Micro-Fragmenting as a Method of Reef Restoration using *Montipora capricornis*

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Abstract

Micro-fragmenting is a process currently being used as a method of reef restoration for coral reefs, but there have been few studies quantifying the effects of this process on growth rate. The purpose of this project was to find the ideal size to micro-fragment coral. If one large piece of *Montipora capricornis* is micro-fragmented into smaller pieces, ranging from 0-6 sq. cm cross-sectional area, then the larger pieces will have a faster growth rate compared to the smaller pieces. To perform this project, one piece of *Montipora capricornis* was cut, using a saw blade, into 48 fragments. Each fragment was attached to ceramic disks using cyanoacrylate adhesive. Fragments were placed in a 29-gallon tank equipped with lights, a filter, a heater, twelve *Calcinus spp.* (red-legged hermit crabs), twelve *Margarites pupillus* (Margarita Snails), and live rock. Supplements were added accordingly. The fragments were grown for nine weeks with measurements taken approximately every two weeks. Exact measurements of fragments were found using the computer imaging program, GIMP. There was a polynomial relationship between the initial coral size and growth rate, with an r-squared value or 0.9219.

Introduction

While working with pieces of coral at MOTE Marine Laboratory in the Florida Keys, David Vaughan accidently broke a piece of coral that had attached itself to the bottom of the tank, and he thought nothing of this. A week later he came back to this tank and discovered that this small piece of coral was not only still alive, but had also grown. He had discovered the process of micro-fragmenting. Today MOTE Marine Laboratory uses the process of micro-fragmenting to restore reefs, but there has not yet been any research that quantifies the difference in growth rate between micro-fragmented pieces of coral and large, mature pieces of coral. Coral is a keystone species in coral reefs and is what physically builds the reef. Coral reefs have many benefits environmentally and economically that people take for granted. Currently we are killing coral faster than it can grow and recover. Because coral grows at such a slow rate it cannot restore any of the damage that humans have caused, even if we immediately halted all damage being done to coral. This is why there is a large project of reef restoration in the Florida Keys. Micro-fragmenting coral is the only known method of stimulating coral growth, therefore giving coral reefs the opportunity to recover and possibly be returned to their former state of existence.

Literature Review

The Importance of Coral Reefs

Coral reefs and their structural complexity have started experiencing a global degradation, however, because of the limited scale and replicability of reefs many studies have been restricted and are incapable of having a complete understanding of the role of coral in this complex ecosystem. A qualitative and quantitative analysis of the current literature presented, in regards to the importance of structural complexity of coral reef ecosystems, is offered by this study. The number of publications about coral reef complexity has increased over the past forty years, with an increase in the different methodologies used to evaluate the structure.

Existing data shows a negative relationship between structural complexity and algal cover, this could prove the importance of coral complexity which enhances herbivory through reef fishes. The area of total live coral and branching coral was positively related to structural complexity. Habitat characteristics, such as this, have a collinear relationship with structural complexity, but there is evidence of improved coral recovery from disturbances when there is already a high complexity of coral in the reef. Urchin densities were negatively correlated with the structural complexity of a reef. This suggests that urchins are eroding the reef structure, or the social behavior of urchins when in an open area disturbs the reef.

A strong positive relationship between structural complexity and fish density and biomass was found. This is likely due to the density-dependent competition between fish and refuge from predation of larger fish that is offered by a complex reef. There was a variation in the relationship between individual fish families. Each family examined had a positive relationship with structural complexity, but only approximately half of these relationships were significant. Qualitative data also showed that structural complexity increased ecosystem

services, including tourism and shoreline protection. Structural complexity is necessary in coral reef ecosystems and needs to be incorporated into monitoring programs, as well as management objectives (Graham & Nash 2012).

Structural complexity is the physical three-dimensional structure/ shape of an ecosystem. This structure is often formed by the physical shape and complexity of the living organisms, such as grass, trees, kelp, and corals, often known as "ecosystem engineers." However, structural complexity can be shaped by other element of the environment, including geological features and dead matrices formed by organisms. Structural complexity creates various microhabitats in ecosystems and often leads to a greater biodiversity and copiousness amounts of associated animals. This is because as the structural complexity increases there in an increase of these slightly different microhabitats. The effects of structural complexity on species richness and abundance has been shown in a variety of ecosystems, such as forests, seagrass, and kelp beds (Graham & Nash 2012).

Early studies have shown the significance of structural complexity in coral reefs, indicating the importance of complexity for reef fishes. However, the increase in disturbance and degradation of coral reefs has brought about the issue and importance of structural complexity. Studies have shown that initial small disturbances that cause coral mortality, but do not affect the reef structure, can have a limited effect on other components of the ecosystem. However, if the structural complexity of the reef is disturbed, there are negative impacts on fish and other marine life. Data in this area has grown, and analyses of the effect of a disturbance in coral reefs on fish has exaggerated the importance of the complexity of the reef. Overall, the loss of live coral in regions, such as the Caribbean, has been complemented with a loss of reef structural complexity. Knowledge of the importance of structural complexity and the loss of it

in coral reefs has led to the understanding of the importance of structural complexity in the coral reef ecosystem (Graham & Nash 2012).

The only well documented examples of the importance of reef complexity and its correlation to the health of a reef are studies performed on fish abundance. Most studies found a positive correlation between structural complexity of coral and the diversity, quantity, and/or biomass of reef fishes. The strength of this relationship is varied across studies. The importance of reef complexity on corals, algae, and other invertebrates has been understudied and data is less conclusive. Reef structural complexity can also influence fish biomass for fisheries, as well as increasing shoreline protection by dissipating wave energy (Graham & Nash 2012).

A search of ISI Web of Science data-base was done using these keywords: coral reef AND rugosity OR complexity OR topography OR structure OR shoreline protection OR matrix AND structure. The results were 158 publications, after a thorough check for pertinence to coral reefs. The methods used for measuring the structural complexity of the reef were found from primary research. The relationships between structural complexity and coral reef communities, or human activities were drawn from each study and classified as positive, negative, or neutral. Studies that utilized a rugosity test that could be calculated using RI = linear/ surface, where linear is the distance covered by a taught chain/ rope and surface is measured when the chain/rope is laid over the structure and shape of the reef. The study also had to record the density, or biomass of different components of the reef. Information about six different components of the reef ecosystem: algal cover, coral cover, branching coral cover, urchin density, fish density, and fish biomass, were then related to the structural complexity of the reef. Fish density was calculated using per m^2 and biomass was calculated as kg per hectare (Graham & Nash 2012).

Reef management has an astounding impact on reef fish communities, and it therefore could affect the strength of the relationship between structural complexity and other reef communities. The management of the area was then also investigated and its influence recorded. Access to the coral reef was divided into four categories: open access with no restrictions, restrictions on types of fishing gear used, protected areas mixed with open fishing areas, and no-take protected areas. No-take areas prohibit anyone form fishing or removing anything from that area (Graham & Nash 2012).

Technological advances have allowed for the quantification of structural complexity to improve, with some studies focused on the colony level, and others using side-scan sonar to assess reef complexity. The relationship between increased structural complexity and ecosystem service have positive effects due to structural complexity. Structural complexity was also analyzed to have positive effects on tourism, as well as shoreline protection. A strong negative relationship between algal cover and coral reef complexity was found.

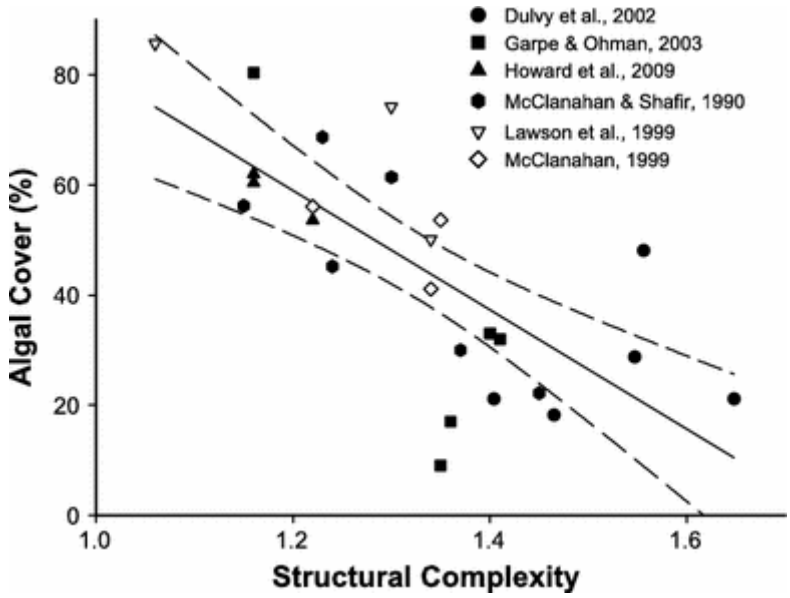

Figure 1. Relationship between percentage algal cover (turf & macroalgae) and structural complexity (RI). Open symbols are studies from the Caribbean, while closed symbols are studies from the Indo-Pacific (Graham & Nash 2012)

The coral cover of each site was also positively related to the structural complexity, but it only correlated for Indo-Pacific reefs. Caribbean reefs showed a neutral relationship, but the range of coral for the Indo-Pacific region was much higher than the Caribbean. Also, many of the studies did not have enough data points to be analyzed. There was a stronger correlation between the structural complexity and the coral cover of branching coral.

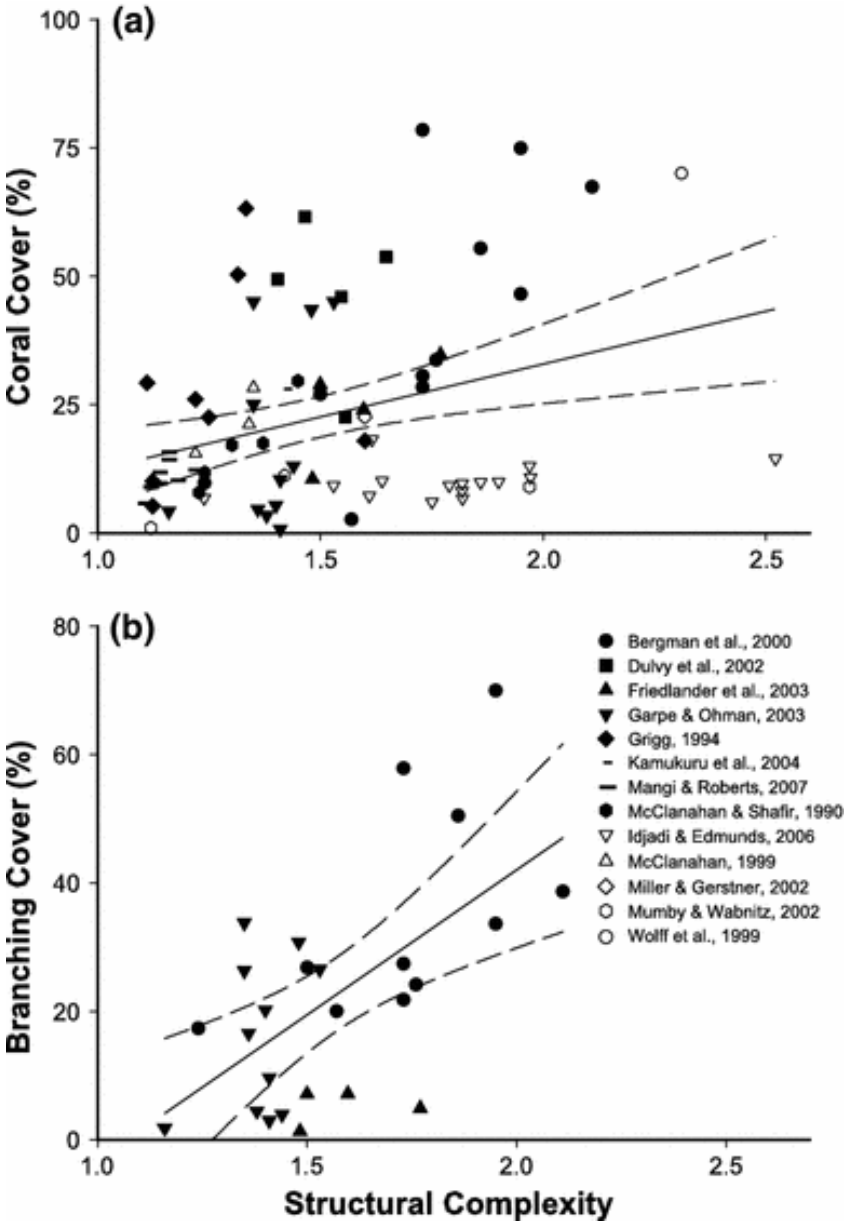

Figure 2. Relationship between a percentage of total live coral cover and b percentage of branching coral cover and structural complexity (RI). Open symbols are studies from the Caribbean, while closed symbols are studies from the Indo-Pacific (Graham & Nash 2012).

There was also a positive correlation between fish density and structural complexity within no-take areas, or partial no-take areas, as seen in figure 2, as well as a strong correlation between structural complexity and fish biomass. The biomass for fish overall was greater at mixed managed sites than those that allowed fishing. There was not enough data to draw accurate conclusions about the biomass in no-take areas (Graham & Nash 2012).

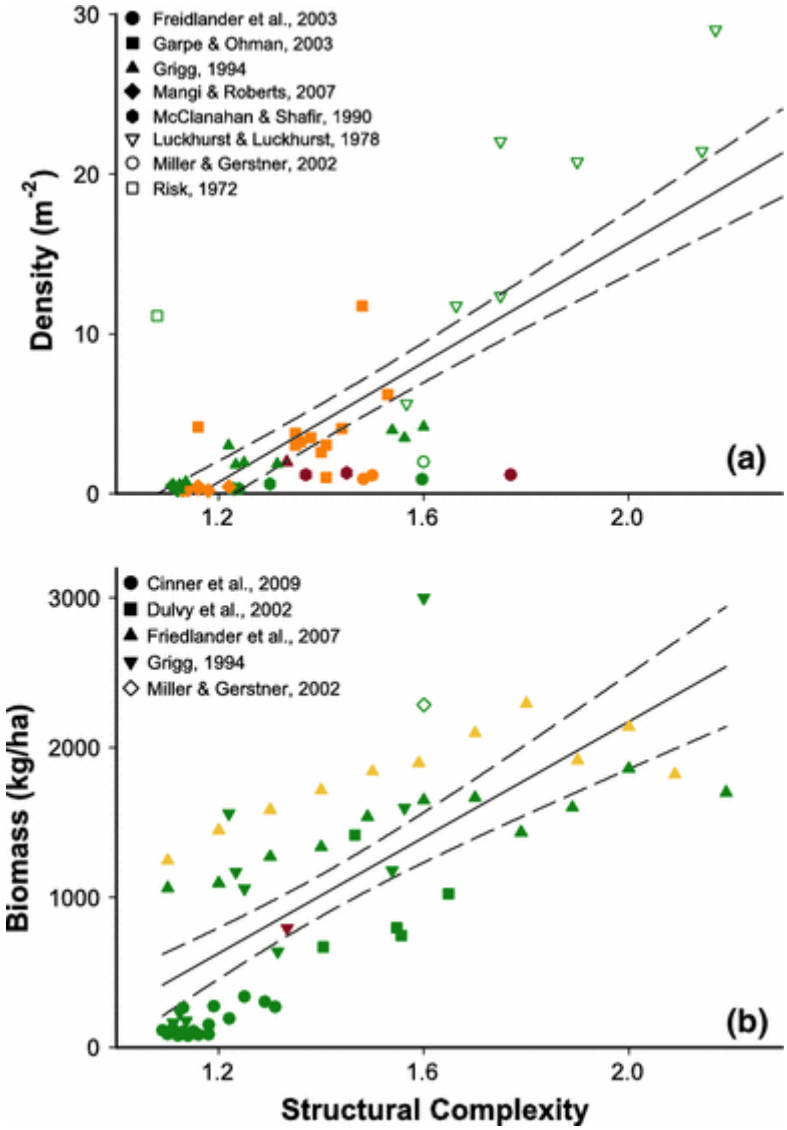

Figure 3. Relationship between a fish density (no. m−2) or b fish biomass (kg/ha) and structural complexity (RI). Colors represent management regime: green sites are open to fishing, orange sites are subject to gear restrictions, yellow sites have a mix of open and protected areas, red sites are no take. Open symbols are studies from the Caribbean, while closed symbols are studies from the Indo-Pacific (Graham & Nash 2012)

Coral is Dying

J.E.N. Veron of the Australian institute of Marine Science has discovered and documented more than 20% of the coral species in the oceans. He began his investigation on coral when he noticed that there were slight differences between the same species at different locations. After travelling around the world and talking to locals he came to the conclusion that corals species intermix and produce new hybrids of species formed connected to their parent species. Through this research Veron found an overarching problem, that coral was becoming extinct. He reviewed previous analyses of coral reef extinctions and discovered the effects of changing sea levels, temperature stresses, and human-influenced changes in nutrient levels. All of these increased his concern for the health of the world reefs. Another concern that Veron had was crown-of-thorn starfish, which eat coral. Veron thought that the populations of these destructive starfish were soaring because of the decrease in predators, but it turned out that it was because the crown-of-thorn starfish larvae thrive in polluted waters (McCalmon, 2014).

Before scientists started studying coral reefs people had taken the ocean and its inhabitants for granted, and thought that they were imperishable. Unfortunately, that was not true, and many locations do not have laws to protect coral, such as in the Central Indo-Pacific. Here coral reefs degenerated to masses of coral skeletons by the time Veron arrived. This was most likely due to coral bleaching, which has come in waves over the past few decades since the 1980s. This first mass bleaching was recorded between 1981 and 1982, and the next between 1997 and 1998. These each affected reefs in over 50 countries. The worst mass bleaching event

to date was between 2001 and 2002, in connection to the El Niño weather patterns. Global warming had been on a steady incline and finally coral's weakness to increased temperature and sunlight warned scientists of these climate changes (McCalmon, 2014).

Coral bleaching occurs when the temperature of the water increase by two or three degrees Celsius, and/or there are increased levels of sunlight. Coral bleaching occurs when the algae that live in coral tissues, Zooxanthellae, which provides coral with its color and energy, produces an excess amount of oxygen through photosynthesis that is lethal to the coral polyps. The coral has to expel the algae, which it normally lives in symbiosis with, in order to survive. The coral is then left with its white calcium carbonate skeleton. Coral is able to regain its algae and color if the water temperature returns to normal within a few weeks, and the water quality remains healthy. The amount and intensity of these mass bleaching are at such a high occurrence that the amount of reef lost due to coral bleaching will likely increase as time goes on (McCalmon, 2014).

Coral bleaching is not the only problem that reefs are faced with currently. Because coral grows so slow, coral reefs are able to keep a very accurate record of prior oceanic events. Using coral skeletons scientists can measure the chemical levels of the ocean throughout the past hundreds of years. This is found through fossil typography, which has shown that four of the five previous mass extinctions came after a large amount of ocean acidification. Acidification is a process in which the ocean absorbs excessive amounts of carbon dioxide and methane, therefore decreasing the pH of the ocean. This has negative effects on not only coral, but also all marine life. Currently the oceans have absorbed approximately one third of their maximum capacity. Scientists are saying that the ocean has shown a sign of commitment, and there will inevitably be a clear destruction of coral reefs and marine life due to acidification as early as 2050. At this

point the oceans may be so acidic that coral skeletons become soluble in seawater. Phytoplankton, the bottom of every marine food chain, will be affected as dramatically as coral if this intense acidification occurs, leading to destruction for all marine animals (McCalmon, 2014).

Coral is also often physically harmed by human interactions, including motor boats, scuba divers, overfishing, pollution, and eutrophication, the enrichment of an ecosystem with nutrients, commonly nitrogen and phosphorous (Osinga, et al, 2011).

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How to Micro-Fragment Coral

Coral propagation has gained interest because knowledge of procedures involved in simple divisions of reef invertebrates has become a common practice. Passive induction included strategies of division that do not necessarily create a free-living clone. These techniques are used to stimulate budding through fission. Examples of this include slicing the periphery of stolen mat of hardy soft corals, including Star Polyps. This stimulates the coral to grow at a faster rate. Captive coral propagation is done through a variety of influence and imitations of natural reef dynamics (Calfo, 2002).

The most frequent coral propagation is imposed fragmentation of coral which is done by cutting, breaking or sawing the coral. These actions are on purpose and used to increase the asexual reproduction of coral. This form of propagation will most likely be the common aquaculture technique used until sexual reproduction can be utilized in aquarium growth (Calfo, 2002).

New aquarists should learn what species of coral are good for cutting into. Certain corals do not act in conformance to the rest of the family. For example, “Leather” corals are a member

of the *Alcynoniid* family which tend to produce mucous when stimulated. Optimum conditions are needed to give corals the best opportunity to grow. *Trachyphyllia* has been shown to have amazing success when fragmented. Aquarists have fragmented whole *Sarcophyton* individuals into 1/4 and 1/2" fragments that were thrown into a rubble trough to produce many hundreds of daughter colonies from the single parent colony (Calfo, 2002).

Before fragmenting coral the ideal technique for fragmentation must be decided on. Many corals when fragmented will produce clones of the original colony, as well as have an increase in growth rate. Fragmenting also makes the coral more susceptible to disease. One major consideration before fragmenting is how much mucous the species will produce. The heavy mucous species tend to be worse subjects and do better with less sudden techniques for propagation, or a more passive techniques, especially for LPS species. The *Acropora* corals are an exception and tend to react very positively to fragmentation (Calfo, 2002).

Mushroom and toadstool corals tend to be sensitive to handling and do not fare well when fragmented. A *Sarcophyton* coral is very hardy and near indestructible when cut. Plastic cable tis can be used to attach mucous producing corals to substrates. When mucous is produced it stimulates the growth of bacteria already on the exterior of the coral and this could lead to an infection before the cut coral has time to heal(Calfo, 2002)..

Sceleratin corals need a similar consideration, in regards to mucous production. Morpphology is also an important concern because some stony corals are excessively easy to fragment, such as Euphylliids which are branching corals. Separating the branches allows for an increase in light and water flow to the branches of the colony. With SPS corals the same technique could be used. Massive and encrusting corals have a lower success rate with micro-fragmenting. This included brain corals, such as *Favia* and *Favites*, which are very similar but

contrast vastly when fragmented. *Favia* have distinct polyp walls, whereas *Forites* corals have connected wall, giving the aquarist a challenge when trying to cut between polyps. *Favia* corals can be easily fragmented by cutting between the polyp walls. Othe scleractinian corals that are fragmented well include *Blastomussa merleti* and *Galaxea* species which have tubular corallites that are connected through calcareous "plates" that can be separated by a saw blade. These corallites appear to be dependent on each other, but they in fact can live independently (Calfo, 2002).

For scleactinian corals a fine toothed, high-speed, masonry blade works most effectively although other less expensive equipment could work. Scleractinian skeletons could shatter when cut, so protective eyewear should be worn. A hand-held rotary tool either a steel wheel is very versatile and can be used for smaller more porous skeletons, but a stone composite blade will not work for fragmenting because they are prone to shattering. Large corals and high density skeletons may need to be fragmented by a table, wire, or band saw (Calfo, 2002)..

After the identification of attributes of the coral that will be fragmented to process of fragmenting the coral is actually very straightforward. Soft reef invertebrates are cut best using razors, scalpels, knives, or scissors. Scleractinians with less dense skeletons can be fragmented with pliers, scissors, poultry shears, or letter openers. Fragmentation by force with a hand is not suggested because of the stress it puts the coral under unnecessary pressure put on the coral polyps from the hands. Fragmentation using a saw is often what is needed for dense skeletons. Scleractinian coral Goos corals to fragment this way includewith thick columns could favor being fragmented by saws rather than violent break. Coral species that do well with this form of fragmenting include*: Favia, Galaxea, Hydnophora, Blastomussa, Turbinaria*, *Fungia*, and *Pavona.* Also, many Pocilloporids (*Seriatopora, Stylophora and Pocillopora*) and Acroporids do

well when fragmented. Soft corals that tend to respond well after fragmented include: *Lobophytum, Sarcophyton,* and *Sinularia* species (Calfo, 2002)..

When beginning fragmentation the largest division is the best option. Fast, clean cuts should be made with a razor or scalpel instead of using scissor which crush the coral. Very sharp scissors could be used with caution (Calfo, 2002).

Micro-fragmenting as a method for reef restoration

Micro-fragmenting is a method discovered accidentally by David Vaughan. It involves cutting massive corals, such as brain, star, boulder, and mounding corals into small square centimeter pieces and attaching them to pucks. These micro-fragmented pieces grow nearly 25 times as fast compared to if they were not fragmented (Morin, 2014).

Coral, a keystone species of coral reefs, needs to utilize fragmentation and colony fusion in order to recover from reef disturbances. Small fragmented pieces of coral were observed to spread tissue and fuse over artificial substrates, this led to experiments which characterized Atlantic and Pacific corals under various conditions. These began with coral from the same colony being fragmented into small pieces (approximately 1-3 cm^2) and evenly spaced on ceramic tile. The fragments rapidly grew and eventually reached isogenic fusion, the fusion of several fragments from the same genet (parent colony), was reached. Growth as high as 63 cm^2 for *Orbicella faveolata*, 48 cm^2 for *Pseudodiploria clivosa*, and 23 cm^2 for *Porites lobata* was noted each month. Growth was measured by the increase in area encrusted and covered by live tissue. Larger fragments tended to grow at a faster rate. The likelihood of small fragmented coral to encrust and fuse on a variety of substrates could be used for further applications, including coral cultivation, assays for coral growth, and reef restoration (Forsman, Page, Toonen & Vaughan, 2015).

For many different organisms, size directly corresponds to survivorship, fecundity, and the outcome of competitive interactions. Clonal organisms -a group of genetically identical individuals, that have grown in a given location, all originating from a single ancestor- such as coral, have a higher mortality rate the smaller they are. This leave the smallest classes, such as larvae, newly settled planulae, and small fragments at a high risk. The energy of these smaller classes of coral is concentrated on the asexual reproduction, to increase their size as quick as possible and therefore lower their mortality rates. Once coral colonies reach a certain size the energy of coral switches from asexual reproduction to sexual reproduction. Similarly, if a sexually mature reef is fragmented into a smaller size then its resources are concentrated on re-growing, not reproducing. This is the basic idea behind coral fragmenting, taking a large piece of coral and requiring it to put its energy into increasing the size of the fragment (Forsman, Page, Toonen & Vaughan, 2015).

Fragmentation and fission (division of the colony) commonly occur naturally due to a variety of causes: physical disturbance, wave damage, erosion, predation, sedimentation, disease, parasitism, and partial bleaching. Fusion, portions of coral growing together, also naturally occurs and is a valuable strategy for small reefs. It gives them more access to shared resources, a competitive advantage by occupying more space, regaining sexual maturity and reproductive capacity, and escaping vulnerability associated with small colonies. Fusion can occur among genetically identical fragments, or settled larvae. Juvenile cnidiarians can fuse with kin, conspecies, or even conheners, creating chimerism, fusion between genetically different colonies, which has often been connected to struggles among partners. But, it also has been shown to create benefits by allowing expression of alternate phenotype in dissimilar environment (Forsman, Page, Toonen & Vaughan, 2015).

Previous experiments have shown that fusion in juvenile coral could reduce size induced mortality. If conditions are controlled then the survivorship of small colonies could increase. Small culture fragments (~1 cm^2) as well as juvenile colonies can combine with genetically identical colonies through fusion and have the possibility to increase growth for coral aquaculture. Being able to promote growth over a pre-determined substrate could help in a variety of applications, including proliferation of rare coral species, developing standard growth assays, coral aquaculture, and reef restoration. Fusion rates of *Orbicella faveolata* and *Pseudodiploria clivosa* were used to calculate the rates of coverage increase. Another similar experiment was performed on *Porites lobata* to characterize the tissue spreading and determine if abiotic and biotic factors in two different environments influence the rates of growth. Additionally, qualitative and quantitative observations of isogenic colony fusion was compiled on a myriad of coral species in the Atlantic and Pacific Oceans (Forsman, Page, Toonen & Vaughan, 2015).

Five ramets of similar sizes from the same original colony of *Orbicella faveolata* were fragmented into 0.86 ± 22 cm^2 (average ± stdev) pieces and then epoxied to 5 ceramic 20 × 20 cm tiles. Attachment of these fragments was performed with cyanoacrylate gel and fragments were spaced out evenly, approximately 1 cm apart from each other. Twenty to twenty-three fragments were placed on each tile. For *Pseudodiploria clivosa,* five, separate colonies were fragmented into 3.05 ± 1.02 cm^2 (average ± stdev) pieces and then attached to 5 different 20 × 20 cm tiles. Fragments were attached in a similar fashion, placed 1.5 cm apart with 9 fragments on each tile. A shallow 340 liter raceway tank was used to perform this experiment. Water flowed at a rate of 2.5 lpm from a 24 m deep saltwater well. Temperature was consistently between 22˚C and 26˚ C and maintained by constant seawater turnover, as well as four air stones (4 cm) that

helped circulate and aerate water. The shore snail *Batillaria minima* was used to control algal growth, as well as daily siphoning of detritus, and manual removal of encroaching algae. Removal of algae was focused on the area between fragments so that it would not prevent the fusion of fragments, or inhibit growth. Also, freshly hatched *Artemia sp.* were broadcast in the tank on a weekly basis. Photographs of each tile were taken from a fixed point, top down using a 1 cm cube in the frame for reference on 9/2/2014, 12/1/2014, and weekly after that. These tiles were measured for 139 days (Forsman, Page, Toonen & Vaughan, 2015).

Overall, common Atlantic and Pacific corals had a growth rate, throughout all observations that was ~20 cm^2/ month ± 25 cm^2/month (average ± stdev). *Solenastrea bournoni* grew the slowest at a rate of 0.2 cm^2/month, and *Orbicella faveolata* grew the fastest with a rate of 63.2 cm^2/month. These observations were seen during a variety of testing periods as well as with various sampling conditions. Some of these factors were examined more closely in experiment specifically with *Orbicella faveolata, Pseudodiploria clivosa,* and *Porites lobata.*

Table 1. Growth rate of coral species in experimentation

Genus	Species	n	Start area (cm^2)	End area (cm^2)	Obs. Period (days)	Rate (cm^2/month)	% increase
Orbicella	*faveolata*	104	89.0	382.0	139	63.2	329
Pseudodiploria	*clivosa*	45	136.0	345.0	132	47.5	154

In 139 days *O. faveolata* fragment had increased by 329% and 13.5% had fused together, whereas *P. clivosa* fragments had an increase of 154% in size and 31.1% of the fragments fused. None of the fragments detached from the substrate, nor did any fragments die. The growth rate of both species seemed to be linear, explaining the 86% variation for *P. clivosa,* and 88% variation for *O. faveolata* fragments. Another order of polynomial regression expressed the 94% variance

for *P. clivosa* and 97% variance of *O. faveolata.* Linear regression between the initial size of fragments and size of the fragments after the experiment showed that the growth rates have a correlation with colony size. Larger fragments grew faster, justifying the 56% variation in *O. faveolata* and 79% in *P. clivosa.* Figure 4 below shows the linear (gray lines) and polynomial (red lines) growth rate values of *Orbicella faveolata* (black diamonds) and *Pseudodiploria clivosa* (black circles) from 9/2/2014 to 1/19/2015 and Porites lobate (black squares) from 6/25/2006 to 1/17/2007. Figure 4 below that shows (A) initial size of *Orbicella faveolata* versus size after 132 days of growth; (B) initial fragment size of *Pseudodiploria clivosa* versus 139 days of growth; (C) initial size fragments of *Porites lobata* versus size after 38 days of growth (Forsman, Page, Toonen & Vaughan, 2015).

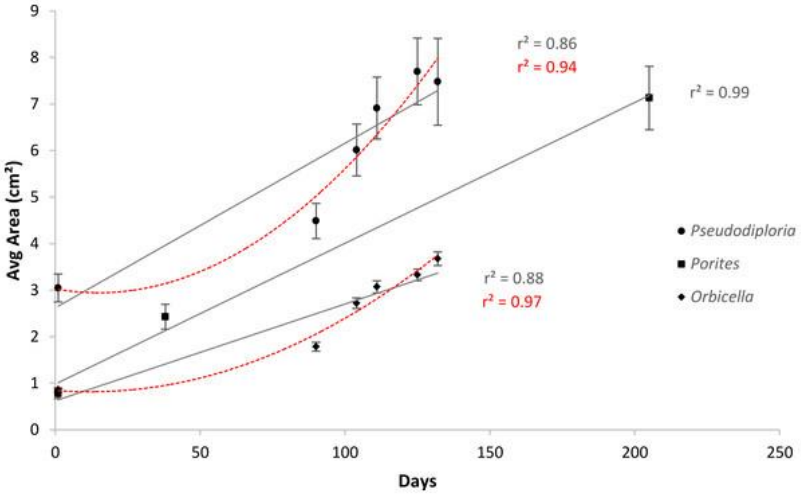

Figure 4. Average increase in coral area over ceramic tiles (Forsman, Page, Toonen & Vaughan, 2015)

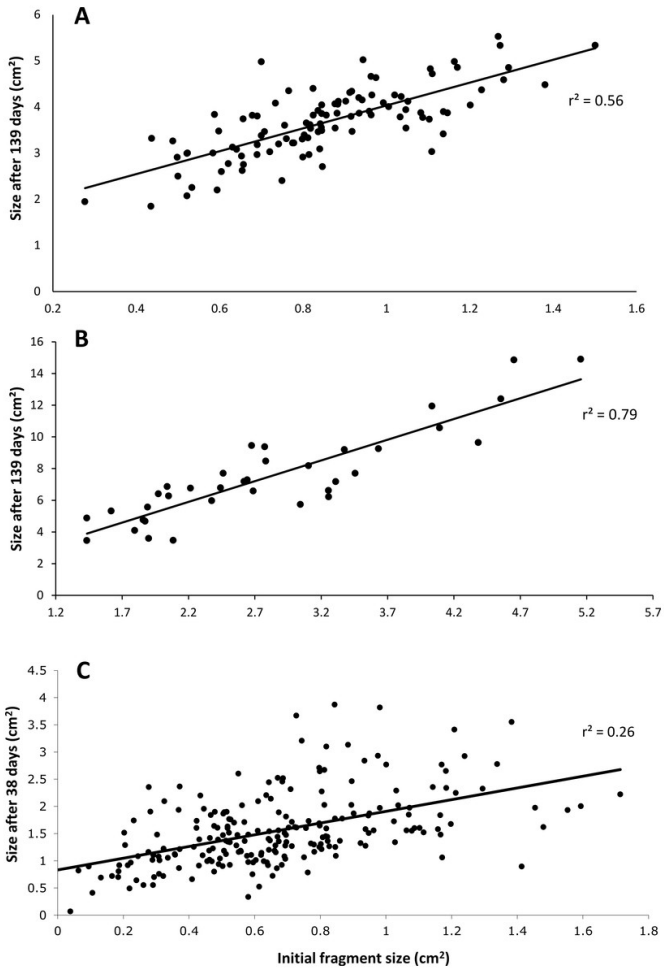

Figure 5. Relationship between initial and final size (Forsman, Page, Toonen & Vaughan, 2015)

During a 4 month time period micro-fragments of *O. faveolata* increased by 293 cm^2 and *P. clivosa* fragments increased by 222 cm^2. This approximates to ~11 cm and ~9 cm of increased colony diameter, assuming circular colony growth. This study measured change in area covered by thin sheets of live encrusted tissue, which would not be comparable to many field studies because they quantify change in maximum diameter or linear extension, for example many Caribbean corals grow 0.5-1 cm per year. Nonetheless, 89 cm^2 of *O. faveolata* live tissue, and 136 cm^2 of *P. clivosa* tissue, resulted in a 329 cm^2 increase of tissue and 154% area increase over four months (Forsman, Page, Toonen & Vaughan, 2015).

The growth rate of both species are within the expected bounds of linear rates of growth, which explains the 86% and 88% variation and the second polynomial curve explained between 94% and 97% of the variance in growth rates. This showed that the growth rates of fragments probably accelerated near the end of the experiment. Difference in growth rate could be explained by a variety of reasons, but the initial fragment size was evidently very important, because smaller fragments grew at a slower rate compared to larger fragments. The scope of the experiment did not consider multiple effects of growth rate, including seasonality, temperature, colony age, or other biotic and abiotic factors. Previous works have shown a clear correspondence between the size of fragmented pieces and growth rates, and that larger fragments grew at a faster rate (Forsman, Page, Toonen & Vaughan, 2015).

Care of a Salt Water Aquarium Tank

Understanding how to properly set up and care for a salt water aquarium was necessary for maintaining a healthy, controlled environment for the coral fragments. Coral is a very fragile species and even if everything is running properly and looks fine one day in twelve hours all of a tank's inhabitants could die (Jason Ryan, personal communication, October 25, 2015). First, set up the tank, install the filtration system, and fill the aquarium with freshwater, preferably treated by reverse osmosis. Untreated city water, if used, should be treated with a de-chlorinator in order to remove any chlorine that could be harmful to aquarium life from the water. Next, add salt following instructions of the salt mix used. A hydrometer can be used to monitor and raise the salinity levels. Install the heater and set it to the desired temperature. Let the system run independently for a few days to guarantee a proper water temperature and that the equipment is functioning properly (Drs. Foster & Smith Educational Staff, 2015).

After the aquarium has run independently for a few days, with the equipment functioning properly start adding aragonite-based substrate and live rock. Adding 2-3 inches of live sand that donates beneficial bacteria and micro-organisms to the aquarium is also suggested. After placing sand and substrate in the tank move onto adding some live rock (Drs. Foster & Smith Educational Staff, 2015).

Live rock is a porous, aragonite-based rock that has been gathered from rubble zones of ocean reefs and hosts large quantities of helpful bacteria and micro-organisms. Additionally, live rock grants fish and other organisms a good hiding spot and assists in preserving healthy water parameters. Live rock provides a tank with an aesthetic appeal as well as a natural, biological filtration, moreover providing a necessary environment for fish and invertebrates. Add approximately 1-1/2 pounds of live rock per gallon of water in the tank. The precise weight should vary depending on the type of rock (Drs. Foster & Smith Educational Staff, 2015).

Before adding fish or invertebrates the live rock must be cured. Curing the live rock takes 4-5 weeks and initiates the Nitrogen Cycle. Certain people will add shrimp to their tanks to increase the ammonia levels and kick-start the Nitrogen cycle of the tank (Jason Ryan, personal communication, November 1, 2015). While this is going on start weekly 25% water changes. To start curing the live rock stack the rock loosely in the aquarium, creating caves for fish to swim through. Make sure to stack the rocks right side up, with the more colorful side facing upward, this will allow for appropriate lighting conditions and ideal conditions for coralline algae, which need a lot of light and sponges, which need minimal lighting. Keep the aquarium dark during this time to enhance algae growth, limiting lighting times to only when checking the tank (Drs. Foster & Smith Educational Staff, 2015).

Once the ammonia and nitrite levels have reached 0 ppm the live rock has fully cured and the biological filtration system of the tank has been established. Set up a lighting system that will be turned on for 10-12 hours a day, mirroring a normal day. There will likely be an algae bloom for the first few weeks after lighting has been added and an algae attack pack can be used to decrease the algae growth. Follow the instructions and the natural biological filtration system should be able to handle new inhabitants because of the fully cured rock system. Test the ammonia and nitrite levels again before adding any fish or invertebrates (Drs. Foster & Smith Educational Staff, 2015).

Potential for reef restoration, growth assays, and coral aquaculture

Being able to promote growth over a pre-determined substrate could help in a variety of applications, including proliferation of rare coral species, developing standard growth assays, coral aquaculture, and reef restoration. The ability for a coral fragment to grow on a benthic surface, or 'self-attach' is necessary for the colony to survive and the transplantation to be successful. In this experiment self-attachment of tissue spreading has been shown through a variety of substrates. This allows for the improvement in transplantation and for the fusion method could be used to increase to increase the likeliness of fusion over the benthic surface. Field trials are currently in development to find procedures that effectively encourage nursery growth coral to fuse and attach itself to the benthic surface. These experiments are testing the utility of this method to restore *O. faveolata, M. cavernosa,* and *P. clivosa* to reefs that have been affected by anomalous cold temperatures that occurred in early 2010.

Coral reefs are declining and this calls for more responsible coastal development. Also, there is an increase in the demand for sustainable resources of coral materials to use for aquacultures, research, mitigation, and restoration projects. As said by Forsman (October 2015)

"The micro-fragmentation- fusion strategy effectively manipulates the surface areas of a coral onto a two dimensional plane, over which small colonies rapidly spread tissue and fuse." Being able to encrust coral onto a myriad of materials allows experiments to be done testing an assortment of fresh methods for coral cultivation and transplantation, such as mass producing 'seedlings.' If a complex three dimensional structure were to be covered, then coral would be able to successfully combine the benefits of reef restoration with artificial reefs. But, to effectively create the maximum beneficial combination long term studies have to determine physiological and reproductive effects of the process, and evaluate the advantages compared to traditional direct transplantations, which often result in small fragments which are susceptible to higher mortality rates. Although, this method could be extended to a larger scale to allow for a more sustainable source of coral material and provide more knowledge on the cultivation of slower growing species of coral. Incorporating in-situ, in the ocean and natural habitat, and ex-situ, in a controlled tank, nursery plans could offer source material at scales formerly not conceivable (Forsman, Page, Toonen & Vaughan, 2015).

Research Plan

A. Researchable question:

How does the original size of micro-fragmented pieces of *Montipora capricornis* affect the two-dimensional cross section area growth rate of the coral?

B. Hypothesis:

If one large piece of *Montipora capricornis* is micro-fragmented into smaller pieces ranging from 0-6 sq. cm cross-sectional area, then the larger pieces will have a faster growth rate compared to smaller pieces of coral.

C. Description in detail of methods or procedures

To perform this experiment, a tank was set up to place the micro-fragmented coral in. After visiting an aquarium store and talking with employees there, who have extensive experience with setting up tanks and maintaining them, the tank that was selected to be used was a 29-gallon bio-cube that contains filters, lights, and everything else needed to maintain a tank, besides a heater incorporated into the tank. The tank was set-up according to the instructions of the aquarium store, and other reliable sources.

One piece of *Monitpora capricornis* will be micro-fragmented into various sizes between 0.5 cm^2, and 6.0 cm^2 using a saw at Jay's Aquatics. The fragments were be epoxied with a marine super-glue on an aragonite substrate plug that was much bigger than fragment, in order for coral fragments to have room to grow. Plugs will be labeled with letters according to size and placed randomly in the tank. The tank was be regulated by snails (*Margaritea pupillus*) and hermit crabs (*Calcinus spp.*) that will clean the algae and act as a bio-filter maintaining the environment within the tank. Once the tank had established a healthy environment, one kenya tree (*Capnella spp.)* was added, to ensure that hard corals, which are more finicky than soft

corals, would survive in the tank. After the soft coral has survived for one week the micro-fragmented coral was brought in.

The process for micro-fragmenting coral involved using a saw to cut between polyps and get an approximate size. The "goal" sizes, between 0.5 cm^2, and 5.0 cm^2, but the exact topographic surface area will be measured for each fragment and recorded. Different sizes were taken from different regions of the coral, and from different pieces of coral to ensure that that fragments did not grow at different rates because of the original poor health of a single coral piece. These fragments were then attached to an aragonite plug substrate with epoxy, then transported to the 29-gallon tank. The fragments were placed in the tank an even distance apart and each size will occupy different sections of the tank.

The tank was be checked daily for temperature, as well as a visual check of the health of the tank, this includes algal growth, checking the filter, and making sure each coral fragment looks healthy. Weekly chemical tests of ammonia, nitrites, nitrates, pH, magnesium, alkalinity, and calcium were performed. If a coral fragment fell off of the substrate then it was included in final calculations of growth rate. Fragments that died were not included in the study. The action of micro-fragmenting is very aggressive and coral can die simply from going through the process, which is why the fragments that died initially were not included. Every two weeks a picture of the coral fragments was be taken with a camera, and a ruler will be placed next to the coral fragments for reference.

At the conclusion of the experiment the percentage increase of each fragment was calculated. The average of each was be calculated to calculate the average percent increase for each original size of coral fragments. The final results will be based on the average growth rate,

not on the percent, and whether or not there is a notable difference between the starting sizes of coral fragments in the species of coral.

Methodology

Setting up the tank

A 29-gallon BioCube® tank designed by Corallife® was acquired from Jay's Aquatics. The dimensions of the tank are 50.80 cm (l) by 53.34 cm (w) by 47.31 cm (h). The tank came with build in lighting, which included one 36 Watt Actinic Blue Straight Pin, one 36 Watt 10,000K Daylight Straight Pin, and one 0.75 Watt Lunar Blue LED Bar. The tank also had a 60 mm, 15.83 CFM, 25.5 Db cooling fan, and a pump with a 1000 L/hour flow rate.

The tank was then slowly filled with pre-made salt water from Jay's Aquatics in 5 gallon increments. After the first 5 gallons were added one 9.072 kg bag of black Nature's Ocean® Bio-Activ Live® Aragonite Reef Sand was placed in the bottom of the tank. Six pieces of live rock weighing a total of 8.50 kg were spread out across the bottom of the tank. The remaining 15 gallons of water were added to the tank. A 150W Marina® Submersible Aquarium Heater was placed in the tank, as well as a Deep Blue Professionals ProTherm™ digital thermometer on the opposite side of the tank. Lastly, 30 mL of API® Aquarium Pharmaceuticals Quick Start® was added to the tank.

Three days after the tank was set up, 2 dead jumbo shrimp were added to the tank in order to jump start the Nitrogen cycle. After 36 hours, the shrimp were removed and the tank took a week after that to "cycle," until the ammonia and nitrite levels were both at 0 ppm. Three days later 12 *Calcinus spp.*, red legged hermit crabs and 12 *Margarites pupillus,* Margarita Snails were added to the tank, and the 0.75 Watt Lunar Blue LED Bar and 36 Watt Actinic Blue Straight Pin were set to stay on for 4 hours a day. The amount of flight was increased over the next week until it was set to 12 hours of light. A *Capnella spp.* coral was placed in the tank to ensure that chemical levels were at the correct level for coral.

Maintaining Tank

Four weeks after setting up the tank measurements of pH, ammonia, nitrites, nitrates, magnesium, alkalinity, and calcium were only taken once a week. The pH, ammonia, nitrates, and nitrites were found using the API™ Aquarium Pharmaceuticals Saltwater Master Test Kit.

Image 1. API™ Aquarium Pharmaceuticals Saltwater Master Test Kit.

The magnesium, alkalinity, and calcium were found using the Red Sea Reef Foundation Pro Test Kit.

Image 2. Red Sea Reef Foundation Test Kit (amazon.com).

Red Sea Reef Foundation C Mg Supplement, Red Sea Reef Foundation B Buffer Supplement, and Red Sea Reef Foundation A Ca/Sr Supplement were added for magnesium, alkalinity, and calcium supplements, respectively, according to the charts. It was calculated that a maximum of 8.0 mL of Mg Supplement could be added per day, 11.1 mL of Buffer Supplement per day, and 8.0 mL of Ca Supplement per day, based on the 20-gallon tank.

Image 3. Testing chemicals in the tank.

Reef Foundation
Mg Supplement C

Concentration: 1 ml /100 liters = 1ppm Mg

Aquarium Type	Soft / Low Nutrient SPS	LPS	SPS Frags
Measured Level (ppm) / Optimal Level (ppm)	1280	1310	1390
	Supplement Dosage: ml / 100 Liters (25 gal)		
1140	140	170	250
1160	120	150	230
1180	100	130	210
1200	80	110	190
1220	60	90	170
1240	40	70	150
1260	20	50	130
1280	-	30	110
1300	-	10	90
1320	-	-	70
1340	-	-	50
1360	-	-	30
1380	-	-	10

Image 4. Reef Foundation C Mg Supplement chart (SwellUK.com)

Reef Foundation
Buffer Supplement B

Concentration: 1 ml / 100 liters = 0.036 meq/L (0.1 dKH)

Aquarium Type	Fish	Soft / Low nutrient SPS	LPS	SPS Frags
Measured meq/ L - dKH / Optimal meq/ L - dKH	2.5 - 7	2.9 - 8.1	4.3 - 12	4.5 - 12.6
	Supplement Dosage: ml/ 100 Liters (25 gal)			
2 - 5.6	14	25	64	70
2.2 - 6.2	8	20	58	64
2.4 - 6.8	3	14	53	58
2.6 - 7.3	-	8	47	53
2.8 - 7.9	-	3	42	47
3 - 8.4	-	-	36	42
3.2 - 9	-	-	31	36
3.4 - 9.6	-	-	25	31
3.6 - 10.1	-	-	20	25
3.8 - 10.7	-	-	14	20
4 - 11.2	-	-	8	14
4.2 - 11.8	-	-	3	8
4.4 - 12.4	-	-	-	3

Image 5. Reef Foundation B Buffer Supplement chart (SwellUK.com)

Reef Foundation
Ca/Sr Supplement A

Concentration : 1 ml / 100 liters = 2 ppm Ca

Aquarium Type	Soft / Low Nutrient SPS	LPS	SPS Frags
Measured Level (ppm) / Optimal Level (ppm)	430	440	465
	Supplement Dosage: ml /100 Liters (25 gal)		
350	40	45	58
360	35	40	53
370	30	35	48
380	25	30	43
390	20	25	38
400	15	20	33
410	10	15	28
420	5	10	23
430	-	5	18
440	-	-	13
450	-	-	8
460	-	-	3

Image 6. Reef Foundation A Ca/Sr Supplement chart (SwellUK.com)

A bi-weekly 25% water change was performed throughout the experiment, and the filter was change every 4 weeks. The pump for the filter was taken out over 4 weeks and cleaned. The two blue lights (36 Watt Actinic Blue Straight Pin, and 0.75 Watt Lunar Blue LED Bar) were left on from 6 am to 6 pm for 12 hours every day, and the white light used occasionally for observation.

Sixty-eight days after the tank was set up, 2.0 mL of API™ Aquarium Pharmaceuticals Algae Fix® Marine Algaecide were added every three days to control the green film algae that had grown on the side of the tank. The algae was also scraped off the side of the tank using a dull razor blade. Four *Trochus maculatus* were added to maintain the algae. The solution was added accordingly when algae started to accrue, but there was minimal usage.

Fragmenting Coral

A decision matrix was utilized to select the best option to micro-fragment coral.

Table 2. Decision Matrix for coral species

Criteria	Weight	*Cyphastrea*	*Astreopora*	*Montipora Capricornis*	*Capnella sp.*	*Seriatopora hystrix*
Polyp size/ shape	7	8	6	9	3	4
natural growth rate	7	5	5	7	8	8
original size	5	7	6	10	5	4
potential for reef restoration	7	7	8	6	2	3
Amount in aquatic store	9	2	0	8	6	4
	Total	193	163	276	170	161

A large piece (15 cm by 25 cm) of *Montipora capricornis* coral was broken off of the rock that it was attached to, using scissors to lever it off. This resulted in seven large pieces which were further fragmented into sizes between 0.73 cm^2 - 5.57 cm^2 using a band saw. A picture was taken of each fragment with a cm ruler in the frame for reference. The 2-D basic measurements of each fragment was also found, and each frag was labelled with an approximate number of square centimeters 1, 1.5, 2, 2.5, and +, for all fragments over 3 cm^2. These

measurements were not used for the actual measurements, only an approximation for identification purposes. The fragments were also labelled with letters, such as 1A, 1B, and so on for individual identification. The identification of each frag was then written on Boston Aqua Farms ceramic reef discs with a graphite pencil. The fragments were then attached to the ceramic reef disks using a small dollop of Seachem® Cyanoacrylate Adhesive Reef Glue™.

Finding Exact Measurements of Coral

The exact measurements of the coral could not have been found using calipers, because of the irregular shape of most of the fragments. In order to find the exact measurements the photo imaging app GIMP was used. The photo of each fragment at each specific timing was imported into GIMP. The area of the circle was then selected, free-hand, using the free-select tool, and the area of the coral found in pixels using the histogram tool. Then the length of 1 cm was found in pixels by measuring 1 cm using the measuring tool. There could be some errors with extremely precise measurements, but it is assumed that if any errors were made then they were repeated errors since the same person was taking all of the measurements, and the results would not be affected, because the error would be made for each analysis of each picture. The total number of pixels was then divided by the length of 1 cm in pixels squared to find the area of each fragment in cm^2.

When taking the original measurements white pieces of coral were not selected, because they did not cover the surface area of the original piece of *M. capricornis*. Later on these white pieces did turn brown, and were then included in the area because it was an increase in the topical area of the piece of coral. Sections of coral that had died were not included, if that piece of coral was continuing to grow. If glue had covered a portion of the coral then that piece was not included in the data set, because the glue inhibited growth and could interfere with the other

sections of the coral, not just the portion that it covered. If it was obvious that a piece of coral had deceased then it was not counted in the results.

Results

The growth rate, as well as the percent growth rate, area increase, and percent area increase, of 39 micro-fragmented pieces of coral was calculated 68 days after the initial micro-fragmentation.

Table 3. Results of the growth rate, percent growth rate, area increase, and percent area increase of fragmented pieces of *Montipora capricornis*.

		<1 cm^2	1-1.5 cm^2	1.5-2 cm^2	2-2.5 cm^2	2.5-3 cm^2	3-4 cm^2	4-6 cm^2
Day 1	cm^2	0.869	1.207	1.740	2.243	2.750	3.109	4.933
Day 68	cm^2	2.850	3.698	4.759	4.952	6.524	7.181	9.407
growth rate	cm^2/day	0.029	0.037	0.044	0.040	0.055	0.060	0.068
% growth rate	% area increase/ days	4.960	4.503	4.043	3.253	3.445	3.394	2.908
area increase	final cm^2 - initial cm^2	1.981	2.491	3.026	2.704	3.759	4.072	4.643
% area increase	final cm^2 / initial cm^2	337.3	306.2	274.9	221.2	234.3	230.8	197.8

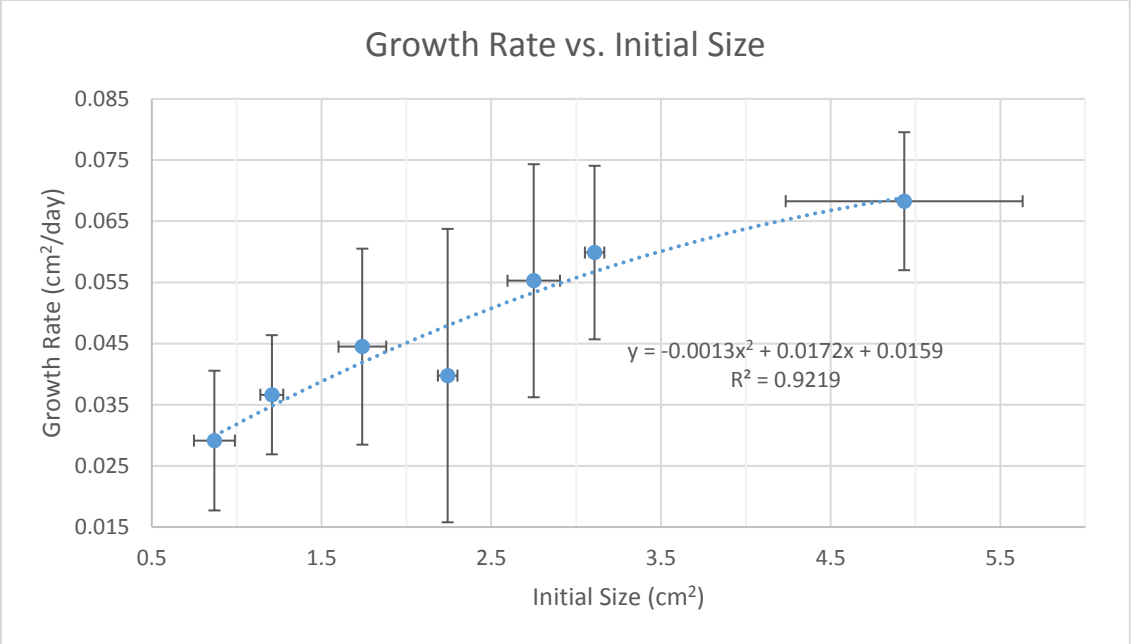

Figure 6. A scatter plot that represents how the growth rate is affected by the initial size.

Overall the large pieces of coral (avg. 4.933 cm^2 ± 0.897 cm^2) had a faster growth rate, at 0.068 cm^2/ day ± 0.011 cm^2/ day compared to the smallest pieces of coral (avg. 0.869 cm^2 ± 0.121 cm^2) which had a growth rate of 0.029 cm^2/day ± 0.011 cm^2/ day. That is 234% faster in the largest setting of micro-fragmented pieces of coral.

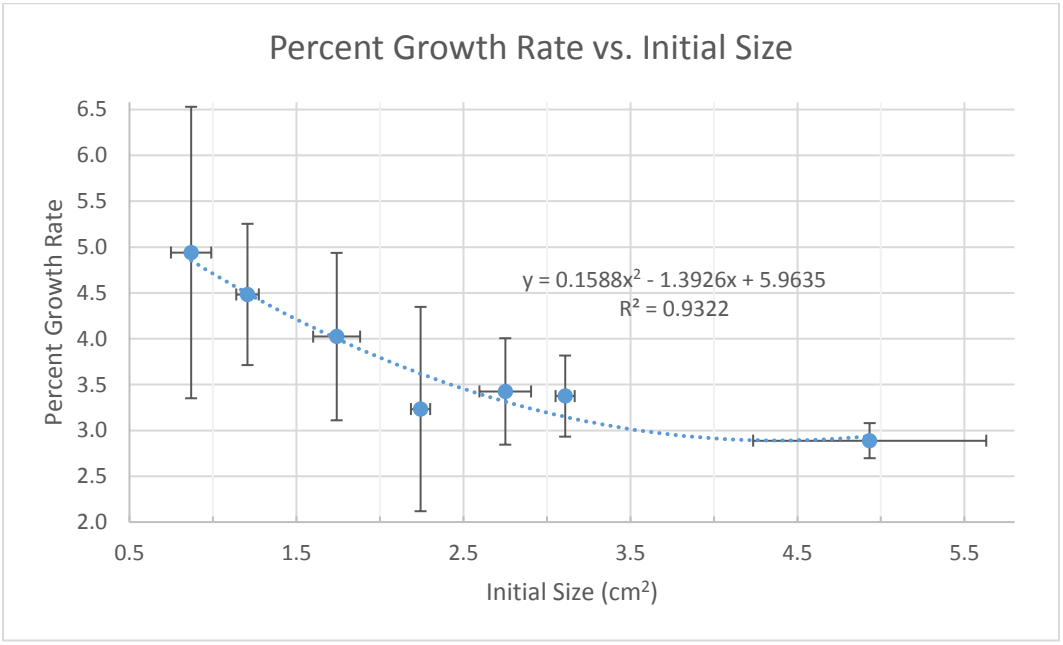

Figure 7. This graph represents the percent that each coral setting grew per day. Percent growth is the percent growth (final size/initial size) over the number of days (68).

As the initial size of the coral increased the percent growth decreased. This relationship resulted in a polynomial line of best fit, with an r-squared value of 0.93.

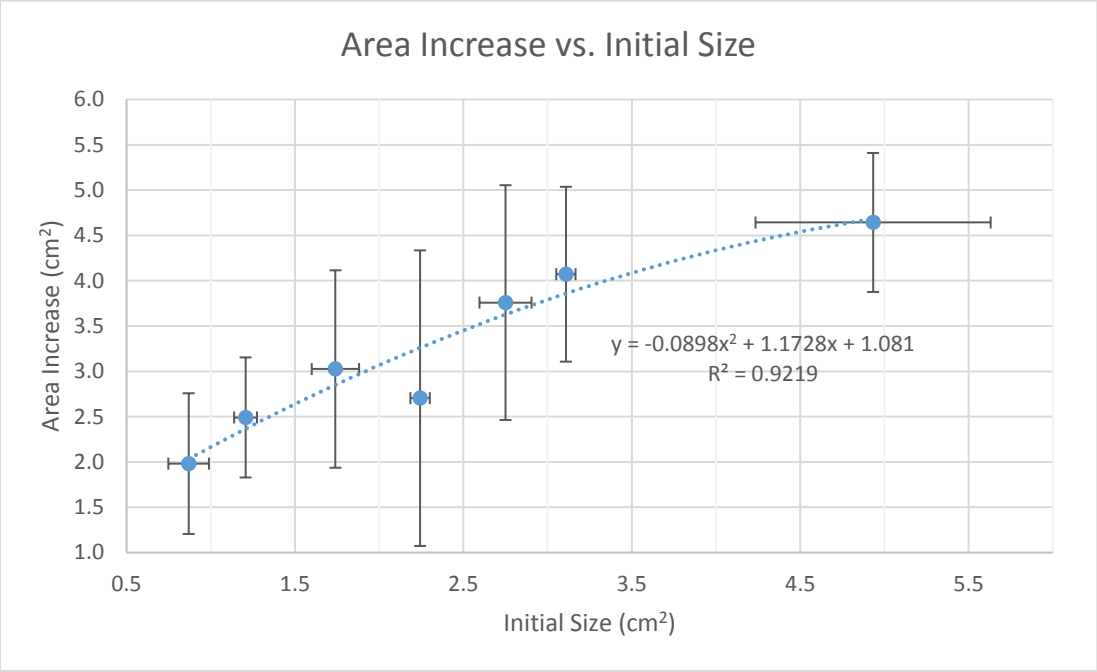

Figure 8. The area increase (cm^2) dependent upon the initial size (cm^2) of the coral fragments.

The area increase (final size cm^2 – initial size cm^2) was dependent upon the initial fragmented size of the coral, with a polynomial relationship that had an r-squared value of 0.92.

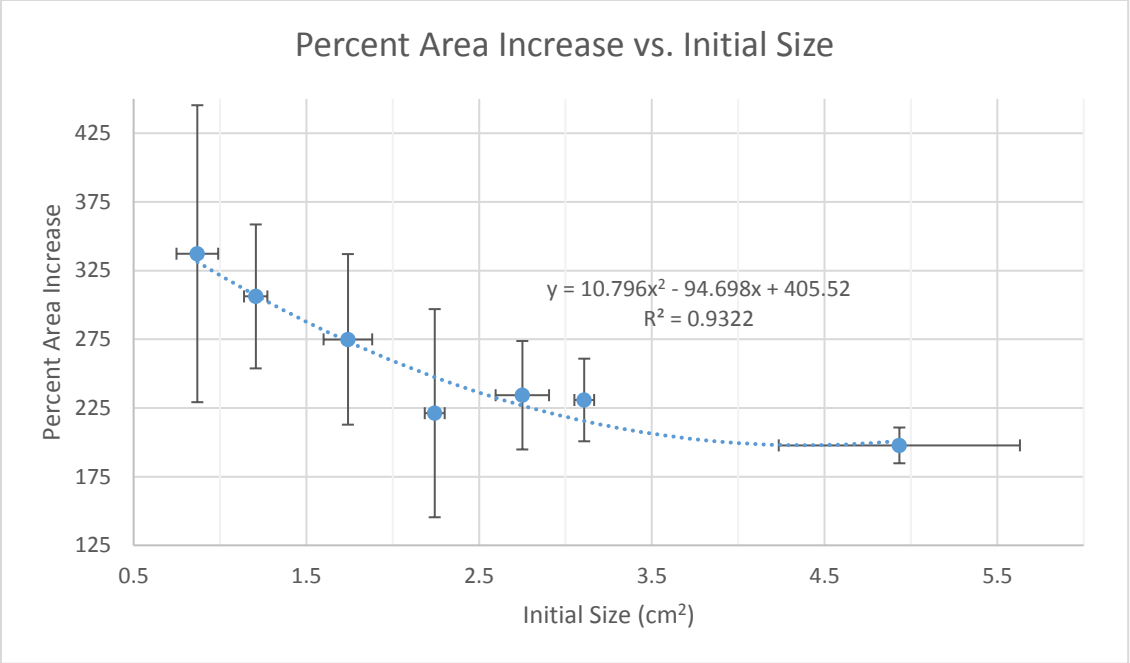

Figure 9. This graph represents the percent increase over the 68 days of the experiment. Percent increase was based on the final size divided by the initial size.

As the initial size of the coral fragments increased the percentage growth decreased, even though they had a higher overall increase in area. There was a strong polynomial relationship between the initial size and the percent area increase, with an r-squared value of 0.93. The largest setting had the smallest percent area increase, which was still almost 200% (actual 197.8% ± 13.02%) of the initial size of the coral fragment.

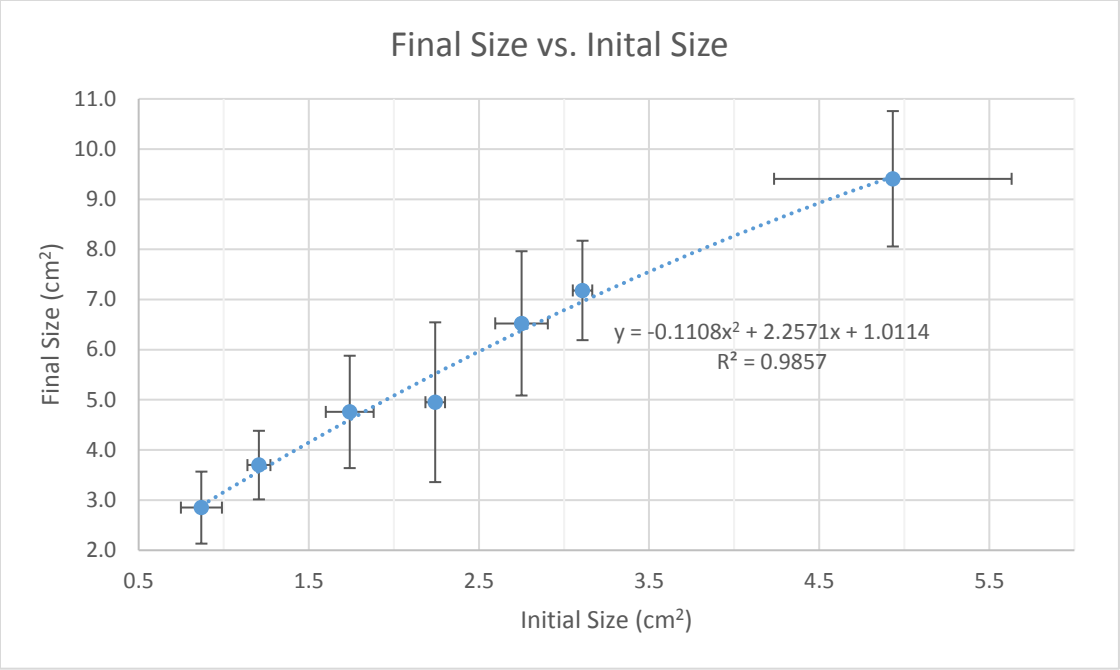

Figure 10. A graph of the initial size of the fragments compared to the final size of the fragments.

The relationship between the initial and final size of coral fragments is a polynomial relationship, with an r-squared value of 0.99.

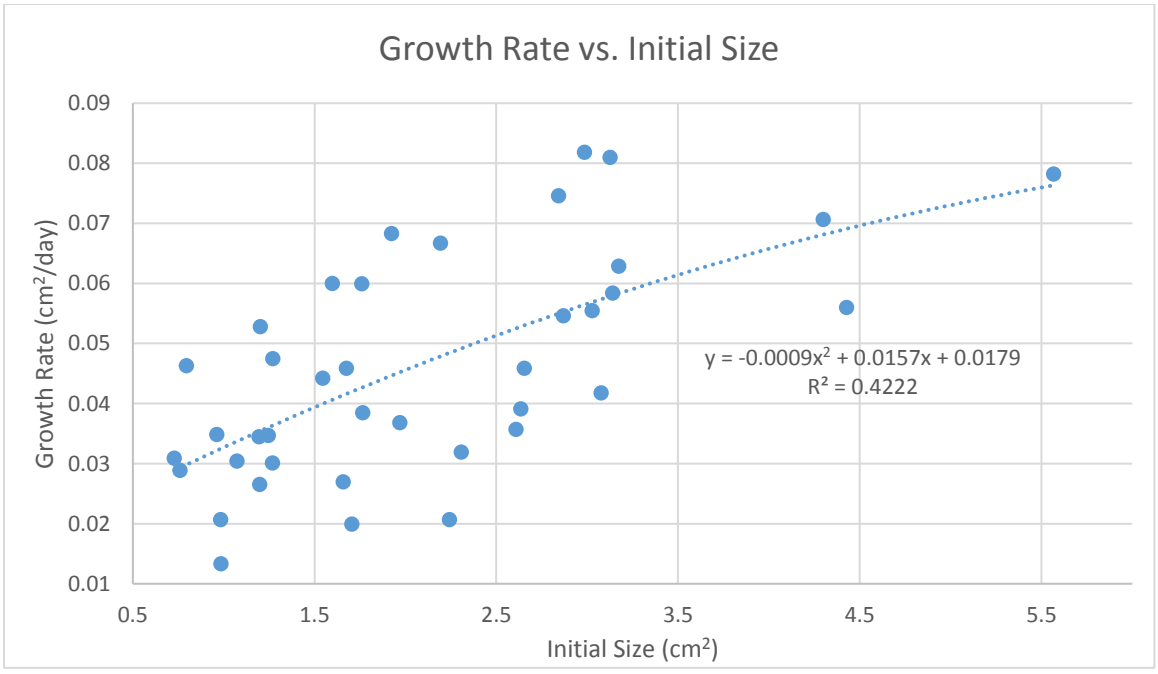

Figure 11. A scatterplot of all the fragments of coral and each individual growth rate after 68 days.

There was a moderate correlation between the initial size of fragments, and the growth rate, with an r-squared value of 0.42. Overall there is a visible trend of an increase in growth rate as the initial size of fragments increased, which is seen more clearly on figure 6.

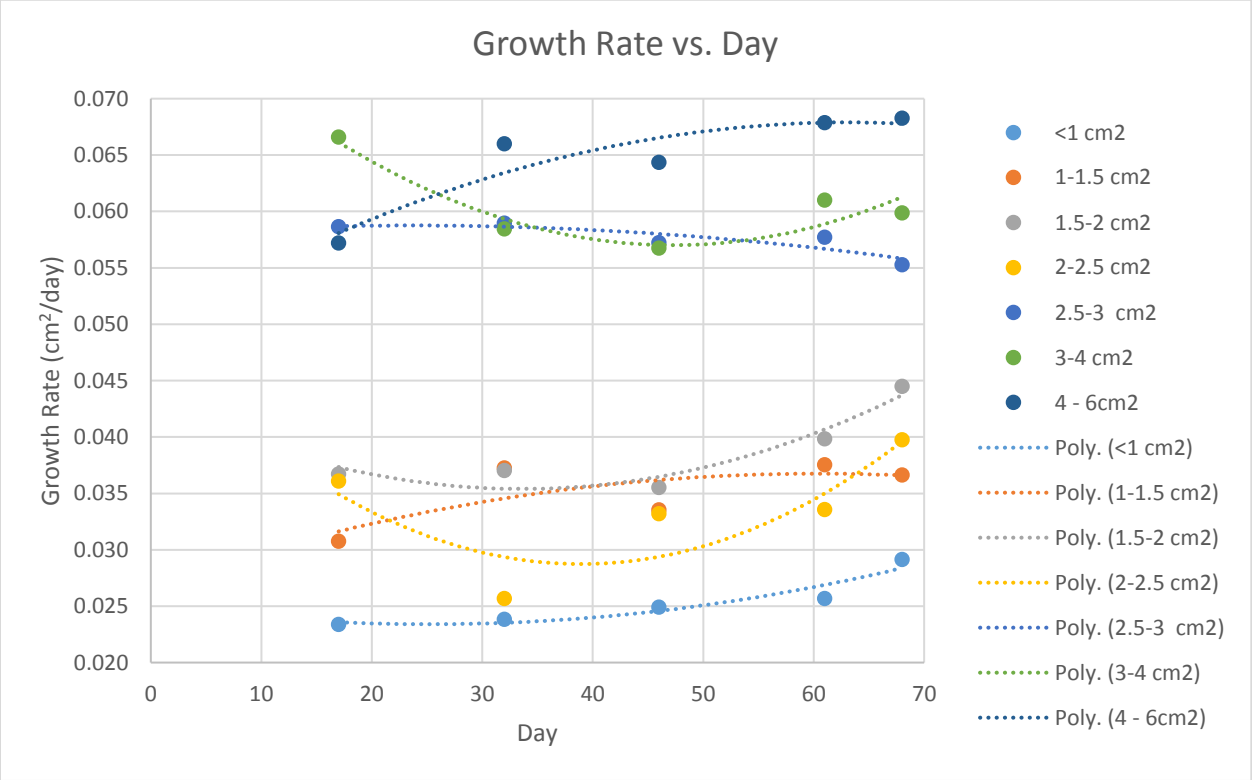

Figure 12. A scatterplot of the growth rate of each range of coral fragments with polynomial lines of best fit.

There was not a consistent change in the growth rate from day 17 to day 68. None of the growth rates remained constant, nor did any of them increase or decrease linearly. Three settings (1–1.5 cm^2, 2.5-3 cm^2, and 4-6 cm^2) had a negative polynomial line of best fit, with r-squared values of 0.56, 0.76, and 0.85, respectively.

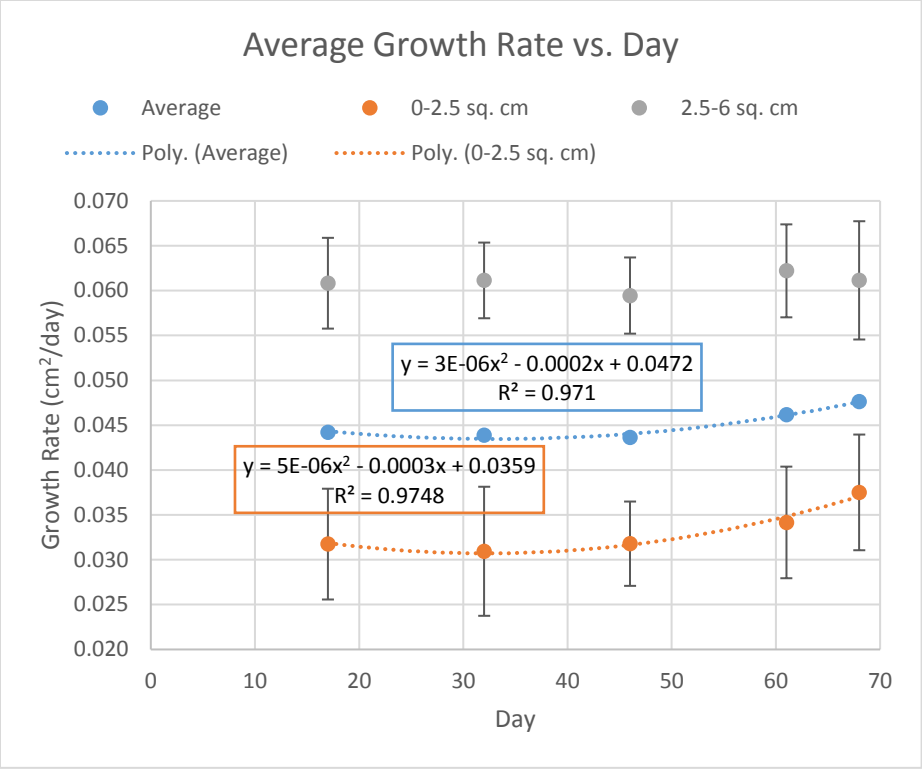

Figure 13. The average growth rate per day of all fragments.

The average growth rate of all the fragments remained mostly constant, approximately 0.044 cm^2/ day for the first 46 days, and only increasing to 0.048 cm^2/day after 68 days.

Data Analysis and Discussions

The data showed that the largest pieces (avg. 4.933 $cm^2 \pm 0.897$ cm^2) grew at a rate 234% faster than the smallest pieces (avg. 0.869 $cm^2 \pm 0.121$ cm^2), and there was a polynomial trend with an r-squared value of 0.92, showing a strong correlation. The percent growth rate decreased as the initial size increased, which is because although the smaller pieces overall had a slower growth rate it was a much larger percent of their original size. Any small increase in smaller pieces of fragments would have a much higher percent increase, just because some of these fragments started at much smaller sizes. This experiment was looking for a faster growth rate, because a faster growth rate meant that the coral would be asexually reproducing faster.

The percent increase is size decreased as the initial micro-fragmented size increased. The smaller pieces had a smaller increase in size, but relative to their original sizes it was larger compared to the larger initial sizes, which is why this trend is seen. The goal of this experiment was to find the ideal size to micro-fragment coral, and the size that had the largest increase in area would be preferable. The percentage is not important, because the overall increase in area has a higher priority than a higher percentage increase.

The maximum value for this polynomial line of best fit is 6.62 cm^2, which is outside of the range of data. The line of best fit for the scatterplot of each individual fragment had a maximum initial size value of 8.72 cm^2. Because the range of data tested did not extend this far it is unsure to conclude that these are the best sizes to micro-fragment pieces of *Montipora capricornis.*

The growth rate did increase overall, from 0.044 cm^2/ day to 0.048 cm^2/day. This was only a 9% increase, and was not seen across the individual fragment settings. It was observed

that the three highest settings (2.5-3 cm^2, 3-4 cm^2, and 4-6 cm^2) overall had a significantly higher growth rate, 184% faster (approximately 0.03 cm^2/ day more). There also did not seem to be a trend between the average of the larger three groups, but there was a strong polynomial relationship between the smaller four settings (<1 cm^2, 1-1.5 cm^2, 1.5-2 cm^2, and 2-2.5 cm^2) and the average growth rate, with r-squared values of 0.97 for both.

Conclusion

The increase in area, both percentage and overall area shows that micro-fragmenting is a legitimate method of propagating coral growth. The polynomial line chosen for the growth rate vs. initial size, with the averaged data, was chosen because it is likely that the curve would decrease once the coral was micro-fragmented to a certain large size. This is because there is a point where fragmented pieces of coral would not be small enough to have the necessity of increasing their size rapidly. The overall increase in size was also calculated to have a positive trend, with the size difference increasing as the initial size increased. This is most likely because the growth rate was higher in the larger pieces of coral. The fragments of coral grew to double, or more of their original size in only 68 days. The growth rate increased overall, but there was no increase in the larger pieces of coral. Contrastingly the smaller pieces increased their growth rate in a polynomial trend upward. The growth rate was higher in the larger fragments, and increased as the size of fragments also increased, which proved the hypothesis, that larger pieces of micro-fragmented *Montipora capricornis* would have a greater growth rate (cm^2/day) compared to smaller micro-fragmented pieces.

Assumptions and Limitations

In order to conduct this experiment assumptions had to be made. First, it was assumed that *M. capricornis* would only grow two-dimensionally, covering a larger area. Also, that the initial 3-D height would not affect the growth rate. The overall shape of the original piece of coral was also assumed to not affect the growth rate. The conditions of the tank were considered to be consistent throughout the floor of the tank, because the fragments were not rearranged during the experiment. It was also assumed that because the fragments came from the same parent coral, with the same DNA, they would all start with the same growth rate, no matter their location within the coral. If the fragmented pieces fell off of the ceramic reef disk it was assumed that it would not affect their growth rate, because they were reattached five days later and the rest of the pieces were not growing, only recovering from the trauma of micro-fragmentation. This project was limited by time constraints, as well as the amount of coral available from a single, large piece at Jay's Aquatic Store.

Future Applications

The work of this experiment suggests that the ideal size to micro-fragment coral is either around or above 5 cm^2. Future applications include using a different species of coral and performing a similar experiment to deduce how the initial micro-fragmented size affected its growth. This experiment could be run again, with a larger, higher range of initial fragment size from 5 cm^2 – 10 cm^2, instead of 0 cm^2 – 5 cm^2. Testing how well the pieces of coral fuse together, and seeing if coral from the same species, but different parent corals would fuse together is another extension of this work. Extending the length of time for a similar project, and seeing how the growth rate changes with time.

Literature Cited

Calfo, A (2002). Coral Fragmenting: Not Just for Beginners. *Reefkeeping.*

Forsman, Zac H., Page, Christopher A., Toonen, Rober J., & Vaughan. (20 Oct. 2015). Growing coral larger and faster: micro-colony-fusion as a strategy for accelerating coral cover. *PeerJ.* doi: 10.7717/peerj.1313.

Graham, N. A. J., & Nash, K. L. (26 Nov 2012). The importance of structural complexity in coral reef ecosystems. *Coral Reefs, 32, 315–326.* doi: 10.1007/s00338-012-0984-y.

McCalmon, Iain. (May 2014). The Coral Grief. *ScietificAmerican.com, May 2014, 66-69.*

Morin, Richard. (2014, November 23). A Lifesaving Transplant for Coral Reefs. *The New York Times.* n.p.

Osinga, R., Schutter, M., Griffioen, B., Wijffels, R. H., Verreth, J. A. J., Shafir, S., Henard, S., et al. (17 May 2011). *The Biology and Economics of Coral Growth.* 10.1007/s10126-011-9382-7.

Williams, Dana E., and Miller, Margaret W. (Nov. 2010). *Stabilization of Fragments to Enhance Asexual Recruitment in Acropora Palmata, a Threatened Caribbean Coral.* Restoration Ecology (pp. 446-451). DOI: 10.1111/ 1526-100X.2009.00579.

Appendix

Table 4. Raw Data after 68 days, organized by size.

	1 B	1.5 C	1 I	1 E	1 F	1 H				AVG	STDEV	% RSD
Day 1	0.7283	0.75893	0.79457	0.96255	0.98313	0.98553				0.86884	0.12067	13.8889
Day 17	0.99057	1.31059	1.5046	1.39683	1.15781	1.23863				1.2665	0.1813	14.3149
Day 32	1.51393	1.79707	1.88923	1.7041	1.49885	1.38448				1.63128	0.19573	11.9985
Day 46	1.90628	2.14597	2.50858	2.269	1.70239	1.55488				2.01452	0.3596	17.8502
Day 61	2.23575	2.49574	3.31944	2.92374	1.77192	1.86589				2.43541	0.60466	24.8277
Day 68	2.82818	2.72096	3.94159	3.33212	2.38839	1.88986				2.85018	0.71779	25.1839
growth rate 17	0.01543	0.03245	0.04177	0.02555	0.01028	0.01489				0.02339	0.0121	51.7278
growth rate 32	0.02455	0.03244	0.03421	0.02317	0.01612	0.01247				0.02383	0.00862	36.1745
growth rate 46	0.02561	0.03015	0.03726	0.0284	0.01564	0.01238				0.02491	0.00934	37.4843
growth rate 61	0.02471	0.02847	0.04139	0.03215	0.01293	0.01443				0.02568	0.01083	42.1722
growth rate 68	0.03088	0.02885	0.04628	0.03485	0.02067	0.0133				0.02914	0.01142	39.1971
% growth rate	5.71068	5.27243	7.29504	5.09082	3.57261	2.82				4.96026	1.58966	32.0479
area increase	2.09988	1.96203	3.14701	2.36957	1.40526	0.90433				1.98135	0.77663	39.1971
% area increase	388.326	358.525	496.063	346.175	242.937	191.76				337.298	108.097	32.0479
	2 I	1 D	1 C	1 A	2.5 G	1.5 E	2 E			AVG	STDEV	
Day 1	1.0722	1.19376	1.19804	1.20212	1.24483	1.26754	1.26938			1.20684	0.06762	5.6032
Day 17	1.29538	1.59159	1.73183	1.53995	1.68711	1.99559	2.26782			1.72989	0.31775	18.3683
Day 32	1.89202	2.17524	2.33673	3.12586	2.09195	2.23634	2.93405			2.39889	0.45576	18.9988
Day 46	2.19135	2.36951	2.58581	3.63101	2.58703	2.41984	3.45966			2.74917	0.56261	20.4647
Day 61	2.74162	3.22264	3.28243	4.57148	3.37207	3.08035	4.20841			3.497	0.65086	18.612
Day 68	3.14003	3.53699	3.00208	4.79078	3.6046	3.3143	4.49587			3.69781	0.68414	18.5011
growth rate 17	0.01313	0.0234	0.0314	0.01987	0.02602	0.04283	0.05873			0.03077	0.01546	50.2547
growth rate 32	0.02562	0.03067	0.03558	0.06012	0.02647	0.03028	0.05202			0.03725	0.01346	36.1357
growth rate 46	0.02433	0.02556	0.03017	0.0528	0.02918	0.02505	0.04761			0.03353	0.01169	34.8692
growth rate 61	0.02737	0.03326	0.03417	0.05524	0.03487	0.02972	0.04818			0.03754	0.01023	27.2458
growth rate 68	0.03041	0.03446	0.02653	0.05277	0.0347	0.0301	0.04745			0.03663	0.00974	26.5949
% growth rate	4.30673	4.35721	3.68503	5.86069	4.25831	3.84521	5.20852			4.5031	0.77055	17.1116
area increase	2.06783	2.34323	1.80404	3.58866	2.35976	2.04676	3.22649			2.49097	0.66247	26.5949
% area increase	292.858	296.29	250.582	398.527	289.565	261.475	354.179			306.211	52.3975	17.1116

	1.5 D	2.5 H	2.5 E	2.5 C	2 F	1.5 B	2.5 B	2 A	+O	AVG	STDEV	
Day 1	1.54447	1.59761	1.65674	1.67437	1.70582	1.76048	1.76415	1.92393	1.97012	1.73308	0.14066	8.11595
Day 17	2.00578	2.47862	2.11718	2.36408	2.31596	2.27795	2.39678	2.95187	2.30888	2.35745	0.26491	11.237
Day 32	2.58268	3.10421	2.41001	2.4659	2.49187	2.97546	3.33941	3.51211	3.37992	2.91795	0.43852	15.0283
Day 46	3.20009	3.31189	2.92375	2.88649	2.74471	3.76645	3.64886	4.05635	3.7614	3.36667	0.46304	13.7537
Day 61	4.16312	4.68446	3.08975	4.14871	2.78238	4.87128	3.784	5.57262	4.37989	4.16402	0.86671	20.8142
Day 68	4.55013	5.67575	3.49014	4.79228	3.06053	5.83676	4.37896	6.56855	4.47468	4.75864	1.12034	23.5433
growth rate 17	0.02714	0.05182	0.02708	0.04057	0.03589	0.03044	0.03721	0.06047	0.01993	0.03673	0.01282	34.894
growth rate 32	0.03244	0.04708	0.02354	0.02474	0.02456	0.03797	0.04923	0.04963	0.04406	0.03703	0.011	29.714
growth rate 46	0.03599	0.03727	0.02754	0.02635	0.02258	0.04361	0.04097	0.04636	0.03894	0.03551	0.00824	23.1902
growth rate 61	0.04293	0.0506	0.02349	0.04056	0.01765	0.051	0.03311	0.05981	0.0395	0.03985	0.01348	33.8348
growth rate 68	0.0442	0.05997	0.02696	0.04585	0.01992	0.05995	0.03845	0.0683	0.03683	0.04449	0.01602	36.0085
% growth rate	4.33248	5.22448	3.09798	4.20903	2.63848	4.87563	3.65029	5.02079	3.3401	4.04325	0.91314	22.5844
area increase	3.00567	4.07814	1.8334	3.11791	1.35471	4.07628	2.61481	4.64462	2.50456	3.02556	1.08946	36.0085
% area increase	294.608	355.265	210.663	286.214	179.417	331.543	248.22	341.414	227.127	274.941	62.0937	22.5844
	2.5 D	+ M	2.5 F							AVG	STDEV	
Day 1	2.19297	2.2427	2.30784							2.24784	0.05761	2.56292
Day 17	2.5649	2.40911	3.60987							2.86129	0.65295	22.8201
Day 32	3.49509	2.25573	3.45689							3.06923	0.70478	22.9626
Day 46	4.52334	2.79031	4.01152							3.77506	0.89039	23.586
Day 61	5.11678	3.634	4.1334							4.29473	0.75444	17.5667
Day 68	6.72775	3.64897	4.47877							4.95183	1.59297	32.1694
growth rate 17	0.02188	0.00979	0.07659							0.03609	0.03559	98.6398
growth rate 32	0.04069	0.00041	0.03591							0.02567	0.02201	85.7373
growth rate 46	0.05066	0.0119	0.03704							0.0332	0.01966	59.2181
growth rate 61	0.04793	0.02281	0.02993							0.03356	0.01295	38.5889
growth rate 68	0.06669	0.02068	0.03193							0.03976	0.02398	60.3167
% growth rate	4.51158	2.3927	2.85393							3.25274	1.11431	34.2577
area increase	4.53478	1.40626	2.17093							2.70399	1.63096	60.3167
% area increase	306.787	162.704	194.067							221.186	75.7733	34.2577
	+N	2.5 A	+ D	+L	+ G	+ F				AVG	STDEV	
Day 1	2.60812	2.63401	2.65442	2.84206	2.86906	2.98595				2.7656	0.15465	5.59181
Day 17	3.28106	3.15246	2.92807	4.21053	4.24449	4.76066				3.76288	0.73886	19.6355
Day 32	3.53949	3.88062	4.32365	5.31629	5.09632	5.75632				4.65211	0.87164	18.7363
Day 46	4.19906	4.42536	4.93098	5.92172	6.47149	6.44498				5.39893	1.01237	18.7512
Day 61	4.58224	5.33414	5.40192	7.83732	6.80416	7.76194				6.28695	1.37433	21.86
Day 68	5.03389	5.29133	5.77328	7.91363	6.58118	8.55191				6.52421	1.43877	22.0528
growth rate 17	0.03958	0.0305	0.0161	0.0805	0.08091	0.10439				0.05866	0.03473	59.2089
growth rate 32	0.02911	0.03896	0.05216	0.07732	0.0696	0.08657				0.05895	0.02259	38.3106
growth rate 46	0.03459	0.03894	0.04949	0.06695	0.07831	0.0752				0.05725	0.01881	32.8561
growth rate 61	0.03236	0.04426	0.04504	0.08189	0.06451	0.0783				0.05773	0.02019	34.9768
growth rate 68	0.03567	0.03908	0.04587	0.07458	0.05459	0.08185				0.05527	0.01905	34.4667
% growth rate	2.83836	2.95419	3.19848	4.09481	3.3733	4.21185				3.44517	0.58054	16.8508
area increase	2.42577	2.65732	3.11886	5.07158	3.71212	5.56597				3.7586	1.29547	34.4667
% area increase	193.008	200.885	217.497	278.447	229.384	286.406				234.271	39.4767	16.8508

	2 C	+ H	+ I	+ E	+ B					AVG	STDEV	
Day 1	3.02762	3.07711	3.12564	3.13987	3.17402					3.10885	0.05722	1.8406
Day 17	4.21096	3.89166	5.34754	3.39303	4.36246					4.24113	0.72132	17.0076
Day 32	5.55357	4.68737	5.85415	4.17302	4.62936					4.97949	0.69873	14.0322
Day 46	5.4479	5.24997	6.84817	4.8748	6.17746					5.71966	0.7892	13.7981
Day 61	6.46735	6.03004	7.76963	6.31741	7.56602					6.83009	0.78403	11.4791
Day 68	6.79762	5.91543	8.63241	7.10939	7.44838					7.18065	0.99144	13.8072
growth rate 17	0.06961	0.04791	0.1307	0.01489	0.06991					0.0666	0.04228	63.485
growth rate 32	0.07894	0.05032	0.08527	0.03229	0.04548					0.05846	0.02268	38.7984
growth rate 46	0.05261	0.04724	0.08092	0.03772	0.06529					0.05676	0.01679	29.5782
growth rate 61	0.05639	0.04841	0.07613	0.05209	0.072					0.061	0.01234	20.2285
growth rate 68	0.05544	0.04174	0.08098	0.05838	0.06286					0.05988	0.01419	23.6941
% growth rate	3.30177	2.82706	4.06149	3.32975	3.45098					3.39421	0.44239	13.0337
area increase	3.77	2.83833	5.50678	3.96952	4.27436					4.0718	0.96478	23.6941
% area increase	224.52	192.24	276.181	226.423	234.667					230.806	30.0826	13.0337
	+A	+ K	+ J							AVG	STDEV	
Day 1	4.29874	4.42727	5.56705							4.76435	0.69812	14.6531
Day 17	4.97466	6.18129	6.05441							5.73679	0.66306	11.558
Day 32	6.57656	7.05625	6.99617							6.87633	0.26134	3.80054
Day 46	7.66778	7.41881	8.08586							7.72415	0.33708	4.36397
Day 61	9.13798	8.45012	9.12647							8.90486	0.39386	4.42294
Day 68	9.10261	8.23499	10.8834							9.407	1.3502	14.3531
growth rate 17	0.03976	0.10318	0.02867							0.0572	0.0402	70.2779
growth rate 32	0.07118	0.08216	0.04466							0.066	0.01928	29.2091
growth rate 46	0.07324	0.06503	0.05476							0.06434	0.00926	14.393
growth rate 61	0.07933	0.06595	0.05835							0.06788	0.01062	15.6496
growth rate 68	0.07065	0.056	0.07818							0.06827	0.01128	16.5236
% growth rate	3.11398	2.73538	2.87495							2.90811	0.19146	6.5838
area increase	4.80387	3.80772	5.31636							4.64265	0.76713	16.5236
% area increase	211.751	186.006	195.497							197.751	13.0196	6.5838

Table 5. Summarized Data with STDEV

		<1 cm^2	STDEV	1-1.5 cm^2	STDEV	1.5-2 cm^2	STDEV	2-2.5 cm^2	STDEV	2.5-3 cm^2	STDEV	3-4 cm^2	STDEV	4 - 6cm2	STDEV
Day 1	cm2	0.869	0.121	1.207	0.068	1.740	0.141	2.243	0.058	2.750	0.155	3.109	0.057	4.933	0.698
Day 68	cm2	2.850	0.718	3.698	0.684	4.759	1.120	4.952	1.593	6.524	1.439	7.181	0.991	9.407	1.350
growth rate	cm2/day	0.029	0.011	0.037	0.010	0.044	0.016	0.040	0.024	0.055	0.019	0.060	0.014	0.068	0.011
% growth rate	% area increase/ days	4.960	1.590	4.503	0.771	4.043	0.913	3.253	1.114	3.445	0.581	3.394	0.442	2.908	0.191
area increase	final cm2 - initial cm2	1.981	0.777	2.491	0.662	3.026	1.089	2.704	1.631	3.759	1.295	4.072	0.965	4.643	0.767
% area increase	final cm2/ initial cm2	337.3	108.1	306.2	52.40	274.9	62.09	221.2	75.77	234.3	39.48	230.8	30.08	197.8	13.02

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