FY24 Activities of the Southeast Florida Coral Reef Restoration Hub

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Final Report

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Management Summary **(300 words or less)**

Our FY24 restoration activities focused on improvements to land-based propagation and field outplanting of recruits, as well as maintenance/establishment of field nurseries, asexual propagation of microfragments to restore large old colonies, and analyses of outplant survival as a function of site characteristics. UM's land-based outdoor facilities have been improved and transitioned to recirculating systems, and sexual recruits produced by the Florida Aquarium were outplanted via these staging facilities. There is promising evidence that a new structure to protect outplanted juveniles (reusable cement "umbrellas" produced by Reef Cells) have very high rates of success, although they need to be removed/recovered and hence require more personnel time. Nursery acclimation played a crucial role in coral survival following outplanting, but we found evidence that that practitioners can potentially outplant coral juveniles earlier in life to decrease cost and time kept in ex situ facilities. We also found that supplementary feeding of early-stage recruits may help increase growth, showing a potentially valuable benefit from this early feeding treatment for a relatively modest investment of effort. Trials of probiotics on early-stage recruits of *Colpophyllia natans* contributed to mounting evidence that these beneficial bacteria are indeed safe for application as a potential treatment against stony coral tissue loss disease, provided they are applied post-settlement, although continued research is still needed. Progress was made to establish a new nursery midway between establish Miami-Dade and Broward nurseries, and analyses were undertaken of site characteristics in Broward that determine outplant success. In addition, 140 fragments of five genotypes were used to initiate reskinning of two large colonies of *O. faveolata* using microfragmentation. Finally, eleven Master's-level students were recruited on 6-month internships to work with partners across the network, and ten new interns began a new set of internships ready for FY25.

Acknowledgments

We thank the Florida Department of Environmental Protection (FDEP) for their continued support of the Southeast Florida Restoration Hub under project C2A66F. UM wishes to thank Giovanni Ceron and Soraida Diaz from the Rosenstiel School's Sponsored Research Office for their budgetary and administrative support since the Hub was founded in 2020.

Executive Summary

Over the past several decades, coral reefs in Florida and the wider Caribbean have suffered tremendous declines in coral abundance and diversity. The Southeast Florida Coral Reef Restoration Hub was created with the overall goal of reversing these declines in Florida and brings together the restoration activities of coral restoration partners in order to better coordinate science-driven restoration activities and help train the next generation of coral reef restoration practitioners. The Hub consists of seven partners (the University of Miami Rosenstiel School, Nova Southeastern University, The Florida Aquarium, SECORE International, the Phillip and Patricia Frost Museum of Science, The Reef Institute, and the Smithsonian Marine Station) in five counties (Miami-Dade, Broward, Palm Beach, St. Lucie, and Hillsborough).

In FY24, activities focused on translating recent research in coral reproduction, land-based propagation, and coral outplanting to increase the recovery potential of Florida's coral populations. New generations of sexually produced corals are needed to replace colonies that have been lost, but natural recruitment to Florida reefs has largely failed in recent decades. As such, assisted reproduction accompanied by juvenile rearing and outplanting is necessary to introduce new genetic diversity to Florida's Coral Reef and facilitate population recovery. At the same time, asexual fragmentation of existing corals can also be exploited to help increase coral cover and also help large colonies recover lost tissue.

Activities included infrastructural improvements to the outdoor coral propagation facilities at the University of Miami's Experimental Hatchery, including a new ozonation system to pre-treat all incoming seawater and facilitate a switch to largely recirculating, rather than open (running seawater), systems (Task 2). Field- and land-based studies of the effect of short-term feeding improvements on early growth of recruits were also undertaken (Task 3), as were field-based studies of nursery acclimation and predation deterrents on the survival of juveniles outplanted to the reef (Task 4). The value of probiotics in enhancing recruit survivorship on the reef was also investigated with trials on one candidate (*Pseudoalteromonas* sp. CNAT2-18.1, that has shown promise in slowing the progression of stony coral tissue loss disease in adult corals), found to be safe to apply to early coral recruits after settlement (Task 7). Progress on establishing a new in-water nursery mid-way between existing nurseries in Broward and Miami-Dade Counties was also made, and is currently pending permitting.

In addition, the importance of various site characteristics such as distance from shore, distance from Port Everglades, sediment depth, and rugosity, on the success of thousands of outplanted corals in Broward County was also investigated (Task 5), as was the success of using microfragmentation to help re-skin some of South Florida's largest and oldest remaining colonies (Task 6). QA/QC and reporting for all of these activities were established and executed (Task 1).

Finally, this project funded 6-month internships for eleven Master's degree-level interns in FY24 across the Hub partners (as well as the first month of new internships for an additional ten FY25 interns). The goal of the internship program is to help train the next generation of restoration practitioners and establish career-long relationships among the FY24 cohort, while also strengthening links across the partners to help coordinate research and restoration activities.

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Task 2 - Install recirculating seawater systems at UM hatchery

The Coral Reef Futures Lab has installed a Telchine Energy Technologies brand ozonation system for sterilization of incoming seawater at the land-based coral nursery located on Virginia Key. Each Telchine ozone sterilization system is custom-built to the specifications and needs of the facility. As such, we have taken some time to work with the manufacturer to optimize the programming and improve the efficiency and user interface.

The system is designed to be both manually and remotely operated. It comprises a 5g/hr ozone production unit that will ensure that all incoming water is ozonated by a set amount that can be determined via a touch screen on the front of the unit. For the moment, we are targeting a treatment level of 600 ORP, but this can be raised/lowered as necessary. Ozone is created via supply of oxygen, stored nearby in a cylinder with gauges to monitor contents and refill as needed. The system is programmed to fill two 180-gallon reservoirs with seawater that has already been filtered down to 5- and then 1-micron. Each reservoir has a fill-level monitor, allowing the system to sense and display the current volume of treated water at any point during the treatment process.

Once filled, the system then begins treating the reservoirs via conversion of oxygen into ozone. The water in the reservoirs recirculates using aquarium pumps to give it multiple passes through the ozone injection point and ensure a homogenous composition in the two containers of water. There are probes in place before and after the water loop to monitor ORP as it increases. Once the ORP has reached the designated treatment set point, the system then enters a decay stage. The water continues to circulate while the ozone is broken down. We have set our low point for this decay at 400 ORP, as that is comparable to the ORP of the seawater in our tanks. Once the system reads that the water is ready for use, icons appear on the touch screen giving the option to begin pump out, or re-treat should the need arise.

The water is then passed through a UV sterilizer, and subsequent 10-foot-tall carbon chamber to ensure the destruction of any residual ozone as well as the removal of any chemical compounds that could arise as a result of this process. There is also a final probe monitoring the ORP as the water leaves the ozone system. Should the water not meet the expected treatment level at any of the monitoring points, or if any of the equipment were to fail during the process, the system is programmed to shut off to avoid any risk of under- or over-treated water entering our coral systems.

The water then feeds directly to the sumps of each of our coral systems via buried PVC plumbing, and each sump is valved so that individual systems can be topped off or receive a water change. This feature allows the aquariums to run on a fully recirculating system, with a 20% weekly water change to make up for minerals lost via absorption. The unit was installed on April 1, 2024, and over the past two months we have been testing the ozonated seawater on one of our ~800 gallon 5-tank coral systems.

Task 3 - Effect of early feeding on subsequent survivorship and size distribution of coral settlers

Recent literature indicates that coral settlers of several species are capable of feeding on live *Artemia* nauplii within just a few days of settlement (Geertsma et al. 2022). During the 2023 spawning season, coral settlers of three of these species were established from both field-collected and land-based spawning at the Florida Aquarium (TFA). These species are *Orbicella faveolata* (field-collected), *Colpophyllia natans* (TFA-spawned), and *Pseudodiploria strigosa* (TFA-spawned). A portion of the settlers from each species were subject to seven feeding bouts within the first 2.5 weeks post-settlement in large mesocosms. The survivorship and change in size of these early-fed and not-fed groups has been quantified in three different settings (UM Hatchery, Frost Science Museum, and a hybrid reef off Miami Beach) to test for potential latent benefits of early post-settlement feeding. Although low survivorship resulted in non-significant results, a consistent pattern was observed in the field site of higher survivorship in the early-fed group.

During the current reporting period (Jan - May 2024) substrates with coral recruits of three species from both early feeding treatments were maintained at the UM Hatchery and at the Frost Museum of Science under varying husbandry conditions. Additionally, a subset of the recruits was outplanted to the field on December 5, 2023 (to a hybrid reef off Miami Beach). Survivorship of recruits on all substrates at the three settings were scored between May 7, 2024 and May 24, 2024, and the size of each surviving recruit was recorded in the two land-based settings. We compared the survivorship among early-fed treatments and settings (two-way factorial analysis) within each species.

Survivors (Sept 23 to May 24; age ~8 months) were observed at both The Frost Science Museum (all three species) and the Miami Beach hybrid reef (only *Colpophyllia natans* and *Pseudodiploria strigosa* were outplanted there; survivors were found of both). No surviving recruits were found on the tagged substrates from either feeding treatment at the University of Miami Experimental Hatchery, although there were other recruits from these cohorts that did survive, but which had not been designated for tracking in this experiment (Fig. 1). Consequently, survivorship varied significantly among these three settings for all three species. More interestingly, although the feeding treatment was not significant in the two-way ANOVA on ranks for any of the species, for recruits outplanted to the field, there was a strongly suggestive pattern of higher survivorship in the early-fed treatment for both of the species outplanted (*C. natans* and *P. strigosa*). *Colpophyllia natans* also showed higher survivorship in the 'early-fed' group at Frost Museum (mean of about 3% compared to ~ zero for the 'not early-feds'. The highest mean survivorship was of *P. strigosa* at Frost Museum, which was ~ 14%.

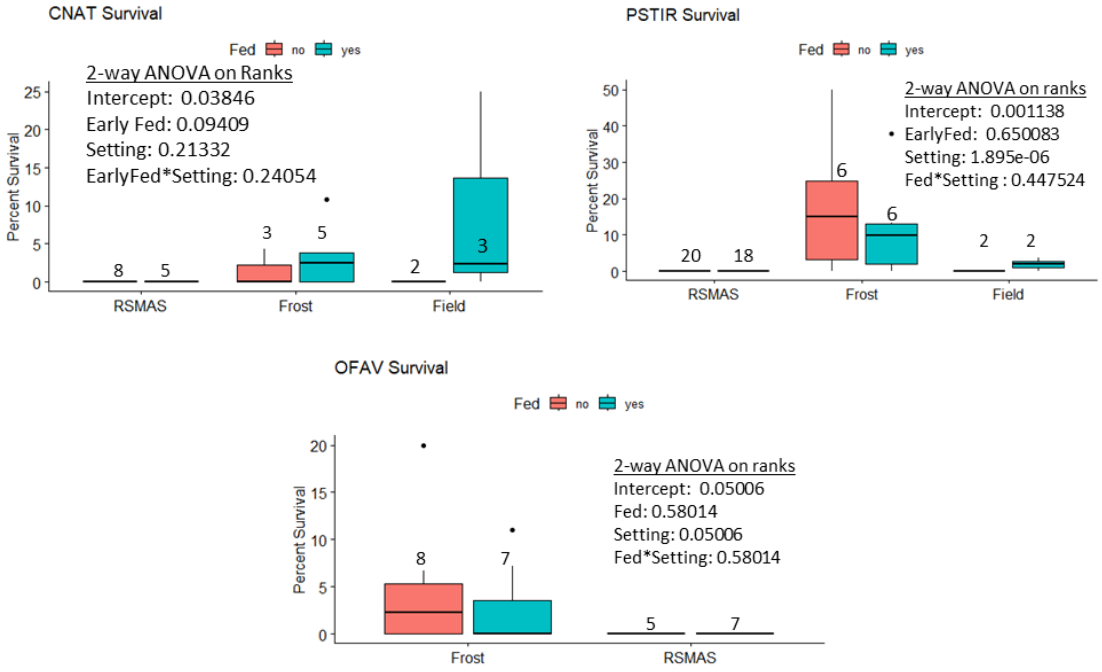

Figure 1. Percent survival over 8 months for larval recruits of three species (Cnat = *Colpophyllia natans*, Pstir = *Pseudodiploria strigosa*, Ofav = *Orbicella faveolata*) in three husbandry settings (x-axis) and two early feeding treatments. P-values -from 2-way ANOVA on ranks is given in each panel and n is given above each bar.

Although the early-fed treatment had a significant effect on the initial size of the settlers for two of the three species (as reported previously), no ongoing (latent) effect of early feeding on surviving recruit size was observed in the recruits under land-based culture (Fig 2; there was no opportunity to measure the recruits in the field setting due to timing and field-work constraints). This is perhaps not surprising, because the overall number of surviving recruits was rather small (n=36 pooled among the three species) and all these experimental substrates experienced ongoing feeding in both land-based husbandry settings.

Overall, this experiment confirmed prior results from Curaçao (Latijnhouwers et al. in prep.) showing that a short-term feeding regime for recent settlers of *C. natans*, and additionally *O. faveolata*, can increase the mean size of these settlers prior to outplanting which is expected to improve their success. For the settlers in this experiment that were outplanted to the field (*C. natans* and *P. strigosa* only), we saw a pattern that is consistent with the prior results from Curaçao with somewhat higher (though not significant overall) survivorship rates in the early fed group. Hence, for coral larval settlers that will be outplanted to the field relatively soon after settlement (ours were outplanted at ~ 3 months age), there is likely benefit from this early feeding treatment, for a relatively modest investment of effort.

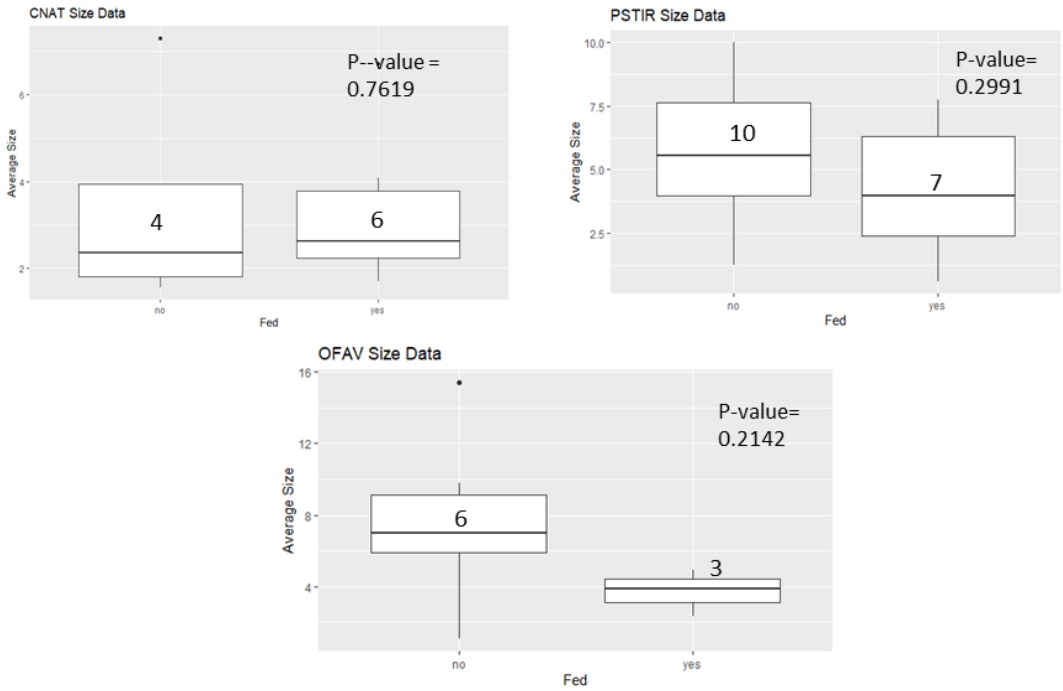

Figure 2: Box plots of recruit size (mm diameter) of surviving recruits of each of three species at ~ 8 months of age. None show a significant effect of the early feeding treatment. N is given in or above each boxplot.

For recruits that were kept under intensive husbandry (including intensive ongoing feeding of all substrates there) at Frost Museum, there was no clear pattern in survivorship between early feeding treatments. However, it was clear that the largest colonies at the end of the study were at Frost (see uploaded photos). Meanwhile, although some recruits did survive at UM, the monitored cohort did not fare well, likely due to differences in the husbandry regime between UM and Frost including water quality (ozonated at Frost but not yet at UM, but see Task 1, UM now has ozonation), light quality/quantity (indoor artificial lighting at Frost, outdoor and shaded at UM), and feeding intensity (daily at Frost and including live *Artemia* and powdered food, twice per week at UM with only powdered food). In discussion with other project partners, we believe the feeding regime may have been the most influential, and hypothesize that the intensive feeding regime throughout the subsequent husbandry period evened out the size advantage of the recruits that were in the early-fed treatment.

Task 4 - Recruit rearing and outplanting

Coral recruit rearing & spawning parent information

Broodstock corals are colonies on loan to TFA from the Florida Fish and Wildlife Conservation Commission/Coral Rescue Team and collected under permit FKNMS-2017-100. These corals have been housed in closed aquaria since December of 2018, May of 2019, and March of 2022. Potential *P. strigosa* parents are listed in Table 1 and spawned on four nights in August and September of 2022. Potential *D. labyrinthiformis* parents are listed in Table 2 and spawned on three separate nights in May 2022.

Tables 1 and 2. Potential parents for crosses

Pseudodiploria strigosa Broodstock
5753_PSTR_001
5830_PSTR_001
6206_PSTR_003
IRMA 109_PSTR_001
IRMA 109_PSTR_002
T1081_PSTR_006
T1081_PSTR_008

Diploria labyrinthiformis Broodstock
10_DLAB-006
22_DLAB_003
22_DLAB_007
3_DLAB_003
39_DLAB_001
5753_DLAB_002
5753_DLAB_003
6206_DLAB_001
6206_DLAB_003
BW16_DLAB_001
BW16_DLAB_002
BW24b_DLAB_001
BW5_DLAB_004
BW5_DLAB_007
IRMA 109_DLAB_001
IRMA 109_DLAB_003
IRMA 109_DLAB_004
T1081_DLAB_008
T1081_DLAB_006

Coral larvae were settled and reared in closed aquarium systems in naturally sun-lit greenhouses in Apollo Beach, Florida. Juvenile coral care includes regular system maintenance, water chemistry testing, heterotrophic feeding, and dosing that is performed according to best husbandry practices. Weekly water chemistry is tested and recorded to maintain target values for coral growth. The holding systems receive a 20% water change weekly. Natural seawater is provided by the Florida Aquarium and has been sourced from the Gulf of Mexico and ozonated for disinfection before use. Corals are fed twice weekly (Reef Roids, Golden Pearls, copepods, rotifers, and enriched with Selcon), supplemented with Aquaforest Amino Acids, Lugol's Iodide, activated carbon, polyfilter, and ammonium chloride dosed to 0.02ppm twice weekly. The first 500 corals were transported from TFA to UM on January 18, 2024. The second round of 500 corals passed their health inspection on April 17, 2024, and were transported on April 24, 2024.

Table 3: Weekly water quality results for closed systems housing juvenile corals at The Florida Aquarium's Coral Conservation and Research Center.

Salinity (ppt)	pH	Ammonia (ppm)	Nitrite (ppm)	Nitrate (ppm)	Alkalinity (ppm)	Calcium (ppm)	Magnesium (ppm)	Phosphate (ppm)
8.09 ± 0.08	8.09 ± 0.08	0.00 ± 0.01	0.005 ± 0.002	1.5 ± 0.3	136 ± 11	441 ± 27	1401 ± 45	0.04 ± 0.05

Two rounds of *D. labyrinthiformis* and *P. strigosa* juveniles from TFA and the University of Miami were outplanted to Paradise Reef. The first round was outplanted on February 16, 2024, and the second on May 8, 2024. For each outplanting, half of the recruits were deployed to the Key Biscayne Nursery for ocean acclimation before being transferred to the reef (Fig. 1), while the other half were directly outplanted to the reef.

In the first round, two predation deterrents were used to determine their effectiveness for juveniles, alongside control groups. Two different predation treatments were chosen: "Umbrella" made by Reef Cells, and "Coral Castle" made by Kyle Pisano (Fig. 2). Each base could hold four recruits. On the reef, each plot contained one Umbrella base, one Coral Castle, and one Control group (consisting of 3-4 corals cemented directly to hard bottom).

For the second round, results indicated that the Coral Castle was less effective than the Umbrella base. Consequently, juveniles were outplanted using only the Umbrella bases, maintaining multiple controls at each plot (Fig. 3). The first round of juveniles acclimated at the nursery for three months before being outplanted to the reef, while the second round will be outplanted after two months at the nursery (Fig. 4).

Table 4: Ages of the coral juveniles at the time of outplanting in each round.

	DLAB	PSTR	PSTR_UM
Round 1	21 months	17 months	5 months
Round 2	24 months	20 months	8 months

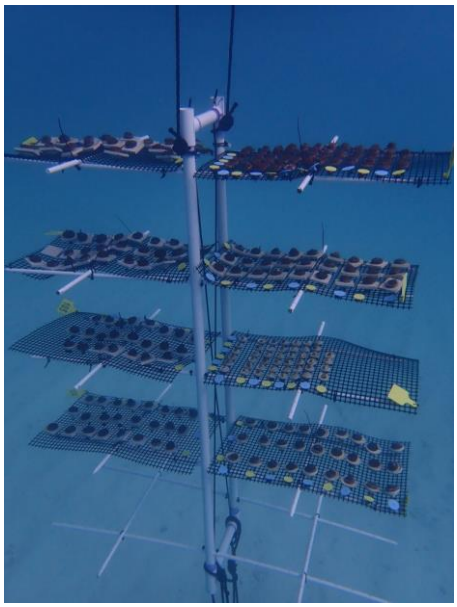

Figure 3. The nursery tree where half of all corals were deployed to condition them to the ocean prior to outplanting onto the reef.

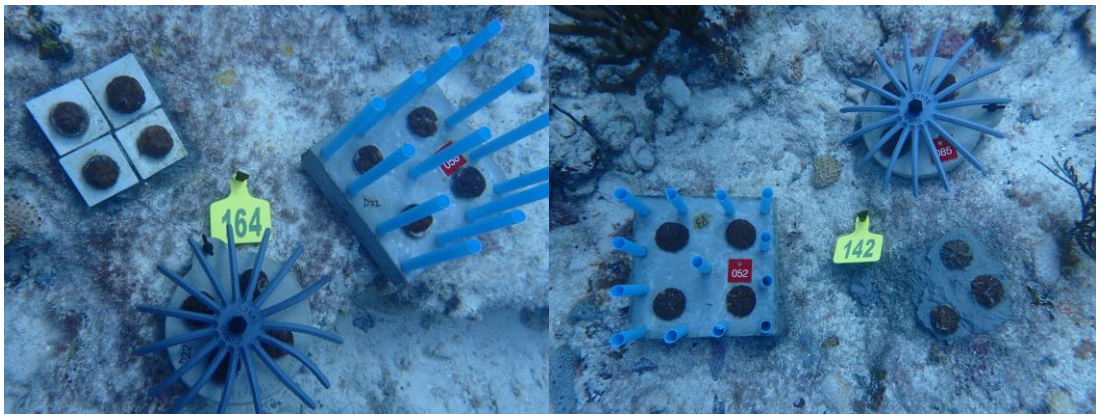

Figure 4. Round 1 of outplanting, using two different predation deterrents ("Umbrella" and "Coral Castle") and controls.

Figure 5. Round 2 of outplanting, without the "Coral Castle" predation deterrent.

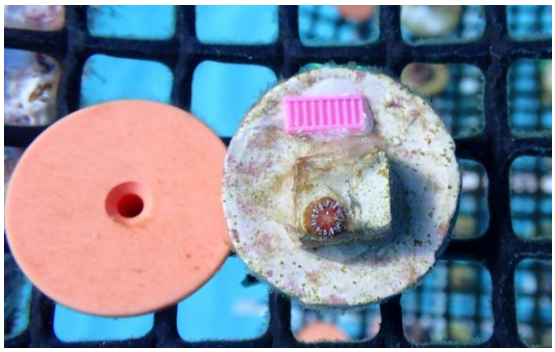

Figure 6. A recruit of *P. strigosa* raised by the University of Miami at the Key Biscayne nursery.

Juvenile survey methods

We conducted juvenile surveys at Paradise Reef and Emerald Reef in early May 2024. The surveys covered an area of 75-100 m^2 using transect lines. A juvenile was defined as any coral < 5cm in diameter. Each diver recorded the species and diameter of each juvenile.

Outplanting survival

D. labyrinthiformis exhibited greater overall survival compared to *P. strigosa* when comparing the corals that came from the TFA (Figure 5, 6, 7, ANOVA, $p < 0.001$). This indicates that these two species have significantly different survival outcomes under the same conditions.

The Umbrella treatment had a higher survival throughout this project compared to both the Coral Castle treatment and the Control treatment with no predation deterrents (Figure 5, 6, 7) (ANOVA, $p < 2.2e\text{-}16$).

Nursery acclimation played a crucial role in coral survival ($p < 2.2e\text{-}16$). Corals that underwent nursery acclimation have different survival probabilities compared to those that did not, however, to date there are only two timepoints to examine this difference, and more observations is needed to accurately determine this relationship.

There was no significant difference between the age of the juveniles and their survival (Wilcoxon, $p > 0.5$), indicating that practitioners can potentially outplant coral juveniles earlier in life to decrease cost and time kept in ex situ facilities. Age was a significant factor in the survivorship of juveniles across all sites, treatments, and species (ANOVA, $p < 5.026e\text{-}07$).

Overall, the Umbrella treatment appears to be the most effective predation deterrent for both species, suggesting it could be a valuable strategy for improving coral survival during reef outplanting. Coral species respond differentially to the same treatments, highlighting the need for species-specific strategies in coral conservation efforts.

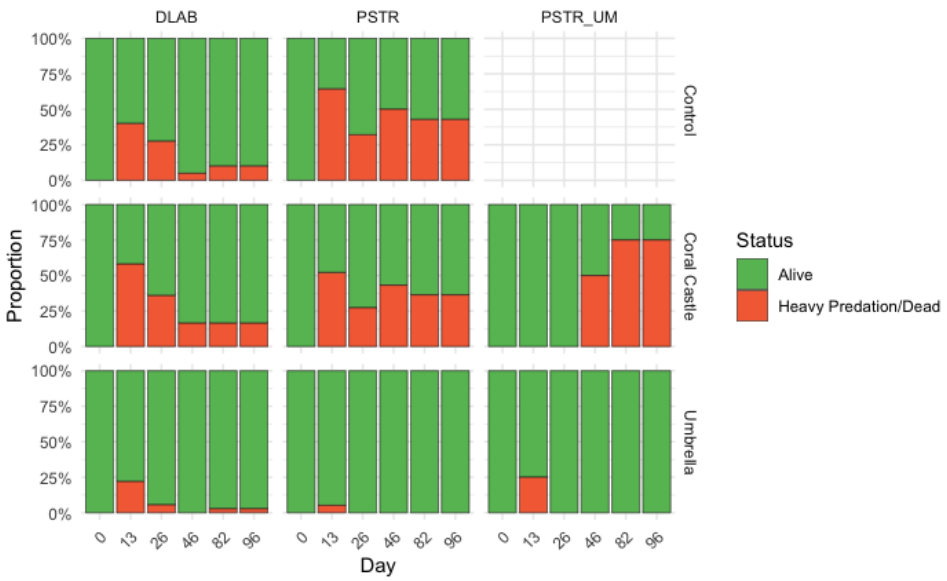

Figure 7. Proportion of *D. labyrinthiformis* and *P. strigosa* juveniles that survived in the first round of outplanting to the reef, using both the Coral Castle and Umbrella as predation deterrents.

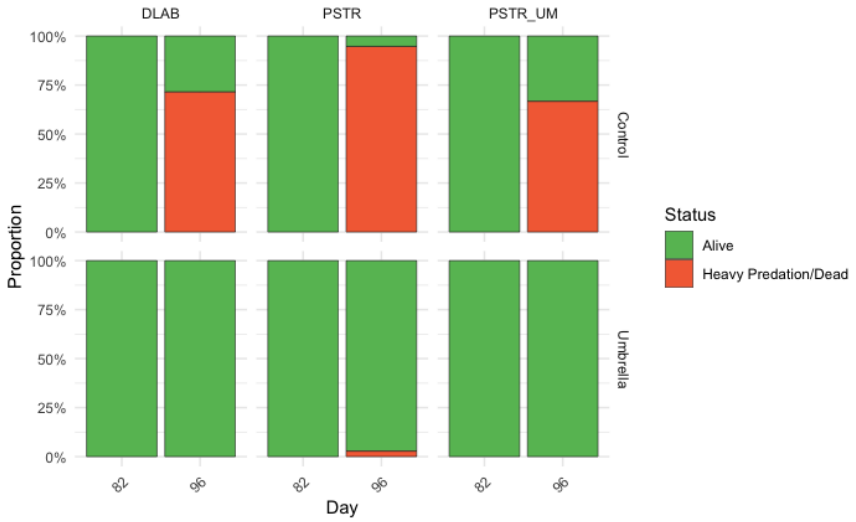

Figure 8. Proportion of *D. labyrinthiformis* and *P. strigosa* and UM *P. strigosa* juveniles that were acclimated at the Key Biscayne Nursery for three months prior to be outplanting. This figure is showing the first day of outplanting (Day 82) and two weeks after being outplanted (Day 96).

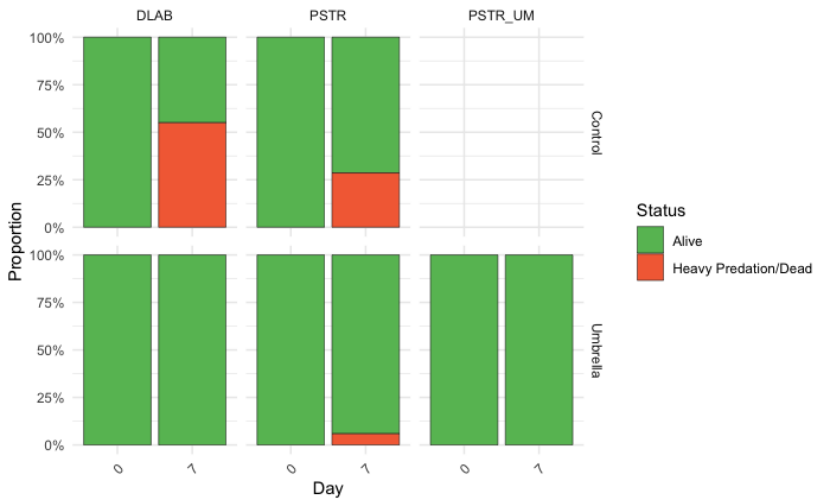

Figure 9. Proportion of *D. labyrinthiformis* and *P. strigosa* and UM *P. strigosa* juveniles that survived in the second round of outplanting to the reef, using the Umbrella as a predation deterrent.

Juvenile surveys

There were a total of four species found in surveys of Emerald Reef and nine species found on Paradise Reef (Figure 8). Both reefs were dominated by the genus *Siderastrea*, with *Siderastrea siderea* having the highest occurrence. This was expected, as brooding coral species tend to produce more juveniles than spawning corals throughout the year. One divergence in the two reefs is that Paradise Reef had a high number of *Porites astreoides* individuals, while Emerald Reef had more M*ontastrea cavernosa.* The reason for the high number of *M. cavernosa* individuals is likely due to the fact that the randomly-positioned transect line did pass close to a large adult colony.

Using the diameter measurements from the surveys, the area of each juvenile coral was calculated assuming that each individual is circular in shape. The size of juveniles between reefs was not found to be significantly different (Wilcoxon, p >0.5) (Figure 9).

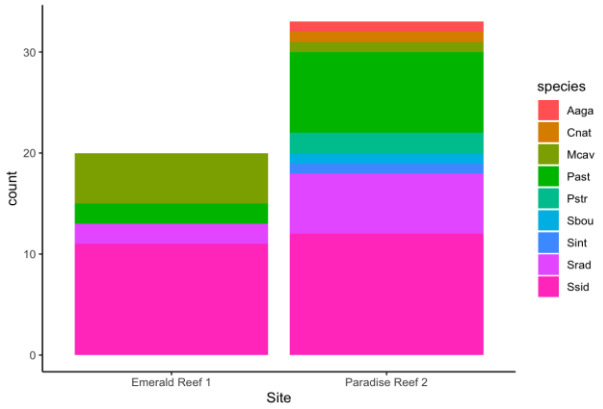

Figure 10. Number of coral recruits recorded by transects at each site, by species.

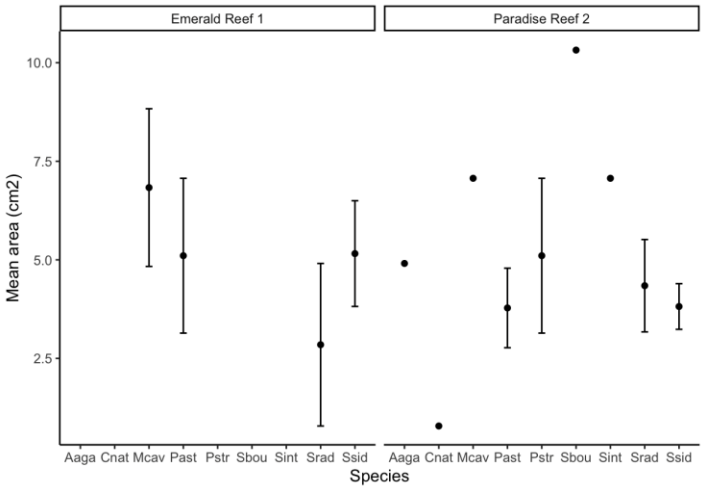

Figure 11. The mean and standard error of recruit area in the juvenile surveys at two reefs.

We have been successful in obtaining funding from FWC (State Wildlife Grant) to continue this project in 2025 and 2026, and we will settle, rear, and outplant 200 individuals from *Colpophyllia natans, D. labyrinthiformis,* and *P. strigosa* at different ages (i.e., 4 and 8 months) onto reefs in Miami-Dade County (and put half at the nursery). We will monitor restoration sites for survival, health, and growth post outplanting and compare survival, health, and growth of outplants to continue studying the question of how and when to best outplant juvenile corals.

Task 5 - Exploring site characteristics to optimize outplant success in the Coral ECA

Restoration has the potential to maintain species populations and genetic diversity, but spatial variability in outplant growth and survival is apparent at multiple scales and creates a barrier to long-term success. Identifying spatial, biological, and habitat characteristics that optimize outplanting success is needed to improve outplant design, particularly as restoration practitioners look to scale up propagation activities. A major determinant of outplant success is choosing the optimal location for outplanting. This occurs at two distinct spatial scales: sites are the broad locations for outplanting (10 to 100s of meters), arrays are distinct plots within sites (meters). Sites act as proxies for temperature, sedimentation, and eutrophication, which can directly influence coral survival. On smaller spatial scales, biological and habitat characteristics around an area, such as wild colony density, size structure, or habitat complexity may indicate the long-term suitability of a site for colony survival and growth. Overall, quantitatively evaluating outplant survival in relation to biological and topographic factors may reveal characteristics that increase outplant success and can guide future restoration efforts.

Since late 2019, the Coral Reef Restoration, Assessment and Monitoring (CRRAM) lab at NSU has outplanted >5,000 *A. cervicornis* fragments, microfragments, and sexual recruits across 11 sites in Broward County, Florida. Within these sites, outplants are attached to the substrate in localized arrays. In this project, variability in outplanting success was assessed by statistically analyzing outplant survival, in relation to spatial,

biological, and habitat characteristics. We specifically asked: 1) Are there broad-scale spatial characteristics which enhance outplant survival (i.e., between sites)? 2) Are there specific spatial, biological, and habitat characteristics which increase survival and limit breakage (i.e., within sites)?

5,647 *Acropora cervicornis* fragments and 2,630 microfragments or sexual recruits (subsequently termed boulder corals) were outplanted in 73 arrays at 11 sites in Broward County, Florida (Fig. 12). Subsequent monitoring to quantify survival was conducted for 1-3 years and survivorship was calculated annually per species at each array and each site. *A. cervicornis* breakage prevalence was quantified during multiple monitoring events conducted during the first-year post outplanting.

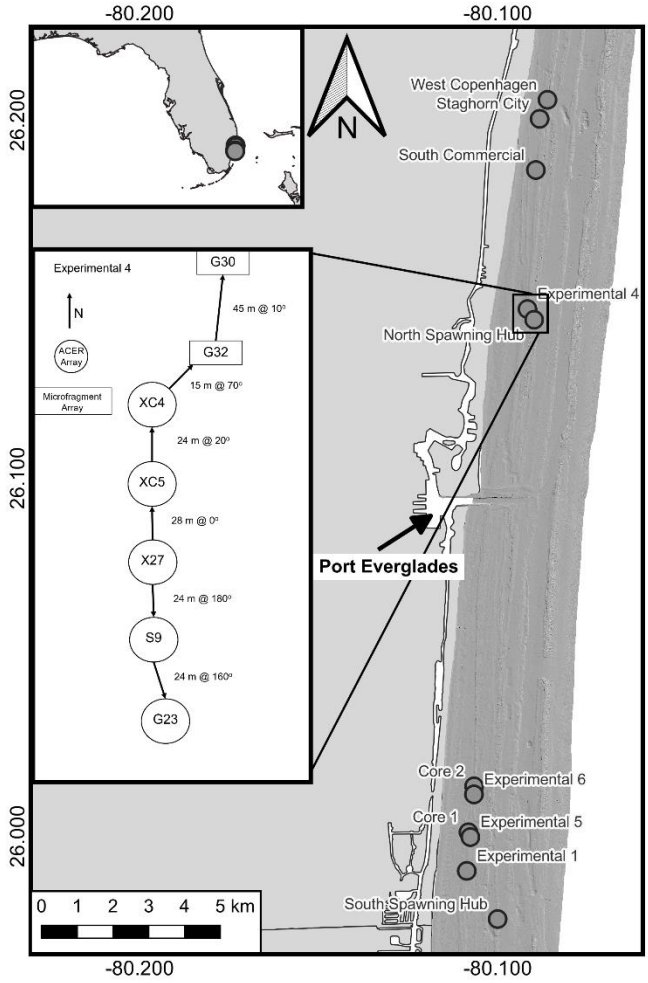

Figure 12. Outplant locations in Broward County Florida; Main: Site locations; Inset top: Study location; Inset Middle: Example site design. Multiple arrays are found at each site, *Acropora cervicornis* arrays (ACER) are labeled with circles, boulder coral arrays are labeled with rectangular boxes. Distance and bearings between arrays listed for reference.

Broad-scale spatial characteristics, latitude, site depth, the distance from shore, and the distance from Port Everglades were measured in QGIS. Specific biological and habitat

predictors were quantified within a 5-meter radius circular plot surrounding each outplant array (Fig. 13) and along a 20 x 1m belt transect running offshore from the array center pin. Within the plot, each stony coral colony (≥4 cm diameter) was identified to species and tallied by size class (4-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 50+ cm). Stony coral density, wild *A. cervicornis* colony density, Shannon-Weaver diversity index, species richness, and evenness (Pielou's J) were then calculated. Log_{10} skewness, kurtosis, and coefficient of variation (CV) of the coral colony size structure were calculated from the mean diameter within each size class. Rugosity was measured by laying out a 10-m rugosity chain along two fiberglass tapes which defined the plot, one running north to south, one running east to west through the center pin. The rugosity index was then calculated (rugosity index = mean (distance covered on tape/length of chain)). Along the 20 x 1 m belt transect, sediment depth was measured every meter, and the maximum substrate height was measured within each 1 m^2 block. From these, the maximum and mean sediment depth and relief height were calculated.

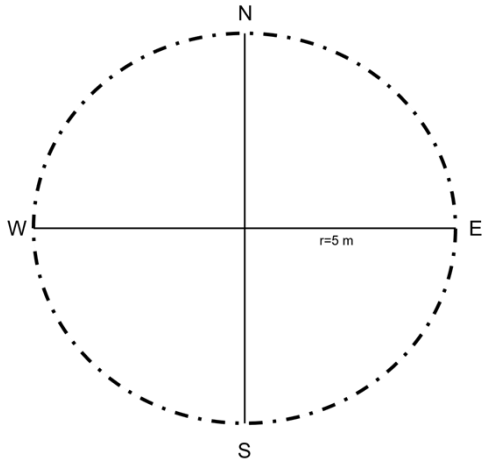

Figure 13. Plot design. Biological and habitat characteristics were collected within the 5 m radius (~78 m^2) plot. The center will be around the center pin marking the outplant array. Rugosity measurements were taken along both the N -> S and E -> W transects.

The relationship between outplant survival prevalence in year 1 (*Acropora cervicornis* or boulder coral) and broad-scale spatial predictors were analyzed at the site level using a Generalized Linear Model (GLM) and a Generalized Linear Mixed Model (GLMM) respectively.

The relationship between the response variables, *A. cervicornis* survival prevalence in year 1, boulder coral survival prevalence in year 1, and *A. cervicornis* breakage prevalence, and the spatial, biological, and habitat predictors were analyzed at the array level in a two-stage process. First, a random forest regression model was used to identify the most important predictors in variation in each response variable. Second, these predictors were modeled using GLMMs, which incorporated any potentially meaningful interactions. If a predictor had evidence of a quadratic relationship in the random forest model, identified from partial regression plots, a centered quadratic term was included in the GLMM. Site was fitted as a random effect in each model and species fitted as a random

effect in the boulder coral model. Model selection was determined using the Akaike Information Criterion (AIC) from multiple candidate models.

Acropora cervicornis fragment survival prevalence was 77.8% (±16.5 SD) after one year, 62.9% (± 22.6 SD) after two years, and 59% (± 20.4 SD) after three years (Figure 3). At the site level, *A. cervicornis* survival declined with distance from shore (GLM, $p = 0.03$) and increased with the distance from Port Everglades (GLM, $p = 0.007$). Boulder coral survival varied strongly by species and site (Fig.14). After one year, outplant survival ranged from 40.6% for *Stephanocoenia intersepta* to 100% for *Colpophyllia natans*, although the latter only had two fragments. Boulder coral fragment survival significantly declined with latitude (GLMM, $p = 0.0003$) and varied strongly by species (conditional $R^2 = 0.19$, marginal $R^2 = 0.07$).

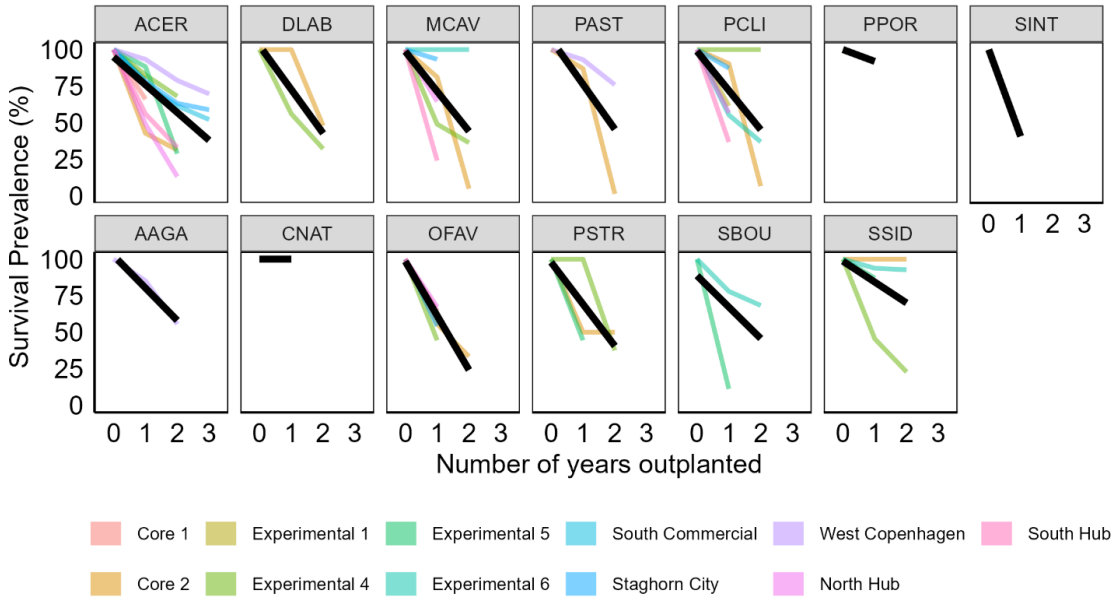

Figure 14. Survival prevalence by species over time. Each colored line represents the mean survival prevalence per site and the solid black line is the mean GLM estimate for all sites. Panel titles represent the species four letter code with the first letter genus and first three letters of the species (e.g., ACER = *Acropora cervicornis*).

Acropora cervicornis fragment survival (Fig. 15a) and breakage (Fig. 15b) also varied widely by array. Random forest regression analysis found that CV, species richness, mean relief height, distance from shore, distance from port, outplant attachment material, and outplant method most strongly influenced *A. cervicornis* survival ($R^2 = 28.4\%$). *A. cervicornis* survival probability was 4 times higher if attached by cement than epoxy (Tukey pairwise, $p < 0.0001$) and significantly higher if attached as a single fragment than a cluster (Tukey pairwise, $p = 0.01$). *A. cervicornis* survival probability increased significantly with distance from Port Everglades (GLMM, $p = 0.002$), had a significant declining quadratic relationship with CV and species richness (GLMM, $p < 0.0001$), and a significant interaction between CV and species richness (conditional $R^2 = 0.26$, marginal

R^2 = 0.18). At mean or below mean CV, *A. cervicornis* declined with species richness but increased with species richness when CV was above average (Fig. 16).

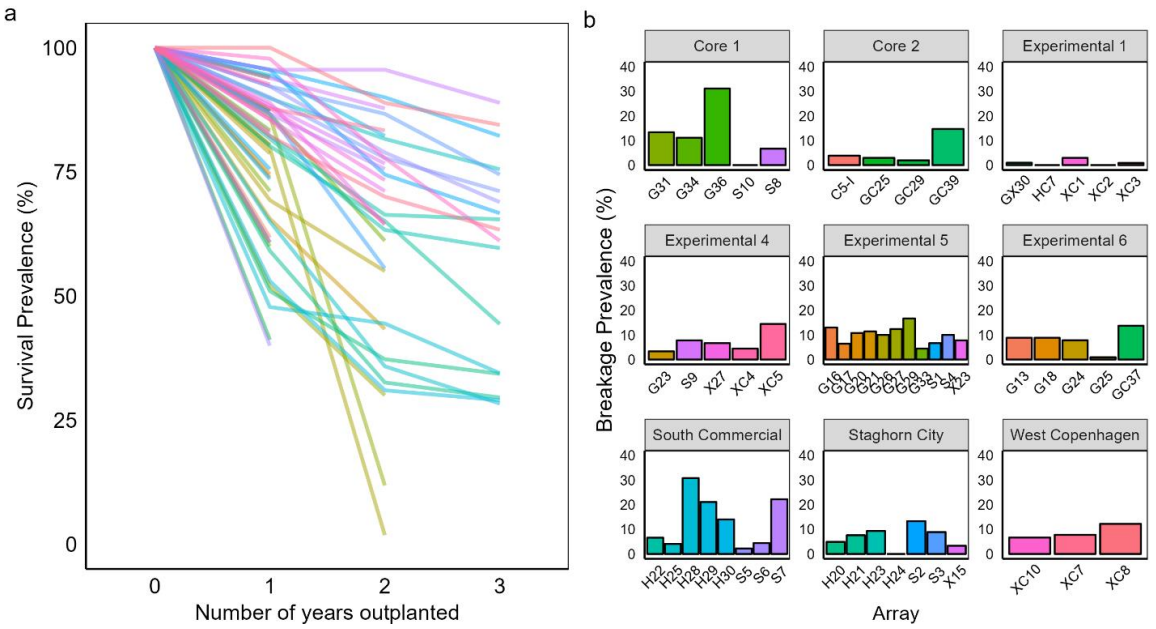

Figure 15. *Acropora cervicornis* a) survival prevalence over time by array; b) Breakage prevalence in year one by array. Each panel represents a site. Colors are consistent between arrays.

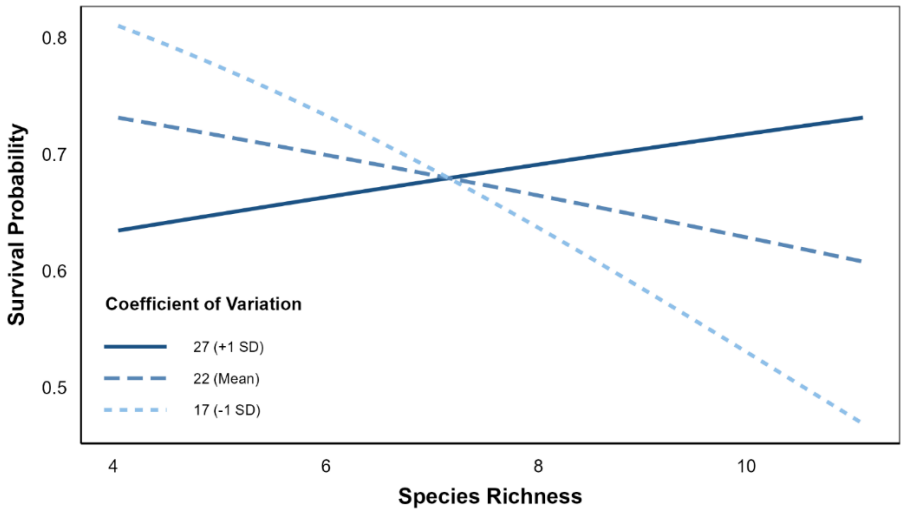

Figure 16. Interaction plot showing the significant relationship between *Acropora cervicornis* survival prevalence and the interaction between species richness and the stony coral community coefficient of variation (GLMM, $p < 0.0001$). Each line shows the relationship between species richness abundance and survival probability at the mean value, 1 SD above the mean and 1 SD below the mean coefficient of variation.

Acropora cervicornis breakage was most strongly influenced by colony abundance, evenness, and maximum relief height, but these only explained a small amount of variation (random forest regression, R^2 = 3.2%). There was a significant interaction between colony

abundance and evenness (GLMM, p = 0.01; conditional R^2 = 0.13, marginal R^2 = 0.03), with an increasing exponential relationship between breakage probability and colony abundance as evenness increased (Fig. 17).

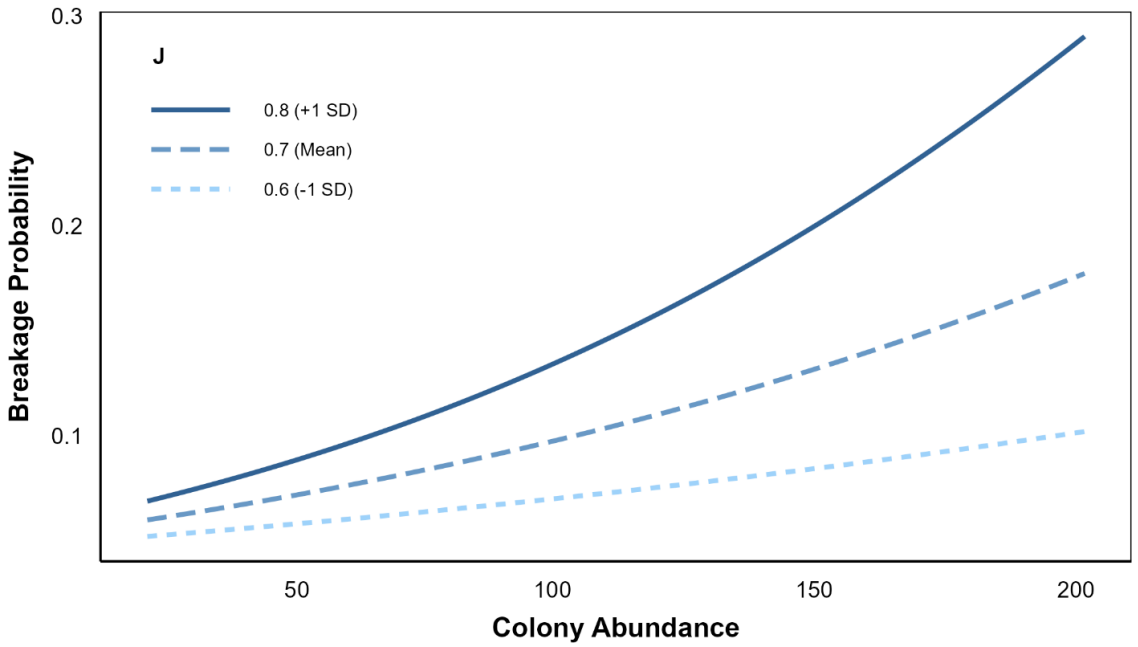

Figure 17. Interaction plot showing the significant relationship between *Acropora cervicornis* breakage prevalence and the interaction between stony coral adult abundance and evenness (Pielou's J; GLMM, p = 0.01). Each line shows the relationship between colony abundance and breakage probability at the mean value, 1 SD above the mean and 1 SD below the mean evenness score.

Boulder coral survival was most strongly influenced by species, latitude, colony abundance, rugosity, mean relief height, and distance from shore (random forest, R^2 = 27.0%). Boulder coral survival declined with colony abundance (GLMM, p = 0.002), there was a significant negative relationship with the mean relief height quadratic (GLMM, p = 0.003) and there was a significant interaction between colony abundance and mean relief height (GLMM, p = 0.002; Fig. 18). At or below mean relief height, fragment survival declined with colony abundance, but increased with colony abundance when mean relief height was above average.

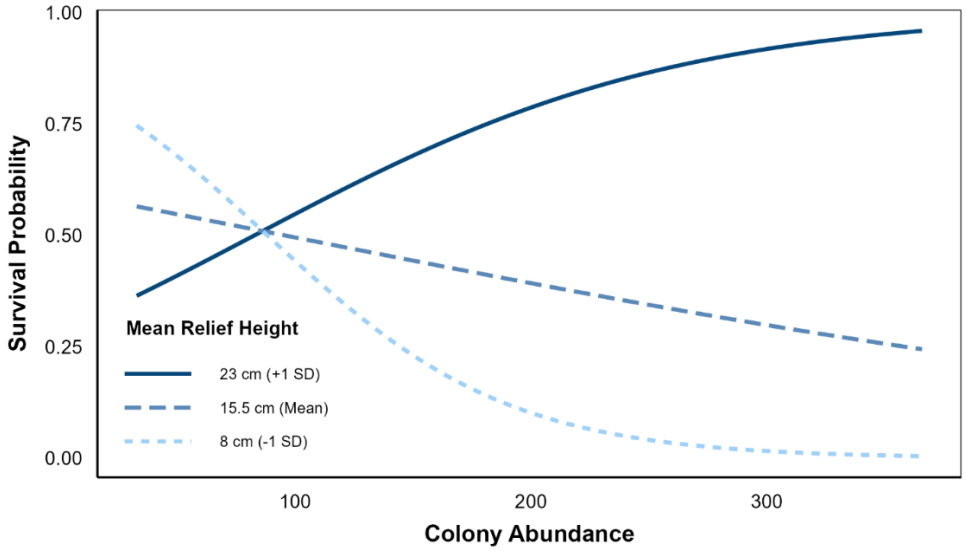

Figure 18. Interaction plot showing the significant relationship between boulder coral survival prevalence and the interaction between stony coral adult abundance and mean relief height (GLMM, p = 0.002). Each line shows the relationship between colony abundance and survival probability at the mean value, 1 SD above the mean and 1 SD below the mean relief height.

Acropora cervicornis fragment survival was significantly higher at sites close to shore and further from Port Everglades while sites further south increased boulder coral survival. *A. cervicornis* fragment survival was four times higher when attached with cement than epoxy and was substantially higher if species richness was higher and there was a large spread in colony size structure or if species richness and coefficient of variation were both low. The presence of wild *Acropora cervicornis* colonies was not found to be an important determinant of fragment survival after one year. *A. cervicornis* fragment breakage was marginally higher as colony abundance increased, and increased exponentially with colony abundance, particularly when evenness was above average, but it only explained a small portion of the variation. This suggests more study is needed to identify the causes of fragment breakage, which limits growth. Boulder coral survival varied widely between species and was significantly influenced by the interaction between colony abundance and mean relief height. Survival probability was particularly high when mean relief height was above average and colony density was above three colonies m^{-2}. Future studies should assess whether these predictors also influence survival probability elsewhere on Florida's Coral Reef.

Task 6 - Maintain coral nursery and reskin large corals

Over 300 corals >2 m in length have been documented in southeast Florida's nearshore habitats, the majority being *Orbicella faveolata* with some dating up to 320 years old (Walker & Klug, 2015). The age and size of these colonies indicate their resilience, having survived numerous natural and anthropogenic pressures. However, since 2015, many have died or lost >90% of their living tissue due to a variety of intrinsic and extrinsic factors, most recently stony coral tissue loss disease (SCTLD) (Walker & Klug, 2015). Once a colony dies, its surface becomes colonized by other organisms, and bioerosion is accelerated (Toth et al 2023).

In response to the degradation of Florida's Coral Reef, coral restoration techniques have become increasingly widespread and shifted to restoring boulder species such as *O. faveolata* through microfragmentation. Microfragmentation is a technique used to propagate massive slow-growing boulder species by cutting them into smaller identical clones, which stimulates a rapid growth response (Forsman et al., 2015; Page et al., 2018). These fragments can then be transplanted onto the reefs to produce new colonies. Yet, restoring the reefs comes with many challenges and best practices for propagating and outplanting corals are still being developed.

One of these challenges is fish predation on outplanted colonies. Complete removal of fragments smaller than 2 cm^2 has been observed within the first week of deployment and significant tissue damage has also been reported on larger fragments (>2 cm^2) within the first 2 weeks. Predation exclusion devices such as caging corals have been used to deter fish within and beyond this critical window (Rivas et al., 2021). However, these devices require significant labor costs post-transplant to maintain and clean the devices of algae and other fowling epiphytes. A newly innovated device constructed of biodegradable polyhydroxyalkanoate (PHA) has shown initial success in the protection of outplanted fragments in their vulnerable initial months and reduced field maintenance costs post-transplant (Pisano, 2023).

The purpose of this project was to assist with the reproduction and propagation of previously identified and mapped, large (≥ 2 m diameter) *O. faveolata* colonies in the Kristen Jacobs Coral Reef Ecosystem Conservation Area. Monthly SCTLD monitoring and treating of over 100 large corals revealed impacts to these corals, where smaller pieces were recently broken off. These activities harvested the recently broken pieces of the resilient colonies as corals of opportunity (COOs), propagated smaller pieces via microfragmentation, and strategically outplanted them to increase the chances of successful sexual reproduction. Outplants were used to try and restore the surfaces of other previous large colonies that recently died from SCTLD to living structures once again. Effects of genotype, region, and predator exclusion devices were also tested. To examine if PHA predation exclusion devices limit predation on the colonies, 140 *O. faveolata* fragments were outplanted on two colonies off Hollywood Beach (70 fragments per colony). Five genotypes were selected to examine variation in survival and growth. This report summarizes the field collection, ex-situ restoration, and outplanting efforts of all genotypes selected for restoration (LC-114, LC-056, LC-119, LC-124, and LC-041).

COO Collections:

This work was conducted under Special Activity Licenses SAL-23-2515-SCRP and SAL-24-2515-SCRP. In total, 17 COOs were collected from offshore expeditions along the SE Florida coast between Fort Lauderdale beach and Key Biscayne (Table 1). The

collection protocol changed throughout the project several times as sources, survivorship, and lead personnel shifted. In general, corals collected were brought up to the surface, measured, labeled, wrapped in bubble wrap, and housed in an insulated cooler for the 30-60-minute transport to shore. The water inside the cooler was changed every 15 minutes or if it deviated ±5 F. Once onshore, corals were dipped in Lugol's solution for 15 minutes – 1 hour as described in the product's instructions, photographed, and drip acclimated into their ex-situ Seacor nursery tanks for quarantine.

Table 5. COO collections for genotypes LC-114, LC-056, LC-119, LC-124, & LC-041.

Date Collected	Label	Spp.	#	Depth (ft)	Tank Temp	Treatment
2/8/2022	LC-041	OFAV	1	18	77F	Lugol for 15min
2/10/2022	LC-124	OFAV	1	23	77F	Lugol for 15min
10/10/2022	LC-114	OFAV	2	19	78 F	Lugol for 15min
10/11/2022	LC-114	OFAV	9	19	78 F	Lugol for 15min
10/21/2022	LC-056	OFAV	1	17	77 F	Lugol for 15min
1/10/2023	LC-119	OFAV	1	27	77 F	Lugol for 15min
8/2/2023	LC-114	OFAV	1	19	78 F	Lugol for 1 hour
1/10/2024	LC-056	OFAV	1	17	78 F	Lugol for 15min

Husbandry Conditions ex-situ:

The outdoor Seacor systems of the NSU coral nursery are in independent 120-gallon acrylic tanks, covered by two layers of rain-resistant shade cloth (80% and 50%) with an optimal PAR reading of 50-200 µmol m^{-2} s^{-1} (Fig. 19). Each tank's salinity is maintained at 35-36 ppt using bi-diurnal reverse osmosis water changes to replace evaporated water. A submersible heater and a chiller are used to maintain the desired temperature of 77° F. Water quality tests are performed weekly to maintain an alkalinity of 7.1-7.7 dKh, with other tests prescribed as needed. Algal growth is controlled using protein skimmers, herbivorous snails and urchins, and manual removal weekly to minimize coral-algae competition.

Microfragmentation:

In total, 27 ramets (large pieces and small-fractured pieces) were cut into 436 fragments during the project (Table 6). Microfragmentation was conducted according to a modified version of the methods outlined in Forsman et al., 2015. In general, microfragment pieces (or "Frags") were cut into 1 – 4 cm² fragments using a Gryphon© 37" AquaSaw Diamond Band Saw under a sunshade (in days of high solar irradiance), glued to 1 - 1.25-inch diameter ceramic and limestone pucks, and grown in the above-described recirculating tanks until they reached sufficient size and health for outplanting. The provenience of each piece was recorded to allow using micro-fusion techniques and produce larger size colonies to use in future restoration efforts. Initially, pieces were cut into 1 cm² fragments, but due to significant mortality, the area was increased to an area of 4 cm² in May 2022. Survivorship increased as a result.

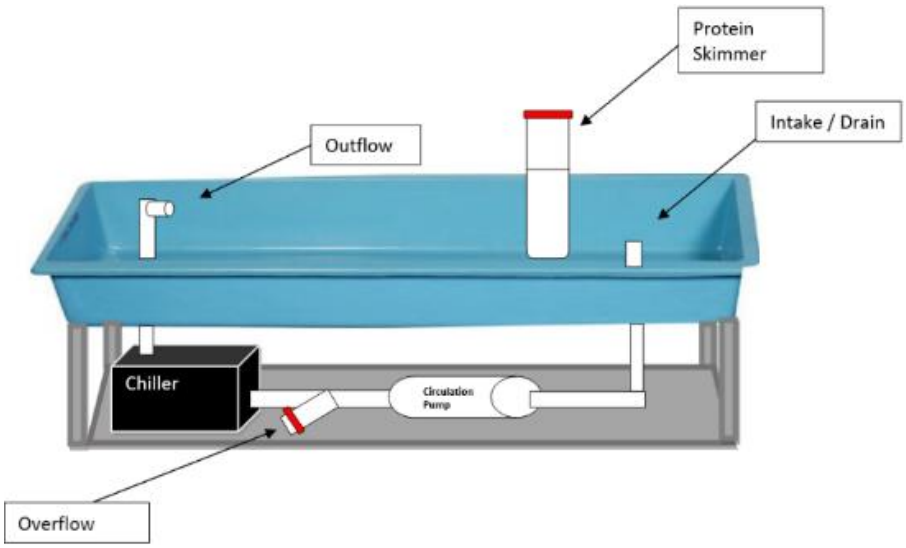

Figure 19. Seacor Tank Diagram.

Table 6. Fragment treatment schedule.

Date	Spp.	Label	Ramet	Made	Treatment
10/28/2021	OFAV	2-119-B	1	3	Frag Recover
3/14/2022	OFAV	LC-124	1	2	Lugol
3/24/2022	OFAV	LC-041	1	3	n/a
5/10/2022	OFAV	LC-041	1	18	Lugol, Frag Recover
5/10/2022	OFAV	LC-124	1	15	Lugol, Frag Recover
10/26/2022	OFAV	LC-114-A	1	26	Frag Recover
11/11/2022	OFAV	LC-114-B	3	72	Frag Recover
12/22/2022	OFAV	LC-114-C	9	51	Lugol, Frag Recover
12/22/2022	OFAV	LC-056-A	1	31	Lugol, Frag Recover
1/24/2023	OFAV	LC-114-D	1	40	n/a
2/7/2023	OFAV	LC-124-A	2	45	n/a
6/14/2023	OFAV	LC-114-E	1	22	n/a
6/14/2023	OFAV	LC-124-B	1	43	n/a
6/15/2023	OFAV	LC-114-E	1	22	n/a
6/15/2023	OFAV	LC-041-B	2	43	n/a

Outplanting:

Two denuded colonies, LC-010 and LC-011 located off Hollywood Beach, Florida, were selected as outplant colonies. LC-010 and LC-011 are approximately 81.07 m from each other, with LC-010 located slightly north of LC-011. Both colonies had a smoother surface texture, low presence of *Cliona* and disease, as well as low live tissue and benthic coverage. In 2023, COOs were outplanted on these colonies by flushing the fragments directly to the colony skeleton. No predation exclusion devices and significant fish predation and removal of fragments was observed. To assess a different outplanting

method, fragments were outplanted on cement convex domes and half of the domes were designed with the PHA predation exclusion devices to test the effectiveness in limiting predation on these colonies.

Twenty cement convex domes constructed with Portland Limestone Cement Type 1 L (Titan America) cast in an 18 cm-diameter, 2 cm-high plastic bowl was determined as the easiest and most effective way to create the outplanting molds (Fig. 20). Domes were designed so that a circular array of seven fragments of the same genotype could fit countersunk within it. This design gave the best chance that the fragments will fuse together and quickly create a reproductive colony. Ten of the domes were constructed with 22.86 x1.27 cm biodegradable PHA straws. The straws were shortened to 15 cm and cast as part of the dome to ensure robustness. A Standard Operating Procedure manual was developed. Divers were trained on the protocols before outplanting. After outplanting, fragments were monitored at 48 hours, 1 week and 2 weeks. Photos were taken at each monitoring event and predation, survivorship, disease, paling, or bleaching were noted if present. Damage to the PHA straws was also reported.

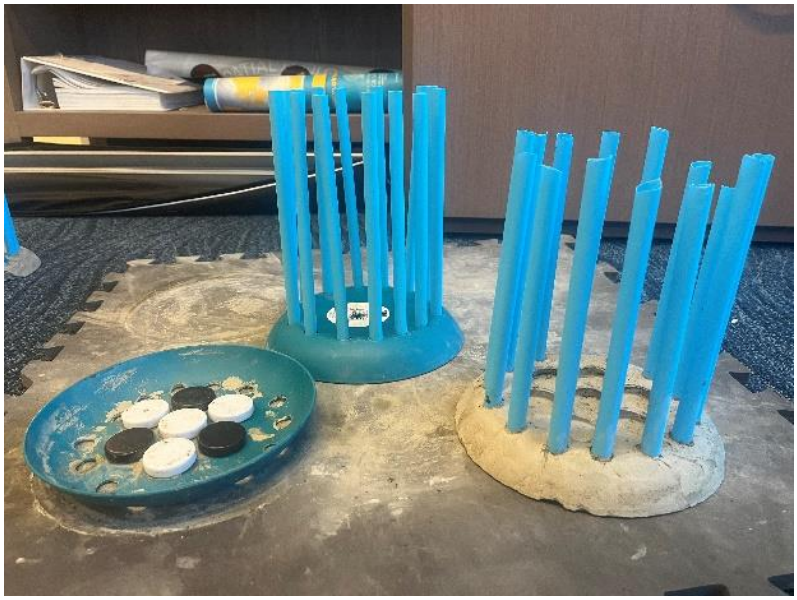

Figure 20. Process of creating cement domes fitted with PHA straws.

On March 21, 2024, LC-010 and LC-011 were visited and prepped for outplanting. Surveys to assess disease within the areas were conducted. No disease was reported for either area. Colonies were also prepped using an underwater Nemo angle grinder to smooth and clear benthic fauna from the surface of the colony. On April 30, 2024, a Coral Nursery Special Activity license was issued to Brian Walker for the purposes of harvest, propagation, and release of marine organisms for coral research and restoration. On May 2, 2024, NSU received a coral health certification for 271 *O. faveolata* fragments. On May 3 and May 6, 2024, 7 fragment plugs were epoxied flushed to each of the 20 cement convex domes using 2-part Apoxie Sculpt Modeling Compound (Fig. 21). The cement domes were then placed in ex-situ nursery tanks until ready to outplant. On May 7, 2024, 140 fragments were outplanted to the two large coral skeletons, LC-010 and LC-011 (Fig. 22). Microfragments

were transported on racks in an insulated cooler filled with *ex situ* nursery water to the outplanting sites. Ten cement domes were outplanted on each skeleton totaling 20 arrays. Five genotypes were outplanted on each skeleton (two arrays per genotype). Half of the arrays were designed with the PHA predation exclusion devices to examine the effectiveness of limiting fish predation on the colonies.

Figure 21. *O. faveolata* fragments epoxied to cement outplant domes.

Figure 22. Arrays outplanted on LC-011 (left). Photo of graduate research assistant, Alex Wagner, securing cement domes to LC-011 (right)

Preliminary results indicated that 100% of the fragments remained attached and 14% showed signs of minor predation with scrapped or subtle damage on a few polyps after 2 weeks (Figure 5). Minor predation was observed on the unprotected controls (Figure 6). The proportion of fragments with predation was significantly different between 48 hours and 2-week monitoring period (p value=0.016). There was no significant difference in proportion of fragments with predation between colony or genotype. The majority of the PHA straws remained attached to the dome after 2 weeks with only one straw becoming unattached during initial transport to outplanting site.

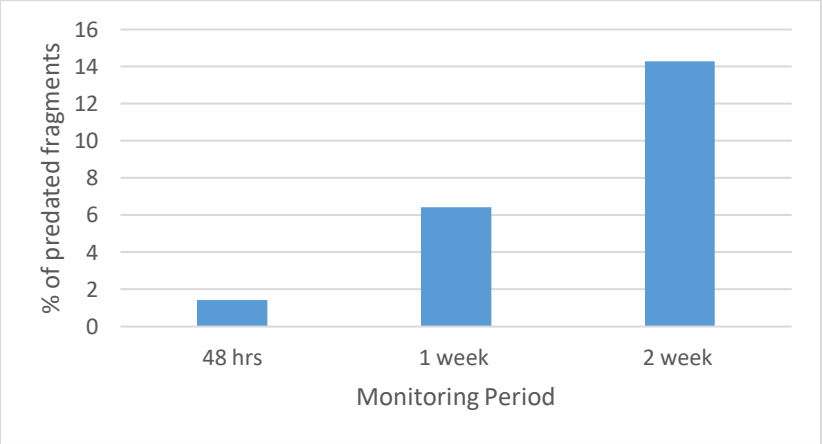

Figure 23. Percentage of total predated fragments for LC-010 and LC-011 colonies.

Figure 24. Photo of minor predation on colony LC-011 (left) and on colony LC-010 (right) after 2 weeks post outplant.

Restoring healthy coral tissue to these structures is vital to reinstate ecological functionality, stave off bioerosion, maximize chances of sexual reproduction, and produce a generation of disease-resistant coral. Evaluating and developing best practices for outplanting is important in scaling up *O. faveolata* restoration efforts.

Task 7 - Effects of probiotic bacteria on survival and growth of early life history stage corals

Microbiomes play a critical role in the health of coral holobionts (Rosenberg et al., 2007) and efforts to manipulate microbiomes to enhance resilience are becoming increasingly recognized as an important tool in the restoration toolbelt (Zhang et al., 2021). Treatment with probiotic bacteria can increase thermotolerance (Santoro et al., 2021), enhance disease resistance and reduce or stop disease lesion progression in corals (Ushijima et al., 2023). Many spawning corals do not develop their microbiome until after settlement. Therefore, early life history stages provide a good opportunity for microbiome manipulation.

The objective of this task was to determine the feasibility of using bacteria as settlement enhancers and/or probiotics during coral early life history stages for restoration by examining the growth and survival of <2-year-old corals treated with bacteria compared to untreated controls.

Colpophyllia natans spawned on September 7, 2023, at The Florida Aquarium and larvae were transported to the Smithsonian Marine Station on September 9, 2023 (Table 7). Larvae were maintained in polystyrene takeout containers (20 cm^2) in filtered seawater with water changes every 2-3 days until use in experiments.

Table 7. Source of larvae and recruits, species and age during experiments. Number of recruits at the beginning of each experiment are given for each treatment are given.

Experiment	Coral Species	Source	Age	Treatments
Early recruits	*C. natans*	The Florida Aquarium	Larvae to 3 months	1. Control (no treatment) (n=145 recruits) 2. Probiotic treatment during settlement (n = 39 recruits) 3. Probiotic treatment 6 weeks after settlement (n = 130 recruits)
1-year-old recruits	*C. natans*	The Florida Aquarium	1-year-old	1. Control (no treatment) (n = 21 recruits) 2. Probiotics treatment (n = 21 recruits)

Larvae (~110) were added to experimental containers (20 cm^2 polystyrene) in 400 mL of filter-sterilized seawater (FSW). Each container had two unconditioned and two conditioned settlement tiles from the Reef Institute. There were six replicate containers for each of two treatments: probiotics treatment during settlement, probiotics treatment at 6-week post settlement and 12 containers of untreated controls. There was no settlement after 48 h, therefore tiles and larvae were transferred to new, clean containers with 400 mL of seawater from an ex-situ coral habitat at the Smithsonian Marine Ecosystems Exhibit to encourage settlement. An additional ~30 larvae were added to each container and probiotics (*Pseudoalteromonas* sp. CNAT2-18.1) were added to the during settlement probiotics treatment. After 72 h, settlement was scored for all treatments and tiles were

rinsed and transferred to a common aquarium in FSW with aeration for holding prior to transferring to The Reef Institute 72 h later. At The Reef Institute, corals were held in a shared 300-gallon recirculating tank (Cradle) until the 6-week treatment took place on November 2, 2023. Probiotics were prepared and applied to corals as described in Ushijima et al. (2023).

Approximately 6 weeks after transferring the tiles to The Reef Institute, all tiles were placed in treatment containers (5 L aquaria) with aeration for the final treatment, 3 containers per treatment. Probiotics were added to the 6-week post-settlement treatment. Year-old recruits were also treated at this time, with untreated control aquaria set up as well. After 72 hours, tiles were rinsed and distributed to three holding tanks as evenly as possible: Sys4 (650 gallons), Sys2 (650 gallons) and Obs (100 gallons). Water quality parameters were monitored every 1-3 days; these data are summarized in Table 8.

Table 8. Summary of water quality parameters in holding tanks at Reef Institute from September 25, 2023 – May 15, 2024.

	n	mean	sd	median	min	max	se
Salinity (ppt)	554	35.5	1.5	35.5	24.0	39.8	0.1
Temperature (F)	554	77.1	5.5	77.4	7.9	86.0	0.2
pH	554	8.3	5.1	7.9	6.3	79.6	0.2
Alkalinity (dKH)	554	7.6	0.6	7.6	5.6	10.6	0.0
Calcium (ppm)	521	425.7	29.5	420.0	345.0	500.0	1.3
Magnesium (ppm)	509	1635.1	124.2	1680.0	1280.0	1960.0	5.5
Nitrate (ppm)	543	3.8	1.4	4.0	0.0	12.3	0.1
Phosphate (mg L^{-1})	544	0.1	0.1	0.0	0.0	0.2	0.0

Survival and growth were assessed every month from December to May, with the exception of February. At each time point, photos were taken of each live recruit and size was determined using ImageJ.

Early Recruits

Settlement was significantly lower in corals treated with the probiotic *Pseudoalteromonas* sp. CNAT2-18.1 compared to those without probiotics added ($p = 0.0279$, Figure 25).

Survival was assessed using a Cox proportional hazards model, incorporating probiotic treatment as a time-dependent variable and the initial number of spat per tile (initial count) and the grow out tank (tank) as covariates. Both initial count and grow out tank failed to meet the assumption of proportional hazards because the effects of these factors increase with time. The model was adjusted for the time-dependent coefficients and the resulting model indicated that there is no significant effect of treatment ($p = 0.1566$, Fig. 26). Early recruits had very low survival within the first three months of the experiment. Of the 329 (184 treated, 145 untreated) recruits that settled, only 12 (3.6%) survived to three months of age, therefore survival analysis was discontinued after the 3-month (85-day) assessment.

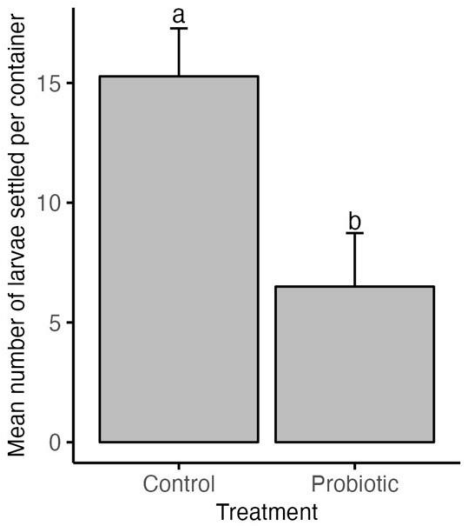

Figure 25. Mean ± SE number of *C. natans* larvae settled in containers treated with (Probiotic) and without (Control) probiotic. Letters above bars indicate significant differences between treatments based on a t-test, p = 0.0279, n = 6 for Probiotic, n = 12 for Control.

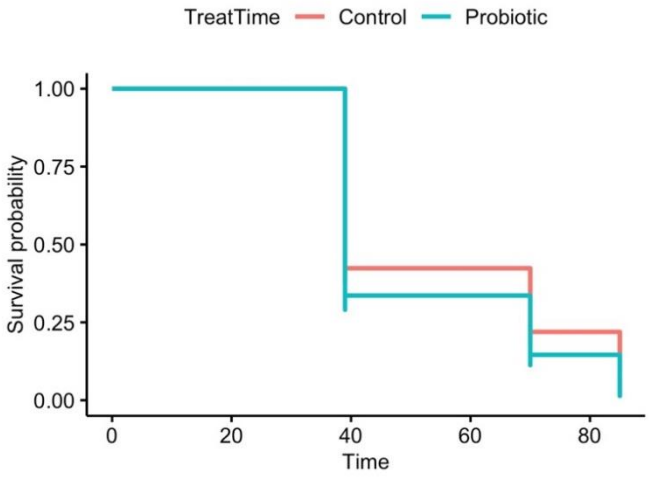

Figure 26. Survival probability over time of *C. natans* larvae treated with (Probiotic) and without (Control) probiotic. Survival was surveyed 39-, 70- and 85-days post settlement.

1-year-old recruits

A total of nine out of 42 one-year-old recruits died during this experimental period. Five were a result of light failure in one of the holding tanks and have been eliminated from the analysis. Photos were taken and ImageJ measurements of surface area were completed for the remaining 33 recruits monthly for 6 months (with the exception of month 3). Recruits grew significantly over time (Fig. 27, repeated measures ANOVA, p = 0.016), but there was no effect of probiotic treatment on recruit size (Fig. 27, repeated measures ANOVA, p = 0.117). There was no interaction between treatment and time on surface area (Fig. 27, repeated measures ANOVA, p = 0.692). Similarly, there was no effect of probiotic

treatment on overall growth rate from the beginning to the end of the experiment (Fig. 28, one-way ANOVA, p = 0.777).

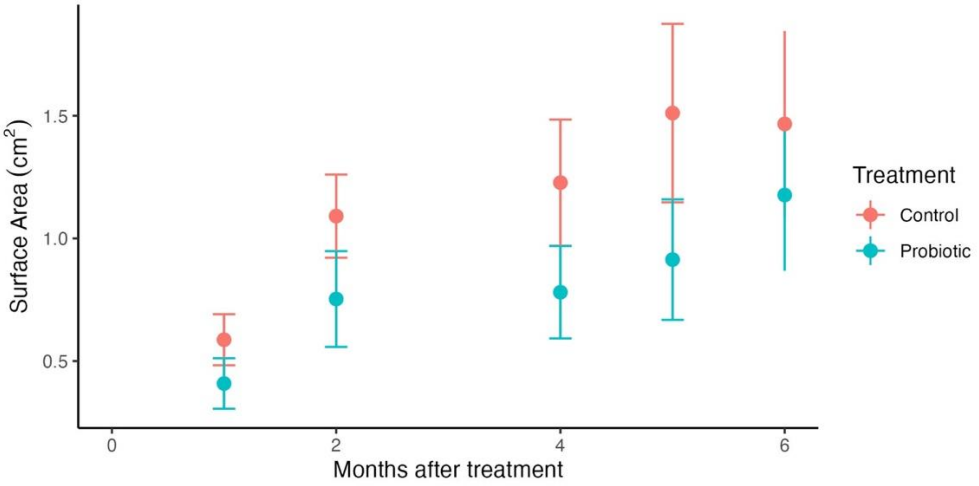

Figure 28. Mean surface area ± SE (cm^2) over time of 1-year-old *C. natans* recruits treated with (Probiotic) and without (Control) probiotic. Size was measured 1, 2, 4, 5, and 6 months after treatment. $N_{Control}$ = 16, $N_{Probiotic}$=17.

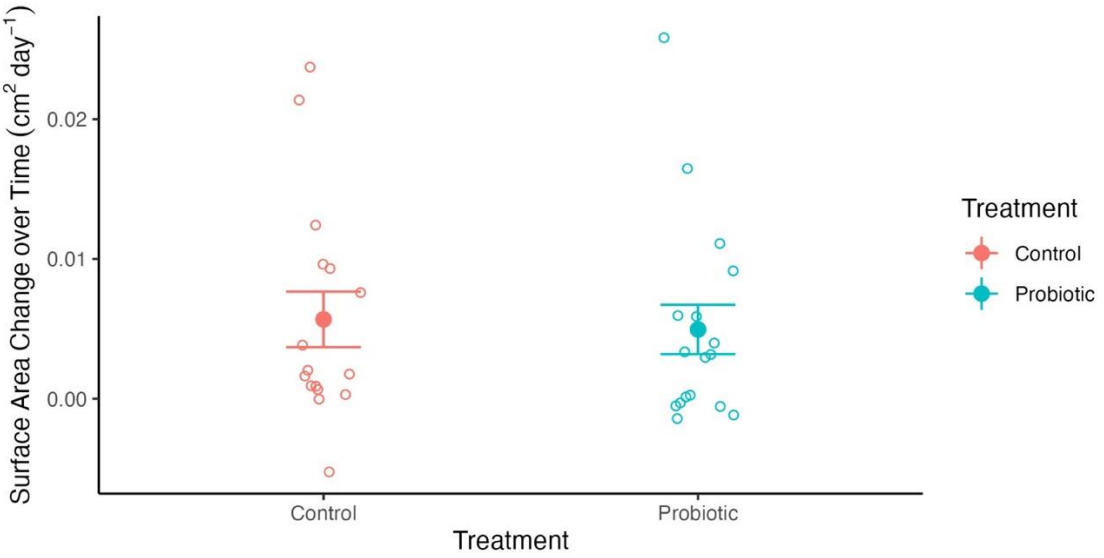

Figure 28. Total growth of 1-year-old *C. natans* recruits treated with (Probiotic) and without (Control) probiotic from month 1 post-treatment to month 6 post-treatment. Closed circles represent mean surface area change over time (cm^2 day^{-1}) ± SE. Open circles represent individual recruits. $N_{Control}$ = 16, $N_{Probiotic}$=17.

ImageJ analysis confirmed a significant negative correlation between recruit growth rate after 6 months and the number of recruits per tile (p < 0.001, Figure 29).

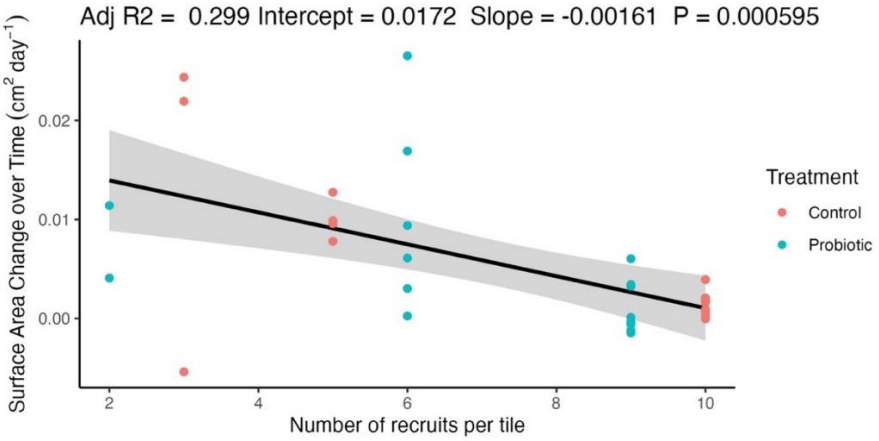

Figure 29. Linear regression showing the correlation between the number of 1-year-old recruits per tile and the growth rate of the recruits. Recruits treated with probiotics are shown in blue, and untreated controls are shown in red. N = 4 tiles per treatment, total number of recruits at 6 months = 33.

Pseudoalteromonas sp. CNAT2-18.1 has shown promise in laboratory and field assays as a potential probiotic to slow the progression of stony coral tissue loss disease. Treating early life-history stage recruits with this probiotic did not result in any benefit to the recruits in this study in terms of growth or survival, but it also did not have any detrimental impacts on the corals. In addition to previous studies that were done with adult corals, this provides evidence that treatment with *Pseudoalteromonas* sp. CNAT2-18.1 is safe for *C. natans* at early life-history stages as well. Assessing the disease susceptibility of recruits after treatment with probiotics was outside the scope of this project. However, the results presented here suggest that treatment at early life history stages is feasible. If, like adults, these recruits have some protection from disease, this could be beneficial in increasing the resilience of corals reared ex-situ for restoration.

These results also demonstrate that the timing of treatment is important. Treatment during settlement resulted in decreased settlement. Therefore, treatment should take place following the settlement and metamorphosis of corals.

Because of the overall poor survival in both control and probiotic-treated newly settled corals, it would be beneficial to repeat this portion of the study. This work should also be repeated with other coral species. Finally, the effects of probiotic treatment at early life-history stages on disease susceptibility should be investigated.

Task 8 - Midway Coral Nursery at the Phillip and Patricia Frost Museum of Science

The goal of this task was for The Phillip and Patricia Frost Museum of Science (Frost Science) to maintain the Midway coral nursery, adding 100 corals to the nursery, conducting presentations to South Florida community groups, and hosting an MPS intern.

With the primary goals of supporting ex-situ coral rearing, outreach to the public, and assisting the career development of UM interns, Frost Science installed a coral nursery, developed and conducted a PowerPoint presentation to community groups and hosted a

UM MPS intern for 6 months. These activities were managed and supervised by the Frost Science Conservation Team in close collaboration with the Husbandry department.

Frost Science Midway Nursery

After receiving permits from DEP, FWC, and the Army Corps of Engineers, we installed the Midway nursery halfway between UM's Rescue a Reef nursery and NOVA Southeastern's nursery off Port Everglades. The Midway Nursery lies approximately 3 miles north of Haulover Inlet in North Miami and approximately 1 mile offshore. This location provides a midway point between two existing coral nurseries to facilitate coral rearing and outplanting in the north Miami/south Broward region. It is also less than a quarter mile from an existing natural reef area that is planned to be utilized as an outplanting site and spawning hub. Work completed to date includes the installation of ten coral nursery trees and regular maintenance of the trees. As per many discussions with DEP, even though we were permitted by FWC to install the nursery, we are still waiting for their permit to allow us to place corals in the nursery. It is anticipated that we will receive this permit in the very near future as the application was submitted over 1 year ago. Once we receive our coral permit, we will begin relocating coral recruits settled on SECORE substrates last summer that are currently being held in our wet lab facility. We will also relocate corals of opportunity from nearby Graceland Reef in north Miami and, when available, relocate corals from UM, NOVA, and Reef Renewal.

Community Presentations

To facilitate a better understanding of issues facing Florida's Coral Reef and to encourage active participation in its protection and restoration, Frost Science has developed a PowerPoint presentation for local community groups. Talks have been conducted and are continuing to be conducted to engage this important stakeholder group. Once our FWC permit is in hand we will amend the presentation to include information on how stakeholders can assist in maintaining the Midway Coral nursery through an Adopt a Tree program. We anticipate volunteer divers and dive shops participating in this program.

The significance of this work is still to be fully realized, though the installation of the nursery and its subsequent maintenance has laid the groundwork for future efforts. Once corals are held and grown out in the nursery, outplanting to the north Miami and South Broward reef areas will be significantly enhanced. In addition, the nursery will provide an opportunity for comparison of grow-out techniques including the "halfway house" holding of coral reared in the lab but not yet ready for full outplanting on the reef. Finally, the outreach and public engagement of this effort will provide significant opportunities for active participation by stakeholders in helping to restore local reefs.

The next steps for this project are to continue to pursue our FWC coral holding permit; to continue to maintain the nursery, and to continue to engage the public through talks and presentations. We have already designed a study for SECORE settled substrates to compare in-situ and ex-situ growth and condition between our wet lab and the nursery once permits are issued.

Task 9 - Internship program

The Southeast Florida Coral Reef Restoration Hub recruited eleven internship positions across the four partner organizations. Each of the positions was for six months and varied in start date due to organizational needs. Of the twelve interns, six graduated from the MPS program by May 2024, two interns are slated to graduate by the Summer or Fall of 2024 due to delayed starts, and three interns transferred from the MPS program to the thesis-based master's program and are planning to graduate in the Fall of 2024. In addition remaining internship funds were used to support the first month (June 2024) of the FY25 DEP intern cohort (these internships generally begin June 1st, but FY25 DEP funds cannot be incurred before July 1st), solving the problem of the mis-alignment between internshop start dates and funding start dates that has complicated internship recruitment since we began the hub internship program.

The following students graduated from the MPS program in Fall 2022 and presented their internship projects:

1. Alex Pitre (NSU – Figueiredo): *Testing vital stains as an effective way to track early life stages of Caribbean Corals*
2. Eliana Galindo (SECORE – Miller): *Increasing early-stage post-settlement survivorship of corals: a co-culture experiment*
3. Julia Cafiero – (UM – Baker): *Optimizing solar irradiance for the survival and growth of corals in an outdoor land-based facility*
4. Victoria Stewart (NSU – Renegar): *Examining the effects of toluene or MC252 oil on* Porites divaricata
5. Sam Schneider (UM – Baker): *A non-invasive method for identifying algal symbionts (Family Symbiodiniaceae) in scleractinian coral recruits*
6. Samantha Thomas (Frost Science – Akins): *The evelopment of a Florida Coral Reef hands-on educational program*

The following students will be graduating from the MPS program in the Summer 2024 or the Fall 2024:

1. Lucia Gil (UM – Baker): *Utilizing media and video science communication to scale-up education and accessibility of novel coral restoration intervention techniques*
2. Mason Fitzgerald (SECORE – Miller): *Predation impacts on* Acropora cervicornis *from* Hermodice carunculata *and* Coralliophila abbreviata*: Does predator culling improve coral survivorship?*

The following students transferred to the MS program and will graduate in the Fall of 2024:

1. Bautista Tobias – (UM – Lirman): *Coral tissue production: the role of fragment size and grow-out location*
2. Erin Weisman – (UM – Lirman): *Developing stress-hardening techniques to increase efficacy of massive-coral restoration under high fish predation*

3. Cailyn Joseph – (UM – Baker): *Elucidating factors underlying variation in bleaching response on reefs in Miami-Dade County and the Upper Florida Keys during the 2023 marine heatwave*

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