Tukey's HSD p<0.05) summary table for growth rates between zones including all corals (n=74). p-Values denoted with an * are significant.

Zone	Zone	p-Value
Forereef	Inshore	<0.0001*
Offshore	Inshore	0.0246*
Mid-Channel	Inshore	0.0512
Forereef	Mid-Channel	0.2235
Forereef	Offshore	0.3302
Offshore	Mid-Channel	0.9945

Table 9: Upper Keys region statistic post-hoc test (Tukey's HSD p<0.05) summary table for growth rates between zones excluding corals with breakage or complete mortality (n=56). p-Values denoted with an * are significant.

Zone	Zone	p-Value
Forereef	Inshore	0.0096*
Mid-Channel	Inshore	0.017*
Forereef	Offshore	0.2115
Mid-Channel	Offshore	0.2979
Offshore	Inshore	0.8363
Forereef	Mid-Channel	0.9938

A one-way ANOVA of growth rate by genotype for all outplanting zones pooled together in the Upper Keys region revealed that genotypes had significantly different growth rates (p>0.0202; F=2.7975; df=6) (Figure 15; Table 10). This data was analyzed excluding corals with breakage or complete mortality (n=56) (Table 11). Growth rates ranged from 0.90 to 3.65 cm/month, and only two genotypes differed significantly. Genotype 6 had a significantly higher growth rate (3.65 ± 1.70 cm/month) than genotype 5 (0.90 ± 0.89 cm/month) (Figure 15).

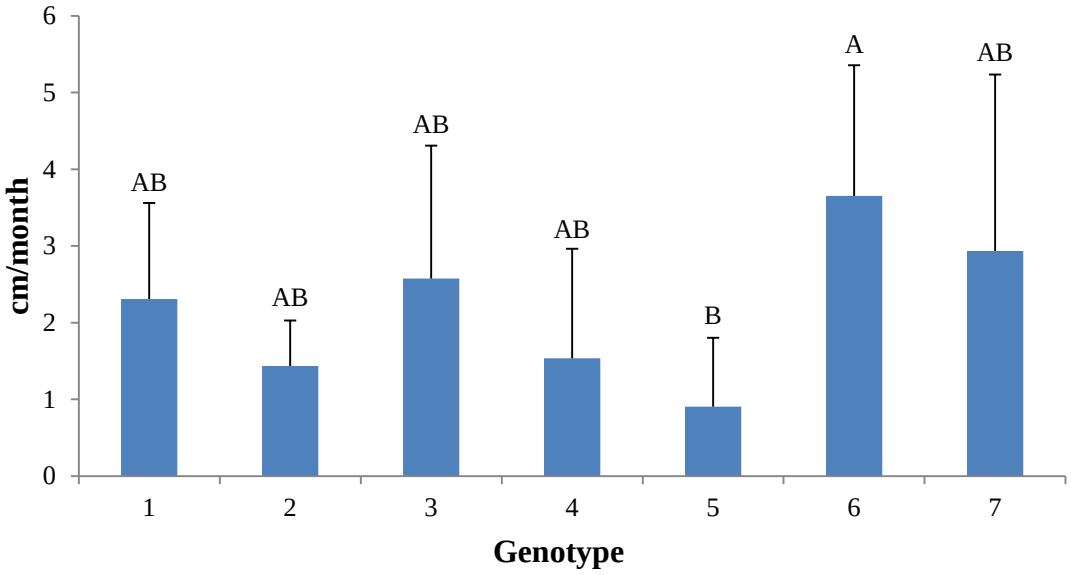

Figure 15: Upper Keys region mean growth rate (cm/month ± SD) by genotype, May 2008 - November 2008. Means with different letters are significantly different from others in the same group (Tukey's HSD $p<0.05$).

Table 10: Upper Keys region statistic post-hoc test (Tukey's HSD $p<0.05$) summary table for growth rates between genotypes excluding corals with breakage or complete mortality (n=56). p-Values denoted with an * are significant.

Genotype	Genotype	p-Value
6	5	0.0264*
6	2	0.096
6	4	0.1615
7	5	0.1808
3	5	0.4822
7	2	0.4683
1	5	0.5808
7	4	0.603
6	1	0.4854
3	2	0.8218
6	3	0.8189
3	4	0.8944
1	2	0.9106
1	4	0.9585
6	7	0.9525

Table 10: (Continued)

Genotype	Genotype	p-Value
4	5	0.9923
7	1	0.9685
2	5	0.9962
7	3	0.9992
3	1	0.9998
4	2	1

Table 11: Upper Keys region number of each genotype outplanted to each zone that were not excluded due to breakage and/or complete mortality (n= 56) during the monitoring period (May 2007- November 2007) (G=genotype)

Zone	G1	G2	G3	G4	G5	G6	G7	Total
Inshore	2	3	2	2	2	2	3	16
Mid-Channel	4	2	3	1	0	3	3	16
Offshore	2	0	1	2	1	1	2	9
Forereef	3	2	1	1	3	3	2	15
Total	**11**	**7**	**7**	**6**	**6**	**9**	**10**	**56**

4.1.2 SURVIVORSHIP

Nursery corals were outplanted in May 2007 and survivorship was recorded in November 2007 (n=74). Overall survivorship of all corals was 89.2% (Table 12). The offshore zone had the lowest survivorship at 68.8%, while the other three zones were approximately 95% (Table 12). White-band disease occurred at the offshore outplant site in June 2007, right after outplanting occurred which greatly contributed to mortality (5 of 16 corals).

Table 12: Table of Upper Keys outplanted corals percent survivorship per zone. Data was collected from May 2007 through November 2007.

Region	Zone	Initial n (May 2007)	Final n (November 2007)	% Survivorship
Upper Keys	Inshore	19	18	94.7
Upper Keys	Mid-Channel	21	20	95.2
Upper Keys	Offshore	16	11	68.8
Upper Keys	Forereef	18	17	94.4
Upper Keys	**All Zones**	**74**	**66**	**89.2**

4.2 LOWER KEYS REGION

4.2.1 GROWTH

Fragments from nursery corals were outplanted to four sites in the Lower Keys region with one site in each of four reef zones (inshore, mid-channel, offshore and forereef) in May 2008. Nursery coral fragments were 3-5cm in length. All outplanted corals (n=113; Table 13) were measured for linear extension in May 2008, immediately after outplanting, and three month and six month after outplanting. There were no significant differences between mean initial outplant coral size among genotypes ($p>0.05$; $F=.8875$; $df=9$). Ten different genotypes were outplanted to each site, and each genotype had 2 or 3 replicates (Table 13).

Table 13: Lower Keys region number of each genotype outplanted to each zone and the number of broken corals and corals with complete (dead) mortality identified through the monitoring period (May 2008- November 2008) (G=genotype)

Zone	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	Total	# Broken	# Dead
Inshore	3	3	3	3	3	3	3	3	3	3	30	6	5
Mid-Channel	3	2	3	3	3	3	2	3	3	3	28	1	8
Offshore	3	3	3	3	3	3	2	3	3	3	29	1	2
Forereef	3	2	2	3	3	2	2	3	3	3	26	3	6
Total	**12**	**10**	**11**	**12**	**12**	**11**	**9**	**12**	**12**	**12**	**113**	**11**	**21**

Throughout the monitoring period (May 2008-November 2008) 11 corals experienced breakage due to storms or other unknown causes and 21 experienced complete mortality (Table 13). These factors negatively affected linear extension (measured growth rate), and therefore, two data sets were analyzed: 1) including all corals (n=113) and, 2) only corals that did not experience fragmentation or complete mortality (n=81).

The overall mean growth rate was 0.45 ± 0.58 cm/month for all corals (n= 113). There were significant differences in growth rates among zones ($p<0.002$ $F= 7.0214$; $df=3$). The offshore (0.51 ± 0.55 cm/month) and forereef (0.76 ± 0.61 cm/month) zones had significantly higher growth rates than the inshore zone (0.11 ± 0.38 cm/month) (Figure 16; Table 14). The mid-channel zone (0.47 ± 0.58 cm/month) did not have significantly different growth rates than any of the zones. After excluding corals which experienced breakage or complete mortality from the analysis, significant differences in growth rates among zones was still determined ($p<.0001$ $F= 7.8168$; $df=3$). However, the zones which had significantly different mean growth rates changed, the mid-channel (0.74 ± 0.51 cm/month) was now significantly different from the inshore zone (0.32 ± 0.28

cm/month) (Figure 16; Tables 14 and 15). This data showed that the forereef (0.95 ± 0.44 cm/month), offshore (0.75 ± 0.43 cm/month) and mid-channel (0.74 ± 0.51 cm/month) zones had significantly higher mean growth rates than the inshore (0.31 ± 0.28 cm/month) zone (Figure 16; Table 15). The forereef zone also had the highest mean growth rate (0.95 ± 0.44 cm/month) (Figure 16).

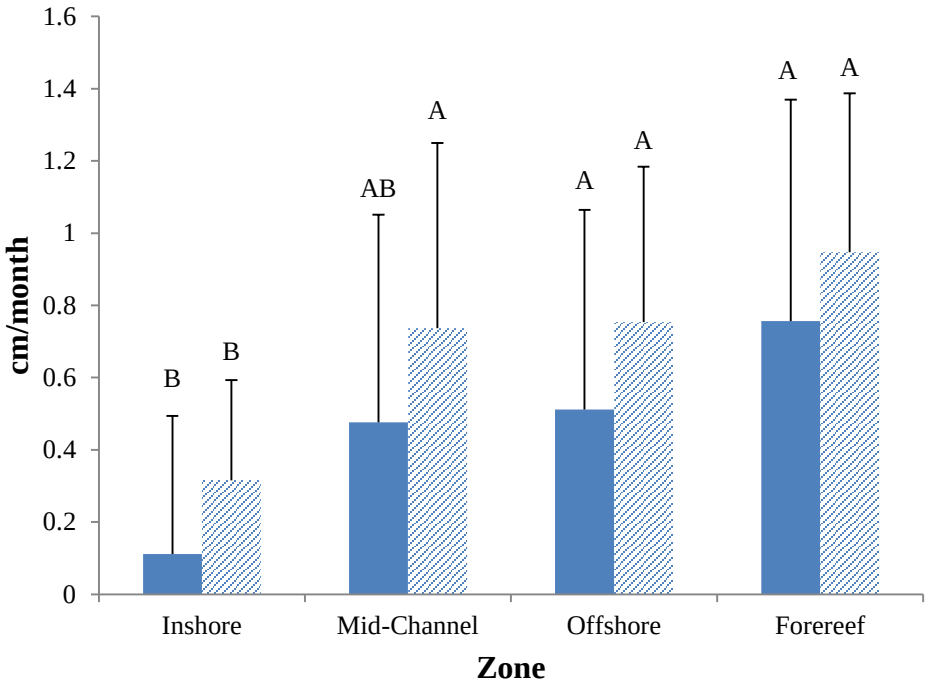

Figure 16: Lower Keys region mean growth rate (cm/month ± SD) by zone, May 2008 - November 2008. Means with different letters are significantly different from others in the same group (Tukey's HSD $p<0.05$). Solid bars include all corals. Hashed bars only include corals without fragmentation or partial or full mortality.

Table 14: Lower Keys region statistic post-hoc test (Tukey's HSD p<0.05) summary table for growth rates between zones including all corals (n=113). p-Values denoted with an * are significant.

Zone	Zone	p-Value
Forereef	Inshore	<0.0001*
Offshore	Inshore	0.0246*
Mid-Channel	Inshore	0.0512
Forereef	Mid-Channel	0.2235
Forereef	Offshore	0.3302
Offshore	Mid-Channel	0.9945

Table 15: Lower Keys region statistic post-hoc test (Tukey's HSD p<0.05) summary table for growth rates between zones excluding corals with breakage or complete mortality (n=81). p-Values denoted with an * are significant.

Zone	Zone	p-Value
Forereef	Inshore	<0.0001*
Offshore	Inshore	0.0089*
Mid-Channel	Inshore	0.0160*
Forereef	Mid-Channel	0.3965
Forereef	Offshore	0.4491
Offshore	Mid-Channel	0.9993

With each genotype pooled among zones, no genotypes had significantly different growth rates (p>0.127 F=1.6355; df=9) (Figure 17). This data was only analyzed excluding corals experiencing breakage and/or complete mortality (n=81) (Table 16).

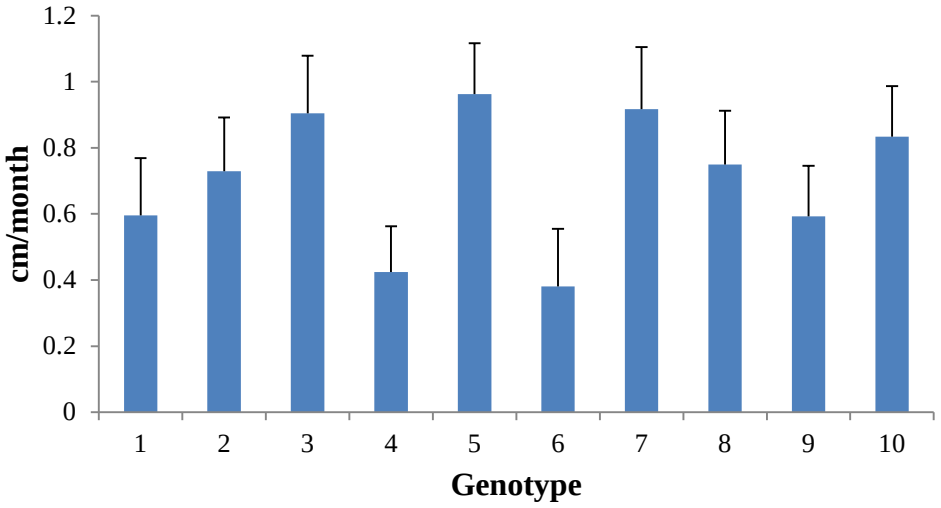

Figure 17: Lower Keys region mean growth rate (cm/month± SD) by genotype, May 2008 - November 2008. No significant difference was determined among genotypes ($p>0.127$ $F=1.6355$; $df=9$).

Table 16: Lower Keys region number of each genotype outplanted to each zone that were not excluded due to breakage and/or complete mortality (n= 81) during the monitoring period (May 2007- November 2007) (G=genotype).

Zone	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	Total
Inshore	2	3	2	2	2	2	1	1	2	2	19
Mid-Channel	1	2	2	3	2	2	2	2	1	2	19
Offshore	2	1	1	3	2	2	2	2	3	3	21
Forereef	2	2	2	3	3	1	1	3	3	2	22
Total	**7**	**8**	**7**	**11**	**9**	**7**	**6**	**8**	**9**	**9**	**81**

4.2.1.1 GROWTH BY GENOTYPE WITHIN THE NURSERY

Linear extension of all corals (n=100) was measured quarterly in the Lower Keys nursery from May 2007 – May 2008. A one-way ANOVA analysis of growth rate (cm/month) by genotype within the Lower Keys nursery showed that genotypes had significantly different growth rates ($p<.0001$ $F=6.5642$; $df=9$) (Figure 18; Table 17). This data was analyzed excluding corals with breakage and/or complete mortality (n=76). Genotypes 6 and 8 had significantly higher growth rates than other genotypes at 0.79 ± 0.32 cm/month and 0.67 ± 0.23 cm/month (Figure 18; Table 17).

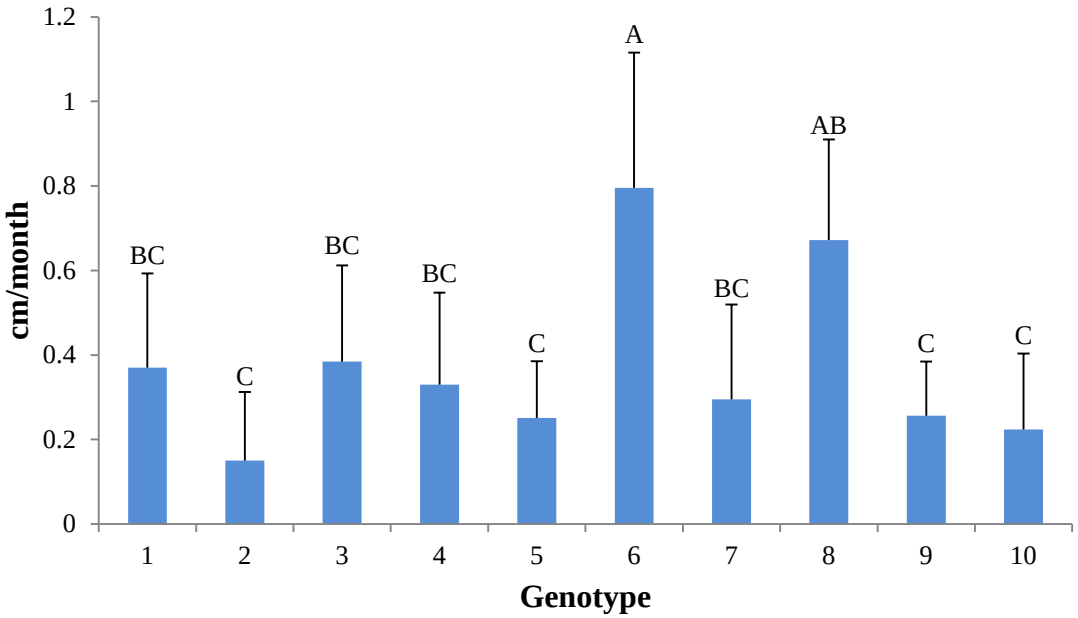

Figure 18: Lower Keys region nursery mean growth rate (cm/month± SD) by genotype, May 2008 - November 2008. Means with different letters are significantly different from others in the same group (Tukey's HSD $p<0.05$).

Table 17: Lower Keys region nursery statistic post-hoc test (Tukey's HSD $p<0.05$) summary table for growth rates between genotypes excluding corals without breakage or complete mortality (n=76). p-Values denoted with an * are significant.

Genotype	Genotype	p-Value
6	2	<.0001*
6	10	0.0005*
6	5	0.0001*
6	9	0.0001*
8	2	0.0066*
6	7	0.0005*
6	4	0.0006*
8	10	0.0353*
6	1	0.0025*
8	5	0.0211*
8	9	0.0239*
6	3	0.0126*
8	7	0.0592
8	4	0.0896
8	1	0.1998
8	3	0.3558
3	2	0.7067
1	2	0.7022
4	2	0.8823
3	10	0.9589
1	10	0.9652
7	2	0.9743
3	5	0.9725
3	9	0.9786
6	8	0.9849
1	5	0.9769
1	9	0.9827
9	2	0.9972
4	10	0.9963
5	2	0.9981
3	7	0.9985
4	5	0.9989
1	7	0.9992
10	2	0.9999
4	9	0.9993
7	10	0.9999
3	4	1
7	5	1
1	4	1

Table 17: (Continued)

Genotype	Genotype	p-Value
7	9	1
4	7	1
9	10	1
5	10	1
3	1	1
9	5	>1

4.2.2 SURVIVORSHIP

Survivorship of outplanted corals was monitored from May 2008 - November 2008 (n=113). Survivorship was highest in the Lower Keys forereef zone at 92.3%, and lowest in the Mid-Channel zone at 71.4% (Table 18).

Table 18: Percent survivorship of outplanted corals within the Lower Keys region per zone. Data was collected from May 2008 through November 2008.

Region	Zone	Initial n (May 2008)	Final n (November 2008)	% Survivorship
Lower Keys	Inshore	30	25	83.3
Lower Keys	Mid-Channel	28	20	71.4
Lower Keys	Offshore	26	24	79.3
Lower Keys	Forereef	29	23	92.3
Lower Keys	All Zones	113	92	81.4

4.3 BISCAYNE REGION

4.3.1 GROWTH

Fragments from nursery corals were outplanted to four sites in the Biscayne region with one site in each of four zones (inshore, mid-channel, offshore and forereef) in July 2008. All outplanted corals (n=96) were measured in July 2008 for linear extension immediately after outplanting and in November 2008. No three month monitoring event occurred. The outplant corals were 7-10cm in length. Despite these slight size differences there were no significant differences between mean initial coral size among genotypes ($p>0.0723$; $F=1.9449$; $df=7$).

A total of 96 corals were outplanted to four sites, representing seven distinct genotypes in the Biscayne region. Each of the seven genotypes was replicated (3 corals) at each site (Table 19). Throughout the monitoring period (July 2008-November 2008) 56 fragments were broken due to storms or other unknown causes (Table 19). Ten fragments experienced complete mortality. These factors negatively affect linear extension (measured growth rate), and therefore, two data sets were analyzed: 1) including all corals (n=96) and, 2) corals without complete mortality and/or breakage (n=30).

Table 19: Biscayne region number of each genotype outplanted to each zone and the number of broken corals and corals with complete (dead) mortality identified through the monitoring period (July 2008- November 2008) (G=genotype).

Zone	G1	G2	G3	G4	G5	G6	G7	G8	Total	# Broken	# Dead
Inshore	3	3	3	3	3	3	3	3	24	11	4
Mid-channel	3	3	3	3	3	3	3	3	24	16	1
Offshore	3	3	3	3	3	3	3	3	24	18	2
Forereef	3	3	3	3	3	3	3	3	24	11	3
Total	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**96**	**56**	**10**

The overall mean growth rate was -0.4 ± 1.23 cm/month for all corals (n=96) due to the large number of broken corals (Table 19). There were significant differences in growth rates among zones ($p<0.0201$ $F= 3.4377$; $df=3$). The offshore zone (-1.07 ± 1.01 cm/month) was significantly different from the inshore zone (0.01 ± 1.32 cm/month), but not the mid-channel (-0.35 ± 0.90 cm/month) or forereef (-0.22 ± 1.58 cm/month) zones (Figure 19; Table 20).

After excluding broken corals (n=30), the data was not significant ($p>.0796$; $F= 2.5240$; $df=3$) showing that no zones had significantly different growth rates (Figure 19).

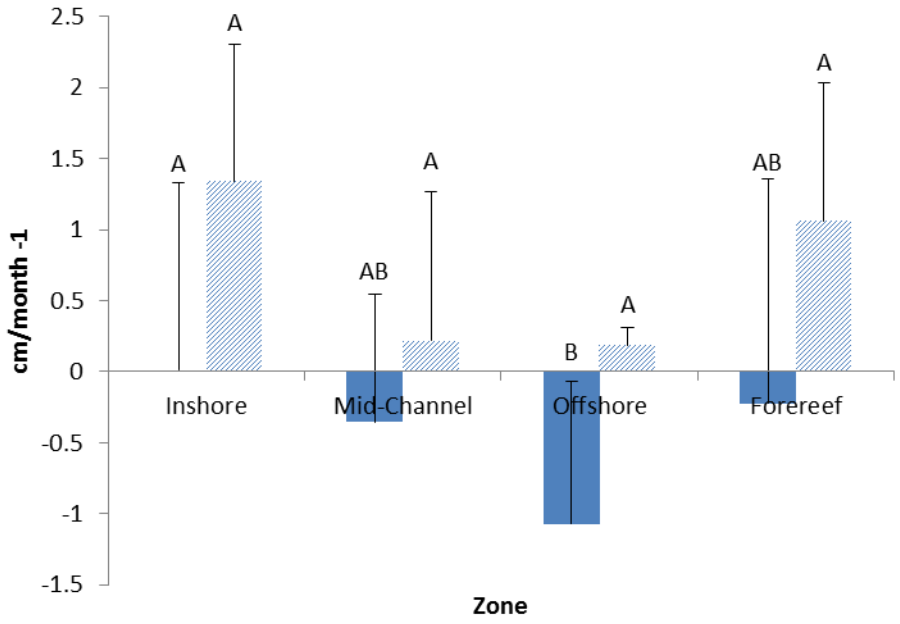

Figure 19: Biscayne region mean growth rate (cm/month ± SD) by zone, July 2008 - November 2008. Means with different letters are significantly different from others in the same group (Tukey's HSD $p<0.05$). Solid bars include all corals. Hashed bars only include corals without breakage or complete mortality.

Table 20: Biscayne region statistic post-hoc test (Tukey's HSD $p<0.05$) summary table for growth rates between zones including all corals (n=96). p-Values denoted with an * are significant.

Zone	Zone	p-Value
Inshore	Offshore	0.016*
Forereef	Offshore	0.0868
Mid-Channel	Offshore	0.1871
Inshore	Mid-Channel	0.7397
Inshore	Forereef	0.914
Forereef	Mid-Channel	0.9835

A one-way ANOVA analysis of growth rate by genotype for all outplanting zones in the Biscayne region showed that genotypes did not have significantly different growth rates

(p>0.9241; F=0.3448; df=7) (Figure 20; Table 21). This data was analyzed excluding corals with full mortality and/or breakage (n=30).

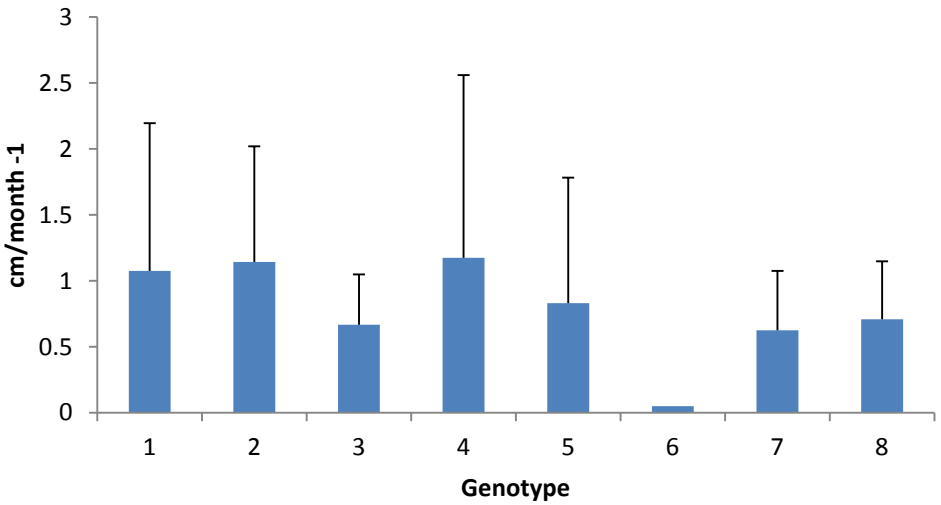

Figure 20: Biscayne region mean growth rate (cm/month ± SD) by genotype for all zones, July 2008 - November 2008. No significant difference was determined among genotypes (Tukey's HSD p<0.05).

Table 21: Biscayne region number of each genotype outplanted to each zone that were not excluded due to breakage and/or complete mortality during the monitoring period (July 2008- November 2008) (G=genotype)

Zone	G1	G2	G3	G4	G5	G6	G7	G8	Total
Inshore	1	2	1	3	1	0	0	1	9
Mid-channel	2	2	0	1	1	0	1	0	7
Offshore	1	0	0	0	1	1	0	1	4
Forereef	1	3	2	0	1	0	2	1	10
Total	**5**	**7**	**3**	**4**	**4**	**1**	**3**	**3**	**30**

4.3.2 SURVIVORSHIP

Nursery corals were outplanted in July 2008 and recorded for survivorship in November 2008 (n=96). Survivorship was highest in the Biscayne mid-channel zone at 95.8% and lowest in the inshore zone (83.3%) (Table 22).

Table 22: Table of Biscayne outplanted corals percent survivorship per zone. Data was collected from July 2008 through November 2008.

Region	Zone	Initial n (July 2008)	Final n (November 2008)	% Survivorship
Biscayne	Inshore	24	20	83.3
Biscayne	Mid-Channel	24	23	95.8
Biscayne	Offshore	24	22	91.7
Biscayne	Forereef	24	21	87.5
Biscayne	All Zones	96	86	89.6

4.4 ALL REGIONS (UPPER KEYS, LOWER KEYS AND BISCAYNE)

4.4.1 GROWTH

Fragments from nursery corals were outplanted to 12 reef sites located with one site in each of the four zones (Inshore, Mid-Channel, Offshore and Forereef) within the Upper Keys, Lower Keys, and Biscayne regions. All outplanted corals (n=283) were measured for linear extension. Corals were outplanted at a length of 2-50cm within all regions. Despite size differences between initial fragment size of outplanted corals in different regions, when zones between the regions were pooled, there were no significant differences between mean initial fragment size between zones ($p>0.2013$; $F=1.5523$; $df=3$).

A total of 283 corals were outplanted to 12 sites, representing 25 different genotypes. Throughout each of the regions monitoring periods, 76 corals were broken due to storms or other unknown causes. Biscayne had the highest number of broken corals (58%), with 56 of 96 corals. Thirty-nine corals experienced partial or full mortality. These factors negatively affected linear extension (measured growth rate), and therefore, two data sets were analyzed: 1) including all corals (n=283) and, 2) corals without breakage and/or complete mortality (n=167).

The overall mean growth rate was 0.72 ± 1.68 cm/month for all corals (n= 283). There were significant differences in growth rates among zones ($p<0.0020$; $F= 5.1955$; $df=3$) (Figure 21). The forereef zone (1.24 ± 2.08 cm/month) had significantly higher mean

growth rates than the inshore zone (0.32 ± 1.05 cm/month), but not the mid-channel (0.95 ± 1.78 cm/month) or offshore (0.36 ± 1.67 cm/month) zones (Figure 21).

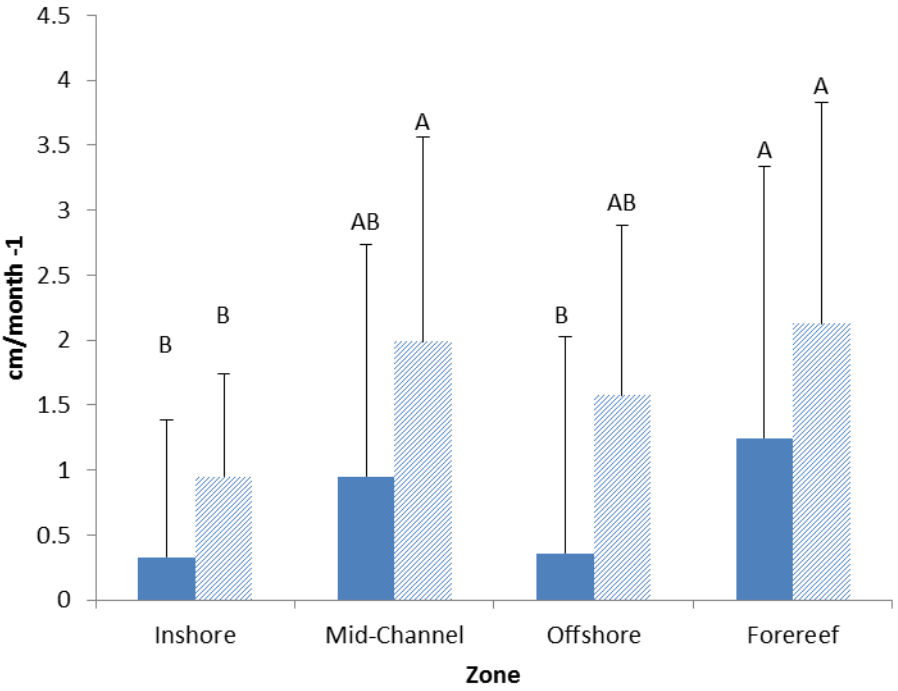

Figure 21: All regions (Upper Keys, Lower Keys and Biscayne) mean growth rate (cm/month ± SD) by zone, May 2008 - November 2008. Means with different letters are significantly different from others in the same group (Tukey's HSD $p<0.05$). Solid bars include all corals. Hashed bars only include corals without breakage or complete mortality.

After excluding corals with breakage and/or complete mortality (n=167), the data was still significant ($p<0.0050$; $F= 6.2967$; $df=3$). The zones which had significantly different mean growth rates also changed, where the forereef (2.12 ± 1.71 cm/month) and mid-channel (1.98 ± 1.58 cm/month) zone were significantly different from the inshore zone

(0.94 ± 0.79 cm/month), but not the offshore zone (1.57 ± 1.31 cm/month) (Figure 21; Tables 23 and 24).

Table 23: All regions (Upper Keys, Lower Keys and Biscayne) statistic post-hoc test (Tukey's HSD $p<0.05$) summary table for growth rates between zones including all corals (n=283). p-Values denoted with an * are significant.

Zone	Zone	p-Value
Forereef	Inshore	0.0073*
Forereef	Offshore	0.0123*
Mid-Channel	Inshore	0.1162
Mid-Channel	Offshore	0.1606
Forereef	Mid-Channel	0.7231
Offshore	Inshore	0.9995

Table 24: All regions (Upper Keys, Lower Keys and Biscayne) statistic post-hoc test (Tukey's HSD $p<0.05$) summary table for growth rates between zones excluding corals with breakage and/or complete mortality (n=167). p-Values denoted with an * are significant.

Zone	Zone	p-Value
Forereef	Inshore	0.0005*
Mid-Channel	Inshore	0.0042*
Offshore	Inshore	0.2061
Forereef	Offshore	0.3081
Mid-Channel	Offshore	0.5876
Forereef	Mid-Channel	0.9658

4.4.1.3 GROWTH BY REGION

A one-way ANOVA analysis of growth rate by region showed that regions did have significantly different growth rates ($p>0.0001$; $F=11.533$; $df=2$) (Figure 22; Table 25). The growth rates of the Upper Keys region (2.33 ± 1.72 cm/month) were significantly higher than the Lower Keys (1.49 ± 1.27 cm/month) and Biscayne (0.91 ± 0.86

cm/month) regions (Figure 22; Table 25). This data was analyzed excluding corals with breakage and/or complete mortality (n=167).

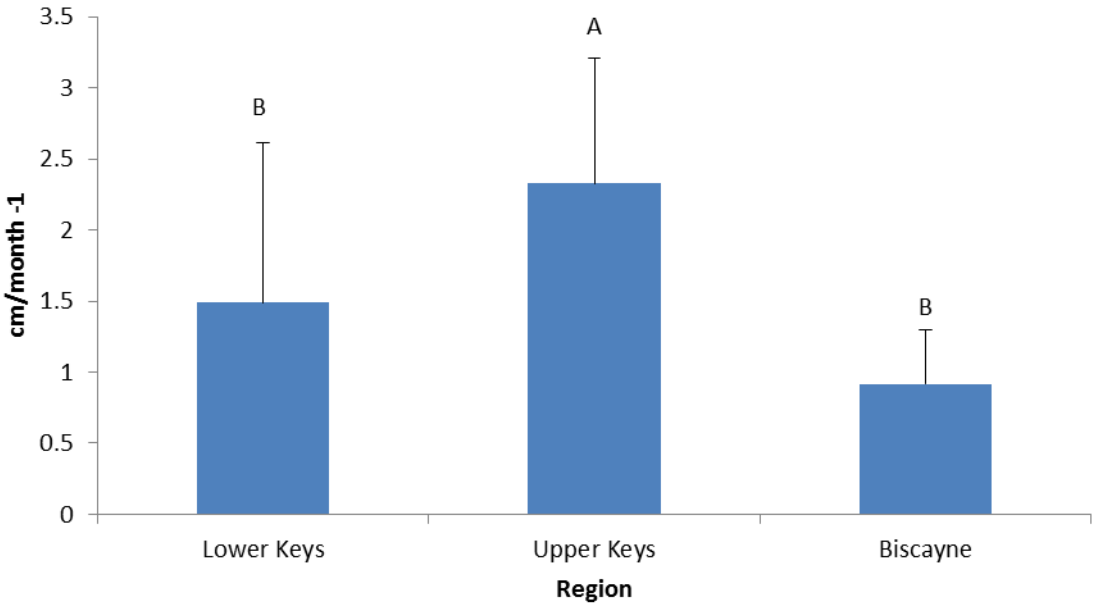

Figure 22: All regions (Upper Keys, Lower Keys and Biscayne) mean growth rate (cm/month ± SD) by region. Means with different letters are significantly different from others in the same group (Tukey's HSD $p<0.05$). The Upper Keys region is significantly different than the Biscayne and Lower Keys regions.

Table 25: All regions (Upper Keys, Lower Keys and Biscayne) statistic post-hoc test (Tukey's HSD $p<0.05$) summary table for growth rates between regions excluding corals with breakage and/or complete mortality (n=167). p-Values denoted with an * are significant.

Region	Region	p-Value
Upper Keys	Biscayne	<.0001*
Upper Keys	Lower Keys	0.0018*
Lower Keys	Biscayne	0.1284

4.4.2 SURVIVORSHIP

Corals were outplanted and recorded for survivorship at six months in the Upper and Lower Keys regions and four months in the Biscayne region (n=329). Survivorship was highest in the Biscayne region at 89.6% (Table 26). Survivorship was lowest in the Lower Keys region at 81.4% (Table 26).

Table 26: Table of percent survivorship of all outplanted corals (n=283) in the Upper Keys, Lower Keys and Biscayne regions.

Region	Zone	Initial n (May 2007, May 2008, July 2008)	Final n (November 2007, November 2008)	% Survivorship
Lower Keys	All	113	92	81.4
Upper Keys	All	74	66	89.2
Biscayne	All	96	86	89.6
All	**All**	**283**	**244**	**86.2**

5.0 DISCUSSION

Due to the continuous decline of coral reefs, coral restoration continues to be a growing field among scientists, managers and environmentalists. *Acropora cervicornis* has proven to be a great candidate for species restoration due to its ease of fragmentation and fast growth rates (Herlan and Lirman 2008; Johnson et al. 2011). The recently released recovery plan for elkhorn and staghorn corals (National Marine Fisheries Service 2015) main goal is to increase the abundance and protect the genetic diversity of these corals throughout their geographic range, and lists "Active Population Enhancement" as an immediate action. Since coral nursery techniques have proven successful over the past decade, focusing more attention on ouptlanting techniques and best practices would appear to be the next step in *Acropora* restoration success.

This study was the first attempt to compare genotypic differences of growth and survivorship of outplanted *Acropora cervicornis* corals across multiple regions of South Florida. The Upper Keys region supported a pilot outplanting project, from where my study was originated. The project design and lessons learned from this project were incorporated into the Lower Keys and Biscayne region projects. Upper Keys corals were outplanted onto EcoReef® modules (not directly to the reef), and supported high growth rates. Artificial reefs provide extra substrate surfaces and can serve as an ideal research platform (Abelson 2006) and were chosen because of these advantages. The EcoReefs® did provide a raised platform for the corals, which may have increased water flow which has been documented to increase survivorship (Nakamura and Woesik 2001). However,

due to the significant breakage of the EcoReef® modules over a short period of time (~6 months), they were not recommended for use within the other regions.

For this study, all nursery and outplanted corals were measured for linear extension. Although this method can be time consuming, it also provides accurate data and appears appropriate for small corals (5-20cm). A minimum size of 5-10 cm has also been recommended to promote better survivorship of transplanted corals (Edwards and Gomez 2007). However, smaller corals were used in the Lower Keys region (3-5cm) which were also successfully outplanted (81.4% survivorship) over a 6 month period. The Lower Keys region also had the least amount of breakage, with <10% which may be attributed to the smaller outplant size. The Biscayne region had the greatest amount of breakage (58% of outplanted corals), but survivorship was actually higher than in the Lower Keys region (89.6% versus 81.4%). This demonstrates that breakage is not always a good indicator when determining the success of outplanted *Acropora* corals, mainly due to their natural fragmentation process. It does however affect results of growth rate analysis.

When comparing growth rates for all regions, the Upper Keys was the highest, then the Lower Keys and finally Biscayne. There was a large variance in initial size of the Upper Keys outplanted corals (8-28cm). This may have greatly affected the outcomes of the growth data for this region, since larger fragments of *Acropora* spp. are known to have higher growth rates (Lirman 2000; Lirman et al. 2014).

When combining all outplant datasets from the three regions (n=283), the forereef and mid-channel zones had significantly higher growth rates than the inshore and offshore zones. When looking at individual regions data, the Lower Keys and Upper Keys forereef zone had the highest growth rates of outplanted corals. In the Lower Keys, the forereef zone had the highest growth rate of 0.9 cm/month^{-1}, or projected growth rate of 10.8cm/year similar to 10-15 cm/year that has been reported in the past (Gladfelter 1984). In Biscayne, the forereef zone also had the second highest growth rate but was not significantly higher than the other two zones. When looking at where wild colonies of *Acropora cervicornis* are naturally found, based on presence/absence data of the Florida Reef Resilience Program surveys, the majority are found within the forereef and mid-channel zones. Focusing restoration within these zones, where they are naturally found, would appear to achieve the highest rates of growth.

Coral genetics are thought to play a role in the success of outplanted nursery corals, and identifying as well as maximizing the genetic diversity of outplants is considered essential (Shearer et al. 2009). All corals were initially genotyped within the nursery, and were distinct within regions. Due to these results, I could not show differences between genotypes among regions. Within the Lower Keys and Biscayne region, there were no differences in growth rates among genotypes. There were however differences in the Upper Keys region. This may be attributed to the large variance in the size of outplanted corals. Within the Lower Keys nursery, there were differences in growth rates among genotypes. In this case, corals were collected from different zones and moved into a nursery, or common environment, similar to other coral experiments employed (Edmunds

1994; Baird et al. 2003; Smith et al. 2007). When the corals were outplanted from the common nursery environment to different zones within the Lower Keys region, there were no differences in growth rate between genotypes across all zones. Choosing coral genotypes that grow well in a nursery environment and expecting them to perform well when outplanted may not be the best restoration approach. It appears that when looking at growth of outplanted corals the zone in which they are outplanted has more of an effect than the genotype of the coral.

Survivorship of all outplanted corals (n=283) was analyzed for all regions. Survivorship was highest in the Biscayne region, but not significantly higher than the other two regions. Due to low sample size and low incidence of mortality, survivorship of genotypes in each region could not be assessed. It appears that since survivorship is high within the first 6 months, it may be more appropriate to monitor these corals over a longer period of time (>1 year). Although breakage did occur within each region, it did not appear to affect the survivorship of outplanted corals. Previous coral outplant studies report survivorship rates between 50-100% (Harriot and Fisk 1988; Clark and Edwards 1995; Gilliam et al. 2001). The overall survivorship of all outplanted corals was 86.2%, which indicates short-term success among and across regions.

6.0 CONCLUSIONS AND RECOMMENDATIONS

In conclusion, outplanted nursery corals of *A.cervicornis* appear to have higher growth rates in the forereef and mid-channel zones within the Lower Keys, Upper Keys and Biscayne regions. Although certain zones supported higher growth rates over this short-term study, how they will perform over the long-term (i.e. after one year) is unclear. By replicating this study at multiple sites within each zone, a better understanding of whether these same zones support higher growth rates would be achieved. Also, understanding what factors influence growth within these zones could be better understood. Using larger size corals appears to increase the rate of growth, and is suggested for future studies. Growth was measured in linear extension for the all three regions. This method was suitable for the small outplanted fragments used (3-5cm), but could prove timely for larger corals over time as they create more branches. When comparing growth rates for all regions, the Upper Keys was the highest, then the Lower Keys and finally Biscayne. Larger fragments were also used in the Upper Keys, which may have attributed to these results. To more accurately compare which region supports the highest growth rates, similar size corals should be used in each region as well as outplanted and monitored during the same time period. Although the Upper Keys corals had high growth rates on the EcoReef® modules, due to the significant breakage of the modules over a short period of time (~6 months), they are not recommended for long-term outplanting studies. It is recommended that corals be outplanted directly onto the reef and avoid this intermediate module phase.

Differences in growth rates among genotypes were only seen in the Upper Keys region that used varying size corals. Therefore, by using a genetically diverse array of corals outplanted to many reef zones, you may be ensuring the maximum chance of growth and survivorship. Choosing corals that grow best in the nursery may not be the best approach to restoration, since the zone to which they are outplanted seems to have more of an effect than the genotype of the coral. Due to low replication (2-3 corals/genotype) at each site and corals being excluded due to breakage or mortality in the analysis, I could not compare growth rates of genotypes within each zone, only across zones. It is possible that there may be differences in growth rates of different genotypes within a zone, rather than across all zones. There was also not enough data to show survivorship differences between genotypes. Collecting more coral tissue in the beginning of the project, or allowing corals to grow longer than one year in the nursery would have increased the amount of tissue and replication of genotypes available for outplanting. Future restoration projects consider all of these factors when designing a project.

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