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Optimization of grow-out of bouldering coral microfragments: land vs. offshore nursery

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Thesis of
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Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science
Marine Science

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Halmos College of Arts and Sciences

April 2021

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Abstract

Corals along the Florida Reef Tract and the wider Caribbean have been declining for decades. Low densities of adult colonies hinder the ability of corals to replenish themselves through sexual reproduction, thus reef managers are focusing on restoration actions that increase coral biomass. Microfragmentation is a way to quickly increase the biomass of bouldering corals by cutting the coral into small pieces which forces the coral to allocate its resources away from reproduction and back into growth, increasing its growth rate. This study assessed the optimal location for grow-out (land vs. offshore nursery) and its synergy with size of fragment in 4 bouldering coral species (*Orbicella faveolata, Siderastrea siderea, Pseudodiploria clivosa* and *Diploria labyrinthiformis*). Survival and growth rates were tracked through monthly pictures of the microfragments to determine differences between the locations, fragment sizes, species, and individuals. Coral microfragments of all species and sizes grown in the land-based nursery had increased survival and growth rates compared to the counterparts grown in the offshore nursery. These results help inform managers to make the best choices for growing coral microfragments. The longevity of coral reefs is dependent on techniques such as this succeeding in increasing coral cover quickly.

Keywords: Coral, microfragmentation, microfragments, restoration, fragmentation, Allee effects

Introduction

Of all marine ecosystems, coral reefs are home to the greatest number of species per unit area (Knowlton et al., 2010). Residents of coral reefs, both transient and permanent, perform many ecologically distinct roles that are invaluable to both marine and terrestrial life including oxygen and food production, carbon and nitrogen fixation, coastal protection, and habitat construction (Magurran & Henderson, 2003; Bellwood et al., 2004; Knowlton et al., 2010; de Groot et al., 2012). Scleractinian corals build reef habitats through the slow accretion of a calcium carbonate skeleton on which the reef food web is supported (Hatcher, 1988; Moberg & Folke, 1999). Economically, coral reefs provide a reliable revenue stream through tourism and recreation, which, coupled with the provisions of ecosystem services such as primary productivity and nutrient cycling, account for approximately half a million USD per km^2 of coral reef globally (Spurgeon, 1992; de Groot et al., 2012; Spalding et al., 2017). Thus, corals are crucial not only for the health of the ocean but as a global economic resource.

Despite their importance, corals are at risk of being lost due to climate change, disease, pollution, and overharvesting of herbivorous fishes (Moberg & Folke, 1999; Hughes et al., 2003; Bellwood et al., 2004; Hoegh-Guldberg et al., 2007; Smith et al., 2010; Precht et al., 2016). The warm temperatures and decreased pH caused by increased greenhouse gas emissions can drive stress responses in corals such as bleaching or even death (Hughes et al., 2003; Bellwood et al., 2004; Hoegh-Guldberg et al., 2007, 2017; Lamb et al., 2014). These environmental conditions can also accelerate the spread of disease, such as Stony Coral Tissue Loss Disease (SCTLD) which has been spreading through the Florida reef tract since 2014 (Precht et al., 2016; Florida Keys National Marine Sanctuary, 2018; Walton et al., 2018). Stony Coral Tissue Loss Disease is characterized by rapid tissue loss that spreads until the whole colony is deceased and many valuable, Caribbean, reef-building corals are susceptible to it (Florida Keys National Marine Sanctuary, 2018; Walton et al., 2018). Other major coral stressors include pollution and sedimentation from terrestrial run-off, which can decrease water quality, increase turbidity, and boost macroalgal growth (Dubinsky & Stambler, 1996; Lapointe et al., 2011; Fourney & Figueiredo, 2017). Overharvesting of herbivores from reefs also contributes to increased macroalgae growth which has been proven to decrease coral growth and fecundity, and lower coral recruitment success (Lirman, 2001; Mumby et al., 2006; Foster et al., 2008; Hoey et al.,

2011). Significant coral loss disrupts local ecosystems, potentially enough to trigger regime shifts to alternate stables state such as macroalgae, sponge, or urchin-dominated ecosystems (Knowlton, 1992; Bellwood et al., 2004; Elmhirst et al., 2009; Graham et al., 2015; Precht et al., 2016; Jones et al., 2020).

Corals have two reproductive modes to increase population size: asexual and sexual reproduction (Shinn, 1975; Highsmith, 1982). Asexual reproduction is the growth of a new colony after fragmentation and reestablishment; however, in nature this is only common in branching species (Lirman, 2000). This method increases coral cover quickly but only maintains the genetic information currently in the population (Shinn, 1975; Highsmith, 1982). Conversely, sexual reproduction involves the meeting of sperm from one colony with an egg from another, creating hundreds to thousands of genetically distinct larvae per reproductive season (Fadlallah, 1983; Harrison & Wallace, 1990). After fertilization, larvae are dispersed through currents into the water column to potentially settle on and recolonize degraded reefs and contribute to reef connectivity (Fadlallah, 1983). Increased genetic diversity provides the raw material for adaptation, and thus is essential for population persistence through novel environmental stressors and changes (Baums et al., 2006; Drury and Lirman, 2017). This gives sexual reproduction the potential to increase coral biomass and community resilience simultaneously (Harrison & Wallace, 1990; Drury and Lirman, 2017).

Unfortunately, coral populations continue to decline, and this impacts the success of recovery through sexual reproduction (Gascoigne & Lipcius, 2004). This phenomenon is termed Allee effect, and it is defined as the decrease in some aspect of fitness as population density decreases (Gascoigne & Lipcius, 2004). Low densities of reproductive corals can lead to lower fertilization rates due to eggs and sperm perishing before meeting, decreasing reproduction rates (Teo & Todd, 2018). With fewer adult corals producing fewer successful larvae to recruit on a reef, human intervention through coral restoration may be necessary to aid struggling species (Gascoigne & Lipcius, 2004; Teo & Todd, 2018). Asexual restoration techniques can provide a means to quickly increase adult colony density, potentially enough to mitigate Allee effects and promote successful sexual reproduction in the future (Shinn, 1975; Gascoigne & Lipcius, 2004; Young et al., 2012).

Asexual restoration techniques have been widely utilized to quickly increase coral cover and density of colonies on the reef. Currently, corals in the *Acropora* genus have been the main focus of restoration through fragmentation; this is due to their quick growth rates and branching structure which makes them easy to fragment repeatedly (Herlan & Lirman, 2008; Young et al., 2012). Fragmentation was not initially used on bouldering corals, likely because the size and shapes of these corals makes fragmentation more difficult, and their growth rates are slower than *Acropora*. However, a new propagation technique, termed microfragmentation, has recently been developed to grow bouldering corals at a faster rate for reef restoration (Forsman et al., 2006; Forsman et al., 2015; Page et al., 2018). This process involves cutting large coral colonies into many small pieces that are then outplanted onto natural reefs (Forsman et al., 2006; Forsman et al., 2015; Page et al., 2018). Cutting the corals into pieces ranging in size from <1 cm^2 to >20 accelerates their growth rate compared to the intact, adult colony, which increases coral cover on the reef in a shorter amount of time (Forsman et al., 2006; Forsman et al., 2015; Page et al., 2018). This was hypothesized to work by temporarily forcing the coral to allocate their resources away from reproduction, which becomes the top priority for corals once they reach maturity, and back into growth (Lirman, 2000; Zakai et al., 2000). A recent study theorized that a larger perimeter to area ratio could facilitate the deposition of more new polyps relative to the initial number of polyps and thus, a single polyp fragment could increase biomass faster than larger fragments (Dornelas et al., 2017). That theory is possible because corals grow by budding new polyps along their perimeter, meaning the coral's perimeter to area ratio could affect growth rate (Buddemeier &, Kinzie 1976; Dornelas et al., 2017). Contrary to this theory, Forsman et al., (2015) found that in a land-based system, fragments cut to 3 cm^2 grew faster than 1 cm^2 fragments. Further investigation is needed to identify optimal size of microfragment to maximize grow-out.

In addition to size, an optimal location for grow-out has not been determined. Previous studies have grown microfragments either exclusively in the field or in land-based nurseries, but it remains to be determined which of these two environments provides the best growth and survival for the microfragments (Forsman et al., 2006; Forsman et al., 2015; Page et al., 2018). Coral microfragments grown in offshore nurseries are exposed to local biotic and abiotic factors including food/prey availability, diseases, storms, predators, and even human-induced local stressors such as increased turbidity. In land-based nursery systems, water quality can be

maintained at optimal levels, and light levels can be maintained at levels to accelerate growth or made to mimic natural annual and diel cycles while eliminating extreme events. The quantity and quality of food available to corals in each environment is also different. A coral's natural diet composition is difficult to accurately replicate in land-based nurseries, so some artificial diets have been shown inhibiting growth compared to natural diets (Conlan et al., 2018). On a reef, food quantity is likely to vary over the course of the day and between wet and dry seasons, which is different than in land-based nurseries, where food can be given in equal quantities daily but likely not be available throughout the day. This would mean a coral in the land-based nursery would only have one window of time a day to feed heterotrophically, whereas corals in the ocean have the chance to feed at any time, although their success is likely more variable. It's unclear whether these diet differences are significant enough to affect growth rates in the context of this study, however, given that increased heterotrophy has been linked to increased growth rates, it is a possible factor (Jacques & Pilson, 1980; Ferrier-Pagès et al., 2003). In contrast to land-based nurseries, corals in offshore nurseries are exposed predators and competition for space which can limit growth, and even cause mortality (Tanner, 1995; Miller & Hay, 1998; Forsman et al., 2006). The size of the coral fragment may also influence how food availability, predation, or competition impact coral growth (Forsman et al., 2006). Despite being postulated to grow faster, a coral fragment of smaller size has a higher chance of mortality in an offshore nursery due to stress, predation, and/or competition, than a larger fragment (Tanner, 1995; Miller & Hay, 1998; Forsman et al., 2006). Understanding the synergistic effects of fragment size and biotic and abiotic factors on the survival and growth is vital to more effectively using microfragmentation to grow corals for restoration.

From a management perspective, grow-out in an offshore nursery requires less labor, time, and money, but less control of water quality and higher exposure to competitors and predators, relative to a land-based nursery. It is imperative to determine if potential growth differences between the two locations are worth the cost and labor differences in order to quickly and efficiently increase coral densities. Coral reef managers have limited resources and funds available, thus knowing how best to utilize them is key to a successful management plan. In this study, four species of Caribbean bouldering corals, *Orbicella faveolata, Siderastrea siderea, Pseudodiploria clivosa* and *Diploria labyrinthiformis,* were used to test the effects of grow-out location on the survival and growth of two different sized microfragments over six months. The

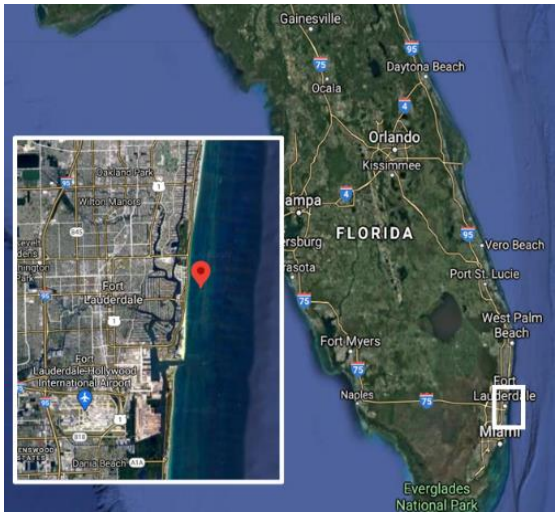
size classes, approximately 1 cm^2 and 3 cm^2, were chosen to maximize the number of fragments per individual, while ideally being large enough to withstand stressors. The overall goal of this study was to determine which location (offshore nursery vs. land-based nursery) leads to better short-term survival and growth of coral microfragments. This study also aims to determine if the ideal initial fragment size is location-specific, and if species grow at different rates between the two locations. Determining the optimal fragment size and grow-out location of reef-building, bouldering corals is essential to maximize coral biomass production and accelerate future large-scale reef restoration efforts.

Methods

Study species and collection

Four Caribbean, reef-building species, *Orbicella faveolata, Siderastrea siderea, Pseudodiploria clivosa* and *Diploria labyrinthiformis* were used to test the effects of grow-out location and microfragment size. *Pseudodiploria clivosa* and *Diploria labyrinthiformis* are considered "highly susceptible" to SCTLD, while *Orbicella faveolata and Siderastrea siderea* are considered "intermediately susceptible" (Florida Keys National Marine Sanctuary, 2018). All of these species have experienced regional declines, indicating urgent need for restoration (Walton et al., 2018).

Colonies of *O. faveolata* and *S. siderea* were collected in 2019 from nearshore reefs off Broward County, FL, USA and maintained in recirculating aquaria prior to the experiment, while *P. clivosa* and *D. labyrinthiformis* were collected one month before the experiment, on February 11th, 2020, from the offshore nursery where they naturally settled years prior (Map 1). The reefs the corals were collected from were located offshore of Broward County, FL at depths of approximately 7-15 m. Collection took place via SCUBA using hammers and chisels, after which the corals were transported to the land-based nursery while wrapped in wet bubble wrap within a cooler. A total of three donor colonies were used for each species to encompass a range of differences among species.



Map 1: Red pin is the location of the offshore nursery, Google ©2021.

Fragmentation

Three colonies for each of the four species were microfragmented (n=3 donor colonies per species), and each individual was distributed equally among all treatments to control for the potential effect of interspecific differences. Six large size class (approx. 3 cm^2) and six small size class (approx. 1 cm^2) microfragments were cut from each donor (Figure 1). Half of the microfragments from each individual colony were raised in the land-based system and half in the offshore nursery. In summary, there were 12 microfragments (6 small and 6 large) per colony, 3 donor colonies per species and 4 species, totaling 144 microfragments of which 72 were reared in the land-based nursery and 72 in the offshore nursery. Thus, in each of the nurseries we had 18 microfragments per species (3 small size class and 3 large size class fragments per genotype, with 3 individual colonies per species). Corals were microfragmented using a Gryphon Aquasaw XL with a diamond tipped blade and QEP 650XT. Microfragments had approximately a week to heal after which they were epoxied to Daltile© unglazed 11 x 11 cm ceramic tiles. All-Fix© 2-Part Marine Epoxy was used to create a slope for the coral to grow over (Figure 2), allowing overhead pictures to capture surface area growth. To be hung in the nursery, tiles for this treatment had three holes drilled before attaching the fragment. This held a strong monofilament looped through the tile that is clamped together with a crimp. The tile was hung on the PVC

"tree" (Nedimyer et al., 2011) so that the coral was parallel to the seafloor and facing up (Figure 2).

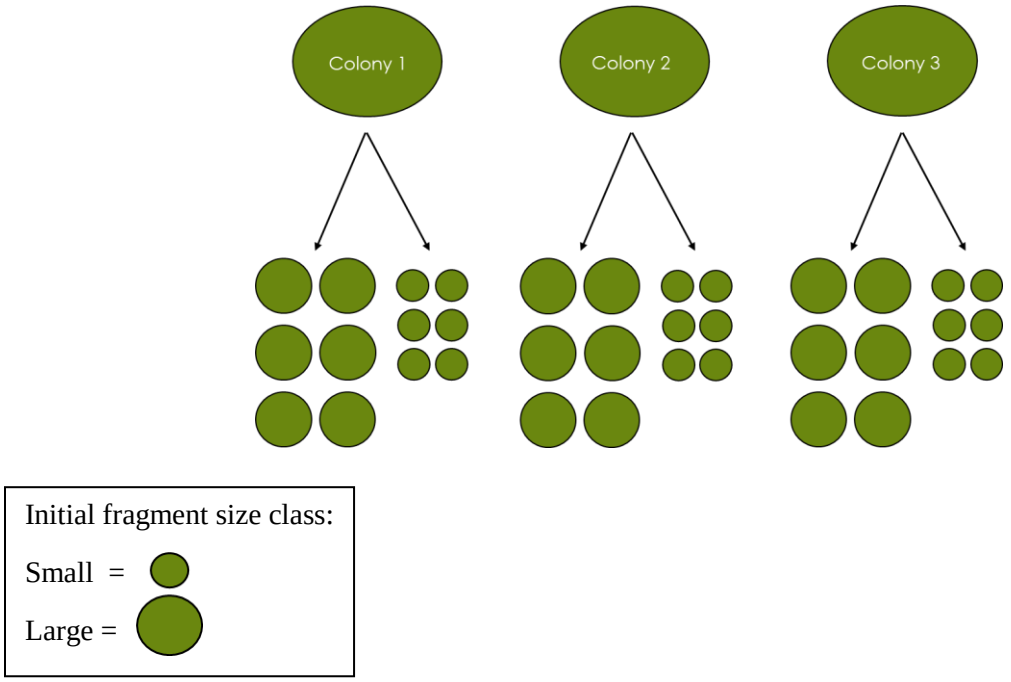


Figure 1: Fragmentation diagram depicting how the original donor colonies are fragmented into the six fragments of each size class. This is repeated for all four species.

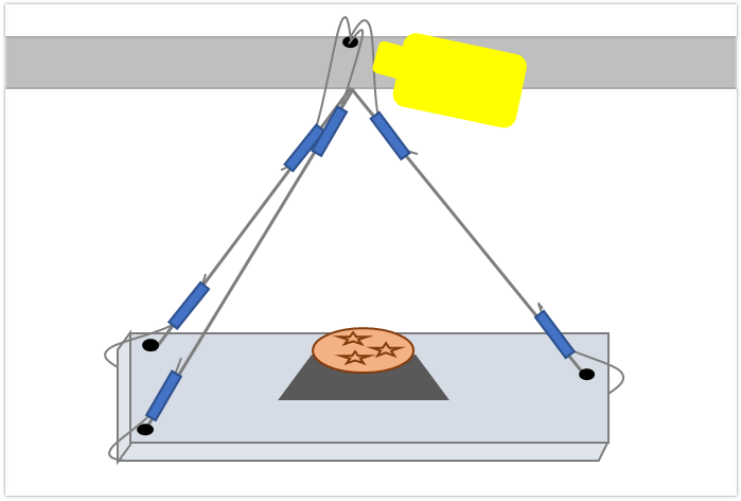


Figure 2: Diagram of how corals were hung from PVC tree in the offshore nursery, with the fragment attached to the tile via epoxy and hung from the tree using monofilament and metal crimps. Cattle tag above for identification.

Experimental treatments

To determine the effect of grow-out location on microfragment survival and growth rate, corals were raised in two locations, a land-based nursery recirculating tank and an offshore nursery.

The offshore nursery was located approximately 1.6 km offshore of Broward County, Florida, USA (26.12453, -80.09703), was 7-9 m deep. This location is less than 3 miles north of a major shipping port, Port Everglades, that experiences a high level of shipping and recreational activity year-round. Temperature loggers present in the nursery provided temperature data for the experiment's duration. The nursery consisted of many PVC coral trees hung approximately 1 m above the seafloor (following the design in Nedimyer et al., 2011), one of which was used to hang coral microfragments for this experiment. Corals in the offshore nursery were grown without human interference, i.e., algae and other benthic competitors were not removed. The corals were hung on the tree (Figure 2) using an arrangement from a random sequence generator that ensured a microfragment of each size, species, and colony was represented on each branch (Figure 3). Cattle tags were secured on the branch over each coral with a zip tie to easily identify and keep track of individual microfragments (Figure 2).

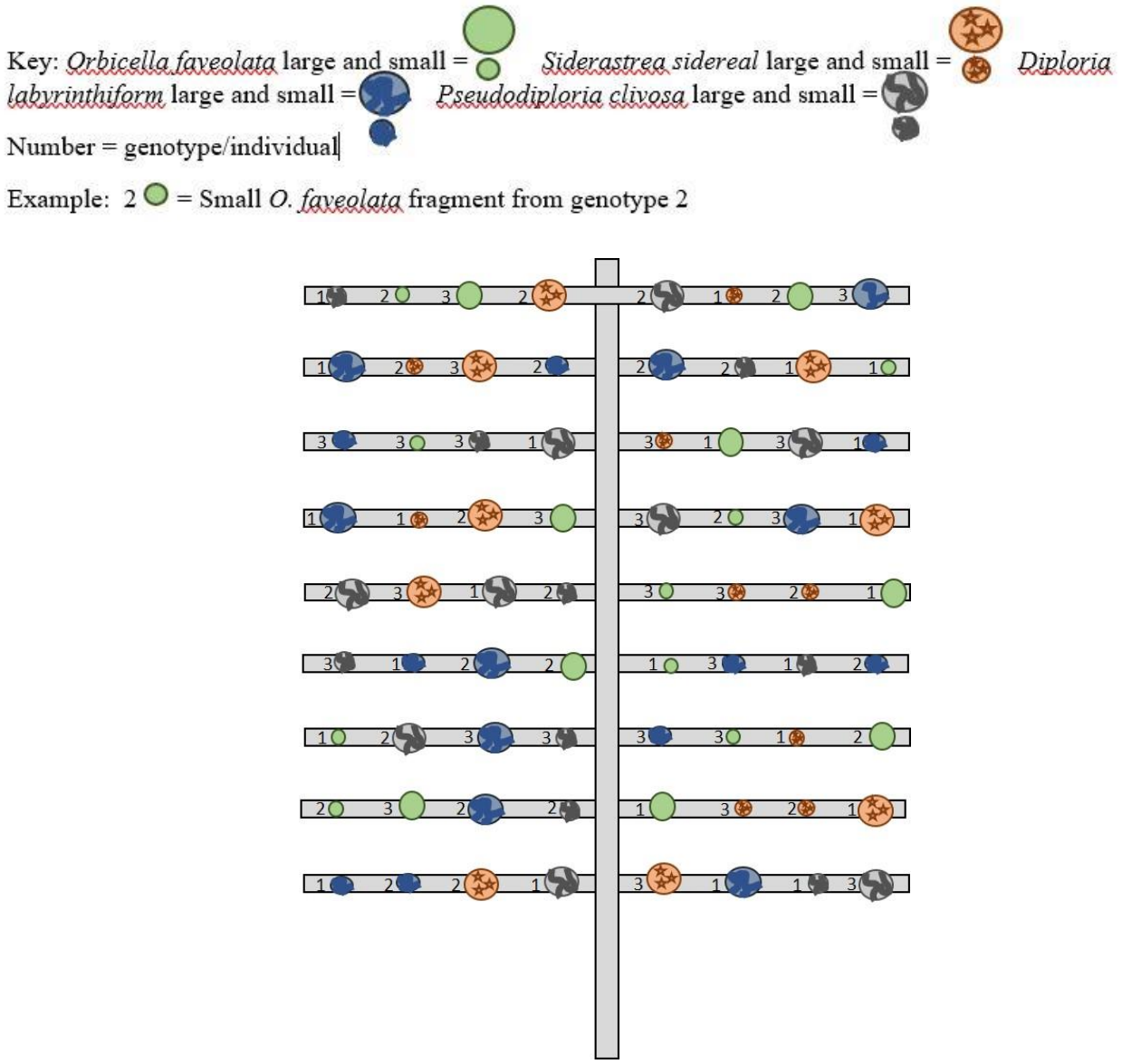


Figure 3: Arrangement of fragments at the coral tree in the offshore nursery. The number corresponds to the donor colony and the circle figures correspond to the species and size class of the fragment, according to the key above.

The land-based nursery was located at Nova Southeastern University's Oceanographic Campus. The outdoor, recirculating system used a 1100 L fiberglass tank a 400 L sump equipped with a Red Sea Reefer RSK-900 protein skimmer, PhosBan Phosphate Reactor 550, and GEO 6x18 calcium reactor. Additionally, bioballs and *Chaetomorpha* algae were used for biological filtration and 100-200 *Lithopoma americanum* snails controlled algae growth. The water

temperature was controlled by Aqua Logic, Inc® NEMA 4X digital temperature controllers connected to Eheim® Jager submersible heaters and an Aqua Logic® water chiller which maintained natural annual temperature variation (23°C in February to 29°C in September) based on data gathered by SECREMP from the southeast Florida Reef Tract from 2007-2016, excluding bleaching years (Gilliam et al., 2017). Aquaforest Reef Salt and reverse osmosis water were used to create the artificial seawater in the tank. Salinity was maintained at 35 ± 1 PSU. Temperature and salinity were monitored each morning with a YSI Pro30 multimeter. A shade cloth covered the tank to simulate light irradiance levels commonly experienced at ocean depths of around 7-15 m, ranging from 150-250 µmol photons $m^{-2}s^{-1}$ (Lesser, 2000). The corals were arranged according to a random number generator on three raised platforms in the tank, ensuring a microfragment of each size, species, and colony is represented on each stand (Figure 4).

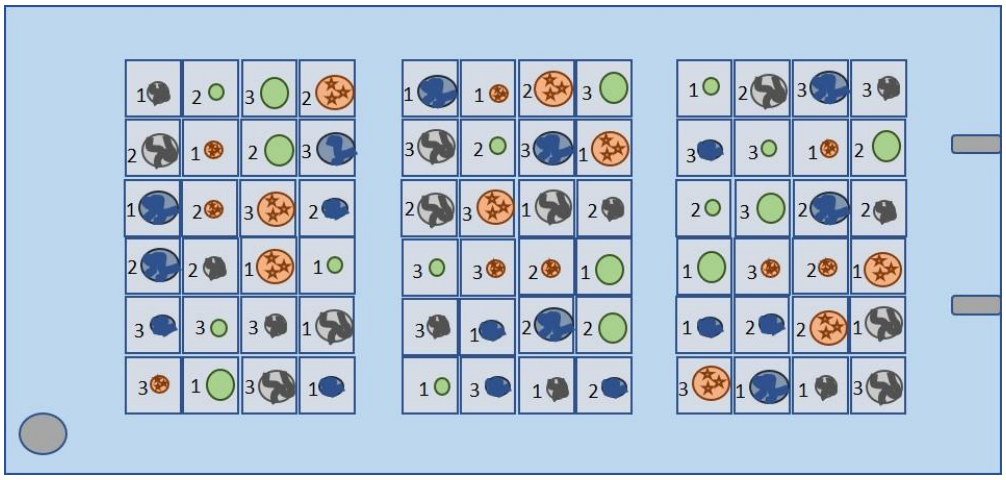


Figure 4: Diagram of land-based recirculating aquarium with input pipes to the right and standpipe to the left. Each square is a tile with the number inside representing the donor colony. The size of the circle represents initial size class, the color corresponds to the species. Green = O. faveolata, red= S. siderea, black = P. clivosa, and blue = *D. labyrinthiformis.*

A novel diet was created for the land-based nursery based on current practices in American Zoological Association certified coral facilities to maximize coral growth while maintaining optimal water quality. The diet consisted of approximately 4,000-7,000 live rotifers (reared with *Nannochloropsis* algae paste and enriched for 1hr before feeding with Rotigrow Plus© algae), 1 tsp. Polyp Lab Reef-Roids©, and 1 tsp. Reef Nutrition Oyster feast© mixed with 500 mL of tank water. Corals in the land-based nursery were fed six times a week by dispersing

the food mixture over each coral microfragment with a turkey baster. Weekly water quality tests were performed to monitor levels of nitrates (0.1 ± 0.1 mg L^{-1}), nitrites (0.005 ± 0.002 mg L^{-1}), ammonia (0 ± 0.01 mg L^{-1}) and phosphate (0.04 ± 0.02 mg L^{-1}) in the tanks using Hach DR900 Colorimeter. Alkalinity was also measured weekly with a Hanna Checker© to maintain values between 130-170 mg L^{-1}. Partial water changes were performed as needed when siphoning debris and waste, usually two-three times per month, using the same brand of artificial seawater to replace what was removed. Once per week, the protein skimmer was cleaned.

To track survival and measure growth, all corals at the land-based and offshore nurseries were photographed at the start of the experiment and once per month for six months with an Olympus Tough TG-5 camera in an Ikelite waterproof case. The camera was mounted to a Nikonos framer with calipers for scaling, ensuring the pictures were at the same scale every time. If no living tissue was observed, the month of mortality was recorded, due to the fact that offshore monitoring only occurred monthly. The monthly pictures were analyzed in ImageJ to estimate live surface area of corals (cm^2).

Data analysis

All statistical analyses were carried out in R (R Core Team, 2020). To assess the effect of location, species, and size class on mortality, a Cox model survival analysis was used, with death as the event of interest (Therneau & Grambsch, 2000). The "survival" package was used to perform the analysis (Therneau, 2021).

To assess the effect of location, species, and initial size class on surface area of the microfragments over time, a Generalized Linear Mixed Model (GLMM) with a log transformed Gaussian distribution was used with month, location, initial size class, and species as categorical predictors. Initial donor colony and individual microfragment were included as random factors. Coral microfragments that had perished were not included in this analysis. The model was created using the package "glmmTMB", and the package "performance" was used to check for multicollinearity (Brooks et al., 2017, Lüdecke *et al.*, 2020). Terms were selected for the model through backwards stepwise selection using AIC scores to remove irrelevant interactions. The "DHARMa" package was then used for model validation (Hartig, 2021). Following, the "emmeans" package was used for post-hoc analysis (Lenth, 2021).

To assess the effect of location, species, and initial size class on the magnitude change, calculated as the ending surface area divided by the initial surface area of the microfragment, a Kruskal-Wallis test was conducted. Location, species, and initial size class were categorical factors and magnitude was the continuous response. For post-hoc analysis, the packages "pgirmess" and "multcompView" were utilized (Giraudoux, 2018; Graves et al., 2019).

To graphically represent the offshore temperature data, the packages "dplyr" and "timelineR" were utilized (Vindhani et al., 2019; Wickham et al., 2020).

Results

Microfragment Survival

Location of microfragment grow-out significantly affected survival (Cox: $z = 2.861$; $p = 0.00422$). Corals grown offshore had 75% survivorship, while corals grown in the land-based nursery had 98.6% survivorship (Figure 5). Both initial size class and species did not have a significant effect on survival (Cox: $z = 1.616$; $p = 0.10613$, $z = 1.080$; $p = 0.28033$, respectively). There was only one mortality in the land-based nursery throughout the experiment. This death occurred less than two weeks into the study, suggesting that this coral never recovered from the stress of being cut and epoxied to the tile. Eighteen corals from the offshore treatment perished before the experiment concluded and some microfragments grown in the offshore nursery had tissue loss and/or damage throughout the experiment, but mortality was not counted until there was no live tissue found. For some microfragments, death was due to the epoxy detaching from the tile, or the fragment detaching from the epoxy. Grazing fish and macroalgae growth were observed in the nursery each visit, but due to the limited time spent there each month, it is impossible to definitively tell what caused the various lesions and tissue losses experienced offshore.

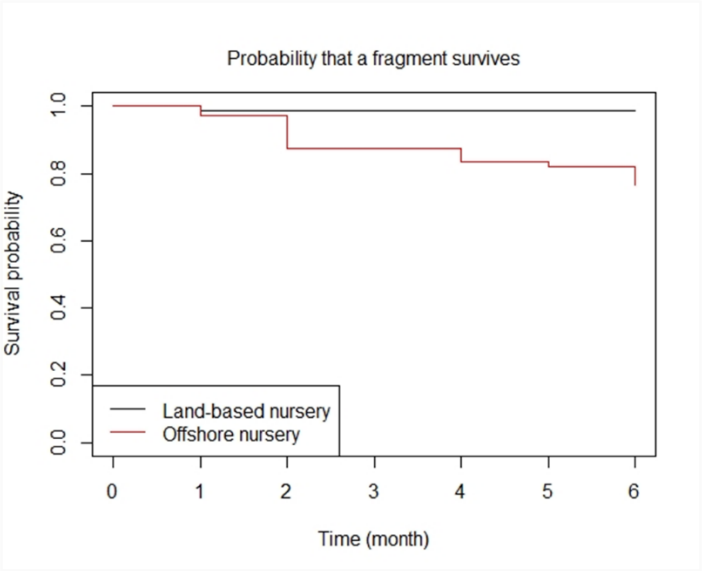


Figure 5. Probability that a fragment will survive in both locations after 6 months.

Microfragment Growth

Overall, average growth rates were higher in the land-based nursery than in the offshore nursery, regardless of initial size class or species (Table 1). Species and location together significantly affect the surface area of the microfragments (Glmm: χ^2= 24.923; df = 3; p = 1.602 x 10^{-5}). Initial size class of the microfragments and species had a significant effect on microfragment surface area (Glmm: χ^2= 14.273; df = 3; p = 0.002556). This model also indicated that the surface area of live tissue was affected by species and time together significantly (Glmm: χ^2= 91.890; df = 18; p = 6.599 x 10^{-12}). The two brain coral species, *D. labyrinthiformis* and *P. clivosa,* were not significantly different from each other (Glmm post hoc: df = 880, p = 0.9970). *S. siderea* and *O. faveolata* were also not significantly different from each other (Glmm post hoc: df = 880, p = 0.4934), but the brain corals were significantly different from the other pair (Glmm post hoc: df = 880, p < 0.0001). The species differences are more pronounced in the land-based nursery (Figure 6). Additionally, location and time significantly interacted to affect microfragment surface area (Glmm: χ^2= 158.521; df = 6; p = 2.2 x 10^{-16}).

Table 1: Average growth rates by species and size class, between locations

		Average growth rate ($cm^2 month^{-1}$) ± S.E.	
Species	**Class size**	**Land-based nursery**	**Offshore Nursery**
O. faveolata	Large	0.162 ± 0.109	0.089 ± 0.066
	Small	0.070 ± 0.054	-0.010 ± 0.039
S. siderea	Large	0.409 ± 0.054	0.107 ± 0.036
	Small	0.182 ± 0.022	0.072 ± 0.036
P. clivosa	Large	1.341 ± 0.196	0.251 ± 0.0814
	Small	1.032 ± 0.185	-0.075 ± 0.028
D. labyrinthiformis	Large	0.936 ± 0.082	0.247 ± 0.242
	Small	0.732 ± 0.060	0.008 ± 0.054

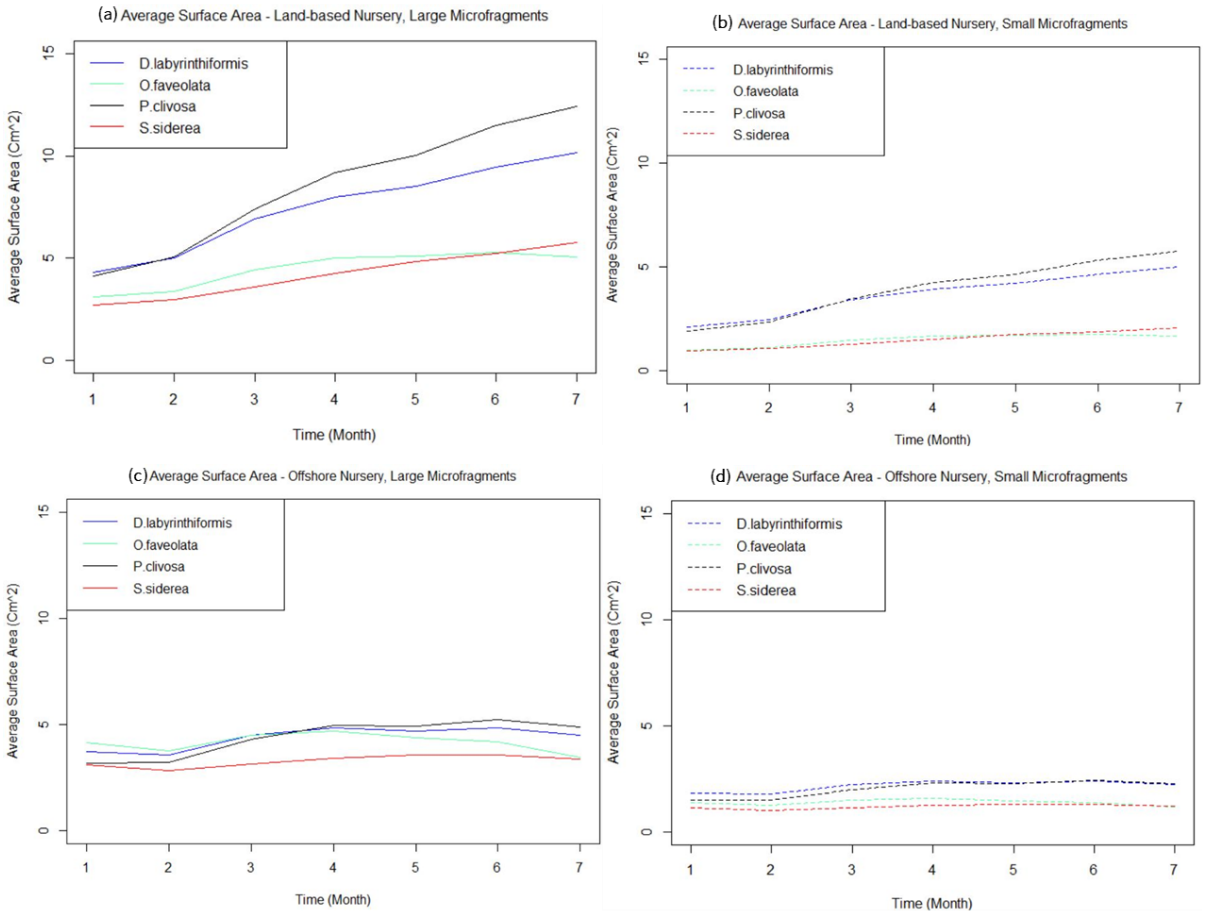


Figure 6. Average growth of the coral fragments over time, by initial size class and location

Microfragment Magnitude

Species, location, and initial size class together significantly affected microfragment magnitude increase (Kruskal-wallis: χ^2= 87.122; df = 15; p = 3.404 x 10^{-12}). Similar to growth rate, magnitude increase was higher in the land-based nursery than in the offshore nursery (Table 2). Post hoc analysis sorted each combination of species, location, and initial size class into four groups that represent significant differences: A, B, C, and D, with many combinations in more than one group (Figure 7). These results show that while the variables all together have a significant effect on magnitude, there are fewer significant differences between the combinations of factors. This is likely due to the similarities between magnitude values and only having a maximum of three replicates per combination, with less if there were mortalities (Table 2).

Table 2: Average magnitude change by species and size class, between locations.

		Average magnitude change ± S.E.	
Species	**Class size**	**Land-based nursery**	**Offshore Nursery**
O. faveolata	Large	1.285 ± 0.179	1.160 ± 0.112
	Small	1.313 ± 0.223	0.969 ± 0.213
S. siderea	Large	1.761 ± 0.102	1.244 ± 0.089
	Small	2.136 ± 0.100	1.365 ± 0.191
P. clivosa	Large	3.234 ± 0.440	1.435 ± 0.180
	Small	4.856 ± 0.793	0.717 ± 0.106
D. labyrinthiformis	Large	2.392 ± 0.095	1.336 ± 0.378
	Small	3.337 ± 0.204	0.957 ± 0.120

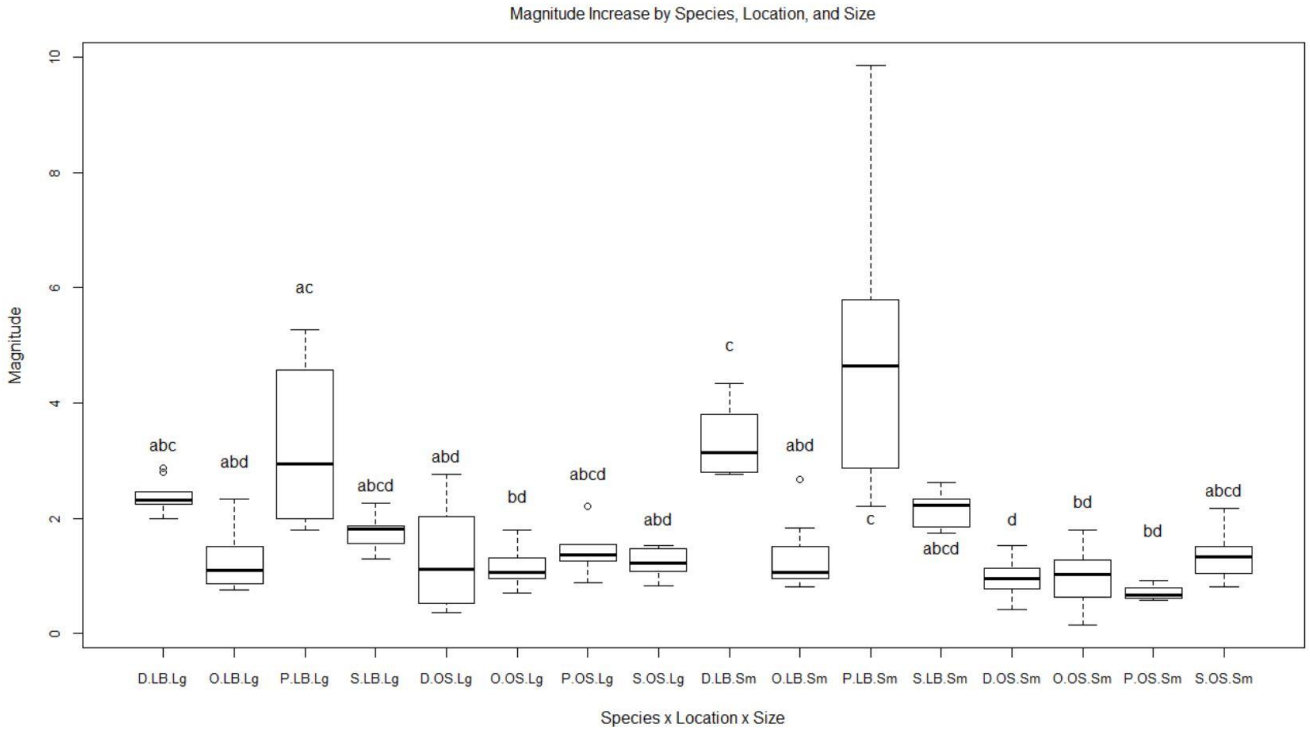


Figure 7: Magnitude increase for all combinations of species, location, and initial size class. The letters above the bars represent groups of statistical significance. O = O. faveolata, S = S. siderea, P = P. clivosa, and D = *D. labyrinthiformis; LB = Land-based nursery, OS = Offshore nursery. Lg = Large initial size class, Sm = Small initial size class*.

Water Quality

Water quality results from the land-based nursery are presented in Figure 8. At approximately month 3, minor equipment issues caused the alkalinity in the land-based system to fluctuate (Figure 8 (*c*)). Troubleshooting the problem and then ordering the required parts meant the issue was not resolved for approximately 8-10 more weeks.

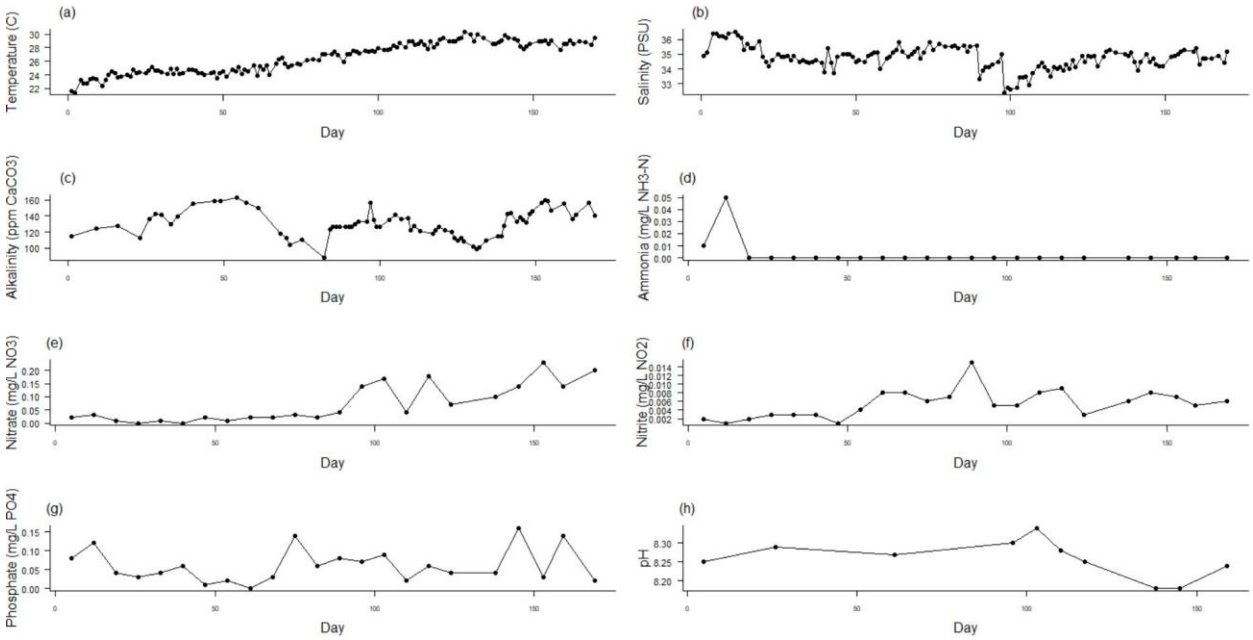


Figure 8: Water quality parameters measured in the land-based nursery throughout the experiment. The x axis for all is the number of days since the experiment began; (a) Temperature (°C); (b) Salinity in practical salinity units (PSU); (c) Alkalinity (PPM of $CaCO_3$); (d) Ammonia (mg/L NH_3-N); (e) Nitrate (mg/L NO_3); (f) Nitrite (mg/L NO_2); (g) Phosphate (mg/L PO_4); (h) pH.

The offshore nursery had a temperature logger present in the nursery throughout the experiment. A small malfunction meant the data in the first 2 weeks of the experiment was lost; however, data was collected every hour beginning on March 13th, 2020 at 15:00:00 (Figure 9).

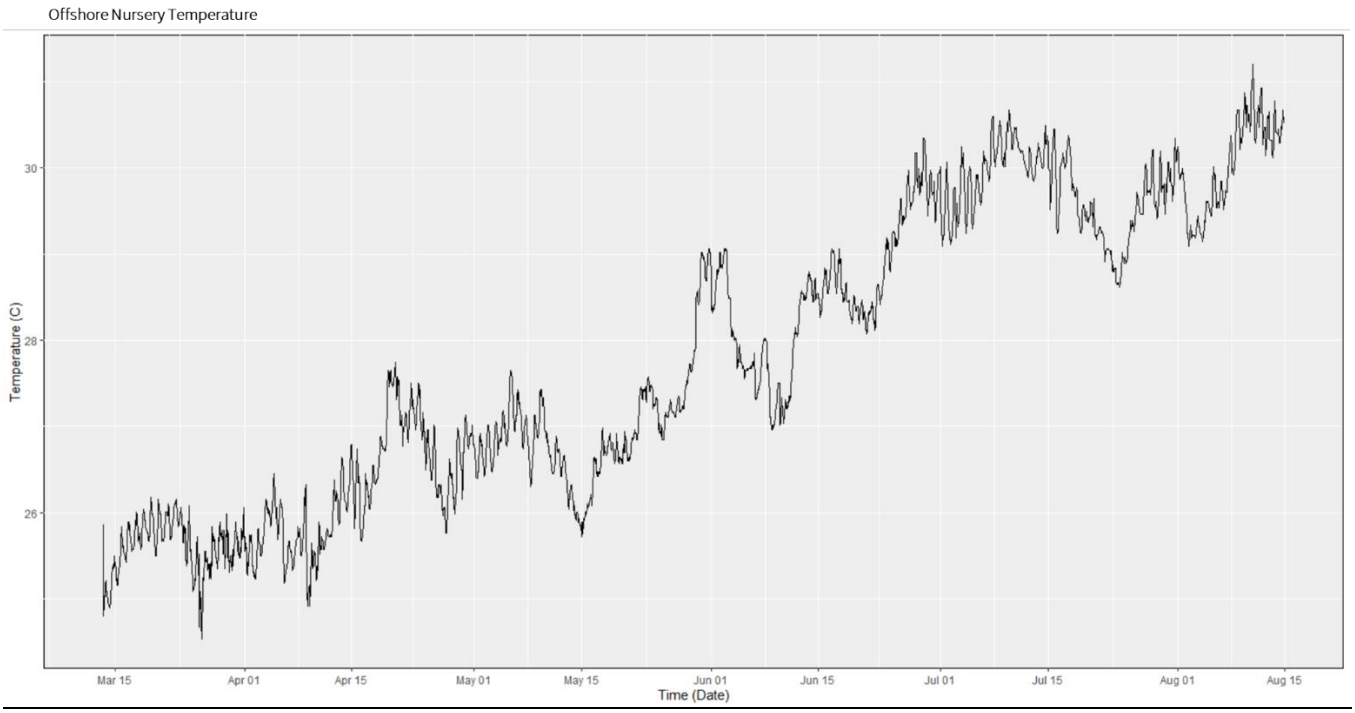


Figure 9: Temperature data collected in the offshore nursery every hour throughout the experiment.

Discussion

The results from this study indicate that coral microfragments of *Orbicella faveolata, Siderastrea siderea, Pseudodiploria clivosa* and *Diploria labyrinthiformis* had significantly greater survivorship and grew significantly faster in the land-based nursery compared to microfragments grown in the offshore nursery. These results were consistent regardless of initial size class or species (Table 1 and Figure 6). The species of the microfragment was found to have a significant effect on growth rate, and the brain corals were found to grow faster than *S. siderea* and *O. faveolata* (Figure 6). These results demonstrate that *D. labyrinthiformis* and *S. siderea* can be successfully microfragmented to these sizes and grown in either location for restoration purposes.

Microfragment Mortalities

Coral microfragments were significantly more likely to die in the ocean than on land where there was only one death throughout the experiment (Figure 5). Of the eighteen

microfragments who perished in the offshore nursery, causes of death for these microfragments can most likely be attributed to either the microfragment becoming unattached from the tile, macroalgal overgrowth, or predation; however, many microfragments had both heavy macroalgae cover and signs of predation throughout the experiment, and so determining the exact cause of death is beyond the scope of this experiment. While in the nursery, fish species such as *Balistes capriscus* and *Sparisoma viride* were observed grazing on or near coral microfragments or the coral "tree" consistently. These observations agree with a recent study on microfragmented corals that concluded that predation is a major problem with microfragments grown in the ocean (Koval et al., 2020). While this recent study and my study both take place off South Florida, worldwide, there are over a hundred species of fish and invertebrates that have been documented predating on corals, suggesting predation likely will be major challenge to overcome regardless of grow-out location (Rotijan & Lewis, 2008).

Offshore predation not only affected survival of coral microfragments, but also growth, with noticeable decreases in growth rates following lesions resembling predation marks. When a coral is damaged from predation, growth rates slow as the coral focuses on healing the lesion, which may not completely close (Meesters et al., 1994). Additionally, calcification can also decrease during healing, and can take months to increase again (Meesters et al., 1994). This indicates that even small amounts of predation in the beginning of the experiment could have long-term effects once the microfragment is outplanted onto a reef.

Effect of Location

Increased growth rates observed in the land-based nursery are likely due to the controlled environment, lack of macroalgae growth, and absence of predators. A recirculating system allows for control over the vital parameters for coral health and growth such as temperature, alkalinity, pH, and water flow to create ideal conditions for coral growth while preventing negative effects from pollutants or turbidity. A land-based nursery is also able to control the timing of feeding, and the amount and quality of food to be similar or more nutritious than corals may experience in the ocean. The results from this study suggest that a varied artificial diet can provide adequate nutrition and not hinder growth when compared to microfragments in the ocean who can eat a natural diet throughout the day (Figure 6). In this study, the corals in the land-based nursery were fed in the daytime, which, according to a study on *Pocillopora damicornis*,

can produce increased growth rates than when feeding at night (Lavorano et al., 2008). Corals in the ocean typically feed the most at night due to increased plankton levels, however, this behavior could have contributed to the slower growth rates seen offshore. Feeding at night has been postulated to slow down calcification due to a buildup of dissolved inorganic carbon, CO_2, inside the polyp which decreases the pH and creates sub-optimal conditions for calcification and growth (Sebens & DeRiemer, 1977; Osinga et al., 2011).

Unlike the land-based nursery, if there were problems with the water quality or pollution in the offshore nursery, nothing could be done about it. Since the offshore nursery used in this study was in close proximity to a busy port, the high traffic of shipping containers, cruise ships, and recreational boats of all sizes may have impacted the local water quality. This is also a port that requires maintenance through regular dredging projects that acutely increase local turbidity (Barnes et al., 2015; Ennis et al., 2016; Water Infrastructure Improvements for the Nation Act, 2016). The combination of high anthropogenic activity and proximity to large vessels increases the chances of gasoline or oil pollution, localized turbidity increases, and physical damage to corals and reefs, all of which can negatively impact the health and consequently, the growth rate, of corals (Lapointe *et al.*, 2011; Browne et al., 2015; Ennis et al., 2016; Fourney & Figueiredo, 2017). Increased port activity has also been shown to correlate with increased macroalgae growth, likely from an input of nutrients, that can smother corals and reduce growth (Tanner, 1995; Dubinsky & Stambler, 1996; Browne et al., 2015; Ennis et al., 2016). Despite the presence of herbivores in the offshore nursery, macroalgae growth was prevalent throughout the experiment and likely contributed to the reduced growth rates observed offshore (Tanner, 1995; Lirman, 2001). While some macroalgae grew in the land-based nursery, the herbivorous snail, *Lithopoma americanum* was present in the tank, which precluded macroalgae from growing near the microfragments while causing minimal to no damage to the microfragment itself.

Effect of Initial size class

While initial size class did not affect survival, initial size class did significantly interact with species to affect growth rates of the microfragments. The interaction suggests that faster growth rates between size classes was different for the four species, not an unexpected result when considering the different morphologies of the species tested (Buddemeier & Kinzie, 1976). Given the conflicting results for magnitude increase, this suggests that the effect of the perimeter

to area ratio on growth rate may not be as strong as theorized, or simply isn't seen with these species or conditions (Dornelas et al., 2017). It is also possible that the perimeter to area ratio difference must be greater than the two size classes tested in this study. To fully determine the effect initial size class has on growth, future research may consider more size classes that have larger size differences.

Effects of Species

Species are compositionally different in terms of polyp structure, tentacle length, gene expressions, symbiont makeup, and, therefore, have different growth rates (Buddemeier & Kinzie, 1976; Yap et al., 1992; Goulet, 2006; Baums et al., 2010). This experiment aimed to look at how these differences unfold under different initial size classes, and different locations, therefore, different conditions. Species differences were more obvious in the land-based nursery, where overall, *P. clivosa* and *D. labyrinthiformis* had noticeably faster growth rates than *S. siderea* and *O. faveolata* despite similar starting sizes (Figure 6, a-b). While the corals were exposed to the exact same conditions and fed the same amounts, feeding efficiency, and thus energy gained from heterotrophy, was potentially different due to the different morphologies. *S. siderea* and *O. faveolata* both have smaller polyp sizes and tentacle lengths than either of the two brain corals who have longer tentacles and larger, interconnected polyps. The longer tentacles on *P. clivosa* and *D. labyrinthiformis* could lead to increased rates of heterotrophy. Heterotrophy has been shown to positively correlate with both photosynthetic capacity and growth rates, indicating that this may be one reason for the growth differences (Jacques & Pilson, 1980; Ferrier-Pagès et al., 2003; Houlbreque et al., 2003; Osinga et al., 2011).

Compared to the other three species, there was a wider range of growth rates observed on land for *O. faveolata*, from -0.15 cm^2 per month to 0.88 cm^2 per month, including five microfragments with negative growth rates. Mid-way through the experiment, equipment issues caused the alkalinity in the land-based system to fluctuate. No other species on land lost tissue during the experiment, suggesting those species may be more resistant to alkalinity changes than *O. faveolata*.

Average growth rates for corals grown offshore were more similar between the species, but had higher variability, with some negative growth rates likely from predation or macroalgae overgrowth, and some microfragments with growth rates as high as some corals in the land-

based nursery (Table 1). Recent restoration research has examined potential predation deterrents to help mitigate these negative growth rates and mortalities, but some level of predation should be expected when outplanting corals in the ocean (Koval et al., 2020). Species differences were less pronounced in the offshore nursery, but like the land-based nursery, *P. clivosa* and *D. labyrinthiformis* had the fastest growth rates with *S. siderea* following (Table 1 and Figure 6). All species had at least one fragment with abrasions that could be predation marks; however, *P. clivosa* and *D. labyrinthiformis* had more microfragments that lost tissue or perished offshore, suggesting a potential preference for a fleshier coral by the local predators. Corallivores have been observed displaying preferences between species, individuals, and even parts of a colony that are more reproductive, which could have impacted the microfragments on a scale this experiment couldn't quantify at its scope (Rotijan, 2007; Rotijan & Lewis, 2005).

Management implications

The observed differences in growth and survival between the two locations suggest that the location best for coral fragment grow out is in a land-based nursery where parameters can be controlled and corals can be monitored easily; however, this is not a cheap or easy endeavor for many facilities. For managers without access to a land-based system, outplanting in an ocean nursery is still a valid option, although there is room for more optimization. The benefits of offshore nurseries are the much larger amount of space available for coastal operations, fewer hours of hands-on work, and potentially cheaper cost, but this clearly can come at a cost of reduced growth rates. Lower growth rates for both size classes offshore may indicate that even the large size class was too small to handle the potential stressors in the ocean, which is confirmed in a recent study stating that fragments approximate 5 cm^2 experienced more predation (Koval et al., 2020). Brain corals that were undamaged throughout the experiment showed high growth rates, leading me to conclude that some offshore grow-out could be successful with some kind of predation deterring device.

The results of this study could be highly location specific, meaning replication at another location could have drastically different results. This location was in close proximity to a major shipping port, but other offshore nurseries could be more pristine with less anthropogenic influence, leading to different water qualities and, likely, growth rates as well (Ennis et al., 2016). Additionally, the location of the study could limit the kind of land-based nursery

equipment and supplies that could be utilized which may impact results. A Hawaii-based study, aiming to test isogenic fusion of coral microfragments, grew microfragments in a land-based flow-through raceway with filtered seawater instead of artificial seawater with great success (Forsman et al., 2015). A different study grew microfragments in a labor-intensive treatment initially, then switched to a simpler tank with reduced labor required and found a significant difference in growth between the treatments (Forsman et al., 2006). Mangers will have to experiment with the environment and materials at their disposal to promote optimal growth rates.

Overall, these results suggest that coral fragments should be grown in a land-based nursery as long as feasible, or at least until they reach a size that is able to better withstand predation or algae overgrowth. This could be a different size and length of time for every species but, given the low growth rates observed in the offshore nursery and the results of an additional study on predation, these should be fragments larger than 5 cm^2 (Koval et al., 2020). Offshore operations could be successful with some species, but maintenance actions such as macroalgae removal may be necessary for success. Future studies can further optimize offshore grow-out and determine a fragment size that is less susceptible to ocean stressors, or other predation deterring methods.

Regardless of location, frequent monitoring is suggested to ensure corals are healthy and growing, and to make changes to their grow-out environment if possible. Certain individual corals may be more susceptible to disease, predation, or more sensitive to parameter changes that can happen in a land-based system, leading to slower growth rates if they are not in the optimal conditions. A recent study on the small-scale progression of SCTLD in the middle Florida Keys noticed differences in the way some individual corals responded to the disease over time, indicating there may be differential disease resistances between individuals or populations of corals (Sharp et al., 2020). In that study, some individuals ceased exhibiting signs of the disease, which would make this individual a desired target for microfragmentation and outplanting to spread that potential disease resistant gene (Sharp et al., 2020).

The final step of this process is the eventual outplanting of coral fragments onto ocean reefs with declining coral populations. As restoration efforts continue, more thought should be put into the locations of the reefs receiving new corals. While it may be important to provide a similar environment to the one the coral settled and grew in, if the water quality could be

detrimental to the coral or likely to degrade over time, other locations should be prioritized. For example, restoring a reef with high larval connectivity would create the potential for compounded restoration benefits over time, as more larvae with different genes could be exchanged (Hughes et al., 2005). With more research and collaboration in upscaling and optimizing coral restoration, improvements in techniques like this could improve coral densities over time. Determining long-term restoration strategies that require little maintenance for success is key to the future of reef restoration.

The coral microfragments used in this study were outplanted onto nearshore reefs off of Broward County, FL, USA in April of 2021 for continued monitoring. The results of their growth and success will provide insights on the crucial next step in restoration beyond grow-out.

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