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Land-Based Coral Nurseries: A Valuable Tool for Production and Transplantation of Acropora cervicornis

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LAND-BASED CORAL NURSERIES: A VALUABLE TOOL FOR PRODUCTION AND TRANSPLANTATION OF *ACROPORA CERVICORNIS*

By

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I. Abstract

Coral nurseries have become a popular and successful method to produce coral fragments for reef-restocking and restoration projects worldwide. Numerous in-situ coral nurseries have been established and many studies have focused on the most effective way to produce coral fragments in offshore nurseries. In contrast, production of coral fragments in land-based nurseries is rarely studied despite a growing knowledge of coral husbandry and coral aquaculture. Little data exist on the success of tank-raised corals when transplanted back into reef environments. This thesis presents the results of a study designed to assess the use of land-based coral nurseries in production of fragments of the Atlantic staghorn coral *Acropora cervicornis* for the purposes of reef re-stocking and restoration.

The first objective of the study was to assess if *A. cervicornis* fragments can be produced in aquarium conditions at comparable rates to offshore nurseries. Fragments from the same wild donor colonies were placed in an offshore nursery and a land-based nursery and monitored for survival, growth, branch production, and branch thickness for 16 months. Survival was lower in the land-based nursery, largely due to a mechanical failure. Linear extension was lower in the land-based nursery until nursery conditions were evaluated and optimized. The optimization process included changes to water quality, temperature control, and lighting. Post-optimization, linear extension in the land-based nursery exceeded the offshore nursery, with a maximum monthly growth rate of 16.0 ± 5.3 mm month^{-1}. The maximum monthly rate in the offshore nursery was 10.6 ± 4.1 mm month^{-1}. Branch number and thickness were also lower initially in the land-based nursery, however both metrics increased rapidly after optimization. This

experiment shows that *A. cervicornis* can be successfully grown in a land-based nursery, and that linear extension and fragment production can be higher than in offshore nurseries if environmental conditions are maintained within optimum ranges. This experiment highlights some of the conditions that promoted high linear extension rates in this species.

The second objective of this study was to examine the success of corals outplanted from land-based nurseries and to determine whether corals reared in a land-based nursery would show the same growth and survival after transplantation as those reared in a traditional offshore nursery. This was examined in two experiments. In the first experiment, small fragments were outplanted from colonies reared offshore and from colonies reared in a land-based system. In the second experiment, larger colonies reared in the two separate land-based systems were outplanted to the same location. All transplanted corals were monitored for survival, growth, branch number, and incidence of predation, breakage, and disease over one year. Two major storm events occurred during this portion of the study, so the potential for differences in breakage or storm damage were also assessed.

There were no significant differences in survival or growth of fragments outplanted from a land-based nursery and an offshore nursery. Colony outplants from one land-based location had better survival and growth than colonies from a second land-based location. Tropical storm activity greatly increased the occurrence of breakage and tissue loss in all groups, resulting in decreases in colony volume and additional mortality. Survival ranged from 85% to 100% after six months, and survival ranged from 70% to 89% after one year and the passing of two tropical storms. Small (5 cm) transplants did not have

significantly lower survivorship than large transplants. Overall, the transplant of fragments and colonies raised in land-based nurseries was successful, as measured by growth and survival rates that were comparable to or exceeded those observed for corals raised in offshore nurseries. Large colony transplants exhibited the best survivorship and extension rates, but were also highly prone to breakage.

Keywords: *Acropora cervicornis*, coral nurseries, coral gardening concept, land-based, aquaculture, transplant, water quality, aquarium, tropical storm, hurricane, breakage, disease, tissue loss, staghorn coral

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III. Preface

The production and transplantation of coral fragments from coral nurseries has become a popular and successful method used for reef-restocking and restoration projects worldwide. In contrast, production of coral fragments in land-based nurseries is rarely studied despite a growing knowledge of coral husbandry and coral aquaculture. Little data exist on the success of tank-raised corals when transplanted back into reef environments. This thesis presents the results of a study designed to assess the use of land-based coral nurseries in production of fragments of the Atlantic staghorn coral *Acropora cervicornis* for the purposes of reef re-stocking and restoration.

This thesis consists of four chapters. Chapter 1 is an overall introduction regarding the current state of coral reefs, an introduction to the topic of reef restoration, and the biology of the study species. Chapter 2 describes a study comparing growth and survival of coral fragments that were taken from wild donor colonies and raised in both a land-based nursery and a more traditional offshore nursery. The purpose of the study is to see how productivity of a land-based nursery compares to the productivity of an offshore nursery. During the study, modifications were made to conditions in the land-based nursery in order to maximize growth rates. This information provides a valuable resource to establish guidelines for land-based production of *A. cervicornis.*

Chapter 3 consists of two experiments designed to assess the success of transplanting corals reared in a land-based nursery to a near-shore reef site. The first experiment compares the growth and survival of small coral fragments that originated in a land-based nursery with those that originated in an offshore nursery. The second experiment compares the growth and survival of larger colony transplants from two separate land-

based nurseries and a group of control colonies that remained in the land-based nursery. Transplants were monitored monthly for the first six months, and again in months nine and twelve. During the year after transplantation, the transplants were heavily affected by two tropical weather systems, and the damage and recovery to transplanted corals is discussed.

Chapter 4 consists of a summary of recommended coral husbandry practices in relation to the production of *A. cervicornis* in land-based nurseries. This chapter presents the "lessons learned" over the course of nearly three years of culture of *A. cervicornis* in a land-based nursery system and may serve as a guide for future work on the subject.

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CHAPTER

1

INTRODUCTION

1.1 Coral Reef Degradation and Future Outlook

Coral reefs are one of the most diverse and productive ecosystems on the planet and provide many resources that are both economically and environmentally important to human populations. Because of this, they are also one of the most heavily exploited ecosystems on the planet. Services provided by coral reef ecosystems include seafood products, biochemical compounds, physical protection of coastlines, and social services such as recreation and tourism (Moberg and Folke 1999). More than 100 countries have coral reefs along their coastlines, and many millions of people depend on reefs for sustenance and income (Spalding et al. 2001, Burke et al. 2011). Coral reef ecosystem services have been valued at $29.8 billion per year globally (Cesar et al. 2003). On a local scale, they have been valued at $3 to 4 billion annually in the Caribbean (Burke and Maidens 2004), and an additional $4 billion per year in southeast Florida (Johns et al. 2003).

Despite worldwide implementation of management plans that aim to conserve coral reefs and their associated ecosystem services, most of the world's reefs have declined at an alarming rate (Gardner et al. 2003, Pandolfi et al. 2005, Bruno and Selig 2007, Wilkinson 2008). Anthropogenic factors such as pollution, overfishing, and global climate change are all associated with the decline of coral reefs and an increased frequency of disease outbreaks and bleaching (Pandolfi 2003, Bruno et al. 2007, Mora 2008). Overfishing is considered one of the greatest local threats, with an 80% increase observed between 1998 and 2008 (Burke et al. 2011). Overfishing is driven by increasing human populations, which also leads to increased land-based sources of pollution. Land-based pollution can

lead to dramatic reductions in species diversity (Edinger et al. 1998, Fabricius et al. 2005), lowered live coral cover (Smith et al. 2001, Bruno and Selig 2007, De'ath et al. 2012), and shifts to a macro-algae dominated community (McManus and Polsenberg 2004, Fabricius et al. 2005, Lapointe et al. 2010).

Recent predictions show the outlook for coral reefs is grim, and it is unlikely that many reefs will to recover to their original state (Pandolfi 2003). Perhaps the most serious future threat to coral reefs is the threat of ocean acidification. Ocean acidification results from an increased level of dissolved carbon dioxide in ocean waters, caused by equilibration with increasing levels of carbon dioxide in the earth's atmosphere. The resulting decrease in ocean pH and decreased carbonate ion availability result in decreased calcification rates by reef-building corals. Within the next few decades, the partial pressure of carbon dioxide in the earth's atmosphere is predicted to be high enough to stop coral growth on many of the world's reefs (Silverman et al. 2009). Predictions such as this show that threats to coral reefs are global as well as local, and conserving coral reefs will require a united effort on many fronts to prevent further reef destruction, as well as to restore what has already been lost.

1.2 Reef Restoration and the Coral Gardening Concept

The continuing global decline of coral reefs has led to the need for a more vigorous approach to coral reef management (Pandolfi et al. 2005, Rinkevich 2008) and the development of a variety of techniques that can be used to restore deteriorated reefs (Precht 2006, Edwards 2010) or to provide mitigation alternatives for anticipated or un-anticipated habitat loss (Seguin et al. 2009, Kilbane et al. 2009). Some restoration

methods aim only to replace or stabilize the three dimensional structure of the reef (Fox and Pet 2001, Fox et al. 2003). This method of restoration may be sufficient in areas where natural coral recruitment is high, as artificial structures can be colonized by coral recruits in as little as 6 months in such areas (Clark and Edwards 1994). In other areas, recruitment onto artificial reef structures may be limited. Coral colonization can be enhanced by transplanting live coral fragments or colonies onto artificial reef structures (Fox et al. 2003, Clark and Edwards 1994, Thornton et al. 2000). In areas where substrate stabilization or reconstruction is not necessary, coral fragments may be transplanted directly to the reef substrate (Bowden-Kerby 2001, Garrison and Ward 2008), or the coral fragments can be used to stabilize the substrate directly (Lindahl 2003).

Early restoration studies utilized corals taken from nearby donor sites, thereby "robbing Peter to pay Paul," and in turn decreasing coral cover at the donor sites. The need for a more sustainable source of coral fragments has led to the development of coral nurseries and the coral gardening concept. The two-step gardening concept involves the creation of a large pool of corals in nurseries established in sheltered ocean areas, followed by the transplantation of nursery-grown fragments to degraded reef sites (Rinkevich 2006). The coral gardening concept has been effectively applied worldwide in a variety of locations, including Tanzania (Mbije et al. 2010), the Philippines (Shaish et al. 2008), the Red Sea (Shafir et al. 2006), and Japan (Omori 2011). Coral gardening can also be used as an economically viable and sustainable means of supplying corals to the ornamental

aquarium trade (Ellis and Sharron 1999, Ellis and Ellis 2002), thus decreasing wild collections.

In response to the success of the coral gardening concept and an increasing demand for fragments for restoration and mitigation projects, numerous in-situ coral nurseries have been established using a wide range of materials, and many studies have focused on the most efficient way to produce coral fragments in offshore nurseries. Offshore nursery structures have been constructed of leg-affixed solid frameworks of metal or plastic (Soong and Chen 2003, Shaish et al. 2008), tethered mid-water lines or mesh to which fragments are tied (Shaish et al. 2008, Levy et al. 2010), or concrete structures or cinder blocks anchored directly to the substratum (Herlan and Lirman 2008). Nurseries can be located over existing reefs, in close proximity to reefs, or in sand channels but are generally located in somewhat protected areas such as back-reefs or lagoons in order to minimize turbulence on small, newly attached fragments. While in the nursery, corals can be plagued by predation by corallivorous gastropods such as *Drupella* sp. (Shafir et al. 2006) or polychaete worms (*Hermodice* sp.). Locating nurseries away from the natural reef or hanging fragments on suspended lines can reduce the impacts of predation (Edwards 2010).

1.3 Reef Restoration in the Western Atlantic

The Western Atlantic region contains only ten percent of the world's coral reefs, and biodiversity is significantly lower than in the Indian or Pacific Oceans. The Atlantic is home to less than 70 species of reef-building corals whereas the Indian and Pacific oceans are home to over 700 species (Veron 2000). The lower species diversity found in

the Western Atlantic basin is largely due to the large number of glacial extinction events that occurred after the closing of the Isthmus of Panama during the Pliocene (Spalding et al. 2001). In the Caribbean, live coral cover has decreased dramatically over the past three decades from an average of 50% to 10% (Gardner et al. 2003). Caribbean reefs also show evidence of lower resilience and recovery (Connell 1997), lower coral recruitment (Richmond and Hunter 1990), and a greater tendency to shift to macro-algal dominated communities (Mumby et al. 2007, Bruno et al. 2009) than their Indo-Pacific counterparts.

Beginning in the late 1970's, widespread mortality of two important Atlantic corals occurred across the Caribbean. Populations of *Acropora cervicornis* and *Acropora palmata*, two primary reef framework-building species, decreased by 80-90% regionally, largely due to disease, but also due to hurricane damage, predation, thermal stress, ship groundings, and anchor damage (Greenstein et al. 1998, Precht et al. 2002). In addition, a major reef-grazing herbivore, the sea urchin *Diadema antillarum* also died in large numbers due to disease between 1983 and 1984 (Lessios et al. 1984). The urchin die-off resulted in a well-documented increase in algal abundance in many areas (Hughes et al. 1987, Levitan 1988). The loss of these critical species from the Western Atlantic reef ecosystem is linked to the ongoing lowered resilience and decline of Caribbean reefs (Roff and Mumby 2012). The dramatic loss of Caribbean *Acropora* led to the 2006 addition of *A. cervicornis* and *A. palmata* to the threatened species list under the U.S. Endangered Species Act. In 2014, an additional five stony coral species found in the region were added to the list of threatened species.

In response to the ongoing plight of Atlantic reefs, many in-situ coral nurseries have been established throughout the region, including the Dominican Republic, Mexico, Jamaica, Puerto Rico, and Florida. These nurseries are established and operated by governmental agencies, universities, and/or private non-profit organizations. Most of these nurseries focus on the fast-growing threatened acroporid corals *A. cervicornis* and/or *A. palmata*. These nurseries act as a source of fragments for transplantation (Johnson et al. 2011 and case studies therein) and also as repositories for genetic material in the face of increasing natural stressors (Schopmeyer et al. 2012). *Acropora cervicornis* fragments produced in offshore nurseries have been used for reef restoration projects in Florida with up to 100% survival over the first year and an average survival of between 80 and 90% (K. Nedimeyer, pers. comm., April 1, 2011).

There is a growing amount of information regarding performance of *A. cervicornis* in a variety of offshore nursery conditions (Herlan and Lirman 2008, Nedimeyer et al. 2011, Bowden-Kerby and Carne 2012, Griffin et al. 2012), and guidelines for nursery and restoration best practices have been established (Johnson et al. 2011). The wealth of information regarding nursery culture and transplantation makes this species an excellent candidate for studying the efficacy of novel nursery techniques.

1.4 Study Species

Acropora cervicornis is a hermatypic, scleractinian coral in the family Acroporidae. The genus *Acropora* is the most speciose of any of the reef-building coral genera, with approximately 180 described species (Veron 2000). *Acropora cervicornis* and all other members of the genus are distinguished by an axial corallite that is found at the tip of

each cylindrical branch. Unlike many Indo-Pacific acroporids, *A. cervicornis* does not show fluorescent accessory pigments, and healthy colonies show brown to tan coloration along the branches with white growing tips. Along with *A. palmata*, it is one of three members of this genus found in the Atlantic Ocean, including *A. prolifera,* a hybrid of *A. cervicornis* and *A. palmata*. It's range extends from southeast Florida to northern Venezuela, and it is found throughout the Caribbean islands and the western Gulf of Mexico. It was historically a dominant reef builder throughout the Pleistocene and Holocene, when well-developed reefs dominated by *Acropora* were found as far north as Palm Beach County, Florida (Lighty et al. 1978). It has been suggested that this species may be able to adapt more readily to changing global climate conditions through a migration of its northern range boundary (Precht and Aronson 2004).

Acropora cervicornis can be found growing from the surface to depths of 30 meters and has rarely been reported as deep as 60 m (Goreau and Goreau 1973). The arborescent colonies often form large monotypic stands, and the species is capable of thriving in many reef zones and in areas of varying wave energy. Early descriptions of reef zonation patterns from Discovery Bay, Jamaica termed the shallow fore-reef areas between 7 and 15 m the "*Cervicornis* Zone" due to the abundance of the species but also noted that it was present in varying amounts in all reef crest and back reef zones (Goreau 1957). The "*Cervicornis* Zone" was also observed in Western Caribbean reefs off Colombia, as well as patch reefs consisting primarily of *A. cervicornis* located in relatively sheltered lagoons (Geister 1977).

The ability of this species to survive in a variety of reef habitats may be due to its ability to grow rapidly, and to adapt its morphology to local environmental conditions such as light and wave energy. Growth rates as fast as 20 cm per year have been reported on individual branches (Tunnicliffe 1983), but more commonly observed average growth rates are around 10-12 cm per year. Growth rates have been correlated with temperature, with maximum growth occurring between 28 and 30 degrees C (Shinn 1966), and lower growth rates occurring during colder, winter months. Branches form from 60 to 90 degrees from the primary stalk, and distance between branches is greater in calmer water, giving the colonies a more "open" appearance (Boulon et al. 2005). Colonies in areas of high wave action are smaller in height with tighter branching and branching at "lower angles" (Bottjer 1980, Vargas et al. 2003). Branch thickness (diameter) ranges from 0.25 to 1.5 cm, and branch diameter is thinner at greater depths and in areas of lower wave action (Boulon et al. 2005, Bowden-Kerby 2008).

Acropora cervicornis is a simultaneous hermaphrodite that has two modes of reproduction: asexual fragmentation and sexual broadcast spawning. Asexual fragmentation is the most common means of local population growth (Tunnicliffe 1981, Highsmith 1982). This is advantageous as it allows the species to colonize habitat quickly and to rapidly recover from breakage (Highsmith et al. 1980). Although natural fragmentation may allow the species to re-colonize local areas, it does not allow for recovery in habitats where the species has been lost entirely as dispersal of fragments may be limited by geographic boundaries. In addition, the monotypic thickets created by natural fragmentation may be at an increased risk of devastation by disease outbreaks due

to lower genetic diversity. Recovery of lost habitat on a larger scale and the long-term genetic stability of the remaining population must occur through a combination of dispersal of fragments of multiple genotypes and recruitment of sexually produced larvae.

Broadcast spawning has been observed in *A. cervicornis* between 2 and 15 days after the full moon of the late summer months, from July to September (Szmant 1986; Vargas-Angel et al. 2006). *Acropora cervicornis* eggs are relatively easily fertilized compared to *A. palmata*, can be self-fertilized, and are viable for up to four hours post-spawning (Fogarty et al. 2012). It is unclear as to whether self-fertilized larvae are equally as fit for settlement and survival. The mean number of *A. cervicornis* sexual recruits in Broward County have been reported to be only 0.01 per m^2 (Vargas-Ángel et al. 2003), indicating that recruitment through sexual reproduction is extremely low in this area. In general for broadcast spawning marine invertebrates, the percentage of fertilized eggs is greatly reduced with increased distance to the closest male individual (Gascoigne and Lipicus 2004). The sparse distribution of the remaining population has led to the suggestion that the remaining population may suffer from Allee effects where the density of colonies is too low to fertilize successfully (Aronson and Precht 2001).

A limiting factor to the recovery of *A. cervicornis* populations is the on-going occurrence of disease. The initial decline of the population was attributed largely to a condition called White-Band Disease (WBD) (Aronson and Precht 2001, Precht 2002), which still occurs in the remaining coral population. Gladfelter (1982) originally described the syndrome on *Acropora palmata* as 'a sharp line of advance where the distally located

zooxanthella-bearing coral tissue is cleanly and completely removed from the skeleton, leaving a sharp white zone about 1 cm wide that grades proximally into algal successional stages.' Ritchie and Smith (1998) described a second type of white-band syndrome on corals in the Bahamas, distinguishing a WBD Type II from the originally described WBD Type I. Type II is described as having a 2-20 cm margin of bleached tissue that precedes the white skeleton zone. A third syndrome reported in the Florida Keys was simply described as "rapid tissue loss" by Williams and Miller (2005). This syndrome is characterized by rapidly spreading and irregular patterns of tissue sloughing, as compared to a somewhat slower and linear progression of tissue loss from the base of the colony outwards in WBD Type I. Recent research has shown that WBD Type I can be spread by the corallivorous snail *Coralliophila abbreviata,* through direct contact of infected tissue, and also through waterborne transmission to injured coral tissue (Kline and Vollmer 2011, Gignoux-Wolfsohn et al. 2012).

The threatened status of *A. cervicornis* and the persistent lack of recovery of the population emphasizes the importance of carefully planned conservation and restoration that minimizes additional damage to the population. Recent genetic research highlights the importance of activities on a local scale in order for the Florida population to be self-sustaining in the future. It has long been thought that broadcast spawning promoted reef recovery through larval dispersal and input of coral recruits from distant populations. Recent research indicates that gene flow is actually restricted over much smaller spatial scales in Caribbean *Acropora* corals (Baums et al. 2005, Vollmer and Palumbi 2007). Populations separated by as little as 2 km show fine-scale genetic differences (Vollmer

and Palumbi 2007), and ongoing recruitment of *A. cervicornis* to the Florida reef tract from other areas of the Caribbean is low (Hemond and Vollmer 2010). It has been suggested that reef restoration efforts should be focused on areas with low natural recruitment (Kojis and Quinn 2001). This strengthens the argument for transplantation of coral fragments to Broward County waters in order to increase the number of individuals of the species and to promote recruitment to local reefs. Transplantation of a single species is appropriate in this case as naturally occurring thickets are generally monotypic (Vargas-Ángel et al. 2003). In addition, fragmentation of donor colonies does not cause mortality of the donor colony and actually results in increased growth on donor branches (Lirman et al. 2010), minimizing the impacts of fragment collection from the remaining population.

1.5 Project Objectives

Offshore *A. cervicornis* nurseries are generally successful in achieving their objectives, but have a number of down-sides, including potential exposure to extreme cold temperatures in winter and extreme warm temperatures in summer, predation, hurricane damage, anchor and vessel damage, and disease. Although each offshore nursery is affected differently, the use of land-based nursery systems may be an equally effective method of culturing fragments of this species. Land-based systems may also provide an additional option in situations where ocean conditions have become so degraded that in situ nurseries cannot be safely established or do not perform well, and corals must be housed in contained systems until such time that local conditions are improved and stabilized.

Acroporid corals are one of the most commonly propagated corals in the aquarium industry (Atkinson et al. 1995, Petersen et al. 2007, Okubo et al. 2010), and *A. cervicornis* is currently exhibited in numerous public aquariums both in the United States and internationally. The species is also maintained in at least five separate land-based coral nursery systems in Florida, including the University of Florida Tropical Aquaculture Laboratory, the Florida Aquarium, Mote Marine Laboratory, University of Miami, and Nova Southeastern University Oceanographic Center. Despite the presence of both offshore and land-based nurseries for *A. cervicornis* in Florida, no study has directly compared the two nursery techniques.

The objective of this study is to evaluate the use of a land-based coral aquaculture system for propagation of fragments of *A. cervicornis* for reef restoration purposes. The study was conducted in two parts. The first part, described in Chapter 2, was to evaluate the use of a land-based nursery for propagating corals. The purpose was to assess if *A. cervicornis* fragments can be produced in aquarium conditions at comparable rates to offshore nurseries. To accomplish this goal, fragments from the same wild donor colonies were placed in an offshore nursery and a land-based nursery and monitored for survival, growth, branch number, and branch thickness.

The second part of the study, described in Chapter 3, was to test the performance of nursery-produced corals when transplanted back to the reef for restoration. The purpose was to assess if *A. cervicornis* fragments produced in aquarium conditions behaved in a similar way as fragments produced in an offshore nursery. The same corals produced

both in a land-based and offshore nursery used for the study described in Chapter 2 were re-fragmented and transplanted to an offshore site along with some larger colonies. In addition, corals produced in a second land-based nursery were transplanted back to the same site. All transplanted corals were monitored for survival, growth, branch number, and incidence of predation, breakage, and disease. Two major storm events occurred during this portion of the study, so the potential for differences in breakage or storm damage were also assessed.

The null and alternative hypotheses of the experiments are as follows:

Ho1: There is no difference in the survival, growth, branch number, and branch thickness of fragments growing in the offshore and land-based nurseries.

Ha1: Survival, growth, and/or branching will differ between fragments cultured in a land-based nursery and an offshore nursery due to differences in the nursery conditions.

Ho2: There is no difference between survival, growth, and branching of large colonies from two land-based nursery locations after transplantation to an offshore restoration site.

Ha2: Survival, growth, and/or branching of large colonies from the two land-based nursery locations will differ after transplantation.

Ho3: There is no difference between survival, growth, and branching of fragments from the NSU land-based nursery and an offshore nursery after transplantation to an offshore restoration site.

Ha3: Survival, growth, and/or branching of fragments from the land-based and offshore nursery locations will differ after transplantation.

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CHAPTER

2

GROWTH AND SURVIVAL OF FRAGMENTS IN LAND-BASED NURSERY CULTURE

2.1 Introduction

2.1.1 Ex-situ Nurseries

Advances in coral husbandry and aquarium technology over the past two decades have promoted the successful propagation of coral fragments in land-based seawater aquarium systems (Borneman and Lowrie 2001). Techniques for maintaining and propagating *Acropora* spp. and other scleractinian corals in aquaria were developed as early as the 1950's and have become commonplace in the aquarium industry (Carlson 1999, Delbeek 2001). Production of coral fragments for trade and sale is now widely practiced by private reef aquarium keepers, public aquaria, and commercial businesses (Olivotto et al. 2011).

A plethora of literature is available on coral husbandry and propagation in aquarium industry publications (Delbeek and Sprung 1994, Borneman 2001, Calfo 2001, Leewis and Janse 2008). Guidelines for coral husbandry are well established, and topics include aquarium system design, optimum water quality parameters, lighting requirements, water flow requirements, and disease treatment. An extensive and detailed review of these topics and others can be found in Delbeek and Sprung (2005) and Leewis and Janse (2008). The knowledge and information available is based largely on personal experiences, anecdotal evidence, and subjective reports rather than controlled scientific study.

Only a few scientific studies have examined the best techniques for culturing corals in ex-situ conditions, and corals cultured in land-based systems are rarely used for reef restoration activities. The most common scientific use of coral fragments in land-based

aquaria is for replicated laboratory experiments, where tank-adapted coral fragments are used to measure the biological response to toxicants (Davies 1995, Shafir et al. 2003), nutrients (Ferrier-Pages et al. 2000, Renegar and Riegl 2005), light (Schutter et al. 2008), and interactions between these and other factors. Although not directly concerned with large-scale production of fragments, the results of these studies can still be used to determine the most appropriate conditions to maximize coral growth in land-based systems.

One advantage of ex-situ aquaculture is the ability to manipulate environmental parameters in order to maximize coral growth rates. Factors such as light intensity, inorganic nutrients, aragonite saturation state, food availability, water flow, genotype, and fragment size all play a role in coral growth and will also be factors in optimization of growth in the aquarium environment (reviewed by Osinga et al. 2011). A small number of studies have examined the effects of different nursery conditions on coral production (Forsman et al. 2006, Schlacher et al. 2007, Sella and Benayahu 2010). Sella and Benayahu (2010) showed that manipulation of light intensity, temperature, and feeding in closed system aquaculture allowed for the maximization of certain traits of the leather coral *Sarcophyton glaucum*, either the organic weight for use in the natural products industry or the physical appearance for use in the aquarium trade. Given appropriate culture conditions, aquarium cultured corals can exhibit growth rates that are equal to or greater than those recorded in the field (Carlson 1999, Atkinson et al. 1995). As ideal culture conditions are likely to be different for each species, more research is necessary to determine the optimal growing conditions for economically or ecologically important species of coral.

A second advantage of ex-situ aquaculture is the ability to propagate corals from very small amounts of material that may otherwise be overgrown in offshore nurseries. This can be especially important in the propagation of rare or endangered species where wild collections must be minimized. One technique is the production of "micro-colonies" that are created from fragments as small as a single polyp (Tambutté et al. 1995, Shafir et al. 2001; Vizel et al. 2011). An increasingly popular technique is to settle and raise sexually produced coral larvae. Ex-situ settlement and survivorship can exceed in situ rates by several orders of magnitude. Settling coral larvae ex-situ may result in up to 100% metamorphosis and settlement (Ben David-Zaslow and Benayahu 1998) and over 30% survival over the first six months (Gateno et al. 2000). Raising coral sexual recruits is a promising way to sustainably stock aquariums (Petersen et al. 2006) and a potential means of creating large stocks of colonies for restoration (Linden and Rinkevich 2011, Ng et al. 2012). Although coral larvae are generally collected from wild colonies, observations of spawning and settlement of both brooded and broadcast spawned larvae have been recorded in public and private aquaria (Petersen et al. 2007, Okubo et al. 2010).

Despite the frequent use of land-based aquarium systems culturing corals and researching coral biology, only one study has looked at the potential for transplanting aquarium-reared fragments to reef restoration sites (Berzins et al. 2008). If coral fragments produced in land-based nurseries are in fact able to acclimate to ocean conditions and survive as well as fragments produced in offshore nurseries, the use of land based nurseries as a more secure means of propagating coral should be considered. Corals in land-based systems are sheltered from environmental extremes that can potentially cause

problems in offshore nurseries, such as high water temperatures, extreme weather events, or pollution spills. Furthermore, predation, disease, and algal overgrowth can be nearly eliminated in land-based nurseries through proper quarantine and maintenance practices.

A common criticism for the use of aquarium systems for culturing corals for restoration is the potential for corals to become adapted to aquarium conditions, to the extent that nursery fragments would be mal-adapted for survival after transplantation. Potential changes to coral biology that may occur under aquarium conditions are shifts in density or make-up of the microbial community or endosymbionts, and morphological or skeletal changes in response to altered light or water flow. Although growth rates may be high, aquarium cultured corals may have decreased skeletal density in comparison to wild colonies (Carlson 1999), which may affect their ability to survive when transplanted back to the reef. More research is necessary on the transplantation of aquarium-cultured fragments to offshore sites, and the potential for aquarium-cultured corals to "re-adapt" to ocean conditions.

The following chapter evaluates the use of a land-based nursery for propagating corals. The objective of this chapter was to assess if *A. cervicornis* fragments can be produced in aquarium conditions at comparable rates to offshore nurseries.

2.2 Methods

2.2.1 Study Area

The reef structure in Broward County, Florida (Ft. Lauderdale area) forms a distinct system of shore-parallel ridges comprised of the nearshore reef complex, the inner reef, middle reef, and outer reef (Goldberg 1973; Banks et al. 2007, Walker 2012).

Scleractinian coral cover is generally low (<6%) with small coral size, and the biological community is dominated by gorgonians, sponges, zoanthids, and macroalgae (Goldberg 1973; Moyer et al. 2003). Despite the high-latitude location and a community structure that is different from other Caribbean areas (Moyer et al. 2003), large thickets of *A. cervicornis* have been documented on Broward County reefs (Vargas- Ángel et al. 2003, Walker et al. 2012). These thickets are surviving despite their close proximity to a highly urbanized coastline and anthropogenic stressors such as nitrification, pollution, and ship groundings (Vargas-Ángel et al. 2003). *Acropora cervicornis* thickets can be a dominant part of the reef community on the nearshore reef complex and are commonly found along the inner reef, yet the species is only occasionally found in lower numbers and smaller size on the deeper reef ridges.

2.2.2 Fragment Collection

Fragments were collected from each of 50 donor colonies on the inner reef and nearshore reef complex in Broward County, Florida from May 17, 2010 through May 20, 2010. In order to maximize genetic variation and minimize the occurrence of overlapping genotypes, each donor colony was located at a different site along the full extent of Broward County waters (**Figure 2.1**). Donor colonies were marked with flagging tape and numeric tags attached to nails placed next to the colony, and GPS coordinates were recorded for monitoring of sampling wound recovery.

Three 10 cm fragments from each colony were collected by SCUBA divers using pruning shears and were transferred underwater into labeled, re-sealable plastic bags. In addition, a small 1 cm fragment was collected and placed into a separate bag for genetic analysis. For this portion of the study, only two of the 10 cm fragments were used. The third

fragment was given to the University of Florida Tropical Aquaculture Laboratory (UFTAL), and results of that study will be discussed further in Chapter 3. Only branches that were free of disease and predation damage were collected. When possible, fragments were chosen so that there were no bifurcations along the collected branch. After returning to the boat, fragments were transferred into plastic fishing tackle boxes divided so that each fragment had its own chamber, and with the lids open, the tackle boxes were submerged in a cooler of seawater. Water changes were performed hourly to maintain water quality during holding and transport.

At the end of each day of collections, the collected fragments were cut into two 5 cm fragments, creating two apical and two central fragments from each donor colony (N=4 from each donor, 400 fragments total). One apical and one central fragment from each donor colony were attached with two-part epoxy (All-Fix®) to square 7.6 cm by 7.6 cm concrete tiles that were numbered with tags and previously affixed to a larger, existing concrete nursery structure ("Layer Cakes," **Figure 2.2**). The concrete nursery structures were located in a sand channel between the nearshore ridge complex and the inner reef. These structures are part of an existing offshore *A. cervicornis* nursery site. The other corresponding apical and central fragments from each colony were returned to the land-based nursery located at Nova Southeastern University Oceanographic Center and attached with the same two-part epoxy to small concrete pyramid blocks, and placed in the land-based nursery. The position of all fragments in both nurseries was previously determined using a random number generator. The total number of coral fragments placed into each nursery was 100, two from each donor colony.

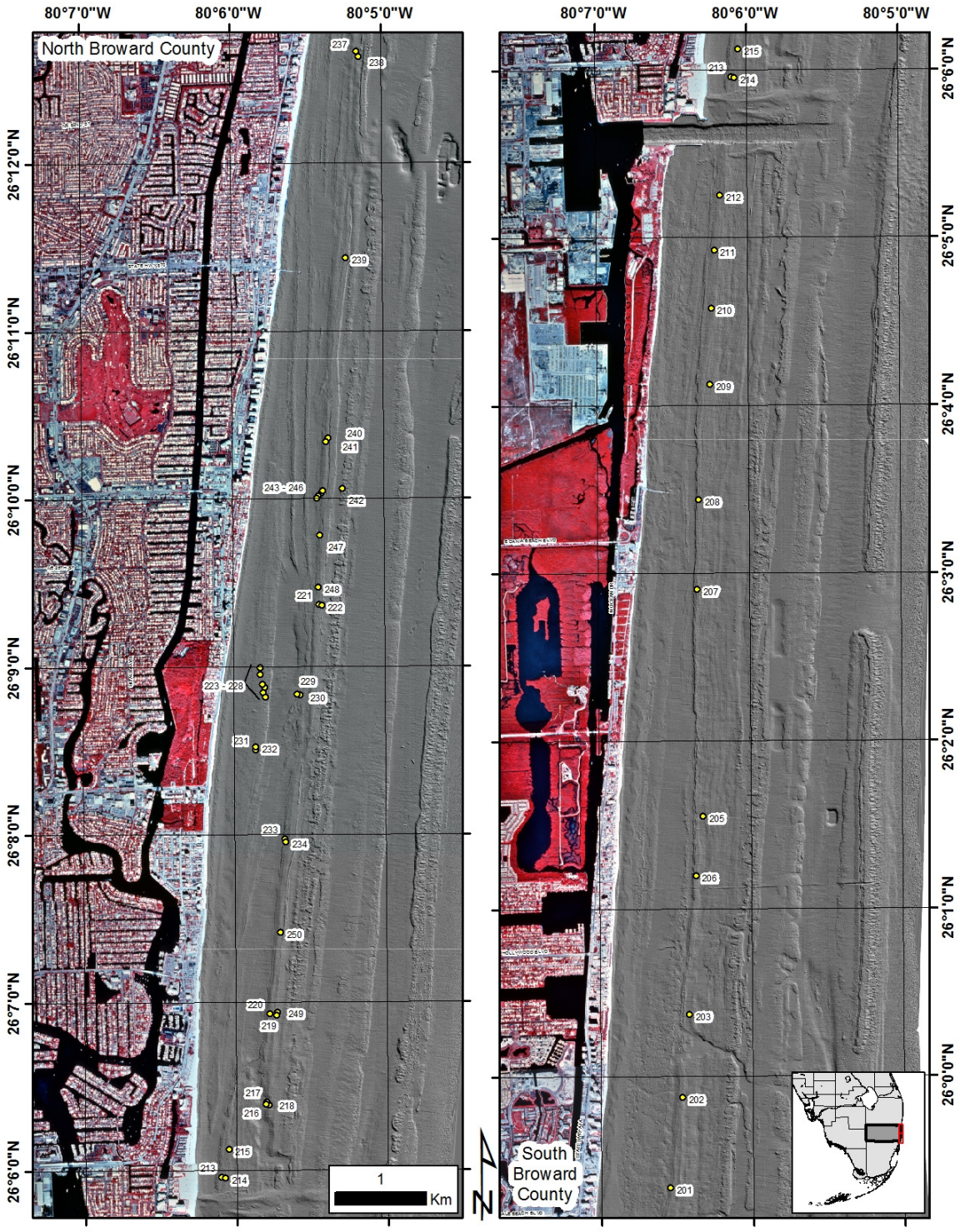

Figure 2.1. Location of the 50 donor colonies, from the northernmost to southernmost extent of Broward County, Florida

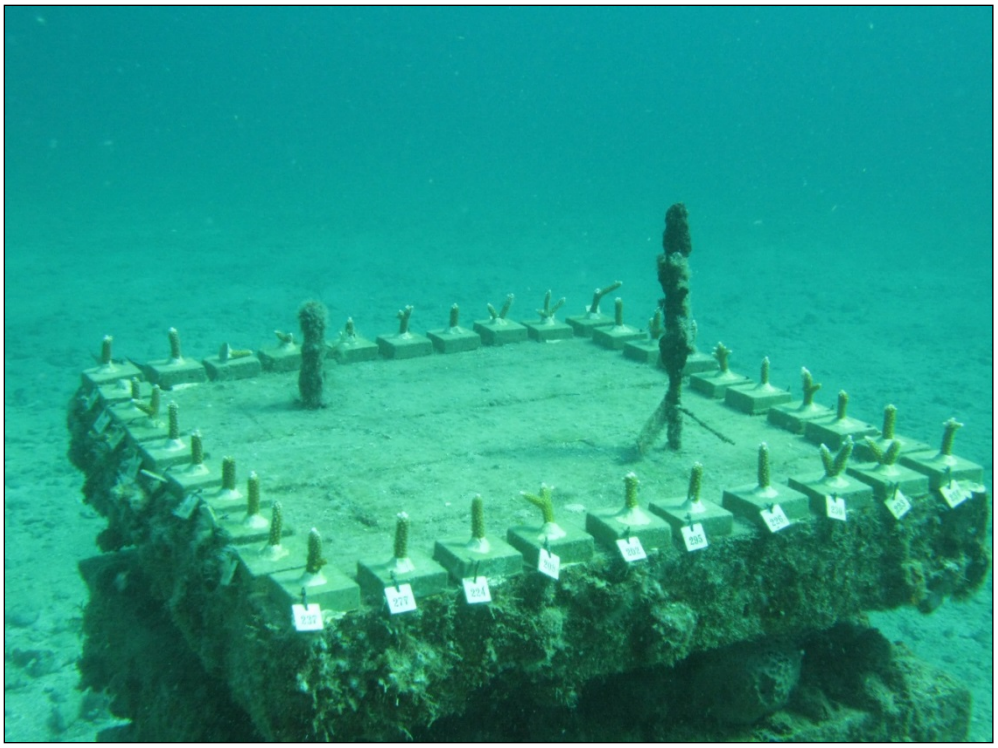

Figure 2.2. One of three "Layer Cake" offshore nursery structures used in the study, shortly after fragment attachment.

2.2.3 Land-Based Nursery System

The land-based nursery used in the experiment was an 8250 L (2180 US Gal) system fed by water from a high-nutrient, low pH saltwater well. The incoming water was aerated and filtered through a large protein skimmer with ozone injection. Temperature of the system was initially regulated by inputs of well water (which is a constant 24 – 25 degrees C), and on days of extreme hot or cold ambient air temperatures, flow-through of well water was almost constant. On days of more moderate ambient air temperatures, the system was largely recirculating. This was referred to as a "semi-open" design. The system consisted of four holding tanks, each tank holding approximately 1400 L (380 US Gal) of water and measuring 2.43 m (96 in) L x 0.91 m (36 in) W x 0.74 m (29 in) H. Only one of the holding tanks was used to house fragments for this project. Water

circulation in the holding tank was provided by two 75 L (20 US Gal) buckets that automatically siphoned into the tank at regular intervals ('Carlson' surge device; Carlson 1996; Watson and Hill 2006) and a closed recirculation loop powered by a magnetic drive water pump that circulated approximately 120 liters per minute (LPM) of water through two 1 inch eductors. Shading was initially provided by a 70% shade cloth structure over the holding tanks. Manual cleaning (removal of algae and *Aiptasia* sp. anemones) was provided as needed, and the system was stocked with herbivorous snails *(Lithopoma* sp., *Cerithium* sp., *Astrea* sp.) and peppermint shrimp (*Lysmata wurdemanni*) for control of algal and anemone growth, respectively.

Temperature, pH, and oxidation-reduction potential (ORP) were recorded daily from an Aquadyne Octopus 4000 automated system monitor. Sensors were calibrated monthly. Water quality parameters were measured weekly at minimum and included ammonia, nitrite, nitrate, phosphate, total alkalinity, and calcium. Ammonia, nitrite, nitrate, and phosphate were measured on a Hach DR840 portable colorimeter. Alkalinity and calcium were tested using LaMotte brand test kits.

2.2.4 Fragment Measurements

One week after collection and attachment, initial measurements were taken on all fragments (May 27, 2010). Coral fragments in the offshore and land-based nurseries were then measured monthly for 18 months. The color and condition of each fragment was recorded based on the scale in Berzins et al. (2008) (summary in **Table 2.1**). Fragments were considered to have survived if given a condition score of 2 – 6, and only considered dead at a condition score of 1. Linear extension (mm/month) of the main

stalk was measured using vernier calipers and measured from the bottom edge of the live tissue to the tip of the live tissue on the vertical fragment (**Figure 2.3**). All growth data are reported as linear extension (mm/month) of the main stalk only. The number and length of all branches were recorded; branch length was measured from the underside point of intersection with the adjacent stalk to the tip of the branch. A branch was determined as a new growth of at least 1 cm off the adjacent stalk. After three months, when a sufficient number of new branches had formed in each nursery, a randomly chosen subset of twenty fragments at each nursery was measured for branch thickness. Only fragments with no original branches were used to ensure that the branch thicknesses were measured on new growth only. Thickness was measured at the point where the branch intersects the adjacent stalk (**Figure 2.3**) and branch thickness was measured every three months. All fragments were photographed during each monitoring event. Additional cleaning and re-attachment at the offshore nursery was done monthly, coinciding with measurements.

Table 2.1. Summary of condition and color scores used to assess health of fragments (Berzins et al. 2008)

Condition Score	Color Score
1 Dead	1 100% bleached
2 < 25% of tissue alive	2 Partial Bleach
3 25-50% of tissue alive	3 Lighter than normal
4 50-75% of tissue alive	4 Good color
5 75-95% of tissue alive	
6 No apparent tissue loss	

Figure 2.3. Measurements taken on fragments. **Left**: Example of linear extension measurement (A) and count of branch number (1-4). **Right**: Example of measurement of branch length and width (all branches on fragment would be included)

2.2.5 Land-Based Nursery Optimization

Early results over the first four months of the experiment showed that linear extension and branch thickness of the fragments were greatly decreased in the land-based nursery. This led to a thorough review of water quality and lighting parameters in the nursery. Levels of photosynthetically active radiation (PAR) were measured at both the offshore nursery and the land-based nursery with an Apogee MQ-200 waterproof quantum sensor. Data were averaged every minute for 30 minutes between noon and 12:30 pm on a clear day in November 2010. Lower light levels in the land-based nursery led to a change of shade cloth density from 75% shading to 40% shading in January 2011. Corals were gradually acclimated to new light conditions over several days.

Weekly water quality testing revealed elevated levels of ammonia and phosphate in the saltwater well supplying the nursery. In response, a 4HP heat pump (Aqualogic Titan HP-4) was installed so that the system could maintain stable temperature without constant inputs of water from the well. As a result, the residence time of water in the nursery was increased, allowing the establishment of biological filtration by nitrifying bacteria and an overall reduction of ammonia in the system. Small (approximately 20%) weekly water changes were conducted with well water after heat pump installation. Phosphate levels in the nursery tank were reduced by periodic additions of lanthanum chloride (SeaKlear® Phosphate Remover), which causes precipitation of phosphate as a fine white solid (lanthanum phosphate) that is removed by sub-micron mechanical filtration and protein skimming.

2.2.6 June 2011 Fragment Collection

In order to re-examine the early survival of corals transplanted into a land-based nursery from offshore conditions after optimization of the land-based nursery was completed, a second set of fragments was taken from the offshore nursery in June 2011. Monitoring of the offshore nursery was completed for one year prior to this fragmenting. Similar to the original collection from wild donor colonies, two 5 cm fragments were taken from each of 56 colonies in the offshore nursery (all of those that were large in size and free of any disease). One of the fragments was kept in the offshore nursery, and the other was transported to the land-based nursery for a total of 56 new fragments in each of the nurseries. These new fragments were also monitored for survival and growth for five months (until the end of the experiment) in order to determine if there were improvements in post-collection stress in the land-based nursery after optimization. The

data from the second set of fragments were used only for comparison with the early period of survival and growth of the original fragments collected from wild donor colonies in 2010, and data from the second set of fragments were not pooled with growth data of the original corals collected in 2010.

2.2.7 Statistical Analysis

Fragment survival in the two nursery locations was compared using the non-parametric Kaplan Meier function (Kaplan and Meier, 1958). Differences in the survival function were tested using a post-hoc log rank test (Mantel, 1966). The count of branches per fragment was not normally distributed in the early portion of the experiment (Shapiro-Wilk, $p<0.05$), and transformation could not achieve normality across all groups. Therefore, a non-parametric Wilcoxon Rank Sum test was used to test for differences in the number of branches per fragment in each nursery location.

For the coral growth data, measurements were analyzed within three growth periods: early (4 months), during optimization (4 months), and post-optimization (4 months offshore, 9 months land-based). Outliers were assessed by inspection of a boxplot for each month's measurements within each nursery location, followed by a review of data and fragment photographs. Outliers resulting from measurement errors and visible breakage events were removed from the dataset, whereas outliers that appeared to be caused by actual extremes in growth were retained in the data. Normality was assessed using Shapiro-Wilk tests. Twenty of the 24 nursery x month combinations were normally distributed, and deviations from normality in the other four combinations were determined to be minor enough to proceed with parametric analyses.

For within-nursery comparisons of growth rate over time, a multivariate repeated measures analysis of variance was conducted. Pairwise comparisons were conducted using paired-sample t-tests after checking for normality of the differences between groups. Due to the potentially high number of multiple comparisons and high chance of Type I error, growth rates were only compared with the next consecutive month and the significance of multiple comparisons was determined using a Bonferroni correction, in which the resulting p-values were multiplied by the number of comparisons being conducted within the family.

For between-nursery comparisons each month, a Levene's test for homogeneity of variances showed that the variance in growth data between the two nurseries for each month was unequal. As a result of the loss of fragments in the land-based nursery during the first month (discussed in results below), the offshore nursery had a greater number of fragments and therefore a greater sample size, resulting in an unbalanced design. However, variance was generally higher in the offshore nursery data. Therefore, comparisons of growth rate between the two nurseries were conducted with a Welch's t-test (Welch, 1947), which does not assume equal variances. After optimization of the land-based nursery, Levene's tests indicated that the variance between the two nurseries was equal during each month, and therefore the data met all assumptions of a standard Student's t-test.

2.3 Results

2.3.1 Survival

Corals in both nurseries suffered a rapid tissue loss (RTL)-like syndrome within the first few days after introduction to each nursery. In the land-based nursery, 16 fragments (16.0%) died completely within the first week after collection. In the offshore nursery, 6 fragments (6.0%) also died of RTL-like symptoms after attachment. One fragment in the offshore nursery was broken off completely after transplant and lost from the experiment. This fragment was considered censored in the Kaplan-Meier analysis.

On day 8 after collection, the NSU land-based nursery suffered a mechanical failure leading to a large portion of water draining from the tank overnight and leaving many corals exposed to air for several hours. This incident led to the loss of an additional 36 corals, for a total loss of 52% of the initial fragments collected, laving 48 corals in the land-based nursery. After this initial event, the population and nursery were stabilized, and no more mortalities were sustained in the land-based nursery until the loss of one individual for unknown reasons in January of 2011. The offshore nursery continued to have occasional mortality throughout the experiment, both from RTL-like symptoms in warmer months and occasional severe breakage. An additional seven fragments were lost between July and October 2011 from a seasonal outbreak of RTL. At the end of the 16 months of monitoring, 85% of corals in the offshore nursery and 47% of corals in the land-based nursery survived (**Figure 2.4**). Survival was significantly lower in the land-based nursery (Kaplan-Meier survival test: Log-Rank X^2=33.605, p<0.01), largely due to the initial mechanical failure (**Figure 2.4**).

The fragments started in each nursery location in June 2011 (post-optimization) also showed a RTL-like syndrome within the first few days after introduction to each nursery. During the first week of the 2011 experiment, 7 of the 56 fragments (12.5%) were lost to tissue loss in the land-based nursery, and 12 fragments (21.4%) were lost from the offshore nursery. One additional fragment died in the offshore nursery in August 2011. Survival after five months in the land-based nursery was 87.5%, and survival in the offshore nursery was 78.5%. Although survival in the land-based nursery was higher in 2011, there was no significant difference in survival between the two nursery locations (Kaplan-Meier survival test: Log-Rank X^2=2.166, p=0.141).

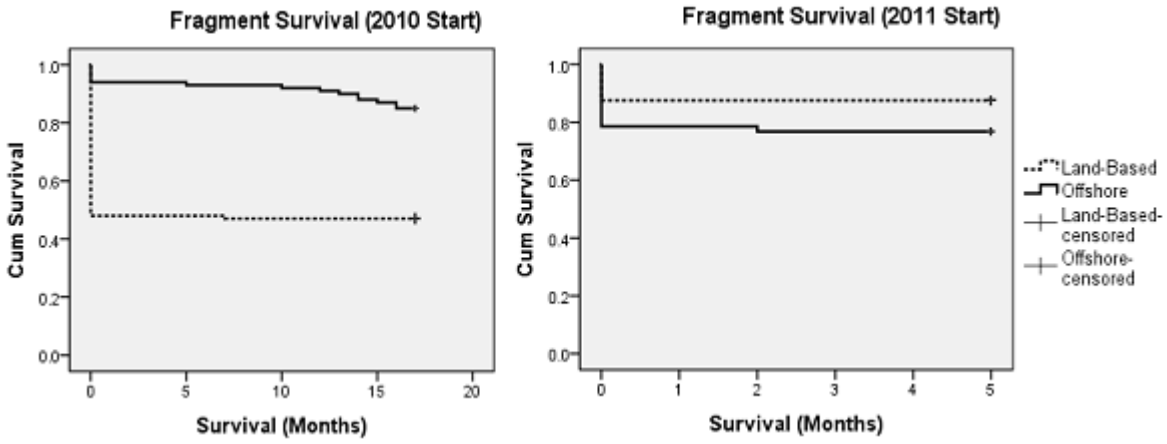

Figure 2.4. Kaplan-Meier survival curves over the course of the entire experiment. Left: Fragments started in May 2010; n=100. Right: Early survival of fragments started in June 2011; n=56.

2.3.2 Occurrence of Predation, Breakage, and Disease

Evidence of predation by the fireworm *Hermodice carunculata* (**Figure 2.5**) was first observed on six corals (6.4% of the 93 remaining fragments) in the offshore nursery at the end of January 2011, 8 months after establishment of the nursery. Predation was

subsequently observed during all monthly monitoring until the end of the experiment, with the highest incidence occurring in April 2011 on 19 corals (20.6% of the remaining fragments; **Table 2.2**). None of these incidents resulted in complete colony mortality. Monthly removal of fireworms from the nursery blocks was conducted during nursery visits beginning in February 2011. No fireworms or fireworm predation was present in the land-based nursery throughout the course of the experiment. One incidence of predation by a brachyuran crab occurred in the land-based nursery in May 2010, shortly after fragment collection. The crab was removed from the nursery. No further predation occurred in the land-based nursery.

A low level of branch breakage occurred regularly in the offshore nursery for unknown reasons between monthly visits, and also occasionally by divers during measurement visits. Three colonies in the land-based nursery had a branch broken during monthly measurements in the later portion of the experiment, as colonies grew large and began to crowd the holding tank, and required shuffling and removal from the tank for measurements. A summary of the number of colonies with broken branches each month is shown in **Table 2.2**. Overall, predation and breakage were greatly reduced in the land-based nursery in comparison with the offshore nursery (**Table 2.2**).

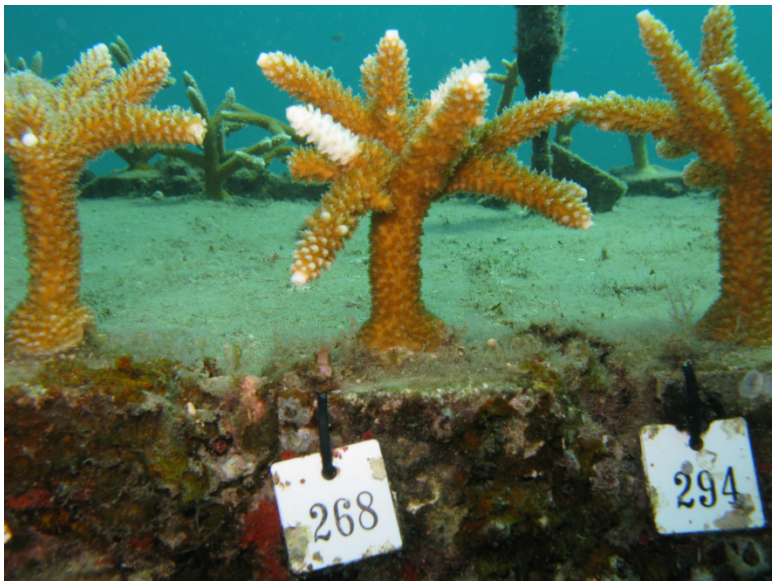

Figure 2.5. Example of predation by the fireworm *Hermodice carunculata* in the offshore nursery. Note white branches where tissue was consumed. Picture taken April 28, 2011.

Table 2.2. Percentage of colonies showing evidence of disease, breakage, and predation during each monthly monitoring event. Disease present in both nurseries was entirely Rapid Tissue Loss (RTL). Predation in the offshore nursery was entirely by fireworms.

	OFFSHORE			LAND-BASED		
	Disease	**Breakage**	**Predation**	**Disease**	**Breakage**	**Predation**
May-10	6.4%	3.2%	-	17.6%	-	1.2%
Jun-10	-	1.1%	-	-	-	-
Jul-10	-	4.3%	-	-	-	-
Aug-10	-	1.1%	-	-	-	-
Sep-10	-	3.2%	-	-	-	-
Oct-10	-	1.1%	-	-	-	-
Nov-10	-	-	-	-	-	-
Dec-10	-	2.2%	-	-	-	-
Jan-11	-	3.2%	6.5%	-	-	-
Feb-11	-	10.8%	10.8%	-	2.1%	-
Mar-11	-	2.2%	9.7%	-	2.1%	-
Apr-11	-	5.4%	20.7%	-	2.1%	-
Jun-11	-	6.5%	9.8%	-	-	-
Jul-11	4.4%	6.7%	7.8%	-	-	-
Aug-11	5.7%	9.1%	3.4%	-	-	-
Sep-11	10.3%	6.9%	1.1%	-	-	-
Oct-11	3.5%	4.7%	1.2%	-	-	-

2.3.3 Early Growth (June-September 2010)

Early in the experiment, linear extension rates (of the main stalk; mean ± SE) in the land based nursery and offshore nursery were 5.1 ± 0.3 mm month^{-1} and 9.8 ± 0.2 mm month^{-1}, respectively. Linear growth rate was significantly lower in the land based nursery (paired *t*-test: $p<0.0001$) when compared over the entire time period. Mean growth rate in each nursery during each month is shown in **Table 2.3 and Figure 2.6**. In the land-based nursery, there were significant differences in growth rate over time (repeated measures ANOVA, $F=40.4$, $p<0.0001$). Multiple comparisons showed that growth was significantly higher in month two and three than in month one (paired *t*, $p<0.0001$), but growth decreased in month 4 and was not significantly different than in month 1 (paired *t*, $p=0.453$). Mean growth rate in the offshore nursery was generally consistent between months; there was no significant difference in growth rate over time in the first four months (repeated measures ANOVA, $F=1.72$, $p=0.169$). Linear extension decreased from a maximum of 10.6 ± 0.4 mm month^{-1}in June (Month 1) to a minimum of 8.7 ± 0.6 mm month^{-1} in September (Month 4). When comparing between the two nursery locations within each month, linear growth in the land-based nursery was significantly lower every month during the first four months of the experiment (Welch's *t*, $p<0.0001$ for each month; **Table 2.4**).

Table 2.3. Mean linear growth rate (mm month^{-1}) and significance level of pairwise comparisons within each nursery location during the early period. Statistically significant differences between months in bold as determined by paired t-tests, significance determined at a Bonferroni corrected alpha of 0.008.

	MEAN ± SE	2 (JUL 10)	3 (AUG 10)	4 (SEP 10)
LAND-BASED NURSERY				
1 (JUN 10)	3.2 ± 0.4	**p<0.0001**	**p<0.0001**	p=0.453
2 (JUL 10)	7.1 ± 0.5		**p=0.004**	**p<0.0001**
3 (AUG 10)	6.4 ± 0.6			**p=0.001**
4 (SEP 10)	3.5 ± 0.7			
OFFSHORE NURSERY				
1 (JUN 10)	10.6 ± 0.4			
2 (JUL 10)	9.9 ± 0.4			
3 (AUG 10)	10.3 ± 0.5			
4 (SEP 10)	8.7 ± 0.6			

Table 2.4. Results of between-nursery comparison of growth rate for each month during the early period. Welch's t-test for unequal variances.

LAND-BASED NURSERY vs. OFFSHORE NURSERY				
Statistic	1 (JUN '10)	2 (JUL '10)	3 (AUG '10)	4 (SEP '10)
Welch's t Ratio	13.0	4.7	6.8	6.2
DF	133.3	122.3	129.9	111.3
Prob > |t|	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

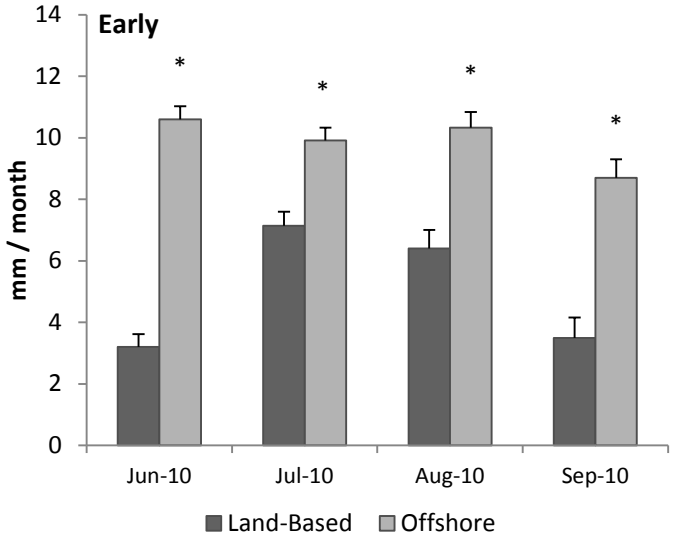

Figure 2.6. Linear extension rate in each nursery location over the first four months of the experiment (early period). Significant differences between the nurseries in each month indicated by asterisk. Error bars are ± SE.

In addition to slower growth, fragments in the land-based nursery produced very few new branches in the early portion of the experiment. By the end of September 2010, the mean (± SE) number of branches per fragment in the offshore nursery was 3.9 ± 2.4. The mean number of branches per fragment in the land-based nursery was 1.9 ± 1.6. The mean number of branches in the offshore nursery was significantly higher starting in July 2010 (Wilcoxon Rank Sum, Z=-3.53, p=0.0004), and the difference between the means increased at each measurement beyond this point (**Figure 2.7**).

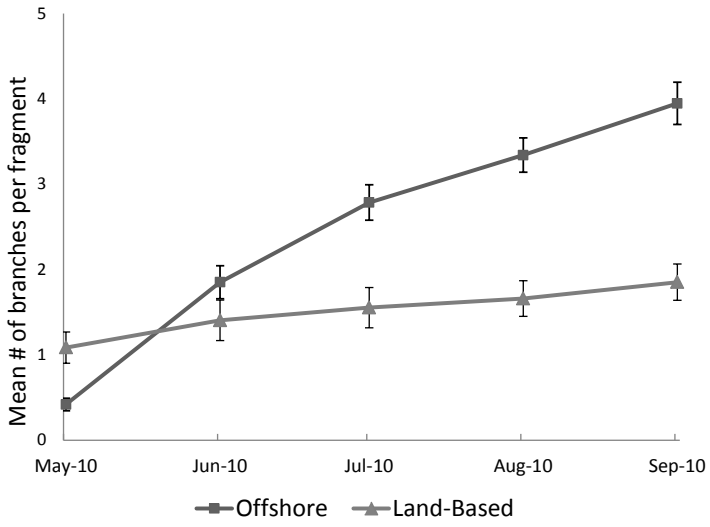

Figure 2.7. Mean number of branches per fragment in each nursery location during the early period. Error bars are ± SE.

The width of new branches in the land-based nursery in the early portion of the experiment appeared to be abnormally thin in comparison with new growth on the fragments in the offshore nursery (**Figure 2.8**). Branch width relative to length was first quantified at the end of September 2010. The maximum branch width in the land-based nursery was 5 mm. The maximum branch width in the offshore nursery was 12 mm. This difference was seen even on branches with the same length. A plot of branch width versus branch length at the end of September 2010 is shown in **Figure 2.9**.

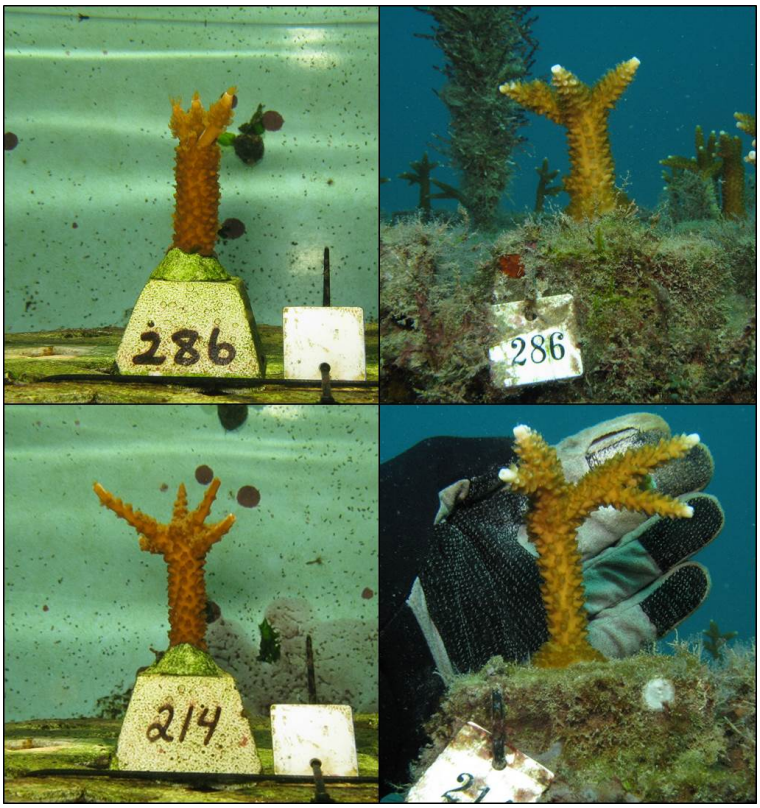

Figure 2.8. Examples of early growth in the land-based (Left) and offshore (Right) nurseries, showing thin branch width in land-based nursery.

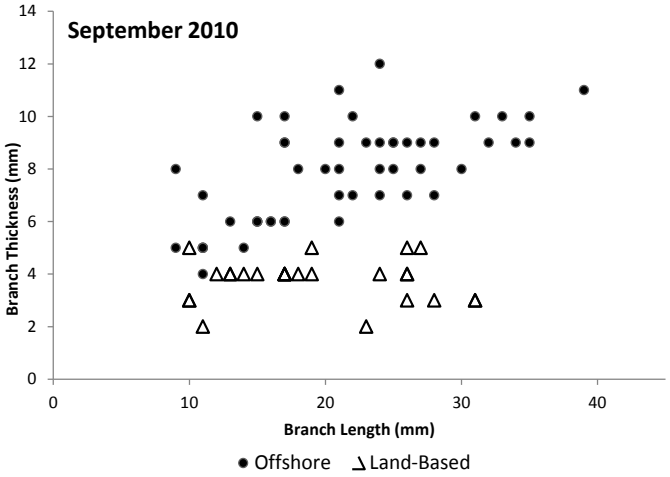

Figure 2.9. Scatter plot of branch length versus width in each nursery location at the end of September 2010 during the early period.

2.3.4 Land-Based Nursery Optimization (October 2010 – January 2011)

Several modifications were made to the land-based nursery between the end of September 2010 and January 2011. The water quality of the saltwater well was determined to be a significant concern, as the incoming well water had a consistently low pH, high total ammonia, and high phosphate levels (**Table 2.5**). Water quality values pre- and post-addition of the heat-pump in the end of September 2010 are shown in **Table 2.5**. The immediate result of allowing the system to re-circulate and avoid constant inputs of well water was an increase in pH and a reduction in total ammonia levels.

Table 2.5. Water quality data from the incoming saltwater well and the land-based nursery during the three growth periods.

	Units	Well	Nursery: Pre-Optimization	Nursery: During Optmization	Nursery: Post-Optimization
Temp	°C	26.1 ± 0.3	27.3 ± 0.5	26.3 ± 0.7	26.8 ± 0.7
pH	-	7.63 ± 0.01	8.00 ± 0.18	8.23 ± 0.13	8.21 ± 0.12
ORP	mV	73 ± 22	271 ± 79	270 ± 28	265 ± 2
Salinity	ppt	35.0 ± 1.0	35.5 ± 0.1	36.0 ± 1.0	36.0 ± 1.0
PO_4^{3-}	mg/l	0.34 ± 0.04	0.30 ± 0.04	0.22 ± 0.11	0.06 ± 0.03
	µM	3.58 ± 0.42	3.16 ± 0.42	2.32 ± 1.16	0.63 ± 0.32
NH_4	mg/l	0.32 ± 0.03	0.28 ± 0.11	0.03 ± 0.06	0.02 ± 0.02
	µM	18.82 ± 1.76	16.47 ± 6.47	1.76 ± 3.53	1.18 ± 1.18
NO_2	mg/l	0.01 ± 0.00	0.08 ± 0.04	0.02 ± 0.03	0.02 ± 0.01
	µM	0.22 ± 0.04	1.74 ± 0.87	0.52 ± 0.63	0.33 ± 0.15
NO_3	mg/l	0.04 ± 0.01	0.21 ± 0.22	0.47 ± 0.16	0.11 ± 0.16
	µM	0.60 ± 0.10	3.39 ± 3.55	7.50 ± 2.61	1.73 ± 2.65
Calcium	mg/l	397 ± 12	389 ± 16	379 ± 20	389 ± 26
Total Alkalinity	mg/l as $CaCO_3$	142 ± 6	141 ± 5	137 ± 9	131 ± 18
	mEq/L	2.84 ± 0.12	2.82 ± 0.10	2.74 ± 0.18	2.62 ± 0.36
	µM	2840 ± 120	2820 ± 100	2740 ± 180	2620 ± 360

Beginning in November 2010, a dilute solution of lanthanum chloride and deionized water was added to the system periodically in order to reduce levels of orthophosphate. Appropriate dosage levels and frequency were determined through frequent testing, and dosing was consistent by the end of December 2010. The target level of orthophosphate was set at 0.05 mg l^{-1}, as gradual reduction by biological processes (i.e., algal growth and coral uptake) was observed to occur, and the desired result was to avoid complete depletion of phosphate between water changes.

During this time, PAR levels in the land-based nursery (as measured at the coral branches) were found to be lower than the offshore nursery (187 ± 16 µmol m^{-2} sec^{-1} and 484 ± 53 µmol m^{-2} sec^{-1}, respectively). This difference was highly significant ($F=258.3$; $p<0.0001$). Incident PAR (as measured outside of the shade structure) at the land based nursery in November 2010 was 1533 ± 74 µmol m^{-2} sec^{-1}. The 75% shade cloth and heavy surface water agitation present in the land-based nursery reduced incident PAR by a total of 88% at the coral branches. To better replicate offshore light conditions, calculations showed that the shade cloth over the nursery should be reduced to a 40% shade level.

During December 2010, prolonged cold temperatures resulted in the need to turn the system back to flow through from the saltwater well in order to maintain the water temperature in the nursery. The minimum air temperature recorded in Fort Lauderdale, FL during this period was 1.1˚C (34˚F) on December 14, 2010 (Global Historical Climatology Network Daily Database; Menne et al. 2012), during which the land-based nursery temperature dropped to only 23.7˚C (74.7˚F). Over the entire month, the average

daily minimum air temperature was 9.6˚C (49.3˚F), and the overall average water temperature in the land-based nursery was maintained at 25.7˚C (78.3˚C). This was the coldest December on record in Fort Lauderdale (National Weather Service, 2010).

By January 2011, water quality parameters were stabilized to within the desired range. The shade cloth covering the tank area was changed from 75% shade to 40% shade on January 11, 2011. Corals were gradually acclimated to the new light levels by keeping an additional piece of 40% shade cloth over the tank during the peak PAR hours from 1100 to 1400 hours. The number of hours of additional shading was gradually decreased over 10 days. The resulting PAR level measured at the coral branches in the land-based nursery at the end of January 2011 was 478 ± 23 μmol m^{-2} sec^{-1}, which was not significantly different than the offshore levels (484 ± 53 μmol m^{-2} sec^{-1}) measured in early December 2010 ($F=0.1$; $p=0.756$). Weather and offshore conditions prohibited re-measurement of PAR levels at the offshore nursery in January 2011.

2.3.5 Growth During Optimization (October 2010 – January 2011)

Coral linear growth rate in the land-based nursery during the optimization period varied significantly over time (repeated measures ANOVA, $F=13.8$, $p<0.0001$). Multiple comparisons showed that growth rate was higher in month six (November 2010) than in month five ($p=0.015$), seven ($p<0.0001$), and eight ($p<0.0001$). There was no significant difference in growth between months five, seven, and eight (**Table 2.6**). Growth in the offshore nursery also varied significantly over time (repeated measures ANOVA, $F=14.4$, $p<0.0001$). Linear growth decreased significantly each month from October through December (**Table 2.6**, **Figure 2.10**), but there was no difference in growth rate between

December and January (p=0.575). Growth in the land-based nursery remained significantly lower than the offshore nursery during each month (Welch's *t*, **Table 2.7**).

Table 2.6. Mean linear growth rate (mm month^{-1}) and significance level of pairwise comparisons within each nursery location during the optimization period. Statistically significant differences between months in bold, as determined by paired t-tests.

	MEAN ± SE	6 (NOV-10)	7 (DEC-10)	8 (JAN-10)
LAND-BASED NURSERY				
5 (OCT-10)	3.9 ± 0.7	**p=0.0147**	p=0.2272	p=0.2611
6 (NOV-10)	5.5 ± 0.5		**p<0.0001**	**p<0.0001**
7 (DEC-10)	3.1 ± 0.5			p=0.7264
8 (JAN-10)	3.4 ± 0.5			
OFFSHORE NURSERY				
5 (OCT-10)	9.0 ± 0.6	**p=0.0020**	**p<0.0001**	**p<0.0001**
6 (NOV-10)	6.8 ± 0.5		**p=0.0159**	**p=0.0071**
7 (DEC-10)	5.3 ± 0.4			p=0.5745
8 (JAN-10)	5.1 ± 0.5			

Table 2.7. Results of between-nursery comparison of growth rate for each month during the optimization period. Welch's t-test for unequal variances.

LAND-BASED NURSERY vs. OFFSHORE NURSERY				
Statistic	5 (OCT '10)	6 (NOV '10)	7 (DEC '10)	8 (JAN '11)
Welch's t Ratio	5.9	2.0	3.9	2.7
DF	113.8	120.2	115.6	131.9
Prob > |t|	**<0.0001**	**0.0426**	**0.0002**	**0.0079**

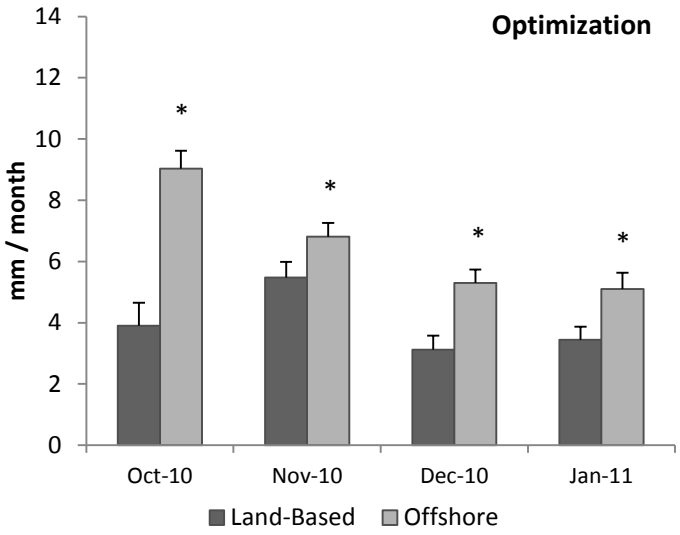

Figure 2.10. Linear extension rate in each nursery location over the period of optimization of the land-based nursery. Significant differences between the nurseries within each month indicated by asterisk. Error bars are ± SE.

The mean (± SE) number of branches per fragment continued to be low in the land-based nursery during the optimization period, while the number of branches per fragment in the offshore nursery steadily increased (**Figure 2.11**). By the end of January 2011, the offshore nursery had 9.0 ± 4.5 branches per fragment, and the land-based nursery had 2.5 ± 2.0 branches per fragment. The thickness of branches in the land based nursery continued to be lower than those in the offshore nursery (**Figure 2.12**), but the maximum branch width increased from 5 mm in the early period to 7 mm in the optimization period. The maximum branch width in the offshore nursery remained at 12 mm.

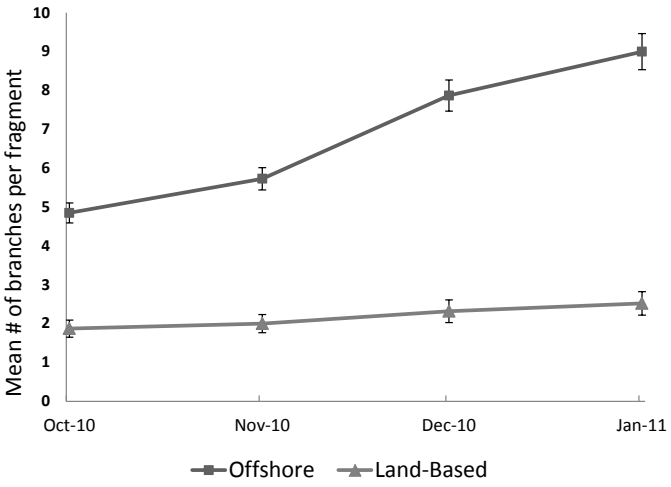

Figure 2.11. Mean number of branches per fragment in each nursery location during the optimization period. Error bars are ± SE.

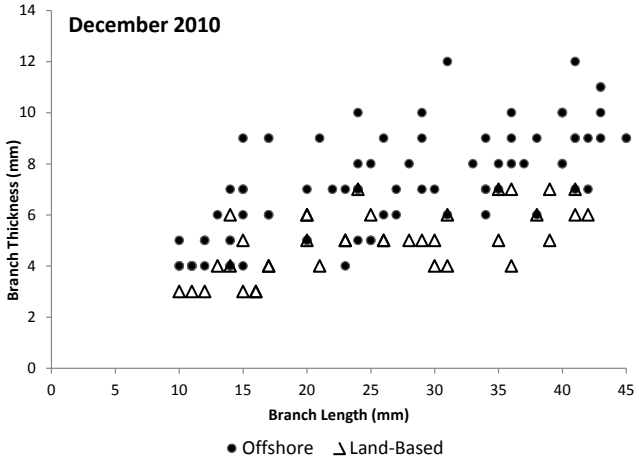

Figure 2.12. Branch length versus width for fragments in the land-based and offshore nurseries, as measured at the end of December 2010 during the optimization period.

2.3.6 Post-Optimization Growth (February 2011 – May 2011)

From the last month of optimization (January 2011) to the first full month post-optimization (February 2011), the linear growth rate in the land-based nursery increased significantly from 3.4 ± 0.4 mm month^{-1} to 9.2 ± 0.8 mm month^{-1} (paired t=9.35;

p<0.0001). Growth rate continued to increase significantly from February to March (p=0.0016; **Table 2.8**), and again from April to May (p<0.0001). During the post-optimization period, growth rate in the land based nursery was significantly higher than in the offshore nursery in every month except for April 2011, when there was no significant difference between the two nurseries (**Table 2.9**).

Table 2.8. Mean linear growth rate (mm month^{-1}) and significance level of pairwise comparisons within each nursery location during the post-optimization period. Statistically significant differences between months in bold, as determined by paired t-tests.

	MEAN ± SE	10 (MAR-11)	11 (APR-11)	12 (MAY-11)
LAND-BASED NURSERY				
9 (FEB-11)	9.2 ± 0.8	**p=0.0016**	**p=0.0168**	**p<0.0001**
10 (MAR-11)	11.1 ± 0.7		p=0.6729	**p<0.0001**
11 (APR-11)	10.9 ± 0.8			**p<0.0001**
12 (MAY-11)	15.1 ± 1.0			
OFFSHORE NURSERY				
9 (FEB-11)	6.4 ± 0.6	p=0.3339	**p=0.0014**	**p=0.0015**
10 (MAR-11)	6.0 ± 0.5		**p<0.0001**	**p<0.0001**
11 (APR-11)	9.4 ± 0.7			p=0.7490
12 (MAY-11)	9.5 ± 0.6			

Table 2.9. Results of between-nursery comparison of growth rate for each month during the post-optimization period. Two-sample t-test for equal variances.

LAND-BASED NURSERY vs. OFFSHORE NURSERY				
Statistic	9 (FEB '11)	10 (MAR '11)	11 (APR '11)	12 (MAY '11)
t Ratio	-2.8	-5.9	-1.5	-5.8
DF	123.0	128.0	126.0	133.0
Prob > |t|	**0.0051**	**<0.0001**	0.1495	**<0.0001**

In the offshore nursery, growth rate increased slightly from a mean of 5.1 ± 0.5 mm month^{-1} in January 2011 to 6.4 ± 0.6 mm month^{-1} in February 2011, but this difference was not significant (t=1.93; p=0.0570). There was also no significant change in growth from February to March (p=0.33), but there was a large increase in April to 9.4 ± 0.7 mm month^{-1}. The growth rates in both April and May were significantly higher than in March (**Table 2.8**).

Although comparison with the offshore nursery ended in May 2011, the land-based nursery corals were measured monthly until October 2011. Growth rate in the land-based nursery continued to exceed 11 mm month^{-1} until the end of monitoring, with a maximum monthly growth rate occurring in July 2011 at 16.0 ± 1.0 mm month^{-1}. The highest monthly growth rate recorded in the offshore nursery over the entire experiment was 10.6 ± 0.4 mm month^{-1} in June 2010. Growth rates recorded during the post-optimization period and continued monitoring of the land-based nursery are shown in **Figure 2.13**.

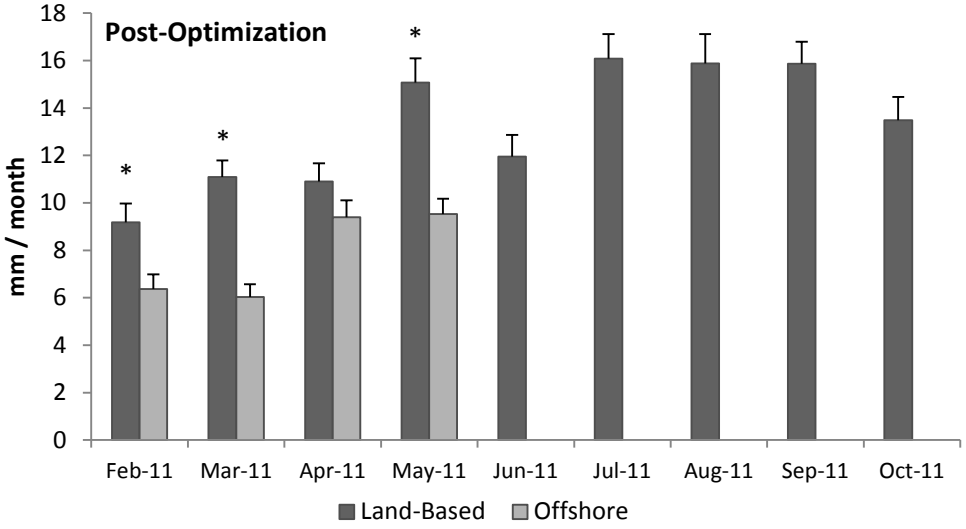

Figure 2.13. Mean linear extension in each nursery location post-optimization of the land-based nursery. Significant differences between each nursery indicated by asterisk over the higher rate. Error bars are ± SE.

During the post-optimization period, the number of branches per fragment in the land-based nursery began to increase rapidly. From the end of January 2011 to the end of February 2011, the mean (± SE) number of branches increased from 2.5 ± 2.0 branches per fragment to 3.8 ± 2.9 branches per fragment, the largest monthly mean increase that had been observed to this point. From February to May, fragments in the land-based nursery formed an average of two new branches per month, resulting in a mean of 10.0 ± 5.1 branches per fragment in the end of May 2011 (**Figure 2.14**). The maximum increase in branches per month in the land-based nursery was observed from July to August 2011, with a mean increase of 6.8 ± 2.8 branches per fragment (**Figure 2.14**). The maximum increase in branches per month in the offshore nursery was from April to May 2011, with a mean increase of 4.6 ± 4.3 branches per fragment; however, the offshore nursery was no longer being monitored after May 2011.

After nursery optimization, fragment branches in the land-based nursery also continued to increase in mean width. By the end of February 2011, the maximum branch width had increased to 10 mm, and continued to increase to 12 mm by the end of April 2011 (**Figure 2.15**). The maximum branch width in the offshore nursery remained consistent at 12 mm during this time. A photographic series from a single fragment in the land-based nursery from the end of nursery optimization to the end of June 2011 is shown in **Figure 2.16**.

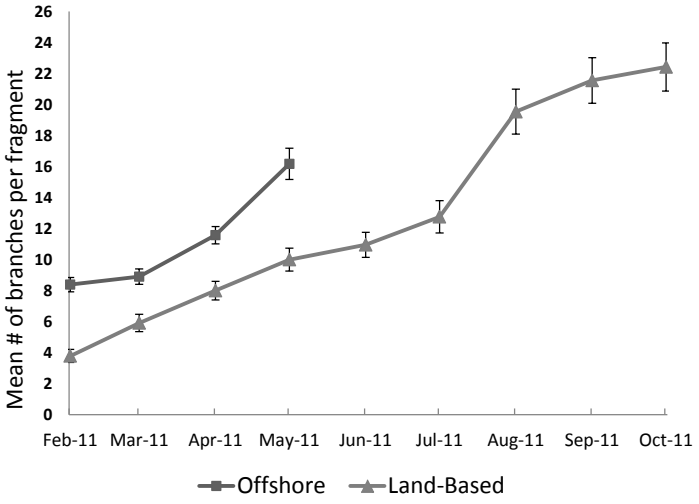

Figure 2.14. Mean number of branches per fragment in each nursery location during the post-optimization period. Fragments in the offshore nursery were not measured beyond May 2011. Error bars are ± SE.

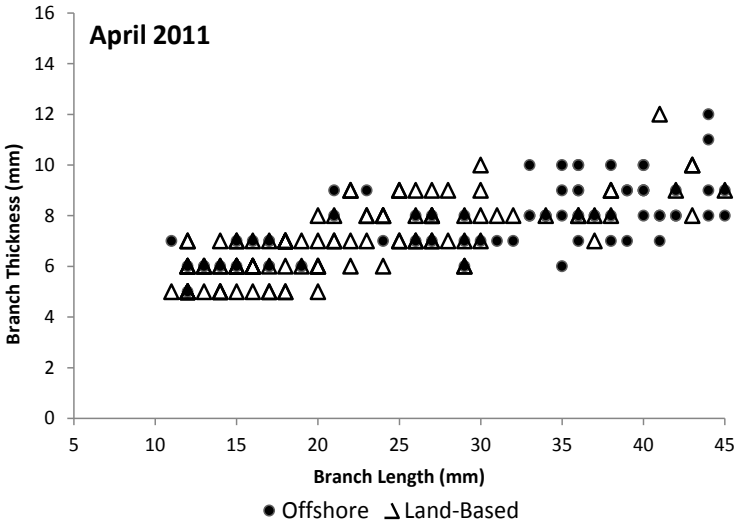

Figure 2.15. Branch length versus width for fragments in the land-based and offshore nurseries, as measured at the end of April 2011 during the post-optimization period.

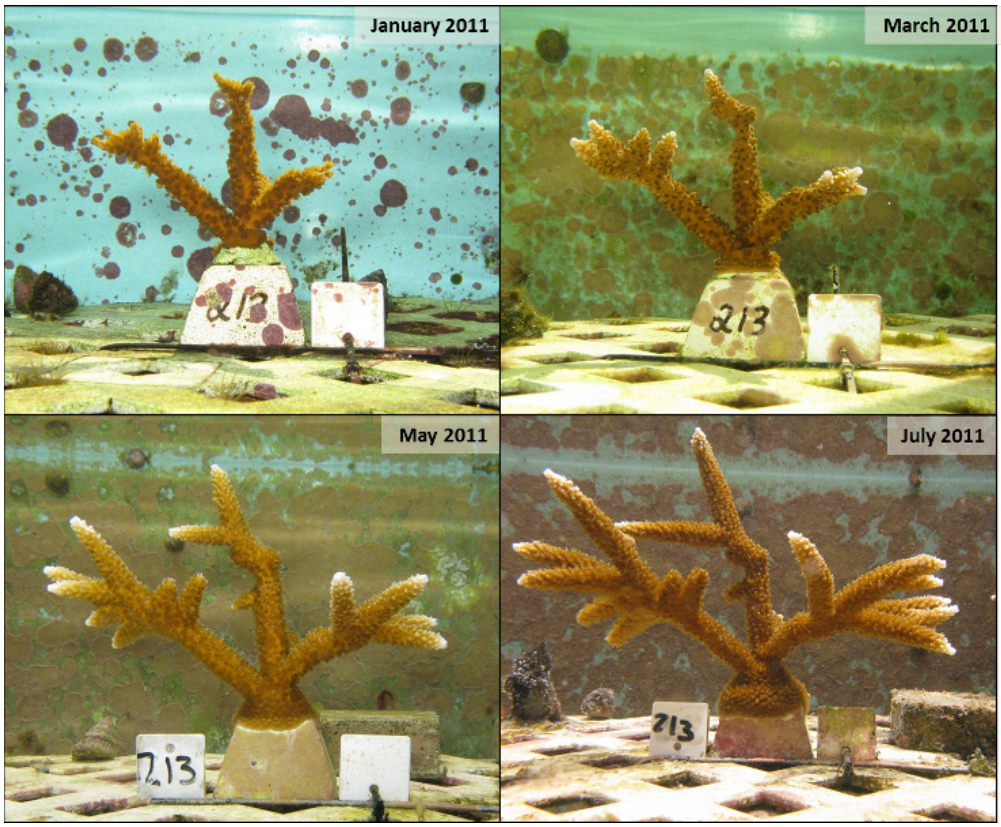

Figure 2.16. Six month time series photographs of Fragment 213 during the post-optimization period: January 2011 through July 2011.

Despite eight months of significantly reduced growth, there was no significant difference in the overall height of colonies in the land-based and offshore nurseries at the end of May 2011 when measurements of the offshore nursery ended. Final coral height in the offshore nursery was 13.6 cm (± 0.4 SE) with a maximum width of 15.1 cm (± 0.5 SE) cm, and 16.2 (± 9.7 SE) branches per fragment. Height of corals in the land-based nursery in the same month was 13.5 cm (± 0.5 SE) with a maximum width of 11.5 cm (± 0.5 SE), and 10.0 (± 5.1 SE) branches per fragment. Maximum width and the number of branches per fragment were lower in the land-based nursery. Photographic comparisons of fragments in the two nursery locations in May 2011 are shown in **Figure 2.17**.

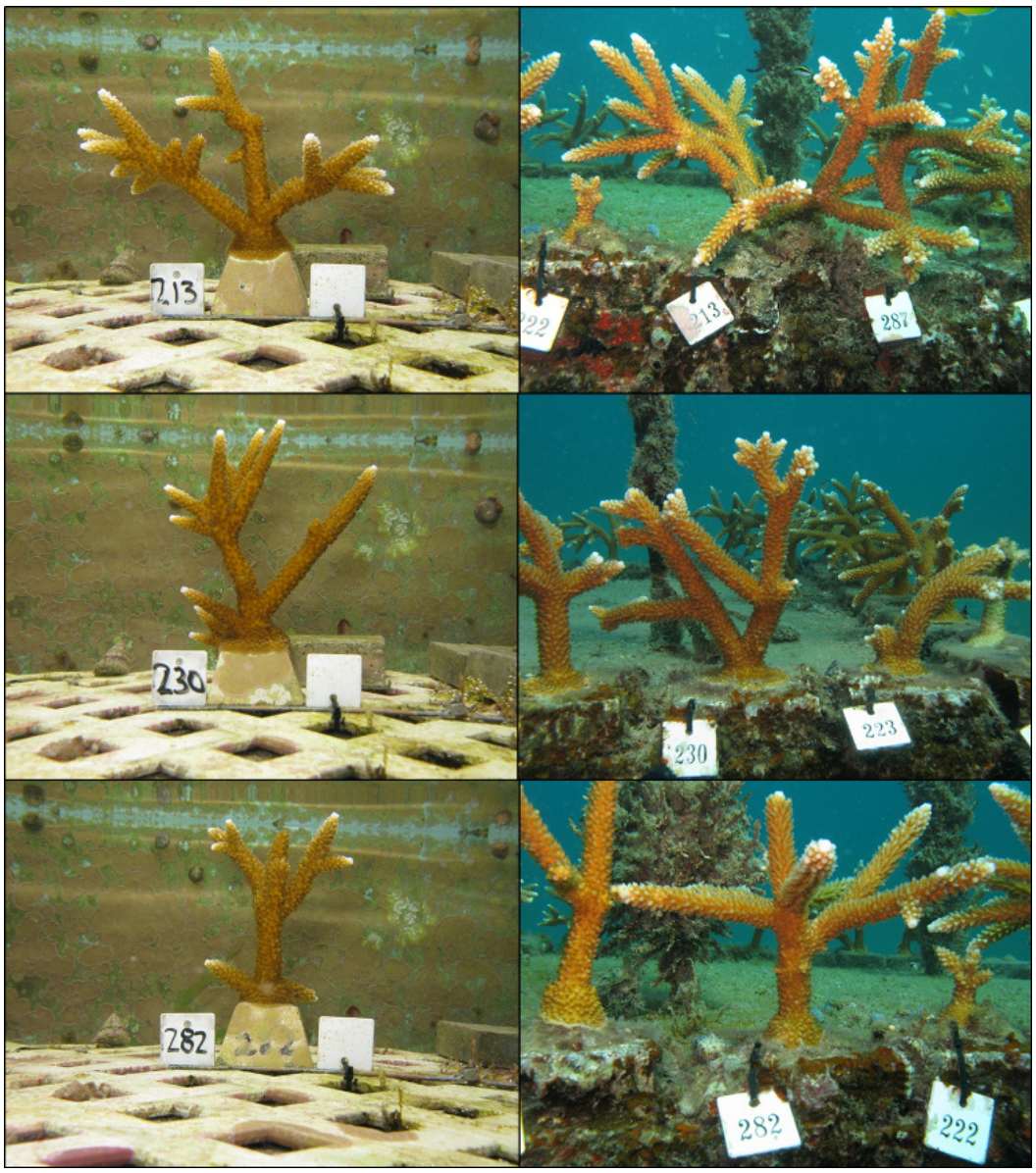

Figure 2.17. Photographs of fragments from the same donor colonies (indicated by numbered tag) taken in May 2011. Left: land-based nursery. Right: offshore nursery.

2.4 Discussion

2.4.1 Survival Between Nurseries

The occurrence of rapid tissue loss in both nursery locations immediately following collection is likely a result of the stress of fragmentation, transport, and re-attachment. Mortality of fragments shortly after attachment is commonly reported in *A. cervicornis* nurseries. Herlan and Lirman (2008) reported 9.2% mortality of *A. cervicornis* fragments in the first three weeks and 17.3% mortality in the first 8 weeks, which is comparable to the mortality observed in this experiment. The percentage of fragments affected in the land-based nursery was higher than the offshore nursery in June 2010, possibly indicating additional stress from being transplanted into an artificial environment. During the early portion of the experiment, water quality in the land-based nursery was not ideal; elevated levels of phosphate and ammonia, and decreased pH levels, may have contributed to additional stress on fragments transplanted in the land-based nursery. However, the percentage of fragments lost to early rapid tissue loss immediately following placement of corals in the land-based nursery was nearly identical pre- and post-optimization (12.0% and 12.5% respectively).

The percentage of fragments lost immediately following fragmentation and placement in the offshore nursery was much greater in June 2011 than in 2010 (21.4% and 6.0% respectively). This is unexpected as the 2011 fragments were generated from corals in the offshore nursery rather than wild donor colonies, were never transported by boat, and were moved less than 1 meter from their original location in the offshore nursery; therefore transplant stress should have been minimal. Larson (2010) found that mortality in newly fragmented *A. cervicornis* was up to 56% in September of 2007 and attributed

this mortality to high water temperatures due to a reduction in mortality (to 22%) in a second transplant event when temperatures were lower in December 2007. Water temperatures at the offshore nursery at the time of fragmenting in June 2011 were slightly higher than in May 2010. However this difference was small, with mean daily water temperature of 27.2 ˚C on May 17-20, 2010 and 28.5˚C on June 13, 2011. Mean water temperatures in the month following fragmentation were similar in both years [29.1±1.3˚C, (maximum 30.5˚C) in 2010 compared to 29.3±0.5˚C (maximum of 30.2˚C) in 2011]. Additional review of photographs and field notes from monitoring of the 2011 fragments in the offshore nursery showed that eleven out of the twelve corals with mortality in the first month had been broken and reduced to a small piece of skeleton only 1 – 2 cm in size at some point between the initial and 1-month monitoring events. It is likely that predation or breakage played a role in the high level of initial mortality in the offshore nursery during this fragmentation.

The loss of 36% of corals in the land-based nursery in 2010 was a result of equipment mechanical malfunction in the nursery relating to a valve being left in a closed position. The mechanical nature of recirculating aquaculture systems makes them prone to the impacts of equipment failure such as power outages, pump seal or motor failures, temperature control malfunction, or human operational error. This has led to the development of a variety of computerized environmental monitoring and control systems that are commonly implemented in intensive aquaculture systems to prevent or minimize losses from such failures (Lee 1995; Simbeye et al. 2014). Automated monitoring and process control can also be used to optimize culture conditions and increase process efficiency (Lee 2000). Such systems are often costly to implement and may not be

included in pilot-scale projects or be affordable in production that is not for financial profit, such as for re-stocking or restoration.

The NSUOC land-based nursery system had limited automated monitoring capabilities; pH, temperature, and ORP readings were local only and not remotely accessible or linked to alarm notifications. Installation of a water level sensor with alarm capabilities could have prevented fragment loss in this situation. Large-scale aquaculture of threatened or endangered species of coral should include computerized, remote monitoring capabilities with notifications to system operators when parameters essential for coral survival are out-of-range. Automated monitoring systems, coupled with daily visual inspection, can greatly reduce the chances of catastrophic losses of stocks due to equipment failure. Daily (7 days per week) observation has been suggested as a best management practice for aquaculture operations in order to reduce the risk of disease outbreaks and to check on the operation of system components in recirculating systems (Florida Department of Agriculture and Consumer Services 2007; Maryland Aquaculture Coordinating Council 2007).

Offshore coral nurseries are not without risk. The final mortality rate of 52% observed in the land-based nursery is comparable or less than those reported in offshore nurseries exposed to risk factors such as extreme water temperatures or hurricane damage (Schopmeyer et al. 2012 [up to 100% mortality]; Larson 2010 [up to 56%]; Quinn and Kojis 2006 [up to 94%]). Whereas the risk of climate extremes and hurricane damage is largely uncontrollable in offshore nurseries, risk can be minimized in land-based nurseries through the use of best management practices and proper system engineering.

2.4.2 Predation, Breakage and Disease

An important benefit of land-based nursery culture is the ability to control environmental parameters and potentially limit losses due to disease, predation, and breakage. Beyond the first month of the experiment, corals in the land-based nursery showed no evidence of disease or predation whereas the offshore nursery experienced persistent predation starting in January 2011 followed by an outbreak of rapid tissue loss in summer and early fall of 2011. Predation has been identified as one of the largest concerns in offshore nursery culture, along with breakage, algal overgrowth, and disease (Young et al. 2012). Controlling predator recruitment and migration into an offshore nursery can be difficult and requires ongoing maintenance. In land-based systems, careful visual inspection of all new additions to the nursery, followed by daily observation, can eliminate the presence of predators. In recirculating aquaculture systems, the use of quarantine tanks and other bio-security protocols can further reduce the possibility of disease outbreaks and the introduction of pest species to the system (Yanong and Erlacher-Reid 2012).

High water temperatures have been linked to oxidative stress (Downs et al. 2002; Lesser 2006), bleaching (Jokiel and Coles 1990; Glynn 1996; Brown 1997; Downs et al. 2002), and disease (Rosenberg and Ben-Haim 2002; Bruno et al. 2007) in scleractinian corals. Cold water temperatures ("cold-stress") can also cause high levels of coral mortality (Lirman et al. 2011; Schopmeyer et al. 2012) and bleaching (Hoegh-Guldberg et al. 2005). The ability to maintain stable year-round water temperatures in land-based nurseries is one advantage over offshore nursery culture. Land-based nurseries located outdoors are more susceptible to seasonal temperature extremes; the temperature of a small body of water can change rapidly with ambient air temperature. Heating and

cooling systems must be engineered to maintain stable water temperatures during extreme weather events in order to minimize stress and mortality of coral stocks.

Although southern Florida generally has a mild tropical climate, the cold event of December 2010 with nightly low temperatures of 1.1˚C could have resulted in devastating losses in the land-based nursery if the ability to return to the flow-through, groundwater well system had not been available. This cold weather event was followed by a cold-water anomaly on nearby reefs with water temperatures below 16˚C for up to 14 days in the Florida Keys in January 2011; nearshore reefs in Broward County remained above 20˚C (Lirman et al. 2011; Schopmeyer et al. 2012). This cold-water event resulted in catastrophic mortality of corals in the Florida Keys (Kemp et al. 2011; Lirman et al. 2011; Colella et al. 2012), including mortality of *A. cervicornis* in several offshore nurseries (Schopmeyer et al. 2012). The design of land-based nursery systems should consider the potential for extreme weather events, and the system should have the heating and cooling capacity to maintain proper temperatures for coral survival during these events.

The occurrence of breakage was greatly reduced in the land-based nursery. *A. cervicornis* is a relatively fragile species with thin, elongated branches that often break when exposed to elevated wave action in the natural environment (Tunnicliffe 1981; 1982). The offshore nursery in the current study was located in a sandy area that was exposed to the nearshore wave climate of southeastern Florida. Broward County is a highly urbanized area, and activity by recreational boaters in the area is high. Observations during monthly visits to the offshore nursery indicated low levels of branch

breakage likely caused by wave action or other biota; large hermit crabs (*Diogenidae* and *Paguridae*) were often seen wedged tightly in between coral branches. There were also several larger breakage events where several adjacent corals were severely damaged at one time. These events may have resulted from entanglement in fishing gear or anchor damage, or possibly damage by recreational divers. The ability to control the occurrence of breakage is another benefit of land-based culture; this benefit is most important in areas where offshore nurseries cannot be placed in sheltered lagoons or where human disturbance is high.

2.4.3 Optimizing Land-Based Nursery Conditions

In the early portion of the experiment, mean linear extension in the land based nursery (5.4 mm month^{-1} or 6.5 cm yr^{-1}) was significantly lower than the offshore nursery and lower than other published extension rates for the species in normal habitat (Shinn 1966 [10 cm yr^{-1}]; Tunnicliffe 1983 [12.0 cm yr^{-1}]). During this time period, corals in the land based nursery were exposed to fluctuating levels of inorganic nutrients, including extremely high levels of ammonia and phosphate. Ferrier-Pages et al. (2000) studied the effects of ammonia and phosphate enrichment on the growth rate of *Stylophora pistillata.* Ammonia levels of 10 µM had no effect on growth rate; however an increase to 20 µM decreased growth by 60% (Ferrier-Pages et al., 2000). A phosphate level of 2 µM also reduced growth rate by 60%, and combined ammonia and phosphate resulted in a 50% decrease in growth (Ferrier-Pages et al., 2000). Mean ammonia level in the land-based nursery in the early period was 16.5 µM, and mean phosphate concentration was 3.2 µM (**Table 2.5**), suggesting that these levels may have been high enough to reduce growth rate.

The reduced number of branches per fragment and smaller width of the branches formed during the early portion of the experiment also have important implications for overall fragment production in the nursery and the ability to use fragments for restoration purposes. The combination of slower growth, with a significantly lower number of branches, would result in a cumulative reduction in the number of fragments produced by the nursery over time. In addition, fragments with a smaller branch diameter may be more prone to breakage following transplant.

Watson and Hill (2006) discussed the importance of maintaining stable conditions that closely mimic those found on tropical reefs when designing recirculating production systems for marine ornamental species, including corals. In order for land-based coral nurseries to be successful, environmental conditions within the nursery must be maintained within the range of natural reefs. Environmental factors that determine the suitability of a habitat to support coral reefs include temperature, nutrients, light, salinity, aragonite saturation state, and water flow (Kleypas et al. 1999, Couce et al. 2012).

Water temperature is considered one of the most important controls on global reef distribution, with the majority of reefs being found where the minimum sea surface temperature is greater than 18˚C (Kleypas 2007). The optimum temperature for scleractinian coral growth is generally considered to be between 25.0 and 29.0˚C and is variable depending on the species and the thermal history of the environment (Clausen and Roth 1975, Coles and Jokiel 1978, Gladfelter 1984). Coral stress and mortality can occur even with short-term deviations from acceptable temperatures. Fitt et al. (2009) observed a reduction in symbiont density and increased mortality in *S. pistillata* after

only 3 days at 32˚C, and Hoegh-Guldberg and Smith (1989) observed visible paling in 2 days at 32˚C and complete mortality within 8 hours at 34˚C.

Coral culture in "high nutrient" water sourced from a saltwater well was reported by Atkinson et al. (1995) at the Waikiki Aquarium. Levels of inorganic nutrients in the NSUOC well water before optimization were almost a full order of magnitude higher than those in Waikiki; total ammonia of 2.4 μM and phosphate levels of 0.60 μM were reported in the well at Waikiki Aquarium (Atkinson et al. 1995). After optimization, levels in the land-based nursery were comparable to Waikiki (1.18 μM total ammonia and 0.63 μM phosphate). The high growth rates observed by Atkinson et al. (1995) and during the latter portion of this study, compared to the reduced rates in the beginning of the current study, suggest a threshold for acceptable nutrient levels that lies between the two observations. In general, levels of dissolved inorganic nutrients should be minimized through both the use of a quality seawater source and removal of accumulated nutrients through biological and chemical filtration.

The effect of light on coral growth and morphology has been studied extensively both *in situ* (Falkowski et al. 1984, Huston 1985, Anthony and Hoegh-Guldberg 2003) and *ex situ* (Schlacher et al. 2007, Schutter et al. 2008, Wijgerde et al. 2012, Rocha et al. 2013). Optimum light levels can be highly species-specific. The Pacific staghorn coral *Acropora formosa* had faster linear extension in 15 m water depth (lower light) than in 5 m but grew with fewer branches and increased branch spacing in deeper water (Oliver et al. 1983). The calcification rate of *A. cervicornis* is correlated with light intensity up to saturating levels (Chalker and Taylor 1975, 1978). Chalker and Taylor (1978) created

light-saturation curves for photosynthesis and calcification of *A. cervicornis*. The irradiance value in which calcification was saturated (I_k) was 330 μE m^{-2} s^{-1}, and maximum calcification rate was not reached until approximately 800 μE m^{-2} s^{-1}, with photoinhibition occurring at higher irradiance levels. The low light levels in the land-based nursery in the early potion of the experiment likely resulted in reduced calcification and may have resulted in reduced branch formation. However, appropriate light intensity should be considered on a case-by-case and species-by-species basis, as Schutter et al. (2012) demonstrated that increasing irradiance and photoperiod had no effect on growth in *Galaxea fasicularis*.

Other factors that can potentially affect coral survival and growth in land-based nurseries that were not specifically monitored in this study include water flow, aragonite saturation state, and the level of dissolved organic material. Water flow in the land-based nursery was adjusted before the experiment began and was measured up to 70 cm s^{-1} depending on location in the nursery and distance from the outlets of the circulation system. Water flow was measured between 1 and 33 cm s^{-1} directly around the coral branches. Under moderate wave conditions, forereef water flow can be in the range of 20 to 40 cm s^{-1}; maximum forereef flow in the surf zone under heavy wave conditions can exceed 100 cm s^{-1} (Sebens et al. 2003). Water flow has been shown to affect the photosynthetic rate (Dennison and Barnes 1988, Lesser et al. 1994), prey capture (Sebens et al. 1997, Sebens et al. 1998), nutrient uptake (Thomas and Atkinson 1997, Atkinson et al. 2001), calcification (Dennison and Barnes 1988), growth (Nakamura and Yamasaki 2005), and bleaching resistance and recovery (Nakamura and Van Woesik 2001, Nakamura et al. 2003, Fabricius 2006, Finelli et al. 2006) of scleractinian corals.

Calcium and alkalinity levels in coral aquariums are typically maintained through addition of chemicals such as calcium hydroxide, calcium chloride, sodium bicarbonate, and/or sodium carbonate, or the use of a calcium reactor in which solid aragonite material is dissolved in a low pH chamber and slowly added back into the system. The ability to increase the aragonite saturation state in the system has been shown to increase the calcification rate in experimental closed systems (Gattuso et al. 1998, Langdon et al. 2000, Schneider and Erez 2006).

Although not measured directly in this study, it is probable that the well water also contained high levels of dissolved organic matter, as the incoming well water was visibly discolored (approximately the color of a weak tea). In addition, scleractinian corals have been shown to release dissolved organic nutrients (Ferrier-Pages et al. 1998), and these nutrients can potentially accumulate over time in closed systems. In aquaculture applications, dissolved and suspended organic material can be removed through foam fractionation (Lemlich 1972, Weeks et al. 1992), also known as protein skimming. The efficiency of protein skimming can be increased by the application of ozone, which helps to promote flocculation of organic material (Sander and Rosenthal 1975). However, the use of ozone must be carefully monitored, especially in the presence of ammonia in seawater. When ozone is applied to seawater, the formation of free bromine (OBr^-) and free chlorine (OCl^-) occurs, which in the presence of ammonia rapidly results in the formation of potentially toxic oxidation by-products (Schroeder et al. 2011).

2.4.4 The Potential for Land-Based Nurseries

The goal of modifying the land-based nursery during September 2010 through January 2011 was to better approximate environmental conditions on offshore reefs, and thereby improve production of the nursery to a level comparable with the offshore nursery. Although it may be nearly impossible to achieve truly oligotrophic conditions in recirculating systems, the growth rate of corals in this study increased dramatically by simple changes to system design and lighting levels. Changes in growth occurred rapidly after conditions were improved; lighting was increased in the end of January 2011, and growth rate had increased to levels significantly higher than the offshore nursery and comparable to reported rates for natural colonies by February 2011. This result indicates that differences in linear growth rate can be seen in as little as a few weeks after changes to environmental conditions.

The maximum growth rate achieved in the land-based nursery (16.0 ± 1.0 mm month^{-1}) equates to a yearly linear extension of 19.2 cm year^{-1}, versus a maximum of 12.7 cm year^{-1} observed in the offshore nursery. Fragments in the land-based nursery ended at an almost identical mean height to those in the offshore nursery in May 2011 (13.5 cm and 13.6 cm respectively), showing that if the rapid growth rate observed from February to May had been maintained throughout the full twelve months of the experiment, the land-based corals would have easily overtaken the offshore corals in size. This difference in extension could result in the production of at least one more fragment per growing tip per colony each year, showing that land-based nurseries have the potential to produce a greater number of fragments per coral than offshore nurseries.

Colonies in the offshore nursery showed a reduction in growth rate beginning in September and ending in March. The minimum offshore growth rate (5.1 ± 4.7 mm month^{-1}) occurred in January and was a 50% reduction from the 10.6 ± 4.1 mm month^{-1} observed in June of the previous year. This reduction coincides with colder water temperatures and shorter length of sunlight and has been observed by other researchers in higher latitude areas (Shinn 1966, Gladfelter 1984, and Larson 2010). Gladfelter (1984) and Shinn (1966) suggested that seasonal reductions in growth of *A. cervicornis* were only observed in areas where water temperatures dropped below 26˚C, suggesting that this may be a suitable minimum temperature for land-based culture of this species. By providing optimal temperatures year-round in a land-based system, fragment production could be increased simply by avoiding seasonal decreases in growth rate.

2.5 Conclusions

Nursery-grown coral fragments have many potential uses including re-stocking of threatened species, supply for the marine aquarium trade, discovery and production of marine natural products, and subjects for controlled experimental research. Although coral fragments in the land-based nursery had reduced growth rates initially, the results of this experiment showed that colonies of *A. cervicornis* can be successfully grown in a land-based nursery, and that linear extension and fragment production can be higher than in offshore nurseries if environmental conditions are maintained within optimum ranges. This experiment highlights some of the conditions that promoted high linear extension rates in *A. cervicornis*, a threatened species that is commonly produced in offshore nurseries in the Western Atlantic. More research is needed to clearly define a set of optimal parameters for production of *A. cervicornis,* and other coral species, in land-

based culture, but this study provides a useful starting point for identifying some parameters that can be targeted for increasing growth of *A. cervicornis* beyond what has been observed in the wild and in offshore nurseries.

As a "proof of concept" study, this research shows that land-based and offshore nurseries can be equally successful, and land-based nurseries may be able to surpass offshore nurseries in terms of maximizing coral growth and production. However, the balance of cost and effort between the two nursery types must be closely examined in order to determine if land-based nurseries can be used on a larger scale at a cost that is comparable to offshore nurseries. Land-based nurseries may be especially valuable in areas where offshore nurseries are regularly exposed to extreme water temperatures, disease, predation, or breakage and as a strategy for spreading the risk by housing corals in multiple locations and settings. In order to minimize the risk of loss in land-based culture, especially when dealing with threatened or endangered coral species, a set of best management practices should be implemented at any facility that is permitted to culture listed coral species for restoration.

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CHAPTER

3

GROWTH AND SURVIVAL OF TRANSPLANTED CORALS

3.1 Introduction

3.1.1 Transplantation of Coral Fragments

Coral transplantation is commonly used as an experimental technique to study the effects of changing environmental variables on corals. Factors such as light, temperature, and water flow can have important biological effects on the coral colony. Moving coral colonies from one light regime to another may result in changes in growth rate (Yap et al. 1998, Anthony and Hoegh-Guldberg 2003), morphology (Muko et al. 2000, Ow and Todd 2010), and zooxanthellae density and clade (Dustan 1982, Baker 2001). Changes in water flow can also have significant effects on coral growth (Sebens et al. 2003), physiology (Nakamura 2010), and morphology (Bruno and Edmunds 1997). It is important to consider such changes any time corals are moved from one location to another. The re-location of corals is not necessarily detrimental to transplants, as the ability of corals to adapt to changing conditions through photo-acclimation (Anthony and Hoegh-Guldberg 2003) and morphological plasticity (reviewed by Todd 2008) has been well documented.

There are many examples of successful transplantation of corals to restoration sites (Edwards 2010 and case studies therein), but environmental conditions can have a measurable impact on the growth and survival of transplants (Shaish et al. 2010a). High sea surface temperatures can increase the initial mortality in newly transplanted coral fragments (Herlan and Lirman 2008, Okubo et al. 2005), and the season of transplantation should, therefore, be considered to minimize the risks of exposing newly fragmented corals to high temperatures. For example, in Broward County, transplanting permits require that corals only be transplanted between October and May, in order to

avoid the summer season where water temperatures are higher. As the transplantation of nursery-grown coral fragments increases, it will be necessary to refine protocols to minimize environmental stress on newly transplanted corals, and maximize the benefits to the local ecosystem (Edwards and Gomez 2007). The ultimate goal of the transplantation project should be thoroughly considered, and the risks and costs of coral transplantation should be weighed against the benefits, on a case by case basis, in order to avoid "misguided meddling" (Edwards and Clark 1999).

3.1.2 Factors Affecting Transplant Survival

Environmental factors are not the only consideration when transplanting coral fragments. A major factor affecting survival of coral transplants is the attachment method. These methods can include nylon cable ties, coated wire, monofilament line, iron nails, underwater epoxy, cement, or various combinations. Garrison and Ward (2008) showed that transplants of *A. cervicornis* and *A. palmata* attached to reef structures with nylon cable ties were prone to mortality by dislodgement during storm swells and suggested this may be due to the ability of nylon to stretch. Long-term survival for that study was only 9% after 12 years, and was 0% for *A. cervicornis* (Garrison and Ward 2012). By contrast, Forrester et al. (2011) showed that there was no difference in survival between fragments attached with two types of epoxy, cement, and cable ties. Although most studies use only one attachment method, one study observed higher survival with cable ties over wire (Bruckner and Bruckner 2001), and another found no difference in survival but a greater incidence of detachment with super glue over epoxies (Dizon et al. 2008). The cost of different attachment methods is also a consideration, with cable ties and wire being much more affordable and easy to work with than epoxies or concrete mixtures.

Fragment size may or may not affect transplant survival or growth, as findings have been mixed. Some studies show that longer/larger fragments have higher growth (Soong and Chen 2003) and survival (Bruckner and Bruckner 2001), but others show no difference in survival between size classes (Plucer-Rosario and Randall 1987). Johnson et al. (2011) suggest that *A. cervicornis* fragments should have at least 5 cm of length when transplanted.

The choice of coral species for transplant can have long-term impacts on the structure of the restored reef community (Muko and Iwasa 2011a, 2011b), and choice will depend largely on the goal of the project (i.e., recovery of an endangered species, immediate increase in rugosity, or re-creation of the previously existing community structure). In order to increase the rate of establishment after transplant, it has been suggested that the best species for use in coral nurseries are "weedy" species, or those with fast growth rates and easy fragmentation, that can re-colonize a reef in a rapid manner (Shaish et al. 2010b). On the other hand, there may be unexpected harmful effects of transplanting fast-growing species, and this may lead to the preclusion of other species (Muko and Iwasa 2011a). Earlier studies showed that branching corals had higher rates of mortality and decreased growth after transplanting relative to control corals (Yap and Gomez 1985, Plucer-Rosario and Randall 1987, Clark and Edwards 1995). If natural recruitment is sufficient, the initial increase in percent cover gained by transplantation may be negligible over longer time periods (i.e., 10 years), and transplantation may not be justified or should focus on massive species (Edwards and Clark 1999).

3.1.3 Transplantation from land-based nurseries

In addition to the factors that must be considered for transplantation from offshore nurseries, there is concern from regulatory agencies over the fact that corals raised in land-based systems may carry diseases back to wild populations when returned to the ocean. At this time there are no data to support or refute this hypothesis. It has been suggested that the microbial community associated with a coral colony may shift while in land-based aquaculture systems. As a result of these concerns, a disease diagnostic and certification system was developed by The Florida Aquarium and The University of Florida Tropical Aquaculture Lab and approved for use by the Florida Fish and Wildlife Conservation Commission (Berzins et al. 2007). It requires all coral fragments to be inspected and given a Health Certificate by a U.S. Department of Agriculture accredited veterinarian that has experience and training in coral health and disease. The inspection and release approval process involves a review of collection information, culture history, biosecurity, and a visual inspection of color and condition within 30 days of release. According to current guidelines, corals must be held in a system that contains only organisms from the county intended for transplantation, to prevent cross-contamination or introduction of foreign microbes.

The purpose of this study was to examine the success of corals outplanted from land-based nurseries and to determine whether corals reared in a land-based nursery would show the same growth and survival after transplantation as those reared in a traditional offshore nursery.

This chapter describes two outplanting experiments. The first experiment compares growth and survival of small fragments taken from colonies reared offshore with

fragments taken from colonies reared in a land-based system. The second experiment compares the growth and survival of large colonies reared in the two separate land-based systems. This research begins to answer some of the many questions that exist regarding the feasibility of using captive-reared corals for reef restocking. This study also provides more data on the survival and growth of coral transplants of different sizes, as both small (5 cm) coral fragments and large (ranging from 8 to 29 cm in height) colonies were transplanted.

3.1.4 Transplant Location

The site chosen for outplanting is located just west of the crest of the shore-parallel, inner reef of Broward County, Florida (**Figure 3.1**). The outplant location is 3.2 km north-northeast of the offshore nursery location discussed in Chapter 2. The restoration site is in close proximity to other healthy thickets of coral as described in Vargas-Ángel et al. (2003; e.g. Cervicornis II and Oakland I), and colonies still remain in relative abundance at these other thickets.

A large thicket of staghorn coral was once present at the restoration site and formerly covered 7,900 m^2 (Vargas-Ángel et al. 2003). The outplant area is located at the northern edge of the previously existing thicket. In June-August of 2002, white band disease affected the thicket, and significant coral mortality occurred. The status of the site shortly before coral outplanting in February 2012 was that most live *A. cervicornis* had been lost at the site, and the majority of the three dimensional structure had been reduced to scattered staghorn rubble. Several healthy colonies were present at the site prior to tranplantation. Although the exact reason for the demise of the former thicket at the restoration site is unknown, it is likely that the thicket was largely comprised of one or a

few genotypes that were more susceptible to disease than genotypes of nearby thickets. The current existence of healthy colonies and lack of active disease during siting dives in 2011 at the restoration site indicates that the habitat is currently suitable for growth of *A. cervicornis*.

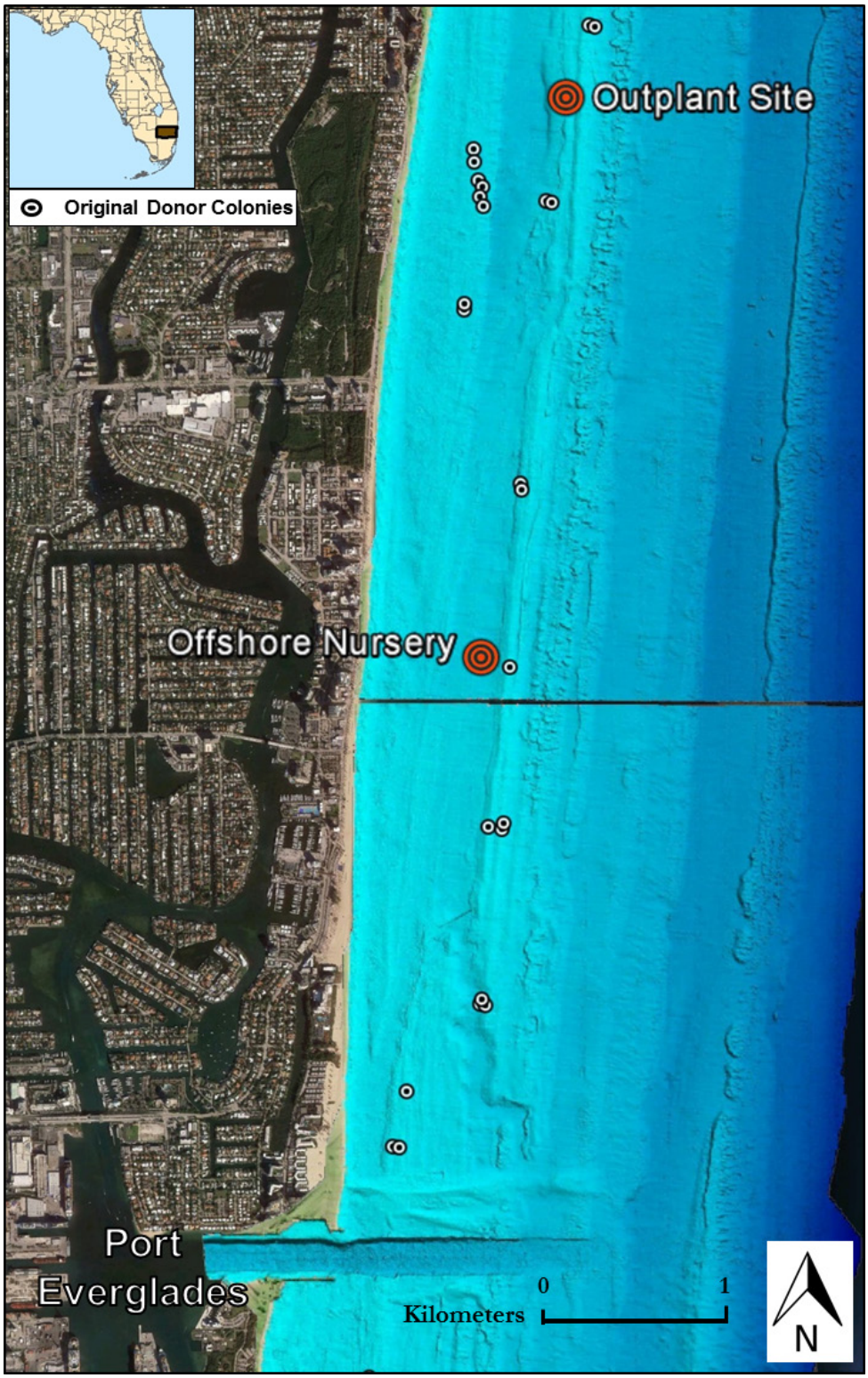

Figure 3.1. A benthic map showing the location of the offshore nursery site, the coral outplant site, and the location of nearby colonies of *Acropora cervicornis*. Bathymetry map provided by United States Geological Survey FLaSH Map Project.

3.2 Methods

3.2.1 Small Fragment Transplant (Experiment 1)

On December 7-8, 2011 fragments were cut from twenty parent colonies in each nursery location (land-based and offshore). The parent colonies in the land-based nursery were accumulated from broken branches from the offshore nursery that were brought into the land-based nursery between December 2010 and April 2011. The cumulative time that these corals spent in the land-based nursery was nine to twelve months before re-fragmenting and outplanting. Fragments were cut from newly grown material. These 20 parent corals represented 17 separate genotypes, and fragments for outplanting were taken from the same genotypes in the offshore nursery. Three fragments 5 cm in length were cut from each parent colony using pruning shears, for a total of 60 fragments from each nursery (120 fragments total).

Each coral was attached to a 2.5 cm cubical base that was constructed of a 50/50 mix of concrete and aragonite sand molded over drywall anchors. Corals were attached with a small amount of All-Fix™ epoxy. The drywall anchors were then mounted to racks made of perforated PVC sheet and the coral fragments were held in their respective nurseries for two months to heal (**Figure 3.2**). During this time, health inspections and release certifications were obtained for the land-based fragments.

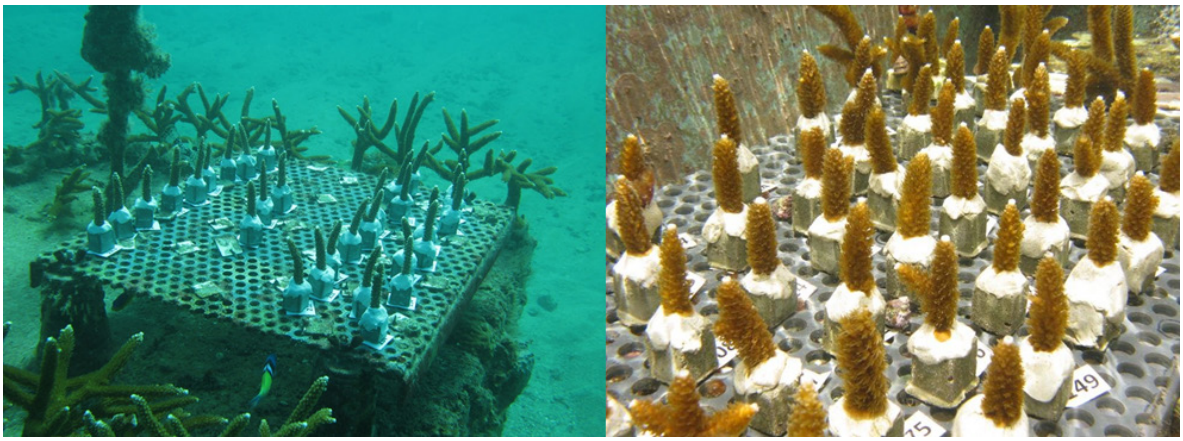

Figure 3.2 Fragments attached to PVC sheet racks in each nursery location (offshore on left, and land-based on right) during a two-month healing period.

In early February 2012, one week prior to transplanting, 7.6 cm concrete tiles with numbered tags were placed in three plots of 40 (4 m x 10 m) at the restoration site. Each tile previously had a hole drilled through the center so that coral fragments could be easily inserted by the drywall anchor attached to the base on which the fragments were growing (**Figure 3.3**). Each plot of 40 fragments was located parallel to the western (landward) edge of the inner reef and were chosen to be as uniform as possible. Each transplant plot consisted of 20 fragments from land-based culture and 20 fragments from offshore culture. Each parent colony by nursery location combination was represented once in each plot. Position of fragments within the plot was determined by a random number generator.

Tiles were spaced approximately 1 meter apart from each adjacent tile in the plot, and the plots were approximately 5 meters apart. Reef substrate was scrubbed with a wire brush, and each tile was attached to the substratum by SCUBA divers with a mixture of hydraulic cement with microsilica additive, seawater, and an anti-washout agent (Rheomac® UW 450, BASF). Tiles were attached prior to coral transplantation to

minimize the time corals had to be handled and held on the day of transplant. In addition, this method allowed for securing the coral fragment without any cement touching live coral tissue and allowed a minimum use of cement during the attachment process.

Figure 3.3. Concrete tiles attached to the substratum prior to coral transplant (left), and a diver securing a coral fragment on the day of transplant (right).

On February 15, 2012 all coral fragments were collected from the nurseries and transplanted to the restoration site. Fragments were held in coolers and supported by perforated PVC racks to keep them upright and not touching. The outplant site is a short (10 minute) boat ride from the offshore nursery site. The corals from the land-based nursery were loaded onto the boat first. Then the ocean-based corals were retrieved, so the land-based corals spent approximately 1 hour longer in the coolers. Fragments were placed into their previously determined position in the plots by SCUBA divers using a small amount of cement mixture in a plastic bag (**Figure 3.3**). The cement mixture used to attach the cubical base of each coral consisted of hydraulic cement with microsilica additive anti-washout agent (RheoMac UW450), and seawater. The microsilica additive

neutralizes the hydroxides released during the cement mixing process, preventing caustic burn to coral tissue.

3.2.2 Large Colony Transplant (Experiment 2)

This portion of the study was done in collaboration with the University of Florida Tropical Aquaculture Laboratory (TAL) in Ruskin, FL and The Florida Aquarium. Corals from the same original donor colonies described in Chapter 2 were raised in two separate land-based nursery systems during the same time period. The first system was located at Nova Southeastern University, and growth of these fragments was described in Chapter 2. The second system was a fully recirculating aquaculture system located in a commercial-style greenhouse at TAL. The roof of the greenhouse consisted of air-inflated double-layered polyethelyene that provided two layers of 30% shading. The aquaculture system consisted of two 1325 liter (350 gallon) tanks and a 1438 liter (380 gallon) sump connected by a 1.0 hp centrifugal pump. Temperature in the system was maintained between 25-27˚C by a 0.5 ton water chiller and by heating of the greenhouse in the winter. Water changes (at least 50%) were made once each month using artificial seawater made by mixing reverse osmosis filtered water with a commercial saltwater mix.

Due to the extensive overgrowth of tissue on the pyramid tiles used for growth of colonies in the land-based nursery at NSU, the large size, and the large number of branches, all of the 48 colonies remaining in the NSU land-based nursery were attached to larger 15 x 15 x 2.5 cm cement plates on January 23, 2012, one month prior to the anticipated transplant (**Figure 3.4**). Each plate was numbered with a plastic tag. Colonies were attached to the plates using a cement mixture containing hydraulic cement

with microsilica, the anti-washout agent, and seawater. Colonies were attached in a flow-thru seawater tray that was completely separate from the nursery system. The tray was continuously flushed with aerated water from the saltwater well. The cement was allowed to harden, and colonies were then returned to the nursery so that cement dust did not enter the nursery filtration system. The large tiles gave the colonies stability and a large attachment point that would allow cementing to the reef without excessive handling and contact with the live tissue. After attachment, veterinary inspection and health certification was obtained in early February 2012.

Figure 3.4. Large colony attached to cement plate one month prior to transplant.

Sixty percent of the 48 large colonies remaining in the NSU nursery (28 colonies) were transplanted into four plots along with 60% of colonies remaining in the corresponding land-based nursery located at University of Florida Tropical Aquaculture Laboratory

(TAL, 41 colonies). Each plot consisted of 7 colonies from NSU and 10 or 11 from TAL, for a total of 17-18 colonies per plot, and 69 corals total.

Colonies from TAL were transported by van to NSU in a large styrofoam cooler on the morning of February 11, 2012. Within the cooler, each colony was bagged with a wet paper towel in the bottom of the bag (**Figure 3.5**). Colonies were immediately loaded onto the boat and transported to the restoration site. Each coral was individually lowered to the reef by SCUBA divers. Designated attachment sites were scrubbed with a wire brush, and all corals were set in place. Colonies were attached to the substratum with a mixture of hydraulic cement with microsilica, the anti-washout agent, and seawater. Due to their smaller size compared to the NSU corals, TAL corals had not been previously attached to concrete plates. The pyramid tiles used for initial attachment in the nursery were still largely not encrusted with coral tissue, allowing for the pyramid base to be attached directly to the substrate without covering live coral tissue.

Figure 3.5. Corals from TAL upon arrival at NSU.

On February 17, 2012 colonies from NSU were loaded into styrofoam coolers with 1-2 colonies per cooler. Coolers were filled to the top with seawater from the nursery in order to submerge as much coral tissue as possible. All coolers were loaded onto the boat and transported to the restoration site. Each coral was hand-carried to the substratum by SCUBA divers and placed in its designated plot. The substratum was again scrubbed with a wire brush, and each cement plate was attached using the same cement mixture that was used to attach TAL corals. After all corals were attached, all 69 colonies were double-checked for solid attachment, and additional cement was added as needed. Initial photographs were taken of all colonies.

3.2.3 Monitoring

The transplanted fragments (experiment 1) and colonies (experiment 2) were monitored by SCUBA divers weekly for the first month (February 2012), monthly through month six (March – August), and again in months nine (November) and twelve (February 2013). During each monitoring event, color, condition, linear extension (height), colony width, and branch number were recorded. Any incidence of disease, predation, or breakage was also recorded.

The color and condition of each fragment and colony was recorded based on the scale in Berzins et al. (2008) described in Chapter 2. Outplants were considered to have survived if given a condition score of 2 – 6 and only considered dead at a condition score of 1, which indicated no living tissue. Linear height of the main stalk was measured from the bottom edge of the live tissue to the tip of the live tissue on the vertical stalk using vernier calipers. Growth of the corals was recorded as the mean extension in mm per

month, with a month standardized to 30.4 days (365 days/12 months). This standardization allowed for easy comparison and conversion to yearly extension rates.

Detached colonies were secured at each monthly monitoring onto their original concrete base. Broken fragments were re-attached to the substratum as they were encountered. If the parent colony could be confirmed, fragments were attached near the original base. If the parent colony could not be confirmed, fragments were re-attached in a designated area outside of the experimental plots. Only the original parent colony was measured for data collection.

3.2.4 Data Analyses

Experiment 1

Comparisons of the initial height and volume between fragments outplanted from the land-based and offshore nurseries were made with t-tests; the initial size and volume data were normally distributed with equal variance. Initial branch number was compared between the two groups with the non-parametric Wilcoxon Signed-Rank test.

Mortality of fragments that originated in two nursery locations were compared using the Kaplan-Meier product-limit method of fitting survivorship curves (Kaplan and Meier, 1958). Differences in the survival function were tested using a post-hoc log rank test (Mantel, 1966). Fragments that were completely detatched and not found were considered lost from the experiment and were censored for the purpose of survival analysis. Fragments that remained attached but died completely, and fragments that were detatched and found dead, were considered as mortalities.

The percentage of broken fragments during each monthly measurement was normally distributed after a square-root transformation. Comparisons of the occurrence of breakage between nursery origins were completed with a paired t-test.

There were no significant effects of plot location on linear extension rate of the three fragment outplant plots (experiment 1, ANOVA, $F=1.27$, $p=0.282$). Growth data from the three plots were, therefore, combined ($N=60$ for each nursery origin). Due to the large amount of branch breakage observed during Tropical Storm Isaac and Hurricane Sandy, the determination of linear extension rate between August and November 2012 was only possible on a few outplants that remained unbroken over this period. Therefore, comparisons of linear extension rates were divided into the pre-storm (March through August 2012) and post-storm periods (November 2012 through February 2013).

The large number of broken outplants resulted in a large number of missing linear extension values. Due to this fact, multivariate repeated measures analysis over the entire pre-storm period was difficult due to the case-wise exclusion of observations resulting in a very low sample size. Analyses were instead done with a univariate mixed model ANOVA, with time, nursery origin, and the interaction of time and nursery origin as fixed effects, and fragment ID as a random effect. The differences between consecutive monthly rates were normally distributed, so therefore post-hoc analyses for significant effects of time and nursery*time interaction were done using dependent sample (paired) t-tests between consecutive months within each nursery origin group. Significant differences from paired t-tests were determined using Bonferroni corrected alpha values determined by multiplying the resulting p-value by the number of pairwise comparisons in each family.

The volume of the fragment outplants was calculated by the method of Kiel et al. (2012) using the volume of an ellipsoid (EV):

$$EV = (4/3) \times \pi \times H/2 \times L/2 \times W/2$$

However, only colony width had been measured, so an equal width and length was assumed, resulting in the formula equivalent to the volume of a spheroid (SV):

$$SV = (4/3) \times \pi \times H/2 \times (W/2)^2$$

Experiment 2

Comparisons of the initial height and volume between large colonies outplanted from the two land-based nurseries and the NSU land-based control colonies were made with t-tests; the initial size and volume data were normally distributed with equal variance. Initial branch number was compared between the two groups with the non-parametric Wilcoxon Signed-Rank test.

Mortality of large colonies that originated in the two land-based nursery locations, and those that remained in the NSU land-based nursery as controls, were compared using the Kaplan-Meier product-limit method of fitting survivorship curves (Kaplan and Meier, 1958). Differences in the survival function were tested using a post-hoc log rank test (Mantel, 1966). In experiment 2, no large colonies were lost completely before storm activity, so no censoring was applied to large colony survival until post-storm analysis. Similar to experiment 1, colonies that were completely detatched and not located were considered lost from the experiment and were censored. Colonies where a portion of the skeleton remained attached but died completely were considered as mortalities.

The percentage of broken colonies during each monthly measurement was normally distributed after a square-root transformation. Comparisons of the occurrence of breakage between nursery origins were completed with a paired t-test.

There were no significant effects of plot location on linear extension rate for the four colony outplant plots (experiment 2, ANOVA, $F=0.34$, $p=0.799$). Growth data from all four plots were, therefore, combined. Analyses were divided in a similar fashion to experiment 1, separating data into pre- and post-storm periods and using a univariate mixed model ANOVA, with time, nursery origin, and the interaction of time and nursery origin as fixed effects, and fragment ID as a random effect. Differences in consecutive monthly extension rates were mostly normally distributed, with minor deviations in some pairs. Post-hoc analyses for significant effects of time and nursery*time interaction were done using dependent sample (paired) t-tests between consecutive months within each nursery origin group. Significant differences from paired t-tests were determined using Bonferroni corrected alpha values.

3.3 Results

3.3.1 Initial Conditions

Experiment 1

The mean (±SD) initial height of fragments from the offshore nursery was significantly larger than those outplanted from the land-based nursery (6.8 ± 1.2 cm and 6.4 ± 1.2 cm respectively; **Table 3.1**). Fragments from the offshore nursery also had a significantly greater initial number of branches than those from the land-based nursery (**Table 3.1**). There was no significant difference in starting volume of the fragments from the two nursery locations (**Table 3.1**).

Experiment 2

At the beginning of experiment 2, there was a significant difference in the initial height, volume and number of branches between large colony outplants from the two land-based nurseries (**Table 3.1**). Colonies from the TAL land-based nursery were smaller and had fewer branches than those that originated from the NSU land-based nursery. There were no significant differences between the NSU land-based nursery outplant and control colonies.

Table 3.1. Initial height, number of branches, and volume of outplants from both experiments with comparisons between initial measurements from the different treatment groups. All errors shown are ±SD.

	Initial Height (cm)	Initial # of Branches	Initial Volume (cm^3)	**Comparison**	Height (t-test)	Branch # (Wilcoxon Rank Sum)	Volume (t-test)
Experiment 1 (Fragments)							
NSU Land-Based Outplant	6.4 ± 1.2	0.5 ± 0.9	219 ± 363	**I.** Land-Based vs. Offshore Fragments	t=2.13	Z=5.26	t=1.97
NSU Offshore Outplant	6.8 ± 1.2	1.5 ± 1.2	180 ± 180		**p=0.035**	**p<0.001**	p=0.052
Experiment 2 (Large Colonies)							
NSU Land-Based Outplant	21.7 ± 4.3	20.2 ± 11.3	55863 ± 22504	**I.** NSU Colony vs. TAL Colony	t=-17.9	Z=6.90	t=-21.99
TAL Land-Based Outplant	9.4 ± 2.4	2.7 ± 1.8	1570 ± 1577		**p<0.001**	**p<0.001**	**p<0.001**
NSU Land-Based Control	22.9 ± 4.8	21.7 ± 10.8	67748 ± 43530	**II.** NSU Colony Outplant vs. Control	t=-0.54	Z=0.54	t=-1.22
					p=0.594	p=0.589	p=0.230

3.3.2 Pre-storm survival

Experiment 1

Of the 60 fragments outplanted from the NSU land-based nursery, three fragments died in the first six months of monitoring. Two mortalities occurred in the first month after transplant, and one mortality occurred in month 5 (July 2012). The first two mortalities were preceded by breakage, followed by tissue-loss. The mortality in month 5 was preceded by a major break in month 4, and only a small piece of tissue remained and was

overgrown by algae. Overall survival of fragments outplanted from the land-based nursery was 95% after six months.

Of the 60 fragments outplanted from the NSU offshore nursery, 11 fragments were lost from the experiment due to complete detachment in the first month after outplanting. Upon inspection of the remaining base where fragments had detached, it was discovered that the epoxy used for attachment of fragments to the cubical base in the offshore nursery had not cured entirely, and the center of the epoxy remained soft although the outside had hardened. Detachment had occurred at the epoxy-coral interface that was made when attaching corals to the bases in December 2012, before the healing period in the nursery (**Figure 3.6**). No fragments had detached during the nursery healing period, so it was not obvious that the epoxy did not cure completely. Conditions on the reef after outplanting may have led to stronger wave action or other incident forces that caused the fragments to fall out of the weak epoxy. All of the cubical bases remained attached to the tiles, and the tiles remained attached to the substratum.

After excluding (censoring) all detached fragments that were not found, considering these as lost from the experiment and not mortalities, the survival of fragments that originated from the offshore nursery was 93.9%. There was no significant difference in survival of fragments that were outplanted from the land-based and offshore nurseries (**Table 3.2**). However the "retention rate," or percentage of corals that remained, for fragments transplanted from the offshore nursery was only 76.7%.

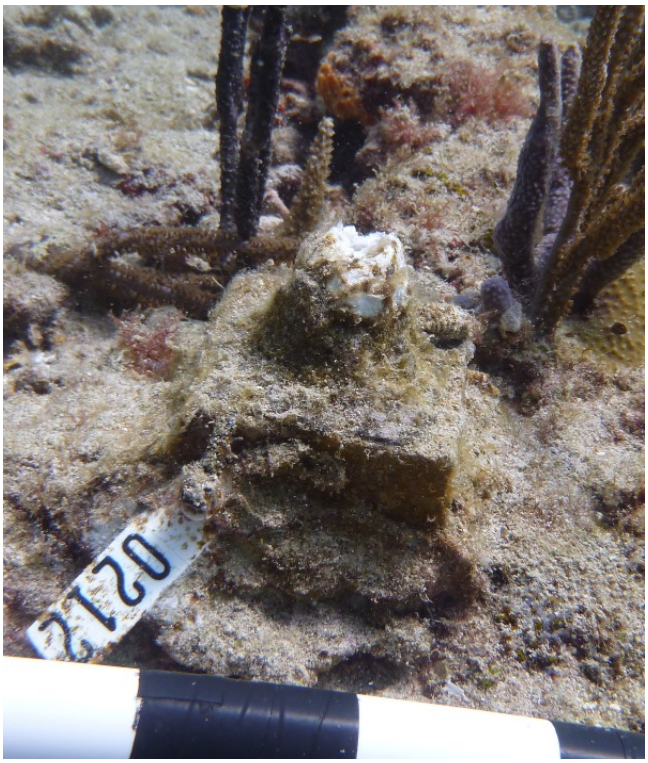

Figure 3.6. Example of detached fragment that originated from the offshore nursery, showing weak epoxy attachment point, and secure concrete attachment from outplanting.

Experiment 2

At the end of August 2012, six months after outplanting, all 28 of the large colonies that originated from the NSU land-based nursery were alive (100% survival), and 35 of the 41 colonies from the TAL land-based nursery were alive (85% survival). Three TAL colonies died of tissue-loss within the first two weeks after transplant (between February 11 and February 24), and the remaining three mortalities, also from tissue-loss, occurred by the end of March. Colonies from TAL were noticeably lighter in color than colonies from the NSU land-based nursery at the time of outplanting. None of the large colonies were lost due to detachment. Survival of large colonies that originated from the NSU

nursery was significantly greater than those that originated from the TAL nursery (**Table 3.2**).

One control colony that remained in the NSU land-based nursery died of tissue-loss in April 2012. This incidence of tissue loss had begun in January after attachment of the colony to the larger cement plates using a hydraulic cement mixture, and progressed slowly across the colony from January to April 2012. There was no significant difference in survival between the outplanted and control colonies in the NSU land-based nursery (**Table 3.2**).

In order to directly compare the effect of size on survival while controlling for nursery origin, fragment outplants from experiment 1 that originated from the NSU land-based nursery were compared to large colonies from experiment 2 that also originated from the land-based nursery. Survival of fragments from experiment 1 was not significantly different from survival of the large colonies from experiment 2 (Log-rank X^2=1.43, p=0.232).

Table 3.2. Survival of outplant and control groups and comparisons of survival curves between nursery origins after 6 months of monitoring, before tropical storm activity.

	N	# Lost	# Dead	6 Month Survival (%)		Log-Rank X^2	df	*p*
Experiment 1								
NSU Land-Based Outplant	60	0	3	95%	I. Land-Based vs. Offshore	0.017	1	0.895
NSU Offshore Outplant	60	11	3	94%				
Experiment 2								
NSU Land-Based Outplant	28	0	0	100%	I. Between Land-Based Locations	4.390	1	**0.036**
TAL Land-Based Outplant	41	0	6	85%				
NSU Land-Based Control	19	0	1	95%	II. NSU Outplant vs. Control	1.474	1	0.225

3.3.3 Occurrence of Predation, Breakage, and Disease

The percentage of outplants from each nursery origin that showed signs of predation, breakage, and disease are shown in **Table 3.3**. Because it is unclear whether tissue loss in each case was caused by stress or disease, any signs of rapidly sloughing tissue, preceded by bare white skeleton with little or no algal colonization were simply referred to as "tissue loss" rather than a particular disease. No signs of white band disease, defined by a slow progression and defined margin of receding tissue, preceded by white skeleton colonized by algae, were observed in the study. Other than the occurrence of tissue loss in six large colonies (7.9%) from the TAL land-based nursery shortly after outplanting, no tissue loss was observed in the large colony outplants for the first six months after outplanting. One fragment that originated from the NSU land-based nursery showed tissue loss at the base of the fragment in August 2012, six months after outplanting.

By the initial monitoring that was conducted approximately one week post-outplanting (February 2012), both the fragment (experiment 1) and colony (experiment 2) outplants experienced breakage. The number of broken branches was highest during the one month post-transplant monitoring (March 2012) in all groups. The large colonies that originated from the NSU land-based nursery had the highest proportion of colonies with broken branches in month one (71.4%), followed closely by the TAL colony outplants at 65.7%. Broken branches were observed in all plots and in all nursery origin groups during all monthly monitoring events, except for the TAL colony outplants in May 2012.

Predation by the fireworm *Hermodice carunculata* was first observed on the fragment outplants (experiment 1) in May 2012, and on the colony outplants (experiment 2) in

August 2012. The percentage of colonies with predated branches did not exceed 8% during any monthly monitoring and was generally less than 5%.

Table 3.3. Occurrence of predation, breakage, and disease in the fragment (experiment 1) and large colony (experiment 2) outplants from each nursery origin prior to tropical storm impacts; LB=Land-Based OS=Offshore

Month	N		% Predation		% Broken		% Tissue Loss	
Experiment 1	LB	OS	Land	Offshore	Land	Offshore	Land	Offshore
Feb-12	60	59	0.0	0.0	41.7	22.0	0.0	0.0
Mar-12	58	46	0.0	0.0	51.7	30.4	0.0	0.0
Apr-12	58	46	0.0	0.0	6.9	10.9	0.0	0.0
May-12	58	47	0.0	2.1	6.9	14.9	0.0	0.0
Jun-12	57	47	3.5	0.0	10.5	14.9	0.0	0.0
Jul-12	57	47	0.0	0.0	17.5	10.6	0.0	0.0
Aug-12	57	46	0.0	4.3	8.8	26.1	1.8	0.0
Experiment 2	NSU	TAL	NSU	TAL	NSU	TAL	NSU	TAL
Feb-12	28	38	0.0	0.0	21.4	18.4	0.0	7.9
Mar-12	28	35	0.0	0.0	71.4	65.7	0.0	0.0
Apr-12	28	35	0.0	0.0	25.0	14.3	0.0	0.0
May-12	28	35	0.0	0.0	14.3	0.0	0.0	0.0
Jun-12	28	35	0.0	0.0	21.4	2.9	0.0	0.0
Jul-12	28	35	0.0	0.0	17.9	5.7	0.0	0.0
Aug-12	28	35	7.1	2.9	35.7	8.6	0.0	0.0

3.3.4 Growth

Experiment 1

The monthly linear extension rate of fragments outplanted from the land-based nursery was similar to fragments outplanted from the offshore nursery (**Figure 3.7**). Fragments from both locations had a somewhat reduced growth rate during the first month after transplant, followed by an increase in month two. There was no significant effect of nursery origin on fragment linear extension (ANOVA, $F=0.87$, $p=0.352$), indicating that fragments originating from the land-based nursery performed equally as well as fragments from the offshore nursery. There was also no significant effect of the

interaction between nursery origin and time (ANOVA, F=0.77, p=0.380), indicating that the extension rate of fragments from both nurseries varied similarly over time.

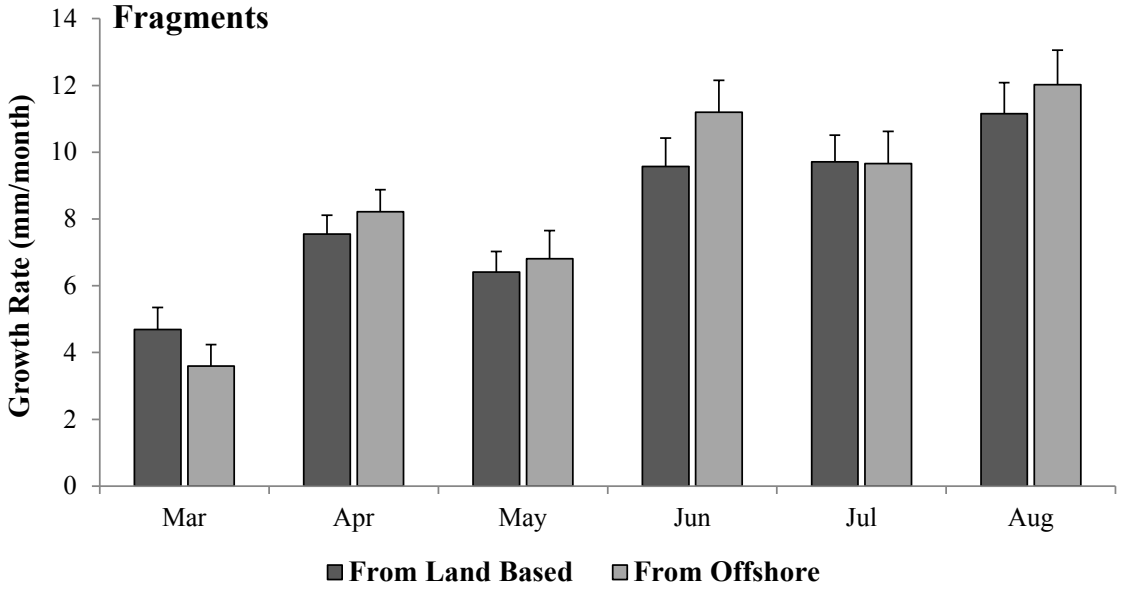

Figure 3.7. Growth rate (mean ± SE; mm month^{-1}) of fragments outplanted from the land-based and offshore nursery locations.

Experiment 2

A univariate mixed model ANOVA with land-based nursery origin (NSU or TAL) and month as fixed effects and colony ID as a random factor showed a significant difference in growth rate between the large colonies that originated from the NSU and TAL nurseries (ANOVA F=85.3, p<0.001). There was also a significant difference in growth rate between the sampling months (ANOVA, F=23.1, p=0.042). The interaction of month and nursery was also significant, indicating that the growth rate of colonies that originated from each land-based nursery varied differently over time (ANOVA, F=5.7, p<0.001).

The growth rate of large colony outplants from each land-based nursery location during each month is shown in **Figure 3.8**. The highest monthly growth rate of 13.5 mm/month (± 1.5 SE) was observed during June 2013 in the NSU large colony outplants, which was similar to the growth rate from April through August 2013. The growth rate of the TAL colony outplants was low during the first two months after outplanting (March: 1.5 ± 0.8 mm/ month, April: 1.4 ± 0.5 mm/month). Post-hoc comparisons showed that the growth rate of the NSU outplants was significantly higher than the TAL outplants in every month except for August, when there was no significant difference in growth between outplants from the two land-based nursery locations (t-test, **Table 3.4**).

The growth rate of the TAL outplants increased over time (**Figure 3.8**); growth rate increased significantly between April and May, and again between May and June, and again between July and August (**Table 3.5**). For the NSU colony outplants, there was no significant difference between any of the consecutive monthly growth rates (**Table 3.5**).

A split-plot univariate mixed model ANOVA with NSU treatment (NSU control or outplant) and month as fixed effects and colony ID as a random effect showed that there was a significant interaction between treatment and month (ANOVA, $F=4.0$, $p=0.047$). There was a significant difference in growth rate between the large colonies that remained in the land-based nursery and those that were outplanted (ANOVA, $F=14.1$, $p<0.001$). The outplanted colonies had a significantly higher growth rate from April through July (**Figure 3.8, Table 3.6**); the growth rate of outplanted colonies and control colonies was the same in March and August. There was no significant difference in the growth rate of the land-based control colonies between months (repeated measures ANOVA, $F=1.13$, $p=0.42$).

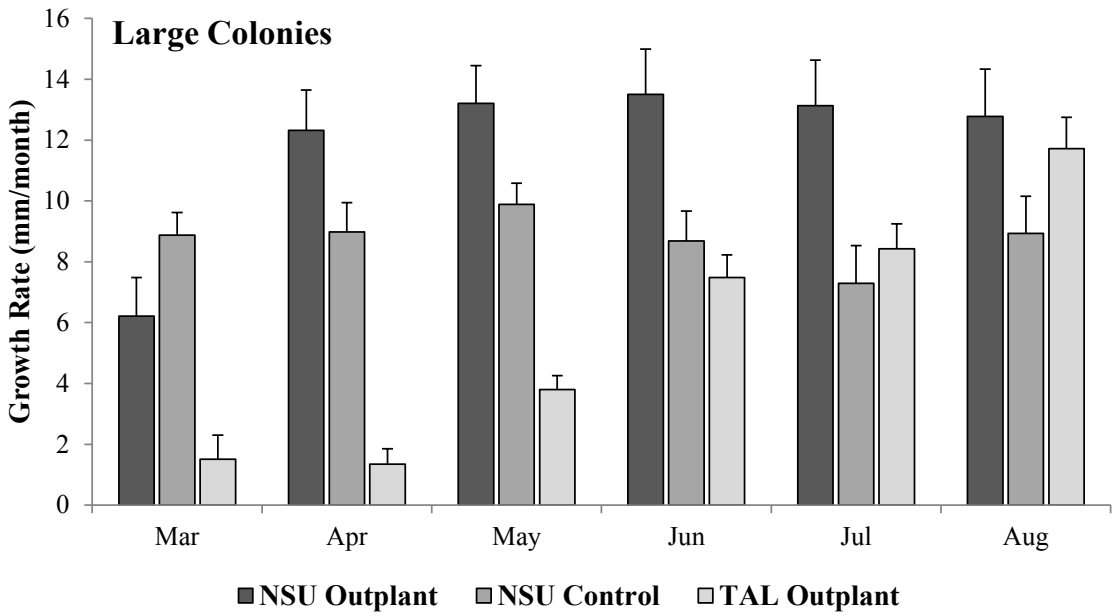

Figure 3.8. Growth rate (mean ± SE; mm month^{-1}) of large colonies outplanted from the two land-based nursery locations and land-based control colonies that remained in the nursery.

Table 3.4. Results of post-hoc t-tests comparing growth rate of large colony outplants from the two land-based nurseries; * indicates assumption of equal variance met; all others show test for unequal variance

	NSU OUTPLANT vs. TAL OUTPLANT					
Statistic	MAR '13	APR '13	MAY '13	JUN '13	JUL '13	AUG '13
t Ratio	-3.2 *	-7.7	-7.1	-3.6	-2.8	-0.6
DF	23.0 *	28.2	21.8	28.6	37.9	53.0
Prob > |t|	**0.004 ***	**<0.001**	**<0.001**	**0.001**	**0.009**	0.559

Table 3.5. Results of post-hoc paired t-tests comparing growth rate of large colony outplants between consecutive monitoring months.

NSU Land-Based Colony Outplants		t-Ratio	DF	p-value
March	**April**	2.24	9	0.052
Apr	**May**	0.43	15	0.675
May	**Jun**	-0.51	14	0.621
Jun	**July**	0.09	17	0.926
Jul	**August**	-0.04	20	0.970
TAL Land-Based Colony Outplants		**t-Ratio**	**DF**	**p-value**
March	**April**	0.50	6	0.636
Apr	**May**	3.49	25	**0.002**
May	**Jun**	5.41	28	**<0.001**
Jun	**July**	0.79	29	0.438
Jul	**August**	3.19	31	**0.003**

Table 3.6. Results of post-hoc t-tests comparing growth rate of large colony outplants and controls; * indicates assumption of equal variance met; all others show test for unequal variance

	NSU OUTPLANT vs. NSU CONTROL					
Statistic	MAR '13	APR '13	MAY '13	JUN '13	JUL '13	AUG '13
t Ratio	-1.9 *	2.0	2.3 *	2.7	3.0	1.9
DF	28.0 *	37.8	34.0 *	31.6	37.8	36.9
Prob > |t|	0.062 *	**0.049**	**0.026 ***	**0.011**	**0.005**	0.060

3.3.5 Storm Damage

Tropical Storm Isaac passed south of the Florida Keys on August 26, 2012 and caused tropical storm force winds and elevated sea conditions throughout the Florida Keys and southeastern Florida. Elevated wind speeds greater than 15 m/s (29 knots) were recorded at a weather station located on the NSUOC campus beginning on August 26 (National Data Buoy Center Station PVGF1). Maximum sustained wind speeds were recorded at 18 m/s (35 knots) with gusts to 24 m/s (46 knots) early on August 27. A waverider buoy located in Fort Pierce, FL recorded elevated significant wave heights ranging from 2.0 to 3.0 m from August 26 through August 28, 2012 (National Data Buoy Center Station 41114). A post-storm damage assessment of the outplant site was conducted on September 7, 2012.

Hurricane Sandy passed over the Bahamas as a Category 1 Hurricane, east of the coast of southeastern Florida on October 26-27, 2012. Although sustained wind speeds in southeastern Florida were only occasionally recorded at tropical storm strength, the wind circulation pattern around the storm led to the development of large surf and significant beach erosion along southeast Florida beaches. Wave data from Fort Pierce, FL recorded elevated significant wave heights of 2.0 m or greater from October 24 through October 27, 2012; significant wave height peaked at 5.5 m on October 26, 2012. The outplant location is approximately 850 m from the coastal shoreline of Fort Lauderdale; the shoreline in this area was severely eroded during the storm, resulting in a breach of the seawall along northern portions of the Fort Lauderdale public beach and flooding and sand intrusion onto local roadways. Elevated wave and wind conditions persisted in the

area before and after the passing of the storm, and post-storm observations of the outplant site were conducted on November 15, 2012.

Observations of predation, breakage, and disease from the post-storm monitoring events are shown in **Table 3.7**. The tropical weather events coincided with a large increase in the occurrence of tissue loss on all outplant groups, with the NSU colony outplants being most heavily affected (21.4% and 37.5% of colonies). A large portion of the outplants were broken, many severely, during the two storm events (**Figures 3.9 and 3.10**). In order to better quantify the loss of coral due to the two storms, the mean colony volume was calculated at each full monitoring event and compared before and after storm occurrence.

Table 3.7. Occurrence of predation, breakage, and disease in the large colony and fragment outplants from each nursery origin after tropical storm impacts; LB=Land-Based OS=Offshore

Month	N		% Predation		% Broken		% Tissue Loss	
Experiment 1	LB	OS	Land	Offshore	Land	Offshore	Land	Offshore
Post-Isaac	54	39	0.0	0.0	31.5	38.5	7.4	2.6
Post-Sandy	48	37	0.0	0.0	22.9	32.4	2.1	2.7
Nov-12	48	37	2.1	0.0	56.3	67.6	0.0	0.0
Feb-13	49	37	0.0	0.0	2.0	2.7	0.0	0.0
Experiment 2	NSU	TAL	NSU	TAL	NSU	TAL	NSU	TAL
Post-Isaac	28	35	3.6	0.0	46.4	37.1	21.4	8.6
Post-Sandy	24	29	8.3	0.0	33.3	34.5	37.5	24.1
Nov-12	24	29	4.2	0.0	41.7	31.0	29.2	13.8
Feb-13	24	29	0.0	0.0	8.3	3.4	0.0	0.0

Figure 3.9. Breakage and sand coverage around TAL colony outplant number 231 from the passing of Hurricane Sandy on October 26-27, 2012. Left: Pre-storm photograph showing extensive rubble surrounding colony. Right: Post-storm photograph showing sand coverage and colony broken at main stalk.

Figure 3.10. Severe breakage and tissue loss observed in one of the large colony outplants from the NSU nursery as a result of tropical weather events. Left: Pre-storm photograph showing colony growth and re-attached branches. Right: Post-storm photograph showing extensive breakage and tissue loss during post-Sandy monitoring.

Experiment 1

In experiment 1, fragment breakage ranged from 22.9% to 38.5% in each post-storm monitoring; however the highest proportion of broken fragments was observed at the end of November 2012 (**Table 3.7**). There was no significant difference between land-based

and offshore nursery origin in the percentage of broken fragments each month (paired *t*-test, t=0.348, p=0.735). Both fragment outplant groups increased significantly in volume over the first six months, then decreased significantly after storm activity (**Figure 3.11, Table 3.8**).

Experiment 2

The large colony outplants showed a pattern of irregular and patchy tissue loss, often observed to be more severe on one side of the colony and appeared to have suffered abrasion and breakage from shifting sediments and rubble during the storm. In addition, during the November 2012 monitoring events (Post-Sandy and at the end of November), a large increase in sand cover and depth was observed on the northern end of the outplant plots. Several of the colony outplants were completely surrounded by sand (**Figure 3.9**).

Between 33.3% and 46.4% of large colonies were recorded as broken during post-storm monitoring, and a large number of broken branches were also recorded at the end of November. Over the course of the entire experiment, the large colonies from the NSU land-based nursery had a significantly higher monthly percentage of broken colonies than colonies from the TAL land-based nursery (experiment 2, paired *t*-test, t=-3.789, p=0.0035).

Due to ongoing breakage, the NSU large colony outplants had not significantly changed in mean colony volume from the initial measurements to month six (August 2012; **Figure 3.12**). The TAL colony outplant group had significantly increased in ellipsoidal volume during this time (**Figure 3.12, Table 3.8**). The NSU colony outplant group also experienced a significant, relatively severe decrease in colony volume between August

2012 and November 2012 whereas the TAL colony outplants did not. The volume of the TAL colony outplants decreased from 4500 ± 4582 cm^3 to 2325 ± 3088 cm^3, but this difference was not significant (**Table 3.8**).

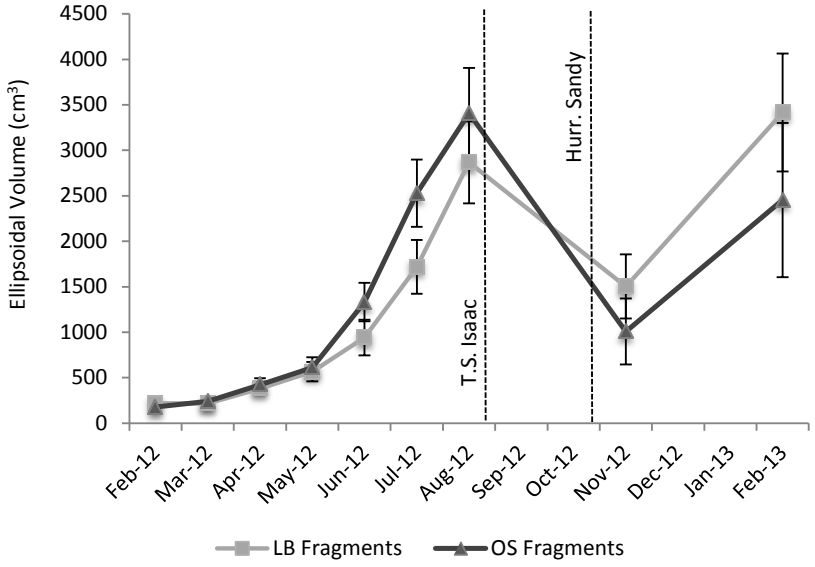

Figure 3.11. Spheroidal volume (mean ± SE) of fragment outplants from the land-based (LB) and offshore (OS) nurseries (experiment 1).

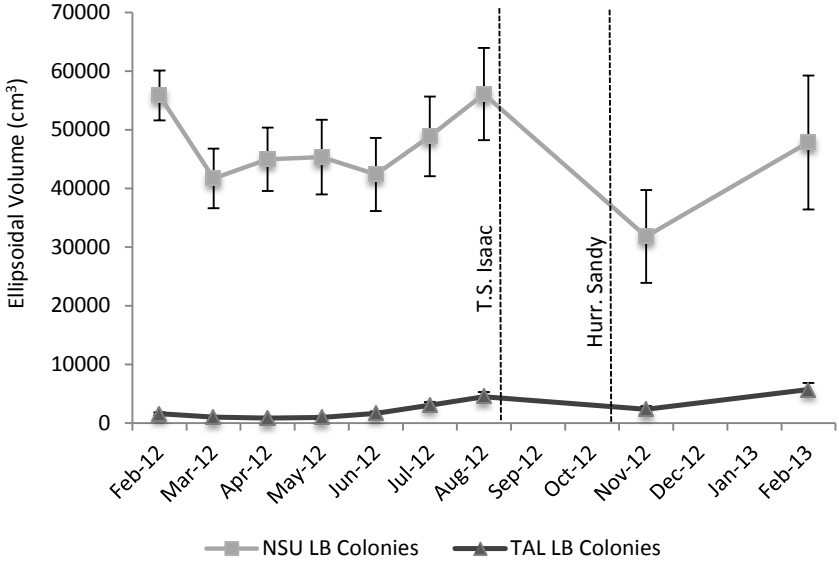

Figure 3.12. Spheroidal volume (mean ± SE) of colony outplants from the two land-based nursery locations (experiment 2).

Table 3.8. Results of paired t-test comparisons of fragment and colony spheroidal volume in selected months after outplanting. Bonferroni corrected p-values for three comparisons within each outplant group.

Experiment 1		**t-Ratio**	**df**	**p**	**Inc/Dec**
Initial vs. 6-month	LB Fragment	-9.78	47	**<0.001**	Increase
	OS Fragment	-6.90	36	**<0.001**	Increase
6 Month vs. 9 Month	LB Fragment	-2.95	47	**0.015**	Decrease
(Storm Damage)	OS Fragment	-4.30	36	**<0.001**	Decrease
Initial vs. 1 Year	LB Fragment	14.87	47	**<0.001**	Increase
	OS Fragment	7.36	36	**<0.001**	Increase
Experiment 2					
Initial vs. 6-month	NSU Colony	0.43	23	0.673	-
	TAL Colony	-4.13	28	**0.001**	Increase
6 Month vs. 9 Month	NSU Colony	-4.18	23	**0.001**	Decrease
(Storm Damage)	TAL Colony	-2.21	28	0.106	-
Initial vs. 1 Year	NSU Colony	-2.19	23	0.117	-
	TAL Colony	4.88	28	**<0.001**	Increase

Due to ongoing construction at the Oceanographic Center, the large control colonies remaining in the NSU land-based nursery were re-located to a new nursery system on August 16, 2012, approximately 10 days before the passing of Tropical Storm Isaac. Electrical power was maintained throughout the storm, and filtration in the new land-based nursery system remained in operation. Excessive rainfall resulted in a decrease in salinity to 29 ppt, which was addressed through large water changes with full-strength seawater from the saltwater well. The new well used for source water for the new nursery had similar water quality properties as the previous well (discussed in Chapter 2), but was filtered with a protein skimmer, ozone injection, sand filtration, and bio-filtration prior to use. However, the demand for water to keep up with necessary water changes to maintain salinity levels in the nursery resulted in a short contact time in the saltwater

filtration and holding system. This resulted in elevated levels of ammonia and phosphate in the saltwater that was used for large water changes to the nursery.

An increased occurrence of tissue loss in the control colonies was observed within several days of relocation to the new nursery system, with three colonies (16.7%) showing signs of tissue loss by August 28, 2012. Ongoing challenges with the newly designed filtration system resulted in several stressor events over the next few months, including temperature reductions to as low as 19.4˚C and periods of elevated dissolved nutrient levels including ammonia and phosphate. In addition, un-secured phosphate filtration media consisting of iron oxide hydroxide was released into both the seawater holding system and the coral nursery. Rainfall from Hurricane Sandy was also managed through water changes, and a drop in temperature in the nursery to 21.7˚C occurred following passing of the storm. Electric power and filtration was also operational throughout Hurricane Sandy. Four control colonies died completely between August 28 and November 29, 2012. Two additional colonies suffered partial mortality with progression that stopped before the entire colony was lost but reduced the colony to only a few living branch tips.

3.3.6 Post-Storm Survival and Recovery

In general, outplants that had even a small amount of live tissue remaining at the end of November 2012 began to heal over broken areas, create new branches, and return to normal extension rates between November 2012 and February 2013. No additional mortalities in any outplant groups occurred after November 2012, and all active tissue recession had stopped by the end of November 2012.

Experiment 1

Six of the fragments from the NSU land-based nursery died of tissue loss after the two storm events, and two were lost completely. Two fragments from the offshore nursery died and seven were lost. Final one-year post outplanting survival was 84.8% for fragments that originated from the land-based nursery (two censored, retention rate 81.7%) and 88.1% for fragments that originated from the offshore nursery (18 censored, 61.7% retention rate). It is likely that a portion of the censored fragments from the offshore nursery resulted in mortalities; however this number is unknown. There was no significant difference in one-year survival between the two fragment outplant groups (Kaplan-Meier Log-Rank, $X^2 = 0.476$, p=0.490).

Linear extension rate also returned to relatively normal winter values in the fragment outplants from November 2012 through February 2013. There was no significant difference in linear extension (± SE) between fragment outplants from the land-based nursery (7.5 ± 0.5 mm month^{-1}) and the offshore nursery (6.1 ± 0.5 mm month^{-1}; t-test unequal variance, t=-1.92, p=0.059) between November 2012 and February 2013.

Experiment 2

As a result of the storm damage and subsequent tissue loss, an additional three large colony outplants from the NSU land-based nursery died, and one outplant was completely lost from the experiment, for a final one-year survival of 88.9% and retention rate of 85.7%. An additional six outplants from the TAL land-based nursery died from tissue loss after storm damage, and one was completely lost, for a final one-year survival of 70.0% and retention rate of 68.3%. Although the one-year survival of large colony

outplants from the NSU nursery was higher, this difference was not statistically significant (Kaplan-Meier Log-Rank, $X^2 = 3.64$, p=0.057). There was no difference in linear extension rate in the large colony outplants from the NSU land-based (8.5 ± 0.9 mm month^{-1}) or TAL land-based nursery (7.6 ± 0.8 mm month^{-1}; t-test, t=-0.781, p=0.438) during this time.

By February 2013, at the end of monitoring, an additional four control colonies showed signs of active tissue loss. Of the 18 control colonies, five had died by the end of the experiment (27.8%), two had suffered major partial mortality that had ceased (11.1%), and four showed active continuing tissue loss (22.2%).

Growth rate in the land-based control colonies was also reduced after re-location to the new land-based nursery. Growth rate in the land-based nursery had remained consistent between 8 and 10 mm month^{-1} from March through August 2012. After re-location, growth rate dropped to 1.8 mm month^{-1} in November 2012 and to 0.5 mm month^{-1} in February 2013 (**Figure 3.13**). There were no significant differences in growth rate from February 2012 through August 2012 before the re-location; growth rate in November 2012 and February 2013 after the re-location were both significantly lower than during August 2012 before the re-location (paired t-test, t= -6.89 and -5.42, p<0.001 for both). Due to the changes in environmental conditions experienced by the land-based nursery controls, the growth of these colonies was not directly compared to outplanted colonies after re-location to the new nursery.

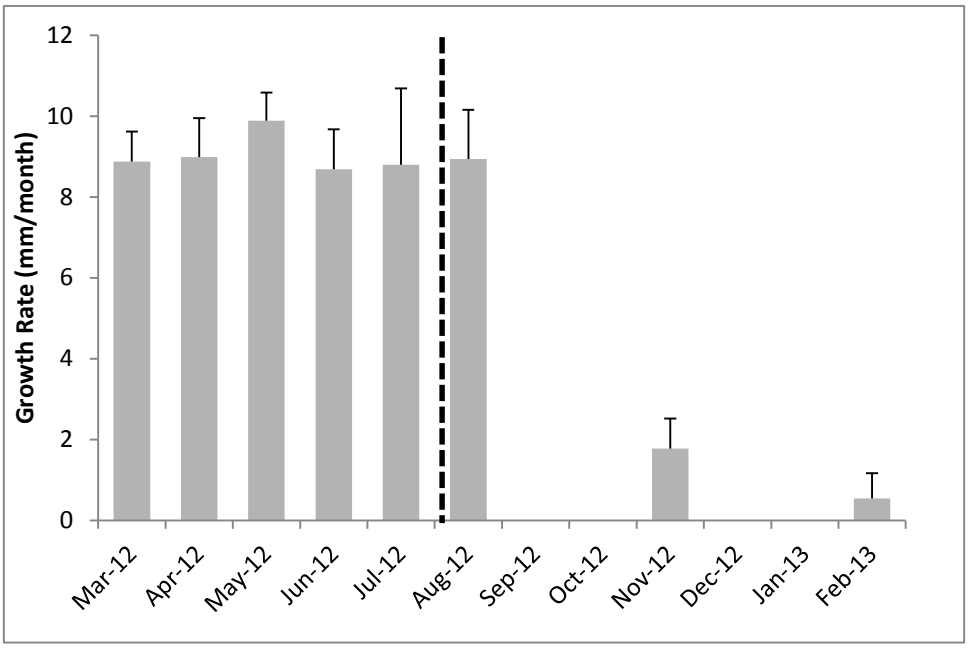

Figure 3.13. Growth rate of control colonies in the NSU land-based nursery. Dashed line indicates re-location of colonies to a new land-based nursery system.

3.4 Discussion

3.4.1 Survival

Survival of corals that originated from land-based nurseries after the first six months ranged from 85% to 100%, which is comparable to or exceeds survival reported in previous studies of the same species outplanted from offshore nurseries (Johnson et al. 2011, Bowden-Kerby and Carne 2012, Hollarsmith et al. 2012, Young et al. 2012) or transplanted from other reef areas (Bowden-Kerby 1997). The highest early survival rate (100% over the first six months) was observed in the large colony outplants from the NSU land-based nursery. Although larger colonies could be expected to undergo a higher level of stress than smaller fragments upon transplant, the greater size allows for partial mortality and breakage without complete colony loss and a greater amount of resources available (i.e. energy reserves) to the colony during acclimation to the new environment.

The lowest early survival (85% in the first six months) occurred in the colony outplants that originated from the TAL land-based nursery. All of these mortalities occurred within the first six weeks after outplanting. Herlan and Lirman (2008) reported an early mortality rate of 17.3% for fragments transplanted from wild colonies into an offshore nursery which is comparable to the 15% mortality rate observed in the current project. Mortality is often associated with recession from the attachment point, however the majority of mortality in the TAL colonies presented as patchy necrosis that often began at the apical end or center of the colony. The significant difference in survival between large colonies from the two nursery locations suggests that the corals from the TAL nursery were more prone to early mortality.

The significantly higher early mortality of colonies from the TAL land-based nursery may have been a result of their initial condition. Colonies from the TAL land based nursery were noticeably lighter in color than colonies from the NSU nursery and had previously shown lower extension rates while in the land-based nursery (unpublished data). In addition, colonies from TAL were transported out of water in sealed plastic bags with a moistened paper towel in the bottom for approximately six to seven hours before being placed into the water at the restoration site. Diver observations on the day of transplant were that the tips of the TAL colonies appeared to be somewhat dried out and that there was no polyp extension upon attachment at the restoration site. The large colonies and fragments from the NSU land-based nursery were transported submerged in water, and were observed to have extensive mucus production and polyp extension shortly after attachment to the substrate at the restoration site.

In the large colony experiment alone, it is difficult to determine whether the effect of colony size influenced survival as the effect of colony size is completely confounded within the nursery origins because the colony outplants from the TAL nursery were all significantly smaller in size than colonies from the NSU nursery. However, the comparison between large colony and fragment outplants from the NSU land-based nursery indicated that there was no significant effect of outplant size on survival in the first six months. This result agrees with the finding of Herlan and Lirman (2008) in which there was no association between fragment mortality and fragment size in an *A. cervicornis* nursery (size classes < 3 cm, 3-5 cm, and >5 cm), although these size classes are much smaller than the current study. In contrast, size-dependent survival was observed by Bowden-Kirby (2001) where large transplants (15-22 cm in length) had

higher survival than small transplants (3-5 cm) in a rubble back-reef environment. However, these corals were not directly attached to the substrate and were only attached to monofilament line secured at each end.

3.4.2 Breakage and Loss

In addition to early mortality, a high percentage of coral fragments that originated in the offshore nursery were lost completely, resulting in a relatively low "retention rate" of only 76.7% before storm activity and 61.7% after the storms. Epoxy is a popular method of coral attachment and is generally considered to be one of the most successful (Hollarsmith et al. 2012, Williams and Miller 2010). It is unclear what factors resulted in the poor curing of the epoxy in the offshore nursery but it is possible that the product may have been past expiration date, the proper ratio of the two parts was not achieved, or that the ambient water temperature was too cold for the epoxy to cure correctly. Although the majority of the detached corals were not located and were considered lost from the experiment, previous studies have shown that greater than 90% of unattached fragments of *A. cervicornis* were able to successfully survive, although survival was size-dependent (Bowden-Kirby 2001). However, more recent evidence suggests that the survival rate may be much lower. Mercado-Molina et al. (2014) found that only 19% and 26% of unattached fragments survived at two sites in Puerto Rico over 18 months.

Although linear extension rates were consistently high in the NSU large colony outplants, colony volume did not increase significantly over the first six months because colony growth was offset by regular breakage that was at times severe. The NSU colony outplants were significantly larger in size than the TAL outplants, which likely contributed to the significantly higher proportion of broken colonies each month simply

because there was a greater number of branches to be broken. In the fragment experiment, there was no difference in the proportion of broken colonies, indicating that the fragments outplanted from the land-based nursery were not more prone to breakage than fragments produced in the offshore nursery.

The branching morphology of *A. cervicornis* is affected by local wave conditions; colonies in low-energy environments tend to grow in a more vertical fashion, have a larger angle between the main stalk and second-order branches, and have no preferred direction of branching (Bottjer 1980). Perhaps one of the most challenging environmental conditions to replicate in a land-based nursery is oscillatory wave action similar to a natural reef. Corals that have grown the majority of their structure in a land based nursery are likely to develop a morphology that would be characteristic of a low wave energy environment. Larger outplants with numerous branches that have formed in a protected tank environment may be prone to breakage simply due to having branch angles and orientations that are not suited to the local wave climate. In this regard, it would be more suitable to outplant smaller fragments with minimal branching in order to allow branch development that is appropriate to the local conditions of the outplant site.

The survival of fragments that are produced through ongoing breakage of outplanted colonies is an important factor in measuring the overall success of a restoration project. The number of coral colonies and the overall restored area can be much greater than the initial transplant effort, depending on the local wave climate, the surrounding benthic substrate, and the survivorship of unattached fragments. Large fragments broken off from the NSU colony outplants were often recovered nearby and re-attached alongside of the original colony when the parent colony could be identified. This resulted in a greater

volume of coral in the overall project area that was not quantified. Regular monitoring and re-attachment of broken fragments may increase the effectiveness of restoration efforts.

3.4.3 Growth and Acclimation

Reduced extension rates in the first month post-outplant were observed in both large colonies and fragments and in outplants from both land-based and offshore nurseries. This indicates that the process of acclimation to the new environment consistently resulted in lower skeletal extension rates across all groups. The acclimation process lasted for an extended period of time in the outplants from the TAL nursery, which showed significantly lower extension rates for up to five months post-transplant. The extended acclimation period may have been related to the initial condition of the coral colonies discussed above in Section 3.4.1.

In order to reach the full potential of land-based nursery aquaculture of coral fragments for restoration purposes, nursery location should not be limited to coastal shorelines with natural supplies of seawater in close proximity to natural reefs, where real estate and operational costs are likely to be higher. In order to achieve this goal, a suitable method of transport from inland areas must be determined so that fragments arrive in the best possible condition before outplanting. Shipping of coral fragments using the "dry method" is often used to save on postage costs (to minimize the weight of water). This method is successful in many cases, and is widely used in the ornamental aquarium trade (Carlson 1999). Becker and Mueller (2001) transported both *A. cervicornis* and *Orbicella* (formerly *Montastraea) faveolata* between reef sites and aquaria using the dry method and reported no adverse effects. However the length of transport was not stated.

The results of this study indicate that the dry shipping method resulted in stress during transport and may have impacted the success (growth and survival) of the TAL colonies post-transplant. For threatened species such as *A. cervicornis*, it is of primary importance to minimize stress and maximize the vitality of the coral at all steps in the culture and transplant process. Therefore, based on the results of this study, wet shipping is recommended for this species.

Edwards and Clark (1999) suggest that transplanted corals are likely to show reduced growth rate for up to one year after transplantation. The results of the current study indicate that the period of reduced growth can be as short as one month given a robust starting condition. The NSU land-based colonies nearly doubled in extension rate from month one to month two post-transplant, increasing from a rate of 6.2 ± 4.4 mm month^{-1} to 12.3 ± 6.4 mm month^{-1}. The extension rate of the fragment outplants also increased significantly in month two, although extension did not exceed 10 mm month^{-1} until month four. The optimization of nursery conditions described in Chapter 2 resulted in environmental conditions in the land based nursery that were well matched to shallow reef sites in terms of light, water flow, and nutrient levels. As a result, transplantation stress was minimized, and the period of acclimation was reduced. The ability to modify conditions in the nursery to match prospective outplant sites, thereby reducing transplant stress, is an advantage of land-based nursery culture.

3.4.4 Storm Damage and Disease

Despite the passing of two major tropical weather events in the first year post-transplant, the overall one-year survival rates of 70 – 88% are excellent, and transplantation can be considered a success by this metric. All mortalities that occurred in the outplants were

due to progressing tissue loss, which peaked after storm activity in the late summer and fall. It is unclear whether some of this mortality may have occurred regardless of storm activity, as outbreaks of disease in offshore nurseries and in wild colonies are often observed in the months of July through October in Broward County, when water temperatures are highest (Chapter 2, Larson 2010, Vargas-Ángel 2003). A concurrent study of disease in outplanted and wild *A. cervicornis* colonies in the Florida Keys also documented a large increase in disease occurrence after the passage of Tropical Storm Isaac in August 2012 (Miller et al. 2014)

Disease is the primary factor driving the decline of *A. cervicornis* populations on a large scale. Before the occurrence of tropical storm Isaac and Hurricane Sandy, the outplants, aside from one fragment, were largely free of any tissue-loss. It appears that the outbreak of rapid tissue loss that persisted from early September until the end of November may have been a result of the extensive storm activity. Outbreaks of disease have been reported following storm activity in several species including *A. cervicornis* (Knowlton et al. 1981, Bruckner and Bruckner, 1997, Brandt et al. 2013, Miller et al. 2014). Significant re-working of sand and rubble at the site was evident in post-storm surveys, and outplants likely experienced significant abrasion and physical impacts from shifting rubble at the site. Tissue damage due to abrasion and breakage may have left corals vulnerable to a disease outbreak. The impacts of tropical storms and disease outbreaks are two limiting factors related to the ongoing *A. cervicornis* restoration efforts.

3.4.5 Control Colonies

During the first six months of growth in the land-based nursery, the growth of control colonies was somewhat lower than the extension rates of outplanted colonies (with the

exception of the first month after transplant). During this time, control colonies reached a maximum mean height of 27.2 cm, and colonies had grown nearly to the surface of the water in the nursery tanks and were nearly touching each other and the walls of the nursery tank. It is possible that space limitations in the nursery impacted extension rates. In addition, the levels of alkalinity and calcium in the nursery system were often depleted, as no calcium reactor was on the system. Manual additions of sodium bicarbonate and calcium chloride were barely able to keep up with the growing demand from coral calcification.

The transition of coral colonies into the new land-based system in August 2012 prevents the direct comparison of control colony growth with outplant growth for the later portion of the experiment. The reduced growth rate and increased mortality in the new land-based nursery can be attributed to several factors. Due to the use of the well water discussed in Chapter 2, initial water quality in the nursery showed elevated levels of ammonia and phosphate. Equipment malfunctions and system design issues resulted in several potentially stressful incidents in the first few months of operations, including salinity and temperature fluctuations, and a spill of finely ground iron oxide based phosphate remover into the system. In addition, low PAR measurements in the new land-based nursery were between 50 and 100 μmol m^{-2} s^{-1}, lower than those in the range determined to be optimum for growth in Chapter 2.

The rapid decrease in growth rate observed between August and November 2012 re-iterates the importance of best practices and system engineering for the design and operation of land-based nurseries. Even short-term variances from optimum ranges can have devastating effects on coral vitality. Over the course of this study, growth rates in

land-based nurseries were highly variable, ranging from a low of 0.5 ± 2.3 mm month^{-1} in February 2013 to a high of 16.0 ± 5.3 mm month^{-1} in July 2011 (Chapter 2). This result shows the potential variability in production of land-based nurseries and the extreme sensitivity of production rate to environmental conditions. More controlled studies on long-term coral growth in recirculating aquaculture systems are needed to refine operational procedures and produce recommendations for system design and maintenance procedures for aquaculture of *A. cervicornis*, but information on water quality, water flow, and light conditions needed to produce good survival and growth were established in this study and can be used as a starting point for future work.

3.4.6 Conclusions

In conclusion, the transplant of fragments and colonies of *Acropora cervicornis* raised in land-based nurseries can be considered successful, as measured by growth and survival rates that were comparable to or exceeded those observed for corals raised in offshore nurseries in this study and others. Large colony transplants exhibited the best survivorship and extension rates, but were also highly prone to breakage, and therefore, colony volume did not increase proportionally with growth. Small (5 cm) transplants did not have significantly lower survivorship, and did increase significantly in volume over time. Tropical storm activity resulted in increased disease occurrence in the outplants, and the occurrence of tissue loss was the primary factor resulting in colony mortality.

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CHAPTER

4

OVERALL CONCLUSIONS AND RECOMMENDATIONS

4.1 Coral Husbandry and Land-Based Nurseries

The results of this thesis show that land-based coral nurseries are a viable option to produce fragments of the scleractinian coral *Acropora cervicornis*. Furthermore, these corals are equally successful when transplanted onto natural reefs as corals that originated in offshore nurseries. The variability in growth rate observed during land-based culture in Chapter 2 combined with variability in growth and survival after transplant in Chapter 3 shows that the success of a given land-based nursery project is highly susceptible to environmental conditions and management practices that occur during production and transplantation. This variation can likely be mitigated by the creation of standardized guidelines for best practices in land-based nursery culture. The science of land-based coral nurseries is in its infancy, yet the science of coral husbandry is practiced by millions worldwide in aquaculture facilities, public aquariums, and in private homes. Essentially, the success of a land-based nursery relies on the application of coral husbandry practices that are founded in knowledge that largely exists in grey literature and industry-based publications. The refinement of these husbandry techniques to maximize production and minimize disease are the subject of the recently expanding field of coral aquaculture, with a growing body of supporting scientific literature (Petersen et al. 2008, Osinga et al. 2011, Schutter et al. 2012, Wijgerde et al. 2012, Sheridan et al. 2013, Leal et al. 2013, 2014).

The current study provides insight into the basic principles for successful aquaculture of *A. cervicornis* along with some detailed information about maximizing production for this particular species through careful husbandry practices. The basis for any land-based nursery project should be founded in a strong knowledge of aquarium principles such as

filtration design and engineering, water quality testing and maintenance, lighting, and careful daily observation. The primary difference between land-based nursery projects and general coral husbandry that is currently practiced worldwide is the need for scientific rigor. The ultimate goal of land-based nurseries is to supply a constant stock of corals for transplant back into natural reef areas, and possibly to also serve as a genetic repository for the species. Attainment of these goals requires not only productive culture on a large scale, but also rigorous experimental design, record keeping, and biosecurity practices in order to minimize risks associated with disease transfer and to maximize the scientific knowledge gained from each project.

Basic principles on the husbandry of scleractinian corals and life support system engineering can be gathered from numerous, widely respected publications regarding these topics, and therefore will not be extensively reviewed here. One of the most relevant and thorough publications regarding husbandry in large-scale aquarium systems is *Advances in Coral Husbandry in Public Aquariums* (Leewis and Janse 2008), a collection of peer-reviewed articles produced by a collaboration of aquarium specialists and coral scientists. Additional references regarding marine aquarium design and husbandry include Spotte (1979, 1992), Delbeek and Sprung (1994, 2005), and Escobal (2000).

4.2 Recommendations for Land-Based Culture

In order to establish best practices for land-based nurseries, general guidelines for life support system design, water quality, lighting, monitoring, quarantine, and biosecurity must be compiled from existing knowledge and publications regarding coral husbandry along with those regarding general aquaculture practices. These general practices must

be combined with species-specific parameters for optimal production that are derived from controlled research on species that are valuable or meaningful for production. The second chapter of this thesis began to define certain parameters that promoted survival and significantly increased extension rates for *A. cervicornis* in land-based culture. A brief description of relevant information regarding coral husbandry along with a summary of the specific conditions that improved the growth of *A. cervicornis* is provided here.

Water Quality and Life Support System Design

The design of a recirculating aquaculture system for production of scleractinian corals is inextricably linked to the specialized environmental needs of these organisms. A simple description of required water quality parameters and their ranges would not be complete without an explanation of how to practically achieve these parameters using available filtration technology. In general, scleractinian corals tend to be intolerant of rapid fluctuations in *any* water quality parameters, so life support system components must also be capable of maintaining stable conditions within the desired range at all times. In turn, system operators must be trained in the maintenance and operation of large-volume filtration equipment and machinery in order to maintain the functionality of system components and recognize potential problems before environmental conditions fall out-of-range. Knowledge of mechanical systems must be paired with a thorough understanding of coral biology in order to visually assess the health and condition of corals and to notice subtle changes that may reflect arising problems with water quality. It is recommended to have an aquarium operator (aquarist) whom is trained in coral

biology and husbandry working closely with facilities operators who are able to quickly repair major mechanical problems before environmental conditions fall out of range.

Appropriate water quality parameters for *Acropora cervicornis* are summarized in **Table 4.1** along with generally accepted ranges for scleractinian corals from published sources. Levels of dissolved inorganic nutrients such as ammonia, nitrate, and phosphate should be limited. However, care must be taken in closed aquaria to not allow nutrient levels to fluctuate too rapidly or to fall to extremely low levels that would be limiting for zooxanthellate growth. Recent evidence shows that decreases in nutrient levels (most importantly phosphate) or imbalanced levels of phosphate and nitrate (high nitrate with low phosphate) can result in bleaching and mortality, especially when corals are exposed to stressors such as high light or temperature (Wiedenmann et al. 2013). A good rule of thumb is that if any parameters are out of range for an extended period of time, they should be brought back into range slowly and with only one parameter change at a time unless corals are in imminent danger. This allows the coral and the associated zooxanthellae community to adapt as needed without providing excessive stress on either the host or its symbionts.

Source Water

A reliable, clean, appropriate source of seawater suitable for coral growth is a necessity for successful culture. This water may be sourced from natural seawater either pumped directly from ocean-based sources or delivered in large volumes for storage at the nursery facility. Artificial seawater may also be used, but must be made from high-quality freshwater treated with reverse-osmosis and deionization mixed with a high-grade salt

mixture (either commercially available or made in-house from food-grade or higher salts). A well-maintained high-output reverse osmosis system is necessary in all applications for the purposes of "topping-off" the system, or replacing lost water due to evaporation. Use of a high-quality seawater source will remove some of the burden on the nursery life support system in that the system can be designed strictly for maintenance of proper water quality rather than removal of excess nutrients brought in by unsuitable source water. The root of the majority of problems associated with the NSU land-based nursery in this thesis stemmed directly from the use of a poor seawater source. By using seawater that was high in inorganic and organic nutrients, coupled with a low pH and anaerobic state, corals were routinely exposed to fluctuating levels of nutrients and poor water quality that required residence time in the nursery to be mediated by the filtration system.

Water Flow

Water circulation in coral aquaria is critical to the removal of waste products, which can be produced rather quickly by fast-growing corals. A high amount of flow should be provided, and flow should be alternating and turbulent if possible. Although water currents were adjusted prior to this study, previous water flow was low in the original nursery, and flow was also highly reduced in the new land-based nursery used after August 2012.

A general "rule of thumb" that has been accepted in the aquarium industry is to provide at least 10 times the tank volume per hour in circulation within the aquarium (Pawlowsky 2008). This is not to say that this entire volume must pass through the filtration, but this

volume of water should be put into motion within each tank. In the NSU land-based nursery used in Chapter 2, water flow originally was less than two times the total tank volume per hour. Addition of a dedicated circulation pump and additional Carlson Surge Devices prior to the start of the experiment increased the circulation rate within each holding tank to approximately 6.5 times the water volume per hour, although addition of eductors to the end of the return lines from the circulation loop would have moved an additional volume of water that was not quantified. This produced measured flow velocity of 0.2 – 0.7 ms^{-1} within the nursery, which proved to be suitable for growth of *A. cervicornis* and compare well to natural reef conditions. As circulation volume is only an estimator of water flow, and tank conditions can have drastic effects on water flow, ideally flow velocity in the vicinity of the corals should be measured directly. Water circulation in the new land-based nursery was observed to be approximately 1.5 volumes of tank water per hour initially, potentially contributing to the stress of relocation and the early tissue loss and mortality observed in this nursery.

Lighting

Other than water quality, lighting levels proved to be one of the most important factors affecting coral growth in the NSU-land based nursery. The natural depth range of *A. cervicornis* is generally limited to less than 30 m, and in Broward County this species is most often encountered in water depths of 10 m or less. In order to achieve optimum growth rates in land-based nurseries, it is critical to supply a level of PAR that is comparable to what is found in the natural reef habitat. For *A. cervicornis*, a single layer of 40% shade cloth provided adequate shade with PAR readings comparable to a 6.7 m deep offshore nursery site. The resulting PAR levels in the land-based nursery were

between 450 and 500 µmol m^{-2} sec^{-1} as measured at the coral branches (approximately 6 inches under the water surface). Prior conditions of less than 200 µmol m^{-2} sec^{-1} showed slow extension with thin branch morphology; however these conditions were also coupled with poor water quality, so the exact effect of each factor is unclear.

The subject of artificial lighting was not addressed in this study, although several studies have begun to address the effects of artificial lighting on coral production (Schlacher et al. 2007, Schutter et al. 2012, Wijgerde et al. 2012), and artificial lights are commonly used for laboratory experiments. It is likely that PAR ranges provided by artificial lighting should still be in the above stated range, but further research is needed into the optimum spectrum, lighting type, and photoperiod to maximize production.

Table 4.1. General recommended ranges for water quality parameters commonly measured in coral aquaculture, recommended ranges for *Acropora cervicornis,* and notes on life support design and maintenance.

Parameter	General Recommended Range	*Acropora cervicornis* Ideal Range	Notes on Life Support Design and Maintenance
Total Ammonia (NH_3)	0 - 0.1 mg L^{-1}	<0.03 mg L^{-1}; Reduced extension seen at 0.28 mg L^{-1}	Well established biological filtration, clean seawater and RO water source should minimize problems
Nitrite (NO_2)	0 - 0.1 mg L^{-1}	<0.03 mg L^{-1}	Well established biological filtration, clean seawater and RO water source should minimize problems
Nitrate (NO_3)	0 - 1.0 mg L^{-1}	<1.0 mg L^{-1}; caution to avoid complete removal or fast reduction	Anaerobic areas such as deep sand or porous rock must be provided or export through algae growth; use of denitrification filters possible but not explored
Phosphate (PO_4)	0.00-0.05 mg L^{-1}; some <0.03 mg L^{-1}	0.02-0.05 mg L^{-1}; caution to avoid complete removal or fast reduction	Dilute lanthanum chloride additions used with no ill effect; caution on un-secured iron oxide hydroxide media potentially causing irritation
Total Alkalinity	3.0 - 4.0 mEq L^{-1} 150-200 mg L^{-1} as $CaCO_3$	Same, maintain a high aragonite saturation state without precipitation	Both calcium and total alkalinity should be maintained through the use of a calcium reactor; additions of kalkwasser, calcium chloride, sodium bicarbonate and/or other buffering agents can also be used but may not be suitable for maintenance in heavily stocked, fast-growing systems
Calcium	350-450 mg L^{-1}	Same, maintain a high aragonite saturation state without precipitation	
Temperature	25.0 - 28.0˚C, minimum 18˚C	Minimum of 26˚C to avoid seasonal decrease in extension rate, maximum of 29˚C to avoid summer temperature stress	Heating and cooling system must be adequately sized to maintain temperature within ±1˚ of setpoint even in extreme weather conditions; temperature manipulations may be necessary to induce gamete production or control disease outbreaks
pH	8.0 - 8.4	>8.20 for best growth	Expected to stay within range if alkalinity values are in range and gas exchange is sufficient
ORP/Ozone	300 - 350 mV	<325 mV; very low dosage of ozone or no ozone use	Negative effects on *A. cervicornis* such as retracted polyps and expulsion of mesenterial filaments were seen at high doses; possibly related to ozone dosage into seawater with ammonium present; higher dosage has potential benefit in disease control if applied only with low N
Salinity	33-36 ppt	unknown, large fluctuations to 30 ppt or less caused stress in new nursery	Stable salinity of seawater source; availability of dry salt and clean RO water for adjustments at all times; benefit of being under shelter to avoid rainfall fluctuations; possible benefit of having a peaked roof if only using shade cloth as cover

Outdoor vs. Indoor

Locating a land-based nursery outdoors has several benefits and drawbacks that were apparent in this study. Several negative aspects encountered included the wide range of ambient air temperature fluctuation, loss of heat due to wind exposure, salinity fluctuations due to rainfall, and the physical breakage of the nursery shade structure due to a strong wind event. Benefits of the outdoor location included natural sunlight and photoperiods. The outdoor location was most heavily affected by extreme cold temperatures coupled with strong winds across the surface of the water in the system. This resulted in the need for a high heating capacity to maintain water temperatures on many winter nights, combined with a high cooling capacity to maintain temperature on hot summer days. Temperature control systems must be designed to account for a "worst-case-scenario" based on historical climate data, not just yearly averages. Although the use of natural sunlight has many benefits, a thorough analysis of the risks and benefits of indoor versus outdoor locations must be considered. It is likely that locating the nursery within a greenhouse could provide the best combination of using natural sunlight while still providing some protection from the elements. However, supplemental lighting may need to be provided to achieve higher PAR values and longer photoperiods if located in northern locations.

Transportation and Handling

Transporting coral fragments over long distances is most commonly practiced by collectors and distributors in the ornamental marine aquarium trade. A variety of techniques have been developed to both save on shipping costs and improve the health of corals upon arrival. These include suspending the coral on a piece of buoyant foam and

floating in shipping water, or even shipping corals wrapped in moist paper towels. The results of chapter three of this thesis indicate that dry shipping may not be an appropriate method for transport of *A. cervicornis*, at least not when colonies have reached a certain size or are going to be out of the water for an extended period of time. Although corals from the TAL land-based nursery were transported with moist paper towels, they also had slower growth and lighter coloration than NSU land-based nursery colonies while being held in the land-based nursery. It is unclear to what extent the initial condition of the TAL corals affected their survival and growth after transplant.

4.3 Overall Conclusion

Land-based nurseries should be considered a useful tool for culture and conservation of threatened coral species worldwide. Land-based nurseries can be equally successful to offshore, ocean-based nurseries as a source of fragments for re-stocking reef areas. In addition, land based nurseries can serve as a repository of genetic material that is separated, and protected, from local stressors and risks that are faced by offshore nurseries. That is not to say that land-based nurseries are risk-free; they present their own unique set of problems that require more research to understand and a set of management guidelines to control. Perhaps the most promising benefit of land-based nurseries is the ability to engineer the environment, and to control what is largely uncontrollable in offshore nurseries. Through careful manipulation of environmental parameters and rigorous standards for design and maintenance, the number of fragments and amount of new tissue per coral produced in land-based nurseries could far exceed that produced in offshore nurseries.

Continued collaboration between aquarium specialists from the public aquarium sector, the private aquarium industry, and the scientific community is necessary in order to produce coral fragments from land-based nurseries at optimal growth rates and to stabilize the currently variable level of success through development of guidelines for best practice. The science of coral husbandry is many faceted; it is an intricate combination of engineering, mechanics, biology, chemistry, and medicine. It will take continued development of each of these facets to maximize the potential of land-based coral nurseries and to maximize the potential for successful restoration of *A. cervicornis*.

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